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United States Environmental Protection Agency

TOXICOLOGICAL REVIEW

OF

1,4-DIOXANE

(WITH INHALATION UPDATE)

(CAS No. 123-91-1)

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

June 2013

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LIST OF ABBREVIATIONS AND ACRONYMS

AIC	Akaike's Information Criterion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
BMC	benchmark concentration
BMCL	benchmark concentration, lower 95% confidence limit
$BMCL_{10}$	benchmark concentration, lower 95% confidence limit at 10% extra risk
BMD	benchmark dose
BMD_{10}	benchmark dose at 10% extra risk
BMD_{30}	benchmark dose at 30% extra risk
BMD_{50}	benchmark dose at 50% extra risk
BMDL	benchmark dose, lower 95% confidence limit
$BMDL_{10}$	benchmark dose, lower 95% confidence limit at 10% extra risk
$BMDL_{30}$	benchmark dose, lower 95% confidence limit at 30% extra risk
$BMDL_{50}$	benchmark dose, lower 95% confidence limit at 50% extra risk
BMDS	Benchmark Dose Software
BMR	benchmark response
BrdU	5-bromo-2'-deoxyuridine
BUN	blood urea nitrogen
BW(s)	body weight(s)
CASE	computer automated structure evaluator
CASRN	Chemical Abstracts Service Registry Number
CFD	computational fluid dynamic
CHO	Chinese hamster ovary (cells)
CI	confidence interval(s)
CNS	central nervous system
CPK	creatinine phosphokinase
CREST	antikinetochore
CSF	cancer slope factor
CV	concentration in venous blood
CYP450	cytochrome P450
DEN	diethylnitrosamine
FISH	fluorescence in situ hybridization
G-6-Pase	glucose-6-phosphatase
GC	gas chromatography
GGT	γ-glutamyl transpeptidase
GST-P	glutathione S-transferase, placental form
HEAA	β-hydroxyethoxy acetic acid
HED(s)	human equivalent dose(s)
HPLC	high-performance liquid chromatography
HSDB	Hazardous Substances Data Bank
Hz	Hertz
IARC	International Agency for Research on Cancer
i.p.	intraperitoneal
i.v.	intravenous
IRIS	Integrated Risk Information System
JBRC	Japan Bioassay Research Center
k_e	1st order elimination rate of 1,4-dioxane
k_{INH}	1st order 1,4-dioxane inhalation rate constant
k_{LC}	1st order, non-saturable metabolism rate constant for 1,4-dioxane in the liver
K_m	Michaelis constant for metabolism of 1,4-dioxane in the liver
k_{me}	1st order elimination rate of HEAA (1,4-dioxane metabolite)
k_{OC}	soil organic carbon-water portioning coefficient
LAP	leucine aminopeptidase
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect-level
MCH	mean corpuscular hemoglobin
MCV	mean corpuscular volume
MOA	mode of action

MS	mass spectrometry, multi-stage
MTD	maximum tolerated dose
MVK	Moolgavkar-Venzon-Knudsen (model)
NCE	normochromatic erythrocyte
NCI	National Cancer Institute
ND	no data, not detected
NE	not estimated
NOAEL	no-observed-adverse-effect-level
NRC	National Research Council
NTP	National Toxicology Program
OCT	ornithine carbamyl transferase
ODC	ornithine decarboxylase
OECD	Organization for Economic Co-operation and Development
PB	blood:air partition coefficient
PBPK	physiologically based pharmacokinetic
PC	partition coefficient
PCB	polychlorinated biphenyl
PCE	polychromatic erythrocyte
PFA	fat:air partition coefficient
PLA	liver:air partition coefficient
POD	point of departure
ppm	parts per million
PRA	rapidly perfused tissue:air partition coefficient
PSA	slowly perfused tissue:air partition coefficient
QCC	normalized cardiac output
QPC	normalized alveolar ventilation rate
RBC	red blood cell
RfC	inhalation reference concentration
RfD	oral reference dose
SCE	sister chromatid exchange
SDH	sorbitol dehydrogenase
SMR	standardized mortality ratio
SRC	Syracuse Research Corporation
TPA	12-O-tetradecanoylphorbol-13-acetate
TWA	time-weighted average
UF	uncertainty factor
UNEP	United Nations Environment Programme
U.S.	United States of America
U.S. EPA	U.S. Environmental Protection Agency
V	volts
VAS	visual analogue scale
V_d	volume of distribution
V_{max}	maximal rate of metabolism
V_{maxC}	normalized maximal rate of metabolism of 1,4-dioxane in liver
VOC(s)	volatile organic compound(s)
WBC	white blood cell
χ^2	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 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 became available during the finalization of the 1,4-dioxane oral assessment that was posted on the IRIS database in 2010 (U.S. EPA, 2010a). In this version of the toxicological review, these studies have been incorporated into the previously posted assessment (U.S. EPA, 2010a). Although the focus of the most recent peer review was on the inhalation toxicity following exposure to 1,4-dioxane, a few comments were received on the oral assessment and were addressed to ensure scientific consistency between both routes of exposure. These comments did not impact the final conclusions of the oral assessment.

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The oral assessment was provided for review to other federal agencies and Executive Offices of the President. Comments were submitted by:

Department of Defense
National Aeronautics and Space Administration
Office of Management and Budget

The oral assessment was released for public comment in May 2009. A summary and EPA's disposition of the comments from the public is included in Appendix A and is also available on the IRIS Web site. Comments were received from the following entities:

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The assessment with the inhalation update was provided for review to other federal agencies and Executive Offices of the President. Comments were submitted by:

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National Aeronautics and Space Administration
National Toxicology Program, National Institutes for Environmental Health Sciences, National Institutes of Health, Department of Health & Human Services
Office of Management and Budget

The assessment with the inhalation update was released for public comment in September 2011 and comments were due on November 15, 2011. The public comments are available on the IRIS Web site. A summary and EPA's disposition of the comments from the public is included in Appendix A and is also available on the IRIS Web site. Comments were received from the following entities:

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1 INTRODUCTION

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

The RfD and RfC, if derived, provide quantitative information for use in risk assessments for health effects known or assumed to be produced through a nonlinear (presumed threshold) mode of action. The RfD (expressed in units of mg/kg-day) is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC (expressed in units of mg/m^3) is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). Reference values are generally derived for chronic exposures (up to a lifetime), but may also be derived for acute (≤ 24 hours), short-term (>24 hours up to 30 days), and subchronic (>30 days up to 10% of lifetime) exposure durations, all of which are derived based on an assumption of continuous exposure throughout the duration specified. Unless specified otherwise, the RfD and RfC are derived for chronic exposure duration.

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

Development of these hazard identification and dose-response assessments for 1,4-dioxane has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983). U.S. Environmental Protection Agency (U.S. EPA) Guidelines and Risk Assessment Forum technical panel reports that may have been used in the development of this assessment include the following *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986c), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986a), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994c), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994a), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2012a, 2000b), *Benchmark*

Dose Technical Guidance Document (U.S. EPA, 2012a, 2000a), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002b), *Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005a)*, *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005c), *Science Policy Council Handbook*: *Peer Review* (U.S. EPA, 2006b), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006c).

In 2010, an updated health assessment for oral exposures to 1,4-dioxane was released (U.S. EPA, 2010b). During the development of the 2010 health assessment, new studies (Kasai et al., 2009; Kasai et al., 2008) regarding the toxicity of 1,4-dioxane through the inhalation route of exposure became available that were not included in the 1,4-dioxane assessment that was posted on the IRIS database in 2010. These new inhalation studies have now been incorporated into the previously posted assessment and inhalation toxicity values are presented in this toxicological review.

The literature search strategy employed for 1,4-dioxane was initially based on the chemical name, Chemical Abstracts Service Registry Number (CASRN), and multiple common synonyms. A subsequent search was completed which focused on the toxicology and toxicokinetics of 1,4-dioxane, particularly as they pertain to target tissues, effects at low doses, mode of action (non-cancer and cancer), and sensitive populations. Following peer review of the assessment, a more targeted search was carried out based on comments received from expert peer reviewers. Additionally, any pertinent scientific information submitted by the public to the IRIS Submission Desk and by external peer reviewers during the Independent Expert Peer Review meeting was also considered in the development of this document.

Selection of studies for inclusion in the Toxicological Review was based on consideration of the extent to which the study was informative and relevant to the assessment, and general study considerations as outlined in EPA guidance documents (*A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002c) and *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhaled Dosimetry* (U.S. EPA, 1994b)).

Primary, peer-reviewed-literature was reviewed through September 2009 for the oral assessment and through April 2013 for the inhalation assessment and was included where the literature was determined to be critical to the assessment. The relevant literature included publications on 1,4-dioxane which were identified through Toxicology Literature Online (TOXLINE), PubMed, the Toxic Substance Control Act Test Submission Database (TSCATS), the Registry of Toxic Effects of Chemical Substances (RTECS), the Chemical Carcinogenesis Research Information System (CCRIS), the Developmental and Reproductive Toxicology/Environmental Teratology Information Center (DART/ETIC), the Environmental Mutagens Information Center (EMIC) and Environmental Mutagen Information Center Backfile (EMICBACK) databases, the Hazardous Substances Data Bank (HSDB), the Genetic Toxicology Data Bank (GENE-TOX), Chemical abstracts, and Current Contents. Other peer-reviewed information, including health assessments developed by other organizations, review articles, and independent analyses of the health effects data were retrieved and may be included in the assessment where appropriate. Studies that had not been peer-reviewed, were not included in the assessment.

The references considered and cited in this document, including bibliographic information and abstracts, can be found on the Health and Environmental Research Online (HERO) website[1] (http://hero.epa.gov). 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.

[1]HERO is a database of scientific studies and other references used to develop EPA's risk assessments aimed at understanding the health and environmental effects of pollutants and chemicals. It is developed and managed in EPA's Office of Research and Development (ORD) by the National Center for Environmental Assessment (NCEA). The database includes more than 400,000 scientific articles from the peer-reviewed literature. New studies are added continuously to HERO.

2 CHEMICAL AND PHYSICAL INFORMATION

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

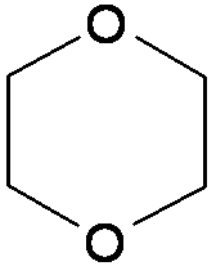


Figure 2-1 1,4-Dioxane chemical structure.

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

CASRN:	123-91-1 (CRC Handbook (Lide, 2000))
Molecular weight:	88.10 (Merck Index (2001))
Chemical formula:	$C_4H_8O_2$ (Merck Index (2001))
Boiling point:	101.1°C (Merck Index (2001))
Melting point:	11.8°C (CRC Handbook (Lide, 2000))
Vapor pressure:	40 mmHg at 25°C (Lewis, 2000)
Density:	1.0337 g/mL at 20°C (CRC Handbook (Lide, 2000))
Vapor density:	3.03 (air = 1) (Lewis, 2000)
Water solubility:	Miscible with water (Hawley and Lewis, 2001)
Other solubilities:	Miscible with ethanol, ether, acetone (CRC Handbook (Lide, 2000))
Log K_{ow}:	–0.27 (Hansch et al., 1995)
Henry's Law constant:	4.80×10^{-6} atm-m^3/molecule at 25°C (Park et al., 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 Handbook (Lyman et al., 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)

1,4-Dioxane is produced commercially through the dehydration and ring closure of diethylene glycol (Surprenant, 2002). Concentrated sulfuric acid is used as a catalyst (Surprenant, 2002). This is a continuous distillation process with operating temperatures and pressures of 130–200°C and 188–825 mmHg, respectively (Surprenant, 2002). During the years 1986 and 1990, the U.S. production of

1,4-dioxane reported by manufacturers was within the range of 10–50 million pounds (U.S. EPA, 2002a). The production volume reported during the years 1994, 1998, and 2002 was within the range of 1–10 million pounds (U.S. EPA, 2002a).

Historically, 1,4-dioxane has been used as a stabilizer for the solvent 1,1,1-trichloro-ethane (Surprenant, 2002). However, this use is no longer expected to be important due to the 1990 Amendments to the Clean Air Act and the Montreal Protocol, which mandate the eventual phase-out of 1,1,1-trichloroethane production in the U.S. (ATSDR, 2007; U.N. Environment Programme, 2000; "Amendments to the Clean Air Act. Sec. 604. Phase-out of production and consumption of class I substances," 1990). 1,4-Dioxane is a contaminant of some ingredients used in the manufacture of personal care products and cosmetics. 1,4-Dioxane is also used as a solvent for cellulosics, organic products, lacquers, paints, varnishes, paint and varnish removers, resins, oils, waxes, dyes, cements, fumigants, emulsions, and polishing compositions (Hawley and Lewis, 2001; 2001; IARC, 1999). 1,4-Dioxane has been used as a solvent in the formulation of inks, coatings, and adhesives and in the extraction of animal and vegetable oil (Surprenant, 2002). Reaction products of 1,4-dioxane are used in the manufacture of insecticides, herbicides, plasticizers, and monomers (Surprenant, 2002).

When 1,4-dioxane enters the air, it will exist as a vapor, as indicated by its vapor pressure (HSDB, 2007). It is expected to be degraded in the atmosphere through photooxidation with hydroxyl radicals (HSDB, 2007; Surprenant, 2002). The estimated half-life for this reaction is 6.7 hours (HSDB, 2007). It may also be broken down by reaction with nitrate radicals, although this removal process is not expected to compete with hydroxyl radical photooxidation (Grosjean, 1990). 1,4-Dioxane is not expected to undergo direct photolysis (Wolfe and Jeffers, 2000). 1,4-Dioxane is primarily photooxidized to 2-oxodioxane and through reactions with nitrogen oxides (NO_X) results in the formation of ethylene glycol diformate (Platz et al., 1997). 1,4-Dioxane is expected to be highly mobile in soil based on its estimated K_{oc} and is expected to leach to lower soil horizons and groundwater (ATSDR, 2007; Lyman et al., 1990). This substance may volatilize from dry soil surfaces based on its vapor pressure (HSDB, 2007). The estimated bioconcentration factor value indicates that 1,4-dioxane will not bioconcentrate in aquatic or marine organisms (Meylan et al., 1999; Franke et al., 1994). 1,4-Dioxane is not expected to undergo hydrolysis or to biodegrade readily in the environment (ATSDR, 2007; HSDB, 2007). Therefore, volatilization is expected to be the dominant removal process for moist soil and surface water. Based on a Henry's Law constant of 4.8×10^{-6} atm-m^3/mole, the half-life for volatilization of 1,4-dioxane from a model river is 5 days and that from a model lake is 56 days (HSDB, 2007; Lyman et al., 1990; Park et al., 1987). 1,4-Dioxane may be more persistent in groundwater where volatilization is hindered.

Recent environmental monitoring data for 1,4-dioxane are lacking. Existing data indicate that 1,4-dioxane may leach from hazardous waste sites into drinking water sources located nearby (Yasuhara et al., 2003; Yasuhara et al., 1997; Lesage et al., 1990). 1,4-Dioxane has been detected in contaminated surface and groundwater samples collected near hazardous waste sites and industrial facilities (Derosa et al., 1996).

3 TOXICOKINETICS

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

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

3.1 Absorption

Absorption of 1,4-dioxane following inhalation exposure has been qualitatively demonstrated in workers and volunteers. Workers exposed to a time-weighted average (TWA) of 1.6 parts per million (ppm) of 1,4-dioxane in air for 7.5 hours showed a HEAA/1,4-dioxane ratio of 118:1 in urine (Young et al., 1976a). The authors assumed lung absorption to be 100% and calculated an average absorbed dose of 0.37 mg/kg, although no exhaled breath measurements were taken. In a study with four healthy male volunteers, Young et al. (1977a) reported 6-hour inhalation exposures of adult volunteers to 50 ppm of 1,4-dioxane in a chamber, followed by blood and urine analysis for 1,4-dioxane and HEAA. The study protocol was approved by a seven-member Human Research Review Committee of the Dow Chemical Company, and written informed consent of study participants was obtained. At a concentration of 50 ppm, uptake of 1,4-dioxane into plasma was rapid and approached steady-state conditions by 6 hours. The authors reported a calculated absorbed dose of 5.4 mg/kg. However, the exposure chamber atmosphere was kept at a constant concentration of 50 ppm and exhaled breath was not analyzed. Accordingly, gas uptake could not be measured. As a result, the absorbed fraction of inhaled 1,4-dioxane could not be accurately determined in humans. Rats inhaling 50 ppm for 6 hours exhibited 1,4-dioxane and HEAA in urine with an HEAA to 1,4-dioxane ratio of over 3,100:1 (Young et al., 1978b; 1978a). Plasma concentrations at the end of the 6-hour exposure period averaged 7.3 µg/mL. The authors calculated an absorbed 1,4-dioxane dose of 71.9 mg/kg; however, the lack of exhaled breath data and dynamic exposure chamber precluded the accurate determination of the absorbed fraction of inhaled 1,4-dioxane.

No human data are available to evaluate the oral absorption of 1,4-dioxane. Gastrointestinal absorption was nearly complete in male Sprague Dawley rats orally dosed with 10–1,000 mg/kg of [^{14}C]-1,4-dioxane given as a single dose or as 17 consecutive daily doses (Young et al., 1978b; 1978a). Cumulative recovery of radiolabel in the feces was <1–2% of administered dose regardless of dose level or frequency.

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

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

3.2 Distribution

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

Mikheev et al. (1990) studied the distribution of [^{14}C]-1,4-dioxane in the blood, liver, kidney, brain, and testes of rats (strain not reported) for up to 6 hours following intraperitoneal (i.p.) injection of approximately one-tenth the median lethal dose (LD_{50}) (actual dose not reported). While actual tissue concentrations were not reported, tissue:blood ratios were given for each tissue at six time points ranging from 5 minutes to 6 hours. The time to reach maximum accumulation of radiolabel was shorter for liver and kidney than for blood or the other tissues, which the authors suggested was indicative of selective membrane transport. Tissue:blood ratios were less than one for all tissues except testes, which had a ratio greater than one at the 6-hour time point. The significance of these findings is questionable since the contribution of residual blood in the tissues was unknown (though saline perfusion may serve to clear tissues of highly water-soluble 1,4-dioxane), the tissue concentrations of radiolabel were not reported, and data were collected from so few time points.

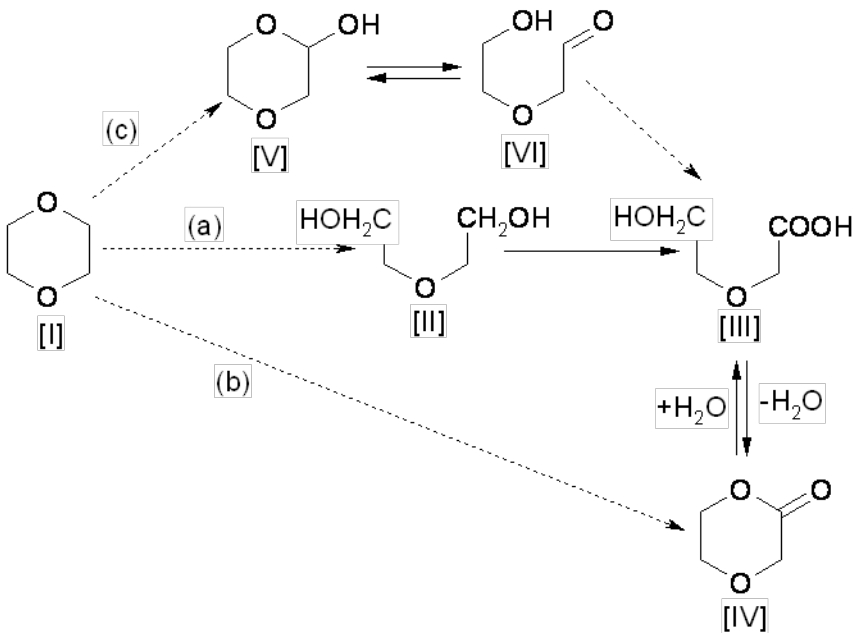
Woo et al. (1977a) administered i.p. doses of [^{3}H]-1,4-dioxane (5 mCi/kg body weight [BW]) to male Sprague Dawley rats with and without pretreatment using mixed-function oxidase inducers (phenobarbital, 3-methylcholanthrene, or polychlorinated biphenyls [PCBs]). Liver, kidney, spleen, lung, colon, and skeletal muscle tissues were collected from 1, 2, 6, and 12 hours after dosing. Distribution was generally uniform across tissues, with blood concentrations higher than tissues at all times except for 1 hour post dosing, when kidney levels were approximately 20% higher than blood. Since tissues were not perfused prior to analysis, the contribution of residual blood to radiolabel measurements is unknown, though loss of 1,4-dioxane from tissues would be unknown had saline perfusion been performed. Covalent binding determined by gas chromatography reached peak percentages at 6 hours after dosing in liver (18.5%), spleen (22.6%), and colon (19.5%). At 16 hours after dosing, peak covalent binding percentages were observed in whole blood (3.1%), kidney (9.5%), lung (11.2%), and skeletal muscle

(11.2%). Within hepatocytes, radiolabel distribution at 6 hours after dosing was greatest in the cytosolic fraction (43.8%) followed by the microsomal (27.9%), mitochondrial (16.6%), and nuclear (11.7%) fractions. While little covalent binding of radiolabel was measured in the hepatic cytosol (4.6%), greater binding was observed at 16 hours after dosing in the nuclear (64.8%), mitochondrial (45.7%), and microsomal (33.4%) fractions. Pretreatment with inducers of mixed-function oxidase activity did not significantly change the extent of covalent binding in subcellular fractions.

3.3 Metabolism

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

A proposed metabolic scheme for 1,4-dioxane metabolism (Woo et al., 1977a) in Sprague Dawley rats is shown in Figure 3-1. Oxidation of 1,4-dioxane to diethylene glycol (pathway a), 1,4-dioxane-2-ol (pathway c), or directly to 1,4-dioxane-2-one (pathway b) could result in the production of HEAA. 1,4-Dioxane oxidation appears to be cytochrome P450 (CYP450)-mediated, as CYP450 induction with phenobarbital or Aroclor 1254 (a commercial PCB mixture) and suppression with 2,4-dichloro-6-phenylphenoxy ethylamine or cobaltous chloride were effective in significantly increasing and decreasing, respectively, the appearance of HEAA in the urine of male Sprague Dawley rats following 3 g/kg i.p. dose (Woo et al., 1978, 1977b). 1,4-Dioxane itself induced CYP450-mediated metabolism of several barbiturates in Hindustan mice given i.p. injections of 25 and 50 mg/kg 1,4-dioxane (Mungikar and Pawar, 1978). Of the three possible pathways proposed in this scheme, oxidation to diethylene glycol and HEAA appears to be the most likely, because diethylene glycol was found as a minor metabolite in Sprague Dawley rat urine following a single 1,000 mg/kg gavage dose of 1,4-dioxane (Braun and Young, 1977). Additionally, i.p. injection of 100–400 mg/kg diethylene glycol in Sprague Dawley rats resulted in urinary elimination of HEAA (Woo et al., 1977c).



Source: Adapted with permission of Elsevier Ltd., Woo et al. (1977a; 1977b).

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

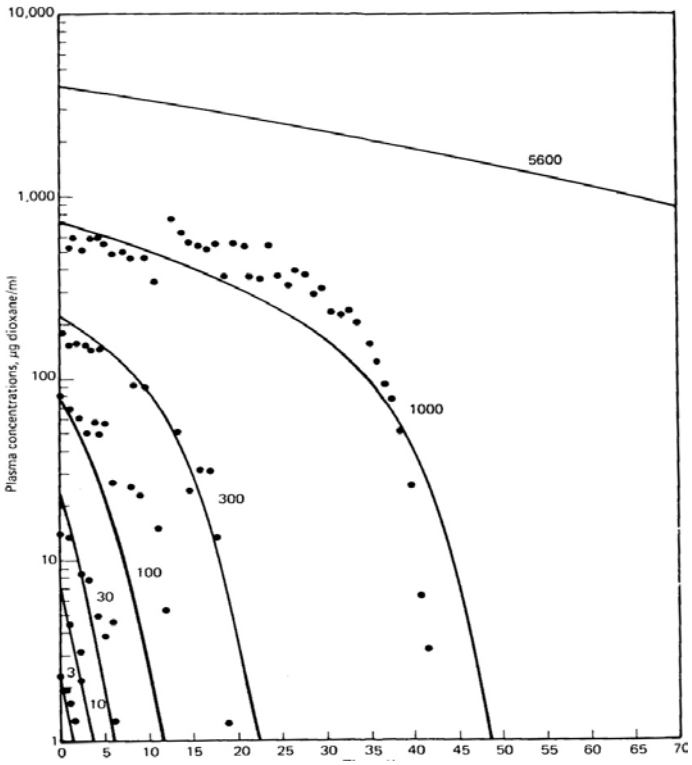
Legend: I = 1,4-dioxane; II = diethylene glycol; III = β-hydroxyethoxy acetic acid (HEAA); IV = 1,4-dioxane-2-one; V = 1,4-dioxane-2-ol; VI = β-hydroxyethoxy acetaldehyde. Note: Metabolite [V] is a likely intermediate in pathway b as well as pathway c. The proposed pathways are based on the metabolites 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 (CO_2) identified in expired air after labeled 1,4-dioxane exposure.

Metabolism of 1,4-dioxane in humans is extensive. In a survey of five 1,4-dioxane plant workers exposed to a TWA of 1.6 ppm of 1,4-dioxane for 7.5 hours, Young et al. (1976a) found HEAA and 1,4-dioxane in the worker's urine at a ratio of 118:1. Similarly, in adult male volunteers exposed to 50 ppm for 6 hours (Young et al., 1977a), over 99% of inhaled 1,4-dioxane (assuming negligible exhaled excretion) appeared in the urine as HEAA. The linear elimination of 1,4-dioxane in both plasma and urine indicated that 1,4-dioxane metabolism was a nonsaturated, first-order process at this exposure level.

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

increased from <1 to 25% as dose increased from 10 to 1,000 mg/kg. Likewise, with multiple daily doses 10 or 1,000 mg [^{14}C]-1,4-dioxane/kg, urinary radiolabel decreased from 99 to 82% and expired 1,4-dioxane increased from 1 to 9% as dose increased. The differences between single and multiple doses in urinary and expired radiolabel support the notion that 1,4-dioxane may induce its own metabolism.

Induction of 1,4-dioxane metabolism was evaluated in a 13 week inhalation study by Kasai et al. (2008). In this study, male and female F344 rats were exposed daily to concentrations of 0 (control), 100, 200, 400, 1,600, and 3,200 ppm. Plasma levels of 1,4-dioxane linearly increased with increasing inhalation concentration, suggesting that metabolic saturation was not achieved during the course of the experiments for plasma levels up to 730 and 1,054 µg/mL in male and female rats, respectively, at the highest exposure concentration (3,200 ppm). In contrast, Young et al. (1978b) estimated from experimentally determined Km values that metabolic saturation occurred near plasma levels of 100 µg/mL. Kociba et al. (1975b) also estimated metabolic saturation near plasma levels of 100 µg/mL in rats following a single i.v. dose. The lack of the metabolic saturation of 1,4-dioxane found in the Kasai et al. (2008) study is likely attributed to enhanced metabolism by the induction of P450 enzymes, including CYP2E1, by 13 weeks of repeated inhalation exposure to 1,4-dioxane at concentrations up to 3,200 ppm (Kasai et al., 2008).



Source: Reprinted with permission of Taylor and Francis, Young et al. (1978b).

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 ×-axis is time (hr)]

1,4-Dioxane has been shown to induce several isoforms of CYP450 in various tissues following acute oral administration by gavage or drinking water (Nannelli et al., 2005a). Male Sprague Dawley rats were exposed to either 2,000 mg/kg 1,4-dioxane via gavage for 2 consecutive days or by ingestion of a 1.5% 1,4-dioxane drinking water solution for 10 days. Both exposures resulted in significantly increased CYP2B1/2, CYP2C11, and CYP2E1 activities in hepatic microsomes. The gavage exposure alone resulted in increased CYP3A activity. Takano et al. (2010) recently tested liver microsome contents from male Sprague-Dawley rats treated with 500 mg 1,4-dioxane/kg BW intraperitoneally (i.p.) for 3 days for CYP450 activities. CYP2B and CYP2E activities were significantly increased ($p < 0.05$) compared to control activity levels, while CYP2C activity was significantly decreased to approximately 50% of control values. This is in contrast to Nannelli et al. (2005b) where CYP2C values increased.

The increase in CYP2C or specifically, CYP2C11 activity reported by Nanelli et al. (2005b) was unexpected, as that isoform has been observed to be under hormonal control and was typically suppressed in the presence of 2B1/2 and 2E1 induction. In the male rat, hepatic 2C11 induction is associated with masculine pulsatile plasma profiles of growth hormone (compared to the constant plasma levels in the female), resulting in masculinization of hepatocyte function (Waxman et al., 1991). The authors postulated that 1,4-dioxane may alter plasma growth hormone levels, resulting in the observed 2C11 induction. However, growth hormone induction of 2C11 is primarily dependent on the duration between growth hormone pulses and secondarily on growth hormone plasma levels (Agrawal and Shapiro, 2000; Waxman et al., 1991). Thus, the induction of 2C11 by 1,4-dioxane may be mediated by changes in the time interval between growth hormone pulses rather than changes in growth hormone levels. This may be accomplished by 1,4-dioxane temporarily influencing the presence of growth hormone cell surface binding sites (Agrawal and Shapiro, 2000). However, no studies are available to confirm the influence of 1,4-dioxane on either growth hormone levels or changes in growth hormone pulse interval.

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

3.4 Elimination

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

Elimination of 1,4-dioxane in rats (Young et al., 1978b; 1978a). was primarily via urine. As comparably assessed in humans, the elimination half-life in rats exposed to 50 ppm 1,4-dioxane for 6 hours was calculated to be 1.01 hours. In Sprague Dawley rats given single daily doses of 10, 100, or 1,000 mg [^{14}C]-1,4-dioxane/kg or multiple doses of 10 or 1,000 mg [^{14}C]-1,4-dioxane/kg, urinary radiolabel ranged from 99% down to 76% of total radiolabel. Fecal elimination was less than 2% for all doses. The effect of saturable metabolism on expired 1,4-dioxane was apparent, as expired 1,4-dioxane in singly dosed rats increased with dose from 0.4 to 25% while expired $^{14}CO_2$ changed little (between 2 and 3%) across doses. The same relationship was seen in Sprague Dawley rats dosed i.v. with 10 or 1,000 mg [^{14}C]-1,4-dioxane/kg. Higher levels of $^{14}CO_2$ relative to 1,4-dioxane were measured in expired air of the 10 mg/kg group, while higher levels of expired 1,4-dioxane relative to $^{14}CO_2$ were measured in the 1,000 mg/kg group.

3.5 Physiologically Based Pharmacokinetic Models

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

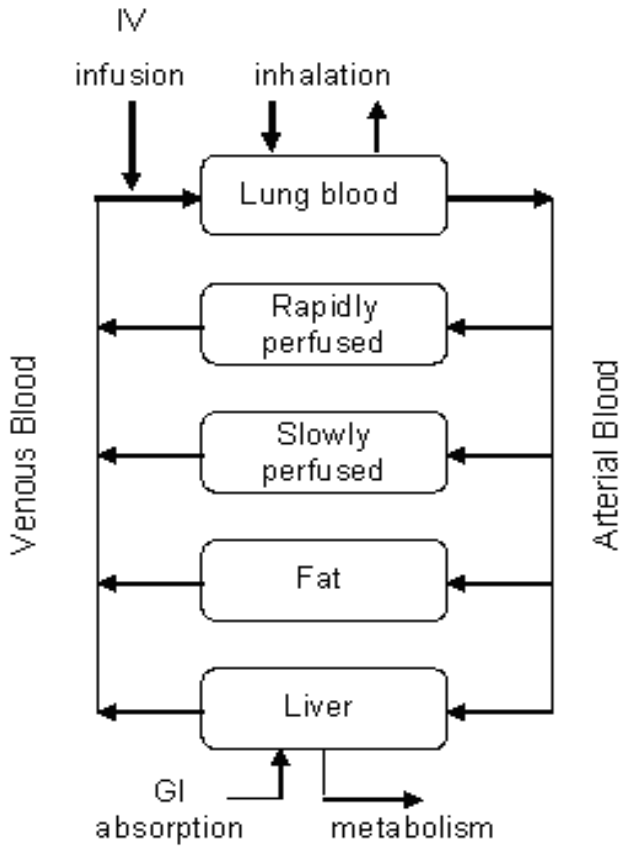


Figure 3-3 General PBPK model structure.

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

Animal and human data sets available for model calibration derive from Young et al. (1978b; 1978a; 1977a), Mikheev et al. (1990), and Woo et al. (1977a; 1977c). Young et al. (1978b; 1978a) studied the disposition of radiolabeled [^{14}C]-1,4-dioxane in adult male Sprague Dawley rats following i.v., inhalation, and single and multiple oral gavage exposures. Plasma concentration-time profiles were reported for i.v. doses of 3, 10, 30, 100, and 1,000 mg/kg. In addition, exhaled $^{14}CO_2$ and urinary 1,4-dioxane and HEAA profiles were reported following i.v. doses of 10 and 1,000 mg/kg. The plasma 1,4-dioxane concentration-time course, cumulative urinary 1,4-dioxane and cumulative urinary HEAA concentrations were reported following a 6-hour inhalation exposure to 50 ppm. Following oral gavage doses of 10–1,000 mg/kg, percentages of total orally administered radiolabel were measured in urine, feces, expired air, and the whole body.

Oral absorption of 1,4-dioxane was extensive, as only approximately 1% of the administered dose appeared in the feces within 72 hours of dosing (Young et al., 1978b; 1978a). Although it may be

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

Saturable metabolism of 1,4-dioxane was observed in rats exposed by either the i.v. or oral routes (Young et al., 1978b; 1978a), and metabolic induction was observed following exposure to high oral daily doses (1,000 mg/kg-day) of 1,4-dioxane. Elimination of 1,4-dioxane from plasma appeared to be linear following i.v. doses of 3-30 mg/kg, but was nonlinear following doses of 100–1,000 mg/kg. Accordingly, 10 mg/kg i.v. doses resulted in higher concentrations of $^{14}CO_2$ (from metabolized 1,4-dioxane) in expired air relative to unchanged 1,4-dioxane, while 1,000 mg/kg i.v. doses resulted in higher concentrations of expired 1,4-dioxane relative to $^{14}CO_2$. Thus, at higher i.v. doses, a higher proportion of unmetabolized 1,4-dioxane is available for exhalation. Taken together, the i.v. plasma and expired air data from Young et al. (1978b; 1978a) corroborate previous studies describing the saturable nature of 1,4-dioxane metabolism in rats (1977a; Woo et al., 1977c) and are useful for optimizing metabolic parameters (V_{max} and K_m) in a PBPK model.

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

Post-exposure plasma data from a single 6-hour, 50 ppm inhalation exposure in rats were reported (Young et al., 1978b; 1978a). The observed linear elimination of 1,4-dioxane after inhalation exposure suggests that, via this route, metabolism follows a first-order process at this exposure level.

The only human data adequate for use in PBPK model development (Young et al., 1977a) come from adult male volunteers exposed to 50 ppm 1,4-dioxane for 6 hours. Plasma 1,4-dioxane and HEAA concentrations were measured both during and after the exposure period, and urine concentrations were measured following exposure. Plasma levels of 1,4-dioxane approached steady-state at 6 hours. HEAA data were insufficient to describe the appearance or elimination of HEAA in plasma. Data on elimination of 1,4-dioxane and HEAA in the urine up to 24 hours from the beginning of exposure were reported. At 6 hours from onset of exposure, approximately 90% and 47% of the cumulative (0–24 hours) urinary 1,4-dioxane and HEAA, respectively, were measured in the urine. The ratio of HEAA to 1,4-dioxane in urine 24 hours after onset of exposure was 192:1 (similar to the ratio of 118:1 observed by Young et al. (1976a) in workers exposed to 1.6 ppm for 7.5 hours), indicating extensive metabolism of 1,4-dioxane. As with Sprague Dawley rats, the elimination of 1,4-dioxane from plasma was linear across all observations (6 hours following end of exposure), suggesting that human metabolism of 1,4-dioxane is linear for a 50 ppm inhalation exposure to steady-state. Thus, estimation of human V_{max} and K_m from these data will introduce uncertainty into internal dosimetry performed in the nonlinear region of metabolism.

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

3.5.2 Published PBPK Models for 1,4-Dioxane

3.5.2.1 Leung and Paustenbach

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

The parameter values for hepatic metabolism of 1,4-dioxane, V_{max} and K_m, were optimized and validated against plasma and/or urine time course data for 1,4-dioxane and HEAA in rats following i.v. and inhalation exposures and humans following inhalation exposure (Young et al. (1978b; 1978a; 1977a)); the exact data (i.e., i.v., inhalation, or both) used for the optimization and calibration were not reported. Although the liver and fat were represented by tissue-specific compartments, no tissue-specific concentration data were available for model development, raising uncertainty as the model's ability to adequately predict exposure to these tissues. The human inhalation exposure of 50 ppm for 6 hours

(Young et al., 1977a) was reported to be in the linear range for metabolism; thus, uncertainty exists in the ability of the allometrically-scaled value for the human metabolic V_{max} to accurately describe 1,4-dioxane metabolism from exposures resulting in metabolic saturation. Nevertheless, these values resulted in the model producing good fits to the data. For rats, the values for V_{max} had to be adjusted upwards by a factor of 1.8 to reasonably simulate exposures greater than 300 mg/kg. The model authors attributed this to metabolic enzyme induction by high doses of 1,4-dioxane.

3.5.2.2 Reitz et al.

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

Alveolar blood levels of 1,4-dioxane were assumed to be in equilibrium with alveolar air at a ratio equal to the experimentally measured blood:air partition coefficient. Transfers of 1,4-dioxane between blood and tissues were assumed to be blood flow-limited and to achieve rapid equilibrium between blood and tissue, governed by tissue:blood equilibrium partition coefficients. These coefficients were derived by dividing experimentally measured (Leung and Paustenbach, 1990a) in vitro blood:air and tissue:air partition coefficients for blood, liver, fat. Blood:air partition coefficients were measured for both humans and rats. The mouse blood:air partition coefficient was different from rat or human values; the source of the partition coefficient for blood in mice was not reported. Rat tissue:air partition coefficients were used as surrogate values for humans. Rat tissue partition coefficient values were the same values as used in the Leung and Paustenbach (1990a) model (with the exception of slowly perfused tissues) and were used in the models for all three species. The liver value was used for the rapidly perfused tissues, as well as slowly perfused tissues. Although slowly perfused tissue:air partition coefficients for rats were measured, the authors suggested that 1,4-dioxane in the muscle and air may not have reached equilibrium in the highly gelatinous tissue homogenate (Reitz et al., 1990a). Substitution of the liver value provided much closer agreement to the plasma data than when the muscle value was used. Further, doubling of the measured human blood:air partition coefficient improved the fit of the model to the human blood level data compared to the fit resulting from the measured value (Reitz et al., 1990a). The Reitz et al. (1990a) model simulated three routes of 1,4-dioxane elimination: pulmonary exhalation, hepatic metabolism to HEAA, and urinary excretion of HEAA. The elimination of HEAA was modeled as a first-order transfer of 1,4-dioxane metabolite to urine.

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

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

3.5.2.3 Fisher et al.

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

3.5.2.4 Sweeney et al.

The Sweeney et al. (2008a) model consisted of fat, liver, slowly perfused, and other well perfused tissue compartments. Lung and stomach compartments were used to describe the route of exposure, and an overall volume of distribution compartment was used for calculation of urinary excretion levels of 1,4-dioxane and HEAA. Blood, saline, and tissue to air partition coefficient values for 1,4-dioxane were experimentally determined for rats and mice. Average values of the rat and mouse partition coefficients were used for humans. Metabolic constants (VmaxC and Km) for the rat were derived by optimization of data from an i.v. exposure of 1,000 mg/kg (Young et al., 1978b) for inducible metabolism. For uninduced VmaxC estimation, data generated by i.v. exposures to 3, 10, 30, and 100 mg/kg were used (Young et al., 1978b). Sweeney et al. (2008a) determined best fit values for VmaxC by fitting to blood data in Young et al. (1978b). The best fit VmaxC values were 7.5, 10.8, and 12.7 mg/hr-kg$^{0.75}$ for i.v. doses of 3 to 100, 300, and 1,000 mg/kg, suggesting a gradual dose dependent increase in metabolic rate over i.v. doses ranging from 3 to 1,000 mg/kg. Although the Sweeney et al. (2008a) model utilized two values for VmaxC (induced and uninduced), the PBPK model does not include a dose-dependent function

description of the change of Vmax for i.v. doses between metabolic induced and uninduced exposures. Mouse VmaxC and absorption constants were derived by optimizing fits to the blood 1,4-dioxane concentrations in mice administered nominal doses of 200 and 2,000 mg/kg 1,4-dioxane via gavage in a water vehicle (Young et al., 1978b). The in vitro Vmax values for rats and mice determined by Sweeney et al. (2008a) were scaled to estimate in vivo rates. The scaled and optimized rat VmaxC values were similar. The discrepancy between the scaled and optimized mouse values was larger, which was attributed to possible induction in mice at the lowest dose tested (200 mg/kg). The ratio of optimized/scaled values for the rat was used to adjust the scaled human VmaxC and Km values to projected in vivo values.

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

3.5.2.5 Takano et al.

More recently, Takano et al. (2010) reported the development of a simplified rat and human pharmacokinetic model. The purpose of this model was to provide a platform for a forward dosimetry calculation using in vivo animal data and in vitro human and animal microsome data to predict the 1,4-dioxane concentrations in humans. The model had three nonphysiological compartments: absorption compartment, metabolizing compartment, and a central compartment. Human metabolic parameters were determined from in vitro data using liver microsomes, coefficients (octanol-water partition coefficient, plasma unbound fraction) derived in silico, and physiological parameters (e..g, hepatic volume and blood flow rate) obtained from the literature. Clearance was described as a first order rate of metabolism from both the metabolizing compartment (e.g. hepatic metabolism) and the central compartment (e.g., renal clearance). This is in contrast to the saturable metabolism used in previous models (Sweeney et al., 2008b; Reitz et al., 1990b).

The rat model outputs of Takano et al. (2010) were compared with 1,4-dioxane blood data at the end of exposure in rats treated for 14 days with an oral dose of 500 mg/kg. The model adequately predicted these rat data and showed a minimal amount of 1,4-dioxane remained in the blood 24hrs after the last exposure. The authors performed an in vitro to in vivo extrapolation to estimate human hepatic intrinsic clearance for the human pharmacokinetic model. The ratio of rat in vivo/in vitro measurements

(0.0244/0.313) was multiplied by the human in vitro determination (22.9 L/h) to yield 1.76 L/h used in the human pharmacokinetic model. The model was then used to simulate hypothetical human exposures; however, no data were compared with model outputs. Thus, the ability of this model to adequately simulate the available human data is unknown.

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

As previously described, several pharmacokinetic models have been developed to predict the absorption, distribution, metabolism, and elimination of 1,4-dioxane in rats and humans. Single compartment, empirical models for rats (Young et al., 1978b; 1978a) and humans (Young et al., 1977a) were developed to predict blood levels of 1,4-dioxane and urine levels of the primary metabolite, HEAA. PBPK models that describe the kinetics of 1,4-dioxane using biologically realistic flow rates, tissue volumes, enzyme affinities, metabolic processes, and elimination behaviors were also developed (Sweeney et al., 2008a; Fisher et al., 1997; Leung and Paustenbach, 1990a; Reitz et al., 1990a). Most recently, Takano et al. (2010) published a pharmacokinetic model utilizing hepatic volume, blood flow, and an in vitro to in vivo extrapolation method for human intrinsic hepatic clearance.

In developing updated toxicity values for 1,4-dioxane the available PBPK models were evaluated for their ability to predict observations made in experimental studies of rat and human exposures to 1,4-dioxane (Appendix B). The Reitz et al. (1990a) and Leung and Paustenbach (1990a) PBPK models were both developed from a PBPK model of styrene (Ramsey and Andersen, 1984), with the exception of minor differences in the use of partition coefficients and biological parameters. The model code for Leung and Paustenbach (1990a) was unavailable in contrast to Reitz et al. (1990a). The model of Reitz et al. (1990a) was identified for further consideration to assist in the derivation of toxicity values, and the Sweeney et al. (2008a) and Takano et al. (2010) models were also evaluated.

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

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

The evaluation consisted of implementation of the Young et al. (1978b; 1978a; 1977a) empirical rat and human models using the acslXtreme simulation software, re-calibration of the Reitz et al. (1990a) human PBPK model, and evaluation of the model parameters published by Sweeney et al. (2008a). Using the model descriptions and equations given in Young et al. (1978b; 1978a; 1977a), model code was developed for the empirical models and executed, simulating the reported experimental conditions. The model output was then compared with the model output reported in Young et al. (1978b; 1978a; 1977a).

The PBPK model of Reitz et al. (1990a) was re-calibrated using measured values for cardiac and alveolar flow rates and tissue:air partition coefficients. The predictions of blood and urine levels of 1,4-dioxane and HEAA, respectively, from the re-calibrated model were compared with the empirical model predictions of the same dosimeters to determine whether the re-calibrated PBPK model could perform similarly to the empirical model. As part of the PBPK model evaluation, EPA performed a sensitivity analysis to identify the model parameters having the greatest influence on the primary dosimeter of interest, the blood level of 1,4-dioxane. Variability data for the experimental measurements of the tissue:air partition coefficients were incorporated to determine a range of model outputs bounded by biologically plausible values for these parameters. Model parameters from Sweeney et al. (2008a) were also tested to evaluate the ability of the PBPK model to predict human data following exposure to 1,4-dioxane.

The rat and human empirical models of Young et al. (1978c, d; 1977b) were successfully implemented in acslX and perform identically to the models reported in the published papers (Figures B-3, B-4, B-5, B-7, and B-8), with the exception of the lower predicted HEAA concentrations and early appearance of the peak HEAA levels in rat urine. The early appearance of peak HEAA levels cannot presently be explained, but may result from manipulations of k_{me} or other parameters by Young et al. (1978c, d) that were not reported. The lower predictions of HEAA levels are likely due to reliance on a standard urine volume production rate in the absence of measured (but unreported) urine volumes. While the human urinary HEAA predictions were closer to the observed data of Young et al. (1977b), no model output was published in Young et al. (1977b) for comparison. The empirical models were modified to allow for user-defined inhalation exposure levels; however, they were not modified to describe oral exposures due to a lack of adequate human or animal data for parameterization. Additionally, the inhalation Young et al. (1977b) model did not provide adequate fits to the subchronic exposure plasma levels of 1,4-dioxane in rats using the data from the Kasai et al. (2008) study, which is likely due to the absence of a model description for metabolic induction.

Several procedures were applied to the human PBPK model to determine if an adequate fit of the model to the empirical model output or experimental observations could be attained using biologically plausible values for the model parameters. The re-calibrated model predictions for blood 1,4-dioxane did not adequately fit the experimental values using measured tissue:air partition coefficients from Leung and Paustenbach (1990b) or Sweeney et al. (2008b) (Figure B-6 and Figure B-7). Use of a slowly perfused

tissue:air partition coefficient 4- to 7-fold lower than measured values produces exposure-phase predictions that are much closer to observations, but does not replicate the elimination kinetics (Figure B-8). Re-calibration of the model with upper bounds on the tissue:air partition coefficients results in predictions that are still 2- to 4-fold lower than empirical model prediction or observations (Figure B-10 and Figure B-11). Exploration of the model space using an assumption of first-order metabolism (valid for the 50-ppm inhalation exposure) showed that an adequate fit to the exposure and elimination data can be achieved only when unrealistically low values are assumed for the slowly perfused tissue:air partition coefficient (Figure B-14). Artificially low values for the other tissue:air partition coefficients are not expected to improve the model fit, because blood 1,4-dioxane is less sensitive to these parameters than it is to V_{maxC} and K_m. This suggests that the model structure is insufficient to capture the apparent species difference in the blood 1,4-dioxane V_d between rats and humans. Differences in the ability of rat and human blood to bind 1,4-dioxane may contribute to the difference in V_d. However, this is expected to be evident in very different values for rat and human blood:air partition coefficients, which is not the case (). Additionally, the models do not account for induction in metabolism, which may be present in animals repeatedly exposed to 1,4-dioxane. Therefore, some other modification(s) to the Reitz et al. (1990b) model structure may be necessary.

Similarly, Sweeney et al. (2008a) also evaluated the available PBPK models (Leung and Paustenbach, 1990a; Reitz et al., 1990a) for 1,4-dioxane. To address uncertainties and deficiencies in these models, the investigators conducted studies to fill data gaps and reduce uncertainties pertaining to the pharmacokinetics of 1,4-dioxane and HEAA in rats, mice, and 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.

The studies conducted by Sweeney et al. (2008a) resulted in partition coefficients that were consistent with previously measured values and those used in the Leung and Paustenbach (1990a) model. Of noteworthy significance, the laboratory results of Sweeney et al. (2008a) did not confirm the human blood:air partition coefficient Reitz et al. (1990a) reported. Furthermore, Sweeney et al. (2008a) estimated metabolic rate constants (V_{maxC} and K_m) within the range used in the previous models (Leung and Paustenbach, 1990a; Reitz et al., 1990a). Overall, the Sweeney et al. (2008a) model utilized more rodent in vivo and in vitro data in model parameterization and refinement; however, the model was still unable to adequately predict the human blood data from Young et al. (1977a). The Takano (2010) model was only tested by the authors using a single dose and route of exposure in rats, so the ability of the model to predict over a range of exposures or exposure routes is unknown. Additionally, the human model (Takano et al., 2010) was not compared to the available published data (1978c, d; 1977b; Young et al., 1976b).

3.6 Rat Nasal Exposure via Drinking Water

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

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

4 HAZARD IDENTIFICATION

4.1 Studies in Humans – Epidemiology, Case Reports, Clinical Controls

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

Several studies examined the effects of acute inhalation exposure in volunteers. In a study performed at the Pittsburgh Experimental Station of the U.S. Bureau of Mines, eye irritation and a burning sensation in the nose and throat were reported in five men exposed to 5,500 ppm of 1,4-dioxane vapor for 1 minute (Yant et al., 1930). Slight vertigo was also reported by three of these men. Exposure to 1,600 ppm of 1,4-dioxane vapor for 10 minutes resulted in similar symptoms with a reduced intensity of effect. In a study conducted by the Government Experimental Establishment at Proton, England (Fairley et al., 1934a), four men were exposed to 1,000 ppm of 1,4-dioxane for 5 minutes. Odor was detected immediately and one volunteer noted a constriction in the throat. Exposure of six volunteers to 2,000 ppm for 3 minutes resulted in no symptoms of discomfort. Wirth and Klimmer (1936), of the Institute of Pharmacology, University of Wurzburg, reported slight mucous membrane irritation in the nose and throat of several human subjects exposed to concentrations greater than 280 ppm for several minutes. Exposure to approximately 1,400 ppm for several minutes caused a prickling sensation in the nose and a dry and scratchy throat. Silverman et al. (1946) exposed 12 male and 12 female subjects to varying air concentrations of 1,4-dioxane for 15 minutes. A 200 ppm concentration was reported to be tolerable, while a concentration of 300 ppm caused irritation to the eyes, nose, and throat. The study conducted by Silverman et al. (1946) was conducted by the Department of Industrial Hygiene, Harvard School of Public Health, and was sponsored and supported by a grant from the Shell Development Company. These volunteer studies published in the 1930s and 1940s (Silverman et al., 1946; Wirth and Klimmer, 1936; Fairley et al., 1934a; Yant et al., 1930) did not provide information on the human subjects research ethics

procedures undertaken in these studies; however, there is no evidence that the conduct of the research was fundamentally unethical or significantly deficient relative to the ethical standards prevailing at the time the research was conducted.

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

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

Only two well documented epidemiology studies were available for occupational workers exposed to 1,4-dioxane (Buffler et al., 1978a; Thiess et al., 1976a). These studies did not provide evidence of effects in humans; however, the cohort size and number of reported cases were small.

4.1.1 Thiess et al.

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

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

Chromosome analysis was performed on six actively employed workers and six control persons (not characterized). Lymphocyte cultures were prepared and chromosomal aberrations were evaluated. No differences were noted in the percent of cells with gaps or other chromosome aberrations. Mortality statistics were calculated for 74 workers of different ages and varying exposure periods. The proportional contribution of each of the exposed workers to the total time of observation was calculated as the sum of man-years per 10-year age group. Each person contributed one man-year per calendar year to the specific age group in which he was included at the time. The expected number of deaths for this population was calculated from the age-specific mortality statistics for the German Federal Republic for the years 1970–1973. From the total of 1,840.5 person-years, 14.5 deaths were expected; however, only 12 deaths were observed in exposed workers between 1964 and 1974. Two cases of cancer were reported, including one case of lamellar epithelial carcinoma and one case of myelofibrosis leukemia. These cancers were not considered to be the cause of death in these cases and other severe illnesses were present. Standardized mortality ratios (SMRs) for cancer did not significantly differ from the control population (SMR for overall population = 0.83; SMR for 65–75-year-old men = 1.61; confidence intervals (CIs) were not provided).

4.1.2 Buffler et al.

Buffler et al. (1978a) conducted a mortality study on workers exposed to 1,4-dioxane at a chemical manufacturing facility in Texas. 1,4-Dioxane exposure was known to occur in a manufacturing area and in a processing unit located 5 miles from the manufacturing plant. Employees who worked between April 1, 1954, and June 30, 1975, were separated into two cohorts based on at least 1 month of exposure in either the manufacturing plant (100 workers) or the processing area (65 workers). Company records and follow-up techniques were used to compile information on name, date of birth, gender, ethnicity, job assignment and duration, and employment status at the time of the study. Date and cause of death were obtained from copies of death certificates and autopsy reports (if available). Exposure levels for each job category were estimated using the 1974 Threshold Limit Value for 1,4-dioxane (i.e., 50 ppm) and information from area and personal monitoring. Exposure levels were classified as low (<25 ppm), intermediate (50–75 ppm), and high (>75 ppm). Monitoring was not conducted prior to 1968 in the manufacturing areas or prior to 1974 in the processing area; however, the study authors assumed that

exposures would be comparable, considering that little change had been made to the physical plant or the manufacturing process during that time. Exposure to 1,4-dioxane was estimated to be below 25 ppm for all individuals in both cohorts. Manufacturing area workers were exposed to several other additional chemicals and processing area workers were exposed to vinyl chloride.

Seven deaths were identified in the manufacturing cohort and five deaths were noted for the processing cohort. The average exposure duration was not greater for those workers who died, as compared to those still living at the time of the study. Cancer was the underlying cause of death for two cases from the manufacturing area (carcinoma of the stomach, alveolar cell carcinoma) and one case from the processing area (malignant mediastinal tumor). The workers from the manufacturing area were exposed for 28 or 38 months and both had a positive smoking history (>1 pack/day). Smoking history was not available for processing area workers. The single case of cancer in this area occurred in a 21-year-old worker exposed to 1,4-dioxane for 1 year. The mortality data for both industrial cohorts were compared to age-race-sex specific death rates for Texas (1960–1969). Person-years of observation contributed by workers were determined over five age ranges with each worker contributing one person-year for each year of observation in a specific age group. The expected number of deaths was determined by applying the Texas 1960–1969 death rate statistics to the number of person years calculated for each cohort. The observed and expected number of deaths for overall mortality (i.e., all causes) was comparable for both the manufacturing area (7 observed versus 4.9 expected) and the processing area (5 observed versus 4.9 expected). No significant excess in cancer-related deaths was identified for both areas of the facility combined (3 observed versus 1.7 expected). A separate analysis was performed to evaluate mortality in manufacturing area workers exposed to 1,4-dioxane for more than 2 years. Six deaths occurred in this group as compared to 4.1 expected deaths. The use of a conditional Poisson distribution indicated no apparent excess in mortality or death due to malignant neoplasms in this study. It is important to note that the cohorts evaluated were limited in size. In addition, the mean exposure duration was less than 5 years (<2 years for 43% of workers) and the latency period for evaluation was less than 10 years for 59% of workers. The study authors recommended a follow-up investigation to allow for a longer latency period; however, no follow-up study of these workers has been published.

4.2 Subchronic and Chronic Studies and Cancer Bioassays in Animals – Oral and Inhalation

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

4.2.1 Oral Toxicity

4.2.1.1 Subchronic Oral Toxicity

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

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

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

An increase in the liver to BW ratio was observed in rats from the high dose group (assumed to be 1,000 mg/kg-day). Histopathological alterations, characterized as minimal centrilobular swelling, were also seen in rats from this dose group (incidence values were not reported). Hepatic DNA synthesis, measured by [^{3}H]-thymidine incorporation, was increased 1.5-fold in high-dose rats. No changes relative

to control were observed for rats exposed to 10 mg/kg-day. EPA found a NOAEL value of 10 mg/kg-day and a LOAEL value of 1,000 mg/kg-day for this study based on histopathological changes in the liver.

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

4.2.1.1.3 Kano et al. In the Kano et al. (2008) study, groups of 6-week-old F344/DuCrj rats (10/sex/group) and Crj:BDF1 mice (10/sex/group) were administered 1,4-dioxane (>99% pure) in the drinking water for 13 weeks. The animals were observed daily for clinical signs of toxicity. Food consumption and BWs were measured once per week and water consumption was measured twice weekly. Food and water were available ad libitum. The concentrations of 1,4-dioxane in the water for rats and mice were 0, 640, 1,600, 4,000, 10,000, or 25,000 ppm. The investigators used data from water consumption and BW changes to calculate a daily intake of 1,4-dioxane by the male and female animals. Thus, male rats received doses of approximately 0, 52, 126, 274, 657, and 1,554 mg 1,4-dioxane/kg-day and female rats received 0, 83, 185, 427, 756, and 1,614 mg/kg-day. Male mice received 0, 86, 231, 585, 882, or 1,570 mg/kg-day and female mice received 0, 170, 387, 898, 1,620, or 2,669 mg/kg-day.

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

Clinical signs of toxicity in rats were not discussed in the study report. One female rat in the high dose group (1,614 mg/kg-day) group died, but cause and time of death were not specified. Final BWs were reduced at the two highest dose levels in females (12 and 21%) and males (7 and 21%), respectively. Food consumption was reduced 13% in females at 1,614 mg/kg-day and 8% in 1,554 mg/kg-day males. A dose-related decrease in water consumption was observed in male rats starting at 52 mg/kg-day (15%) and in females starting at 185 mg/kg-day (12%). Increases in RBCs, hemoglobin, hematocrit, and neutrophils, and a decrease in lymphocytes were observed in males at 1,554 mg/kg-day. In females, MCV was decreased at doses ≥ 756 mg/kg and platelets were decreased at 1,614 mg/kg-day. With the exception

of the 30% increase in neutrophils in high-dose male rats, hematological changes were within 2–15% of control values. Total serum protein and albumin were significantly decreased in males at doses ≥ 274 mg/kg-day and in females at doses ≥ 427 mg/kg-day. Additional changes in high-dose male and female rats included decreases in glucose, total cholesterol, triglycerides, and sodium (and calcium in females), and increases in ALT (males only), AST, ALP, and LAP. Serum biochemistry parameters in treated rats did not differ more than twofold from control values. Urine pH was decreased in males at ≥ 274 mg/kg-day and in females at ≥ 756 mg/kg-day.

Kidney weights were increased in females at ≥185 mg/kg-day with a maximum increase of 15% and 44% at 1,614 mg/kg-day for absolute and relative kidney weight, respectively. No organ weight changes were noted in male rats. Histopathology findings in rats that were related to exposure included nuclear enlargement of the respiratory epithelium, nuclear enlargement of the olfactory epithelium, nuclear enlargement of the tracheal epithelium, hepatocyte swelling of the centrilobular area of the liver, vacuolar changes in the liver, granular changes in the liver, single cell necrosis in the liver, nuclear enlargement of the proximal tubule of the kidneys, hydropic changes in the proximal tubule of the kidneys, and vacuolar changes in the brain. The incidence data for histopathological lesions in rats are presented in Table 4-1. The effects that occurred at the lowest doses were nuclear enlargement of the respiratory epithelium in the nasal cavity and hepatocyte swelling in the central area of the liver in male rats. Nuclear enlargement has not been described elsewhere in published literature; there is a lack of information concerning the nature, severity, and significance of this observation. Thus, the toxicological significance of nuclear enlargement is unknown. Based on these histopathological findings the study authors identified the LOAEL as 126 mg/kg-day and the 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]

[a]Data are presented for sacrificed animals.
[b]$p \leq 0.01$ by χ^2 test.
[c]$p \leq 0.05$.

Source: Kano et al. (2008)

Clinical signs of toxicity in mice were not discussed in the study report One male mouse in the high-dose group (1,570 mg/kg-day) died, but no information was provided regarding cause or time of death. Final BWs were decreased 29% in male mice at 1,570 mg/kg-day, but changed less than 10% relative to controls in the other male dose groups and in female mice. Food consumption was not significantly reduced in any exposure group. Water consumption was reduced 14–18% in male mice exposed to 86, 231, or 585 mg/kg-day. Water consumption was further decreased by 48 and 70% in male mice exposed to 882 and 1,570 mg/kg-day, respectively. Water consumption was also decreased 31 and 57% in female mice treated with 1,620 and 2,669 mg/kg-day, respectively. An increase in MCV was observed in the two highest dose groups in both male (882 and 1,570 mg/kg-day) and female mice (1,620 and 2,669 mg/kg-day). Increases in RBCs, hemoglobin, and hematocrit were also observed in high dose males (1,570 mg/kg-day). Hematological changes were within 2–15% of control values. Serum biochemistry changes in exposed mice included decreased total protein (at 1,570 mg/kg-day in males, ≥1,620 mg/kg-day in females), decreased glucose (at 1,570 mg/kg-day in males, ≥1,620 mg/kg-day in females), decreased albumin (at 1,570 mg/kg-day in males, 2,669 mg/ kg-day in females), decreased total cholesterol (≥ 585 mg/kg-day in males, ≥1,620 mg/kg-day in females), increased serum ALT (at 1,570 mg/kg-day in males, ≥ 620 mg/kg-day in females), increased AST (at 1,570 mg/kg-day in males, 2,669 mg/kg-day in females), increased ALP (≥ 585 mg/kg-day in males, 2,669 mg/kg-day in females), and increased LDH (in females only at doses ≥ 1,620 mg/kg-day). With the exception of a threefold

increase in ALT in male and female mice, serum biochemistry parameters in treated rats did not differ more than twofold from control values. Urinary pH was decreased in males at ≥ 882 mg/kg-day and in females at ≥ 1,620 mg/kg-day.

Absolute and relative lung weights were increased in males at 1,570 mg/kg-day and in females at 1,620 and 2,669 mg/kg-day. Absolute kidney weights were also increased in females at 1,620 and 2,669 mg/kg-day and relative kidney weight was elevated at 2,669 mg/kg-day. Histopathology findings in mice that were related to exposure included nuclear enlargement of the respiratory epithelium, nuclear enlargement of the olfactory epithelium, eosinophilic change in the olfactory epithelium, vacuolic change in the olfactory nerve, nuclear enlargement of the tracheal epithelium, accumulation of foamy cells in the lung and bronchi, nuclear enlargement and degeneration of the bronchial epithelium, hepatocyte swelling of the centrilobular area of the liver, and single cell necrosis in the liver. As noted above, the toxicological significance of nuclear enlargement is unknown. The incidence data for histopathological lesions in mice are presented in Table 4-2. Based on the changes in the bronchial epithelium in female mice, the authors identified the dose level of 387 mg/kg-day as the LOAEL for mice; the 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	5/10b	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]	6/10b	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]

[a]Data are presented for sacrificed animals.
[b]$p \leq 0.01$ by $\chi 2$ test.
[c]$p \leq 0.05$.

Source: Kano et al (2008).

4.2.1.1.4 Yamamoto et al. Studies (Yamamoto et al., 1998a; Yamamoto et al., 1998b) in rasH2 transgenic mice carrying the human prototype c-Ha-ras gene have been investigated as a bioassay model for rapid carcinogenicity testing. As part of validation studies of this model, 1,4-dioxane was one of many chemicals that were evaluated. RasH2 transgenic mice were F1 offspring of transgenic male C57BLr6J and normal female BALB/cByJ mice. $CB6F_1$ mice were used as a nontransgenic control. Seven- to nine-week-old mice (10–15/group) were exposed to 0, 0.5, or 1% 1,4-dioxane in drinking water for 26 weeks. An increase in lung adenomas was observed in treated transgenic mice, as compared to treated nontransgenic mice. The tumor incidence in transgenic animals, however, was not greater than that observed in vehicle-treated transgenic mouse controls. Further study details were not provided.

4.2.1.2 Chronic Oral Toxicity and Carcinogenicity

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

Six of the 26 treated rats developed hepatocellular carcinomas, and these rats had been treated for an average of 452 days (range, 448–455 days). No liver tumors were observed in control rats. In two rats that died after 21.5 weeks of treatment, histological changes appeared to involve the entire liver. Groups of cells were found that had enlarged hyperchromic nuclei. Rats that died or were killed at longer intervals showed similar changes, in addition to large cells with reduced cytoplasmic basophilia. Animals killed after 60 weeks of treatment showed small neoplastic nodules or multifocal hepatocellular carcinomas. No cirrhosis was observed in this study. Many rats had extensive changes in the kidneys often resembling glomerulonephritis, however, incidence data was not reported for these findings. This effect progressed from increased cellularity to thickening of the glomerular capsule followed by obliteration of the glomeruli. One treated rat had an early transitional cell carcinoma in the kidney's pelvis; this rat also had a large tumor in the liver. The lungs from many treated and control rats (incidence not reported) showed severe bronchitis with epithelial hyperplasia and marked peribronchial infiltration, as well as multiple abscesses. One rat treated with 1,4-dioxane developed leukemia with infiltration of all organs, particularly the liver and spleen, with large, round, isolated neoplastic cells. In the liver, the

distribution of cells in the sinusoids was suggestive of myeloid leukemia. The dose of 640 mg/kg-day tested in this study was a free-standing LOAEL, identified by EPA, for glomerulonephritis in the kidney and histological changes in the liver (hepatocytes with enlarged hyperchromic nuclei, large cells with reduced cytoplasmic basophilia).

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

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

Argus et al. (1973a) studied the progression of liver tumorigenesis by electron microscopy of liver tissues obtained following interim sacrifice at 8 and 13 months of exposure (5 rats/group, 574 mg/kg-day). The authors reported qualitatively that the first change observed in the liver was an increase in the size of the nucleus of the hepatocytes, mostly in the periportal area. Precancerous changes were characterized by disorganization of the rough endoplasmic reticulum, an increase in smooth endoplasmic reticulum, and a decrease in glycogen and increase in lipid droplets in hepatocytes. These changes increased in severity in the hepatocellular carcinomas in rats exposed to 1,4-dioxane for 13 months.

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

[a]Precise 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. (1973a).

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

4.2.1.2.3 Hoch-Ligeti and Argus. Hoch-Ligeti and Argus (1970a) provided a brief account of the results of exposure of guinea pigs to 1,4-dioxane. A group of 22 male guinea pigs (neither strain nor age provided) was administered 1,4-dioxane (purity not provided) in the drinking water for at least 23 months and possibly up to 28 months. The authors stated that the concentration of 1,4-dioxane was regulated so that normal growth of the guinea pigs was maintained, and varied 0.5–2% (no further information provided). The investigators further stated that the amount of 1,4-dioxane received by the guinea pigs over a 23-month period was 588–635 g. Using a reference BW of 0.89 kg for male guinea pigs in a chronic study (U.S. EPA, 1988) and assuming an exposure period of 700 days (23 months), the guinea pigs received doses between 944 and 1,019 mg 1,4-dioxane/kg-day. A group of ten untreated guinea pigs served as controls. All animals were sacrificed within 28 months, but the scope of the postmortem examination was not provided.

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

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

Male and female rats in the high-dose group (1% in drinking water) consumed slightly less water than controls. BW gain was depressed in the high-dose groups relative to the other groups almost from the beginning of the study (food consumption data were not provided). Based on water consumption and BW data for specific exposure groups, Kociba et al. (1974a) calculated mean daily doses of 9.6, 94, and 1,015 mg/kg-day for male rats and 19, 148, and 1,599 mg/kg-day for female rats during days 114–198 for the 0.01, 0.1, and 1.0% concentration levels, respectively. Treatment with 1,4-dioxane significantly increased mortality among high-dose males and females beginning at about 2–4 months of treatment. These rats showed degenerative changes in both the liver and kidneys. From the 5th month on, mortality rates of control and treated groups were not different. There were no treatment-related alterations in hematological parameters. At termination, the only alteration in organ weights noted by the authors was a significant increase in absolute and relative liver weights in male and female high-dose rats (data not shown). Histopathological lesions were restricted to the liver and kidney from the mid- and high-dose

groups and consisted of variable degrees of renal tubular epithelial and hepatocellular degeneration and necrosis (no quantitative incidence data were provided). Rats from these groups also showed evidence of hepatic regeneration, as indicated by hepatocellular hyperplastic nodule formation and evidence of renal tubular epithelial regenerative activity (observed after 2 years of exposure). These changes were not seen in controls or in low-dose rats. The authors determined a LOAEL of 94 mg/kg-day based on the liver and kidney effects in male rats. The corresponding NOAEL value was 9.6 mg/kg-day.

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

[a]Rats 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: Reprinted with permission of Elsevier, Ltd., Kociba et al. (1974a).

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

4.2.1.2.5 National Cancer Institute (NCI). Groups of Osborne-Mendel rats (35/sex/dose) and B6C3F$_1$ mice (50/sex/dose) were administered 1,4-dioxane (≥ 99.95% pure) in the drinking water for 110 or 90 weeks, respectively, at levels of 0 (matched controls), 0.5, or 1% (NCI, 1978). Solutions of 1,4-dioxane were prepared with tap water. The report indicated that at 105 weeks from the earliest starting date, a new necropsy protocol was instituted. This affected the male controls and high-dose rats, which were started a year later than the original groups of rats and mice. Food and water were available ad libitum. Endpoints monitored in this bioassay included clinical signs (twice daily), BWs (once every 2 weeks for the first 12 weeks and every month during the rest of the study), food and water consumption (once per month in 20% of the animals in each group during the second year of the study), and gross and microscopic appearance of all major organs and tissues (mammary gland, trachea, lungs and bronchi, heart, bone marrow, liver, bile duct, spleen, thymus, lymph nodes, salivary gland, pancreas, kidney, esophagus, thyroid, parathyroid, adrenal, gonads, brain, spinal cord, sciatic nerve, skeletal muscle, stomach, duodenum, colon, urinary bladder, nasal septum, and skin). Based on the measurements of water consumption and BWs, the investigators calculated average daily intakes of 1,4-dioxane of 0, 240, and 530 mg/kg-day in male rats, 0, 350, and 640 mg/kg-day in female rats, 0, 720, and 830 mg/kg-day in male mice, and 0, 380, and 860 mg/kg-day in female mice. According to the report, the doses of 1,4-dioxane in high-dose male mice were only slightly higher than those of the low-dose group due to decreased fluid consumption in high-dose male mice.

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

[a]Statistically significant trend for increased incidence by Cochran-Armitage test ($p < 0.05$) performed for this review.
[b]Incidence significantly elevated compared to control by Fisher's Exact test ($p < 0.05$) performed for this review.

Source: NCI (1978).

Neoplasms associated with 1,4-dioxane treatment were limited to the nasal cavity (squamous cell carcinomas, adenocarcinomas, and one rhabdomyoma) in both sexes, liver (hepatocellular adenomas) in females, and testis/epididymis (mesotheliomas) in males. The first tumors were seen at week 52 in males and week 66 in females. The incidence of squamous cell carcinomas in the nasal turbinates in male and female rats is presented in Table 4-6. Squamous cell carcinomas were first seen on week 66 of the study. Morphologically, these tumors varied from minimal foci of locally invasive squamous cell proliferation to advanced growths consisting of extensive columns of epithelial cells projecting either into free spaces of the nasal cavity and/or infiltrating into the submucosa. Adenocarcinomas of the nasal cavity were observed in 3 of 34 high-dose male rats, 1 of 35 low-dose female rats, and 1 of 35 high-dose female rats. The single rhabdomyoma (benign skeletal muscle tumor) was observed in the nasal cavity of a male rat from the low-dose group. A subsequent re-examination of the nasal tissue sections by Goldsworthy et al. (1991) concluded that the location of the tumors in the nasal apparatus was consistent with the possibility that the nasal tumors resulted from inhalation of water droplets by 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%)[e]
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%)[c]	8/35 (23%)[c]
Hepatocellular adenoma	0/31 (0%)[f]	10/33 (30%)[e]	11/32 (34%)[e]

[a]Tumor incidence values were not adjusted for mortality.
[b]Group 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).

The incidence of hepatocellular adenomas in male and female rats is presented in Table 4-6. Hepatocellular adenomas were first observed in high-dose females in week 70 of the study. These tumors consisted of proliferating hepatic cells oriented as concentric cords. Hepatic cell size was variable; mitoses and necrosis were rare. Mesothelioma of the vaginal tunics of the testis/epididymis was seen in male rats (2/33, 4/33, and 5/34 in controls, low-, and high-dose animals, respectively). The difference between the treated groups and controls was not statistically significant. These tumors were characterized as rounded and papillary projections of mesothelial cells, each supported by a core of fibrous tissue. Other reported neoplasms were considered spontaneous lesions not related to treatment with 1,4-dioxane.

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

increased incidence of pneumonia and rhinitis in female mice; a NOAEL was not established in this study.

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

Table 4-7 Incidence of hepatocellular adenoma or carcinoma in B6C3F$_1$ 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]

[a]Tumor incidence values were not adjusted for mortality.
[b]$p < 0.001$, positive dose-related trend (Cochran-Armitage test).
[c]$p \leq 0.001$ by Fisher's Exact test pair-wise comparison with controls.
[d]$p = 0.014$.

Source: NCI (1978).

In addition to liver tumors, a variety of other benign and malignant neoplasms occurred. However, the report (NCI, 1978) indicated that each type had been encountered previously as a spontaneous lesion in the B6C3F$_1$ mouse. The report further stated that the incidences of these neoplasms were unrelated by type, site, group, or sex of the animal, and hence, not attributable to exposure to 1,4-dioxane. There were a few nasal adenocarcinomas (1/48 in low-dose females and 1/49 in high-dose males) that arose from proliferating respiratory epithelium lining of the nasal turbinates. These growths extended into the nasal cavity, but there was minimal local tissue infiltration. Nasal mucosal polyps were rarely observed. The polyps were derived from mucus-secreting epithelium and were otherwise unremarkable. There was a significant negative trend for alveolar/bronchiolar adenomas or carcinomas of the lung in male mice, such that the incidence in the matched controls was higher than in the dosed groups. The report (NCI, 1978) indicated that the probable reason for this occurrence was that the dosed animals did not live as long as the controls, thus diminishing the possibility of the development of tumors in the dosed groups.

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

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

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

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

Groups of F344/DuCrj rats (50/sex/dose level) were exposed to 1,4-dioxane (>99% pure) in the drinking water at levels of 0, 200, 1,000, or 5,000 ppm for 2 years. Groups of Crj:BDF1 mice (50/sex/dose level) were similarly exposed in the drinking water to 0, 500, 2,000, or 8,000 ppm of 1,4-dioxane. The high doses were selected based on results from the Kano et al. (2008) 13-week drinking water study so as not to exceed the maximum tolerated dose (MTD) in that study. Both rats and mice were 6 weeks old at the beginning of the study. Food and water were available ad libitum. The animals were observed daily for clinical signs of toxicity; and BWs were measured once per week for 14 weeks and once every 2 weeks until the end of the study. Food consumption was measured once a week for 14 weeks and once every 4 weeks for the remainder of the study. The investigators used data from water consumption and BW to calculate an estimate of the daily intake of 1,4-dioxane (mg/kg-day) by male and female rats and mice. Kano et al. (2009) reported a calculated mean ± standard deviation for the daily

doses of 1,4-dioxane for the duration of the study. Male rats received doses of approximately 0, 11±1, 55±3, or 274±18 mg/kg-day and female rats received 0, 18±3, 83±14, or 429±69 mg/kg-day. Male mice received doses of 0, 49±5, 191±21, or 677±74 mg/kg-day and female mice received 0, 66±10, 278±40, or 964±88 mg/kg-day. For the remainder of this document, including the dose-response analysis, the mean calculated intake values are used to identify dose groups. The Kano et al. (2009) study was conducted in accordance with the Organization for Economic Co-operation and Development (OECD) Principles for Good Laboratory Practice (GLP).

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

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

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

Several hematological changes were noted in the JBRC report (1998): Decreases in RBC (male rats only), hemoglobin, hematocrit, and MCV; and increases in platelets in high-dose groups were observed (JBRC, 1998). These changes (except for MCV) also occurred in mid-dose males. With the exception of a 23% decrease in hemoglobin in high-dose male rats and a 27% increase in platelets in high-dose female rats, hematological changes were within 15% of control values. Significant changes in serum chemistry parameters occurred only in high-dose rats (males: increased phospholipids, AST, ALT,

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

Microscopic examination of the tissues showed nonneoplastic alterations in the nasal cavity, liver, and kidneys mainly in high-dose rats and, in a few cases, in mid-dose rats (Table 4-8 and Table 4-9). Alterations in high-dose (274 mg/kg-day) male rats consisted of nuclear enlargement and metaplasia of the olfactory and respiratory epithelia, atrophy of the olfactory epithelium, hydropic changes and sclerosis of the lamina propria, adhesion, and inflammation. In female rats, nuclear enlargement of the olfactory epithelium occurred at doses ≥ 83 mg/kg-day, and nuclear enlargement and metaplasia of the respiratory epithelium, squamous cell hyperplasia, respiratory metaplasia of the olfactory epithelium, hydropic changes and sclerosis of the lamina propria, adhesion, inflammation, and proliferation of the nasal gland occurred at 429 mg/kg-day. Alterations were seen in the liver at ≥ 55 mg/kg-day in male rats (spongiosis hepatis, and clear and mixed cell foci) and at 429 mg/kg-day in female rats (spongiosis hepatis, cyst formation, and mixed cell foci). Nuclear enlargement of the renal proximal tubule occurred in males at 274 mg/kg-day and in females at ≥ 83 mg/kg-day (JBRC, 1998). As noted previously, nuclear enlargement has not been described elsewhere in published literature; there is a lack of information concerning the nature, severity, and significance of this observation. Thus, the toxicological significance of nuclear enlargement is unknown.

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
Spongiosis hepatis; liver[d]	12/50	20/50	25/50[f]	40/50
Clear cell foci; liver[c,g]	3/50	3/50	9/50	8/50
Acidophilic cell foci; liver[c,g]	12/50	8/50	7/50	5/50
Basophilic cell foci; liver[c,g]	7/50	11/50	8/50	16/50[f]
Mixed-cell foci; liver[c,g]	2/50	8/50	14/50[e]	13/50[e]
Nuclear enlargement; kidney proximal tubule[d]	0/50	0/50	0/50	50/50

[a]Data presented for all animals, including animals that became moribund or died before the end of the study.
[b]Dose levels from Kano et al. (2009).
[c]Data from Kano et al. (2009).
[d]Data 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.
[g]The samples associated with liver hyperplasia for rats and mice in Yamazaki et al. (1994a) and JBRC (1998) were re-examined according to updated criteria for liver lesions and were afterwards classified as either hepatocellular adenoma or altered hepatocellular foci in Kano et al. (2009).

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
Spongiosis hepatis; 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,g]	1/50	1/50	1/50	1/50
Basophilic cell foci; liver[c,g]	23/50	27/50	31/50	8/50[e]
Clear cell foci; liver[c,g]	1/50	1/50	5/50	4/50
Mixed-cell foci; liver[c,g]	1/50	1/50	3/50	11/50[f]
Nuclear enlargement; kidney proximal tubule[d]	0/50	0/50	6/50	39/50

[a]Data presented for all animals, including animals that became moribund or died before the end of the study.
[b]Dose levels from Kano et al. (2009).
[c]Data from Kano et al. (2009).
[d]Data 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.
[g]The samples associated with liver hyperplasia for rats and mice in Yamazaki et al. (1994a) and JBRC (1998) were re-examined according to updated criteria for liver lesions and were afterwards classified as either hepatocellular adenoma or altered hepatocellular foci in Kano et al. (2009).

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

NOAEL and LOAEL values for rats in this study were identified by EPA as 55 and 274 mg/kg-day, respectively, based on toxicity observed in nasal tissue of male rats (i.e., atrophy of olfactory epithelium, adhesion, and inflammation). Metaplasia and hyperplasia of the nasal epithelium were also observed in high-dose male and female rats. These effects are likely to be associated with the formation of nasal cavity tumors in these dose groups. Nuclear enlargement was observed in the nasal olfactory epithelium and the kidney proximal tubule at a dose of 83 mg/kg-day in female rats; however, as noted previously, it is unclear whether these alterations represent adverse toxicological effects. Hematological effects noted in male rats given 55 and 274 mg/kg-day (decreased RBCs, hemoglobin, hematocrit, increased platelets) were within 20% of control values. In female rats decreases in hematological effects were observed in the high dose group (429 mg/kg-day). A reference range database for hematological effects in laboratory animals (Wolford et al., 1986) indicates that a 20% change in these parameters may fall within a normal range (10th–90th percentile values) and may not represent a treatment-related effect of concern. Liver lesions were also seen at a dose of 55 mg/kg-day in male rats; these changes are likely to be associated with liver tumorigenesis. Clear and mixed-cell foci are commonly considered preneoplastic changes and would not be considered evidence of noncancer toxicity. The nature of spongiosis hepatis as a preneoplastic change is less well understood (Bannasch, 2003; Karbe and Kerlin, 2002a; Stroebel et al., 1995). Spongiosis hepatis is a cyst-like lesion that arises from the perisinusoidal (Ito) cells (PSC) of the liver. It is commonly seen in aging rats, but has been shown to increase in incidence following exposure to hepatocarcinogens. Spongiosis hepatis can be seen in combination with preneoplastic foci in the liver or with hepatocellular adenoma or carcinoma and has been considered a preneoplastic lesion (Bannasch, 2003; Stroebel et al., 1995). This change can also be associated with hepatocellular hypertrophy and liver toxicity and has been regarded as a secondary effect of some liver carcinogens (Karbe and Kerlin, 2002a). In the case of the JBRC (1998) study, spongiosis hepatis was associated with other preneoplastic changes in the liver (clear and mixed-cell foci). No other lesions indicative of liver toxicity were seen in this study; therefore, spongiosis hepatis was not considered indicative of noncancer effects. Serum chemistry changes (increases in total protein, albumin, and glucose; decreases in AST, ALT, LDH, and ALP, potassium, and inorganic phosphorous) were observed in both male and female rats (JBRC, 1998) in the high dose groups, 274 and 429 mg/kg-day, respectively.

Significantly increased incidences of liver tumors (adenomas and carcinomas) and tumors of the nasal cavity occurred in high-dose male and female rats (Table 4-10 and Table 4-11) treated with 1,4-dioxane for 2 years (Kano et al., 2009). The first liver tumor was seen at 85 weeks in high-dose male rats and 73 weeks in high-dose female rats (vs. 101–104 weeks in lower dose groups and controls)

(Yamazaki, 2006). In addition, a significant increase ($p \leq 0.01$, Fisher's Exact test) in mesotheliomas of the peritoneum was seen in high-dose males (28/50 versus 2/50 in controls). Mesotheliomas were the single largest cause of death among high-dose male rats, accounting for 12 of 28 pretermination deaths (Yamazaki, 2006). Also, in males, there were increasing trends in mammary gland fibroadenoma and fibroma of the subcutis, both statistically significant ($p < 0.01$) by the Peto test of dose-response trend. Females showed a significant increasing trend in mammary gland adenomas ($p < 0.01$ by Peto's test). The tumor incidence values presented in Table 4-10 and Table 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

	Males				Females			
Dose (mg/kg-day)	**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]

[a]Statistically significant trend for increased tumor incidence by Peto's test ($p < 0.01$).
[b]Significantly different from control by Fisher's exact test ($p < 0.01$).
[c]Significantly different from control by Fisher's exact test ($p < 0.05$).

Source: Reprinted with permission of 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

	Males				Females			
Dose (mg/kg-day)	**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]

[a]Significantly different from control by Fisher's exact test ($p < 0.01$).
[b]Statistically significant trend for increased tumor incidence by Peto's test ($p < 0.01$).

Source: Reprinted with permission of Elsevier, Ltd., Kano et al. (2009).

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

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

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

Table 4-12 and Table 4-13). Commonly seen alterations included nuclear enlargement (toxicological significance unknown), atrophy, and inflammation of the epithelium. Other changes observed included nuclear enlargement 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

[a]Data presented for all animals, including animals that became moribund or died before the end of the study.
[b]Dose levels from Kano et al. (2009).
[c]Data from Kano et al. (2009).
[d]Data 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

[a]Data presented for all animals, including animals that became moribund or died before the end of the study.
[b]Dose levels from Kano et al. (2009).
[c]Data from Kano et al. (2009).
[d]Data 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).

NOAEL and LOAEL values for mice in this study were identified by EPA as 66 and 278 mg/kg-day, respectively, based on nasal inflammation observed in female mice. Nuclear enlargement of the nasal olfactory epithelium and bronchial epithelium was also observed at a dose of 278 mg/kg-day in female mice; however, it is unclear whether these alterations represent adverse toxicological effects. Liver angiectasis, an abnormal dilatation and/or lengthening of a blood or lymphatic vessel, was seen in male mice given 1,4-dioxane at a dose of 677 mg/kg-day.

Treatment with 1,4-dioxane resulted in an increase in the formation of liver tumors (adenomas and carcinomas) in male and female mice. The incidence of hepatocellular adenoma was statistically increased in male mice in the mid-dose group only. The incidence of male mice with hepatocellular carcinoma or either tumor type (adenoma or carcinoma) was increased in the low, mid, and high-dose groups. The appearance of the first liver tumor occurred in male mice at 64, 74, 63, and 59 weeks in the control, low- mid-, and high-dose groups, respectively (Yamazaki, 2006). In female mice, increased incidence was observed for hepatocellular carcinoma in all treatment groups, while an increase in hepatocellular adenoma incidence was only seen in the 66 and 278 mg/kg-day dose groups (Table 4-14). The appearance of the first liver tumor in female mice occurred at 95, 79, 71, and 56 weeks in the control, low-, mid-, and high-dose groups, respectively (Yamazaki, 2006). The tumor incidence data presented for male and female mice in Table 4-14 are based on reanalyzed sample data presented in Kano et al. (2009) that included lesions in animals that became moribund or died prior to the completion of the 2-year study.

Katagiri et al. (1998) summarized the incidence of hepatocellular adenomas and carcinomas in control male and female BDF1 mice from ten 2-year bioassays at the JBRC. For female mice, out of 499 control mice, the incidence rates were 4.4% for hepatocellular adenomas and 2.0% for hepatocellular carcinomas. Kano et al. (2009) reported a 10% incidence rate for hepatocellular adenomas and a 0% incidence rate for hepatocellular carcinomas in control female BDF1. The background incidence rates for male BDF1 mice were 15% and 22.8% for hepatocellular adenomas and carcinomas, respectively, out of 500 control mice in ten 2-year bioassays (Katagiri et al., 1998). Background rates for $B6C3F_1$ mice evaluated by the National Toxicology Program are similar (10.3% and 21.3% for hepatocellular adenomas and carcinomas in male mice, respectively; 4.0% and 4.1% for hepatocellular adenomas and carcinomas in female mice, respectively) to the BDF1 mice background rates observed by JBRC (Haseman et al., 1984). Thus, the BDF1 mouse is not particularly sensitive compared to the commonly used $B6C3F_1$ 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

	Males				Females			
Dose (mg/kg-day)	**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]

[a]Significantly different from control by Fisher's exact test ($p < 0.01$).
[b]Statistically significant trend for increased tumor incidence by Peto's test ($p < 0.01$).
[c]Significantly different from control by Fisher's exact test ($p < 0.05$).
Source: Reprinted with permission of Elsevier, Ltd., Kano et al. (2009).

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

4.2.2 Inhalation Toxicity

4.2.2.1 Subchronic Inhalation Toxicity

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

4.2.2.1.2 Kasai et al. Male and female 6-week-old F344/DuCrj rats (10/sex/group) were exposed to nominal concentrations of 0 (clean air), 100, 200, 400, 800, 1,600, 3,200, or 6,400 ppm (0, 360, 720, 1,400, 2,900, 5,800, 1,2000, and 23,000 mg/m^3, respectively) of vaporized 1,4-dioxane (>99% pure) for 6 hours/day, 5 days/week, for 13 weeks in whole body inhalation chambers (Kasai et al., 2008). Each inhalation chamber housed 20 individual cages for 10 males and 10 females. During exposure, the concentration of 1,4-dioxane vapor was determined every 15 minutes by gas chromatography. In addition, during exposure, animals received food and water ad libitum and the following data were collected: 1) clinical signs and mortality (daily); 2) BW and food intake (weekly); 3) urinary parameters using Ames reagent strips (measured during week 13 of the exposure); and 4) 1,4-dioxane content in plasma from three rats of both sexes (measured on the third day of exposure during weeks 12 and 13 at 1 hour after termination). At the end of the 13-week exposure period or at the time of an animal's death during exposure, all organs were collected, weighed, and evaluated for macroscopic lesions. Histopathological evaluations of organs and tissues were conducted in accordance with the OECD test guidelines, including all tissues of the respiratory tract. Liver sections from male and female rats exposed to 800, 1,600 and 3,200 ppm of 1,4-dioxane were also analyzed for foci (in the absence of tumor formation) by immunohistochemical expression of glutathione S-transferase placental form

(GST-P). Hematological and clinical chemistry parameters were measured using blood collected from the abdominal aorta of rats following an overnight fasting at the end of the 13-week exposure period. The measured hematological and clinical chemistry parameters included: red blood cell count, hemoglobin, hematocrit, MCV, AST, ALT, glucose, and triglyceride. Statistically significant differences (p-value of 0.05) between 1,4-dioxane and clean air exposed groups were determined by study authors using Dunnett's test or χ^2 test.

All rats exposed to 6,400 ppm of 1,4-dioxane died by the end of the first week of exposure; the determined cause of death was renal failure and diagnosed as necrosis of the renal tubules. At concentrations lower than 6,400 ppm, mortality was not observed and all exposed rats were absent of clinical signs. Exposure-related effects on final BWs, organ weights, and hematological and clinical chemistry parameters were reported as compared to controls and these changes are outlined in Table 4-15 and

Table 4-16. Briefly, terminal BWs were significantly decreased in both sexes at 200 ppm; and additionally in females at 800 and 1,600 ppm. Statistically significant increases in several organ weights were observed, including lung (≥1,600 ppm, males; ≥200 ppm, females); liver (≥800 ppm, both sexes), and kidneys (3,200 ppm, males; ≥800 ppm, females). Statistically significant changes in hematological parameters and clinical chemistry were observed in both sexes at 3,200 ppm including increased levels of hemoglobin ALT, RBC, AST ,and MCV. In females only, at 3,200 ppm, increased levels of hematocrit was noted; and in males at this exposure concentration decreased levels of glucose and triglyceride were observed, in addition to slightly decreased urinary protein. However, the urinary protein data were not shown in this study. At 200 ppm, an increased AST level in females was noted. Blood plasma levels of 1,4-dioxane were also evaluated and in both sexes, a linear increase in 1,4-dioxane levels was detected at exposure concentrations of 400 ppm and above. The highest blood levels of 1,4-dioxane were detected in females.

Exposure and/or sex-related histopathology findings also reported by the study authors included nuclear enlargement of the nasal respiratory, nasal olfactory, tracheal, and bronchial epithelium; vacuolic change in the olfactory and bronchial epithelium; atrophy of the nasal epithelium; hydropic change in the proximal tubules of the kidney; and single-cell necrosis and centrilobular swelling in the liver. Table 4-17 presents a summary of these histopathological lesions, including incidence and severity data. Further microscopic evaluation of liver tissue revealed GST-P positive liver foci in both sexes at 3,200 ppm (3/10 males, 2/10 females) and in females at 1,600 ppm (4/10).

The study authors determined nuclear enlargement in the respiratory epithelium as the most sensitive lesion and a LOAEL value of 100 ppm was identified by the study authors based on the incidence data of this lesion in both male and female rats. Nuclear enlargement has only been reported by this research center; there is a lack of information concerning the nature, severity, and significance of this observation. Thus, as with the oral assessment, the toxicological significance of nuclear enlargement is unknown.

Table 4-15 Terminal body weights and relative organ weights of F344/DuCrj rats exposed to 1,4-dioxane vapor by whole-body inhalation for 13 weeks

Males	Males[a]						
	1,4-dioxane vapor concentration (ppm)						
	0 (clean air)	**100**	**200**	**400**	**800**	**1,600**	**3,200**
Body weight (g)	323 ± 14	323 ± 14	304 ± 11[c]	311 ± 19	317 ± 12	312 ± 14	301 ± 11[b]
Lung (%)	0.310 ± 0.011	0.312 ± 0.007	0.325 ± 0.008[c]	0.320 ± 0.009	0.321 ± 0.011	0.333 ± 0.009[b]	0.346 ± 0.017[b]
Liver (%)	2.610 ± 0.069	2.697 ± 0.092	2.613 ± 0.084	2.666 ± 0.080	2.726 ± 0.082[c]	2.737 ± 0.077[b]	2.939 ± 0.101[b]
Kidneys (%)	0.589± 0.016	0.596 ± 0.021	0.612 ± 0.013	0.601 ± 0.020	0.610 ± 0.015	0.606 ± 0.021	0.647 ± 0.026[b]
Females	**Females[a]**						
	1,4-dioxane vapor concentration (ppm)						
	0 (clean air)	**100**	**200**	**400**	**800**	**1,600**	**3,200**
Body weight (g)	187 ± 5	195 ± 8	174± 10[b]	180 ± 5	175 ± 6 [b]	173 ± 8 [b]	168 ± 4[b]
Lung (%)	0.402 ± 0.013	0.402 ± 0.015	0.435 ± 0.018[b]	0.429 ± 0.029[c]	0.430 ± 0.013 [b]	0.454 ± 0.018[b]	0.457 ± 0.016[b]
Liver (%)	2.353 ± 0.081	2.338 ± 0.092	2.395± 0.092	2.408 ± 0.066	2.513 ± 0.076[b]	2.630 ± 0.139[b]	2.828 ± 0.144[b]
Kidneys (%)	0.647± 0.014	0.631 ± 0.019	0.668 ± 0.012	0.662 ± 0.024	0.679 ± 0.018[b]	0.705 ± 0.028[b]	0.749 ± 0.024[b]

[a]Data are presented for 10 sacrificed animals.
[b]$p \leq 0.01$ by Dunnett's test.
[c]$p \leq 0.05$ by Dunnett's test.

Source: Kasai et al. (2008)

Table 4-16 Hematology and clinical chemistry of F344/DuCrj rats exposed to 1,4-dioxane vapor by whole-body inhalation for 13 weeks

Males	Males[a]						
	1,4-dioxane vapor concentration (ppm)						
	0 (clean air)	100	200	400	800	1,600	3,200
Red blood cell (10^6/μl)	9.55 ± 0.17	9.53 ± 0.24	9.54 ± 0.18	9.59 ± 0.26	9.55 ± 0.18	9.58 ± 0.14	9.57 ± 0.37
Hemoglobin (g/dl)	16.0 ± 0.2	16.1 ± 0.4	15.9 ± 0.2	16.1 ± 0.3	16.0 ± 0.3	16.2 ± 0.3	16.4 ± 0.4[c]
Hematocrit (%)	46.2 ± 1.2	46.3 ± 1.3	46.3 ± 0.9	46.3 ± 1.4	46.3 ± 1.1	46.8 ± 0.9	47.3 ± 1.7
MCV (fl)	48.4 ± 0.7	48.6 ± 0.7	48.6 ± 0.4	48.3 ± 0.4	48.5 ± 0.6	48.9 ± 0.6	49.4 ± 0.5[b]
AST (IU/l)	73 ± 8	75 ± 14	73 ± 10	72 ± 5	72 ± 3	70 ± 4	73 ± 4
ALT (IU/l)	27 ± 3	27 ± 4	27 ± 4	28 ± 1	27 ± 2	27 ± 2	30 ± 2
Glucose (mg/dl)	197 ± 17	206 ± 13	192 ± 9	190 ± 12	187 ± 15	184 ± 12	170 ± 11[b]
Triglyceride (mg/dl)	125± 17	148 ± 37	118 ± 33	131 ± 30	113 ± 27	106 ± 24	87 ± 22[c]
Females	**Females[a]**						
	1,4-dioxane vapor concentration (ppm)						
	0 (clean air)	100	200	400	800	1,600	3,200
Red blood cell (10^6/μl)	8.77 ± 0.23	8.69 ± 0.21	8.73 ± 0.25	8.88 ± 0.21	8.68 ± 0.69	8.86 ± 0.16	9.15± 0.12[b]
Hemoglobin (g/dl)[d]	16.2 ± 0.3	16.0 ± 0.3	16.3 ± 0.4	16.2 ± 0.4	16.2 ± 0.6	16.3 ± 0.2	16.6 ± 0.2[c]
Hematocrit (%)[d]	46.0 ± 1.5	45.5 ± 1.2	45.8 ± 1.7	46.5 ± 1.5	45.4 ± 3.6	46.2 ± 0.7	47.5 ± 0.6[c]
MCV (fl)[d]	52.5 ± 0.7	52.3 ± 0.7	52.4 ± 0.7	52.4 ± 0.8	52.3 ± 0.6	52.1 ± 0.5	52.0 ± 0.7
AST (IU/l)[d]	64 ± 6	65 ± 3	74 ± 14[c]	69 ± 5	68 ± 6	70 ± 5	76 ± 5[b]
ALT (IU/l)[d]	23 ± 3	21 ± 2	26 ± 10	25 ± 3	24 ± 4	25 ± 3	30 ± 3[b]
Glucose (mg/dl)[d]	143 ± 18	144 ± 18	137 ± 9	140 ± 15	141 ± 15	139 ± 11	139± 18
Triglyceride (mg/dl)	45± 5	48 ± 6	42 ± 4	47 ± 8	42 ± 6	39 ± 7	42 ± 7

[a]Data are presented for 10 sacrificed animals.
[b]$p \leq 0.01$ by Dunnett's test.
[c]$p \leq 0.05$ by Dunnett's test.
[d]Data were reported for 9/10 female rats.

Source: Kasai et al. (2008)

Table 4-17 Incidence data of histopathological lesions in F344/DuCrj rats exposed to 1,4-dioxane vapor by whole-body inhalation for 13 weeks

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

[a]Data are presented for sacrificed animals.
[b]Values 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.
[e]Data were not reported for male rats.

Source: Kasai et al. (2008)

4.2.2.2 Chronic Inhalation Toxicity and Carcinogenicity

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

Exposure to 1,4-dioxane vapors had no significant effect on mortality or BW gain and induced no signs of eye or nasal irritation or respiratory distress. Slight, but statistically significant, changes in hematological and clinical chemistry parameters were within the normal physiological limits and were considered to be of no toxicological importance by the investigators. Altered hematological parameters included decreases in packed cell volume, RBC count, and hemoglobin, and an increase in WBC count in male rats. Clinical chemistry changes consisted of a slight decrease in both BUN (control—23 ± 9.9; 111-ppm 1,4-dioxane—19.8 ± 8.8) and ALP activity (control—34.4 ± 12.1; 111-ppm 1,4-dioxane—29.9 ± 9.2) and a small increase in total protein (control—7.5 ± 0.37; 111-ppm 1,4-dioxane—7.9 ± 0.53) in male rats (values are mean ± standard deviation). Organ weights were not significantly affected. Microscopic examination of organs and tissues did not reveal any treatment-related effects. Based on the lack of significant effects on several endpoints, EPA identified the exposure concentration of 0.4 mg/L (111 ppm) as a free standing NOAEL.

Tumors, observed in all groups including controls, were characteristic of the rat strain used and were considered unrelated to 1,4-dioxane inhalation. The most common tumors were reticulum cell sarcomas and mammary tumors. Using Fisher's Exact test and a significance level of $p < 0.05$, no one type of tumor occurred more frequently in treated rats than in controls. No hepatic tumors were seen in any rat and the presence or absence of nasal cavity tumors was not evaluated.

4.2.2.2.2 **Kasai et al.** Groups of male 6-week-old F344/DuCrj rats (50/group) weighing 120 ± 5g (mean ± SD) at the beginning of the study were exposed via inhalation to nominal concentrations of 0 (clean air), 50, 250, and 1,250 ppm (0, 180, 900, and 4,500 mg/m^3, respectively) of vaporized 1,4-dioxane (>99% pure) for 6 hours/day, 5 days/week, for 104 weeks (2 years) in whole body inhalation chambers (Kasai et al., 2009). Each inhalation chamber housed male rats individually in stainless-steel wire hanging cages. The authors stated female counterparts were not exposed given data illustrating the absence of induced mesotheliomas following exposure to 1,4-dioxane in drinking water (Yamazaki et al., 1994a). During exposure, the concentration of 1,4-dioxane vapor was determined every 15 minutes by gas chromatography and animals received food and water ad libitum. In addition, during the 2-year exposure period, clinical signs and mortality were recorded daily. BW and food intake were measured once weekly for the first 14 weeks of exposure, and thereafter, every 4 weeks. At the end of the 2-year exposure period or at the time of an animal's death during exposure, all organs were collected, weighed, and evaluated for macroscopic lesions. Additional examinations were completed on rats sacrificed at the end of the 2-year exposure period. Endpoints examined included: 1) measurement of hematological and clinical chemistry parameters using blood collected from the abdominal aorta of rats following an overnight fasting at the end of the 2-year exposure period; 2) measurement of urinary parameters using Ames reagent strips during the last week of the exposure period; and 3) histopathological evaluations of organs and tissues outlined in the OECD test guideline which included all tissues of the respiratory tract. For measured hematological and clinical chemistry parameters, analyses included: red blood cell count, hemoglobin, hematocrit, MCV, mean corpuscular hemoglobin (MCH), AST, ALT, ALP, and γ-GTP. Organs and tissues collected for histopathological examination were fixed in 10% neutral buffered formalin with the exception of nasal cavity samples. Nasal tissue was trimmed transversely at three levels after decalcification and fixation in a formic acid-formalin solution. The levels were demarcated at the following points: at the posterior edge of the upper incisor teeth (level 1), at the incisive papilla (level 2), and at the anterior edge of the upper molar teeth (level 3). All tissue samples were embedded in paraffin, and then sectioned (at 5 µm thickness) and stained with hematoxylin and eosin (H&E). Dunnett's test, χ^2 test, and Fisher's exact test were used by study authors to determine statistical differences (p-value of 0.05) between 1,4-dioxane exposed and clean air exposed group data.

Deformity in the nose was the only clinical sign reported in this study. This deformity was seen at exposure weeks 74 and 79 in one rat each, exposed to 250 ppm and 1,250 ppm of 1,4-dioxane, respectively. Both of these rats did not survive the 2-year exposure with deaths caused by malignant nasal tumors.

Growth rates and survival rates were analyzed. Growth rates were not significantly affected by 1,4-dioxane exposures, but a decreasing trend in growth was observed during the latter half of the 2-year exposure period for all exposure doses (i.e., 50, 250, and 1,250 ppm). Survival rates were significantly decreased following 91 weeks of exposure to 1,250 ppm of 1,4-dioxane. The authors attributed these deaths to increased incidences of peritoneal mesotheliomas, but also noted that nasal tumors could have been a contributing factor. Terminal survival rates were 37/50, 37/50, 29/50, and 25/50 for 0, 50, 250, and 1,250 ppm exposed groups, respectively.

Exposure-related effects on final BWs, organ weights, and hematological and clinical chemistry parameters were reported. Changes in these effects, as compared to control are outlined in Table 4-18 and Table 4-19. Briefly, at 1,250 ppm terminal BWs were significantly decreased and relative liver and lung weights were significantly increased. It is of note that the observed change in terminal body weight was not an effect of food consumption, which was determined by the study authors to be unaltered. Altered hematological and clinical chemistry parameters were also observed with significant changes at 1,250 ppm. Altered endpoints included decreased hemoglobin, MCV, and MCH, and increased AST, ALT, ALP, and γ-GTP ($p \leq 0.01$) levels. In addition, urine pH was significantly decreased in 1,250 ppm exposed rats.

Histopathology findings of pre- and nonneoplastic lesions associated with 1,4-dioxane treatment were seen in the nasal cavity, liver, and kidneys (Table 4-20). At the highest concentration of 1,250 ppm, all pre- and nonneoplastic lesions were significantly increased, as compared to controls, with the exception of clear and mixed cell foci in the liver. At the lowest concentration of 50 ppm, nuclear enlargement of the respiratory epithelium was the most sensitive lesion observed in the nasal cavity. Based on this finding, the study authors identified a LOAEL of 50 ppm in male rats. As noted earlier, the toxicological significance of nuclear enlargement is unknown.

Tumor development was observed in the nasal cavity (squamous cell carcinoma), liver (hepatocellular adenoma and carcinoma), peritoneum (peritoneal mesothelioma), kidney (renal cell carcinoma), mammary gland (fibroadenoma and adenoma), Zymbal gland (adenoma), and subcutaneous tissue (subcutis fibroma). Tumor incidences with a dose-dependent, statistically significant positive trend (Peto's test) included nasal squamous cell carcinoma, hepatocellular adenoma, peritoneal mesothelioma, mammary gland fibroadenoma, and Zymbal gland adenoma. Renal cell carcinoma was also identified as statistically significant with a positive dose-dependent trend; however, no tumor incidences were reported at 50 and 250 ppm. At 1,250 ppm, significant increases in nasal squamous cell carcinoma, hepatocellular adenoma, and peritoneal mesothelioma were observed. At 250 ppm, significant increases in peritoneum mesothelioma and subcutis fibroma were observed. Table 4-21 presents a summary of tumor incidences found in this study. Further characterizations of neoplasms revealed nasal squamous cell carcinoma occurred at the dorsal area of the nose (levels 1-3) marked by keratinization and the progression of growth into surrounding tissue. Peritoneal mesotheliomas were characterized by complex branching structures originating from the mesothelium of the scrotal sac. Invasive growth into surrounding tissues was occasionally observed for peritoneal mesotheliomas.

Table 4-18 Terminal body and relative organ weights of F344/DuCrj male rats exposed to 1,4-dioxane vapor by whole-body inhalation for 2 years

	Males			
	1,4-dioxane vapor concentration (ppm)			
	0 (clean air)	**50**	**250**	**1250**
Number of animals examined	37	37	29	25
Body weight (g)	383 ± 50	383 ± 53	376 ± 38	359 ± 129[b]
Lung (%)	0.45 ± 0.25	0.49 ± 0.27	0.45 ± 0.18	0.46 ± 0.07[a]
Liver (%)	3.57 ± 0.66	3.86 ± 1.05	3.58 ± 0.52	4.53 ± 0.71[b]
Kidneys (%)	0.87 ± 0.21	0.93 ± 0.32	0.81 ± 0.13	0.86 ± 0.12

[a]$p \leq 0.01$ by Dunnett's test.
[b]$p \leq 0.05$ by Dunnett's test.

Source: Kasai et al. (2008)

Table 4-19 Hematology and clinical chemistry of F344/DuCrj male rats exposed to 1,4-dioxane vapor by whole-body inhalation for 2 years

	Males			
	1,4-dioxane vapor concentration (ppm)			
	0 (clean air)	**50**	**250**	**1250**
Number of animals examined	35	35	28	25
Red blood cell (10^6/μl)	7.4 ± 1.8	6.8 ± 1.8	7.9± 1.0	7.0 ± 1.8
Hemoglobin (g/dl)	12.5 ± 3.5	12.0 ± 3.1	13.4 ± 1.9	10.9 ± 2.8[b]
Hematocrit (%)	38.6 ± 8.7	36.9 ± 7.9	40.7 ± 5.1	34.3 ± 7.6
MCV (fl)	52.4 ± 5.7	55.6 ± 8.7	51.8 ± 2.3	49.4 ± 4.0[b]
MCH (pg)	16.9 ± 2.2	17.8 ± 2.4	17.1 ± 1.2	15.5 ± 1.3[a]
AST (IU/l)	67 ± 31	95 ± 99	95 ± 116	98 ± 52[a]
ALT (IU/l)	37 ± 12	42 ± 21	49 ± 30	72± 36[a]
ALP (IU/l)	185 ± 288	166 ± 85	145 ± 171	212 ± 109[a]
γ-GTP (IU/l)	6 ± 3	8 ± 5	10 ± 8	40 ± 26[a]
Urinary pH	7.1 ± 0.6	7.1 ± 0.6	7.1 ± 0.6	6.6 ± 0.4[b]

[a]$p \leq 0.01$ by Dunnett's test.
[b]$p \leq 0.05$ by Dunnett's test.

Source: Kasai et al. (2008)

Table 4-20 Incidence of pre-and nonneoplastic lesions in male F344/DuCrj rats exposed to 1,4-dioxane vapor by whole-body inhalation for 2 years

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

[a]$p \leq 0.01$ by χ^2 test.
[b]$p \leq 0.05$ by χ^2 test.

Source: Kasai et al. (2009).

Table 4-21 Incidence of tumors in male F344/DuCrj rats exposed to 1,4-dioxane vapor by whole-body inhalation for 2 years

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

[a]$p \leq 0.01$ by Fisher's exact test.
[b]$p \leq 0.05$ by Fisher's exact test.
[c]$p \leq 0.01$ by Peto's test for dose-related trend.
[d]$p \leq 0.05$ by Peto's test for dose-related trend.

Source: Kasai et al. (2009).

4.2.3 Initiation/Promotion Studies

Bronaugh et al. (1982b) reported more 1,4-dioxane absorption from occluded than unoccluded surfaces. Due to the volatility of 1,4-dioxane, the unoccluded skin paint studies are unreliable; however, all of the available skin paint initiation/promotion studies are summarized below.

4.2.3.1 Bull et al.

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

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

4.2.3.2 King et al.

1,4-Dioxane was evaluated for complete carcinogenicity and tumor promotion activity in mouse skin (King et al., 1973a). In the complete carcinogenicity study, 0.2 mL of a solution of 1,4-dioxane (purity not specified) in acetone was applied to the shaved skin of the back of Swiss Webster mice (30/sex) 3 times/week for 78 weeks. Acetone was applied to the backs of control mice (30/sex) for the same time period. In the promotion study, each animal was treated with 50 µg of dimethylbenzanthracene 1 week prior to the topical application of the 1,4-dioxane solution described above (0.2 mL, 3 times/week, 78 weeks) (30 mice/sex). Acetone vehicle was used in negative control mice (30/sex). Croton oil was used as a positive control in the promotion study (30/sex). Weekly counts of papillomas and suspect carcinomas were made by gross examination. 1,4-Dioxane was also administered in the drinking water (0.5 and 1%) to groups of Osborne-Mendel rats (35/sex/group) and $B6C3F_1$ mice for 42 weeks (control findings were only reported for 34 weeks).

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

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

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

4.2.3.3 Lundberg et al.

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

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

4.3 Reproductive/Developmental Studies—Oral and Inhalation

4.3.1 Giavini et al.

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

Maternal weight gain was reduced by 10% in the high-dose group (1,000 mg/kg-day). Food consumption for this group was 5% lower during the dosing period, but exceeded control levels for the remainder of the study. No change from control was observed in the number of implantations, live fetuses, or resorptions; however, fetal birth weight was 5% lower in the highest dose group ($p < 0.01$). 1,4-Dioxane exposure did not increase the frequency of major malformations or minor anomalies and variants. Ossification of the sternebrae was reduced in the 1,000 mg/kg-day dose group ($p < 0.05$). The study authors suggested that the observed delay in sternebrae ossification combined with the decrease in

fetal birth weight indicated a developmental delay related to 1,4-dioxane treatment. NOAEL and LOAEL values of 500 and 1,000 mg/kg-day were identified from this study by EPA and based on delayed ossification of the sternebrae and reduced fetal BWs.

4.4 Other Duration or Endpoint Specific Studies

4.4.1 Acute and Short-term Toxicity

The acute (≤ 24 hours) and short-term toxicity studies (<30 days) of 1,4-dioxane in laboratory animals are summarized in Table 4-22. Several exposure routes were employed in these studies, including dermal application, drinking water exposure, gavage, vapor inhalation, and i.v. or i.p. injection.

4.4.1.1 Oral Toxicity

Mortality was observed in many acute high-dose studies, and LD50 values for 1,4-dioxane were calculated for rats, mice, and guinea pigs (Pozzani et al., 1959; HF Jr et al., 1941; Laug et al., 1939). Clinical signs of CNS depression were observed, including staggered gait, narcosis, paralysis, coma, and death (Nelson, 1951; Laug et al., 1939; Schrenk and Yant, 1936; de Navasquez, 1935). Severe liver and kidney degeneration and necrosis were often seen in acute studies (JBRC, 1998; David, 1964; Kesten et al., 1939; Laug et al., 1939; Schrenk and Yant, 1936; de Navasquez, 1935). JBRC (1998) additionally reported histopathological lesions in the nasal cavity and the brain of rats following 2 weeks of exposure to 1,4-dioxane in the drinking water.

4.4.1.2 Inhalation Toxicity

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

Table 4-22 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 (1990a)
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 2,068 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)
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.(1934a)
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)
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)

[a]Lowest 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

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

4.4.2.1 Frantik et al.

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

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

4.4.2.2 Goldberg et al.

Goldberg et al. (1964) evaluated the effect of solvent inhalation on pole climb performance in rats. Female rats (Carworth Farms Elias strain) (eight per dose group) were 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 10 exposure days. Conditioned avoidance and escape behaviors were evaluated using a pole climb methodology. Prior to exposure, rats were trained to respond to a buzzer or shock stimulus by using avoidance/escape behavior within 2 seconds. Behavioral criteria were the abolishment or significant deferment (>6 seconds) of the avoidance response (conditioned or buzzer response) or the escape response (buzzer plus shock response). Behavioral tests were administered on day 1, 2, 3, 4, 5, and 10 of the exposure period. Rat BWs were also measured on test days.

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

4.4.2.3 Kanada et al.

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

4.4.2.4 Knoefel

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

The genotoxicity data for 1,4-dioxane are presented in Table 4-23 and

Table 4-24 for in vitro and in vivo tests, respectively. 1,4-Dioxane has been tested for genotoxic potential using in vitro assay systems with prokaryotic organisms, non-mammalian eukaryotic organisms, and mammalian cells, and in vivo assay systems using several strains of rats and mice. In the large majority of in vitro systems, 1,4-dioxane was not genotoxic. Where a positive genotoxic response was observed, it was generally observed in the presence of toxicity. Similarly, 1,4-dioxane was not genotoxic in half of the available in vivo studies. 1,4-Dioxane did not bind covalently to DNA in a single study with calf thymus DNA. Several investigators have reported that 1,4-dioxane caused increased DNA synthesis indicative of cell proliferation. Overall, the available literature indicates that 1,4-dioxane is nongenotoxic or weakly genotoxic. However, it is important to note that three of the negative studies reported using closed systems to control for evaporation of the test substance (McGregor et al., 1991b; Zimmermann et al., 1985b; Nestmann et al., 1984b).

Negative findings were reported for mutagenicity in in vitro assays with the prokaryotic organisms *Salmonella typhimurium*, *Escherichia coli*, and *Photobacterium phosphoreum* (Mutatox assay) (Morita and Hayashi, 1998; Hellmér and Bolcsfoldi, 1992; Kwan et al., 1990; Khudoley et al., 1987; Nestmann et al., 1984a; Haworth et al., 1983; Stott et al., 1981) (Table 4-23). In in vitro assays with nonmammalian eukaryotic organisms, negative results were obtained for the induction of aneuploidy in yeast (*Saccharomyces cerevisiae*) and in the sex-linked recessive lethal test in *Drosophila melanogaster* (Yoon et al., 1985; Zimmermann et al., 1985a). In the presence of toxicity, positive results were reported for meiotic nondisjunction in Drosophila (Munoz and Barnett, 2002).

The ability of 1,4-dioxane to induce genotoxic effects in mammalian cells in vitro has been examined in model test systems with and without exogenous metabolic activation and in hepatocytes that retain their xenobiotic-metabolizing capabilities. 1,4-Dioxane was reported as negative in the mouse lymphoma cell forward mutation assay (Morita and Hayashi, 1998; McGregor et al., 1991a). 1,4-Dioxane did not produce chromosomal aberrations or micronucleus formation in Chinese hamster ovary (CHO) cells (Morita and Hayashi, 1998; Galloway et al., 1987a). Results were negative in one assay for sister chromatid exchange (SCE) in CHO (Morita and Hayashi, 1998) and were weakly positive in the absence of metabolic activation in another (Galloway et al., 1987a). In rat hepatocytes, 1,4-dioxane exposure in vitro caused single-strand breaks in DNA at concentrations also toxic to the hepatocytes (Sina et al., 1983) and produced a positive genotoxic response in a cell transformation assay with BALB/3T3 cells also in the presence of toxicity (Sheu et al., 1988).

1,4-Dioxane was not genotoxic in the majority of available in vivo mammalian assays (Table 4-24). Studies of micronucleus formation following in vivo exposure to 1,4-dioxane produced mostly negative results, including studies of bone marrow micronucleus formation in $B6C3F_1$, BALB/c, CBA, and C57BL6 mice (McFee et al., 1994; Mirkova, 1994a; Tinwell and Ashby, 1994) and micronucleus formation in peripheral blood of CD1 mice (Morita and Hayashi, 1998; Morita, 1994). Mirkova (1994a) reported a dose-related increase in the incidence of bone marrow micronuclei in male and female C57BL6 mice 24 or 48 hours after administration of 1,4-dioxane. At a sampling time of 24 hours, a dose of 450 mg/kg produced no change relative to control, while doses of 900, 1,800, and 3,600 mg/kg increased the incidence of bone marrow micronuclei by approximately two-, three-, and fourfold, respectively. A dose of 5,000 mg/kg also increased the incidence of micronuclei by approximately fourfold at 48 hours.

This compares with the negative results for BALB/c male mice tested in the same study at a dose of 5,000 mg/kg and sampling time of 24 hours. Tinwell and Ashby (1994) could not explain the difference in response in the mouse bone marrow micronucleus assay with C57BL6 mice obtained in their laboratory (i.e., non-significant 1.6-fold increase over control) with the dose-related positive findings reported by Mirkova (Mirkova, 1994a) using the same mouse strain, 1,4-dioxane dose (3,600 mg/kg) and sampling time (24 hours). Morita and Hayashi (1998) demonstrated an increase in micronucleus formation in hepatocytes following 1,4-dioxane dosing and partial hepatectomy to induce cellular mitosis. DNA single-strand breaks were demonstrated in hepatocytes following gavage exposure to female rats (Kitchin and Brown, 1990a).

Roy et al. (2005a) examined micronucleus formation in male CD1 mice exposed to 1,4-dioxane to confirm the mixed findings from earlier mouse micronucleus studies and to identify the origin of the induced micronuclei. Mice were administered 1,4-dioxane by gavage at doses of 0, 1,500, 2,500, and 3,500 mg/kg-day for 5 days. The mice were also implanted with 5-bromo-2-deoxyuridine (BrdU)-releasing osmotic pumps to measure cell proliferation in the liver and to increase the sensitivity of the hepatocyte assay. The frequency of micronuclei in the bone marrow erythrocytes and in the proliferating BrdU-labeled hepatocytes was determined 24 hours after the final dose. Significant dose-related increases in micronuclei were seen in the bone-marrow at all the tested doses (≥ 1,500 mg/kg-day). In the high-dose (3,500-mg/kg) mice, the frequency of bone marrow erythrocyte micronuclei was about 10-fold greater than the control frequency. Significant dose-related increases in micronuclei were also observed at the two highest doses (≥ 2,500 mg/kg-day) in the liver. Antikinetochore (CREST) staining or pancentromeric fluorescence in situ hybridization (FISH) was used to determine the origin of the induced micronuclei. The investigators determined that 80–90% of the micronuclei in both tissues originated from chromosomal breakage; small increase in micronuclei originating from chromosome loss was seen in hepatocytes. Dose-related statistically significant decreases in the ratio of bone marrow polychromatic erythrocytes (PCE):normochromatic erythrocytes (NCE), an indirect measure of bone marrow toxicity, were observed. Decreases in hepatocyte proliferation were also observed. Based on these results, the authors concluded that at high doses 1,4-dioxane exerts genotoxic effects in both the mouse bone marrow and liver; the induced micronuclei are formed primarily from chromosomal breakage; and 1,4-dioxane can interfere with cell proliferation in both the liver and bone marrow. The authors noted that reasons for the discrepant micronucleus assay results among various investigators was unclear, but could be related to the inherent variability present when detecting moderate to weak responses using small numbers of animals, as well as differences in strain, dosing regimen, or scoring criteria.

1,4-Dioxane did not affect in vitro or in vivo DNA repair in hepatocytes or in vivo DNA repair in the nasal cavity (Goldsworthy et al., 1991; Stott et al., 1981), but increased hepatocyte DNA synthesis indicative of cell proliferation in several in vivo studies (Miyagawa et al., 1999; Uno et al., 1994; Goldsworthy et al., 1991; Stott et al., 1981). 1,4-Dioxane caused a transient inhibition of RNA polymerase A and B in the rat liver (Kurl et al., 1981), indicating a negative impact on the synthesis of ribosomal and messenger RNA (DNA transcription). Intravenous administration of 1,4-dioxane at doses

of 10 or 100 mg/rat produced inhibition of both polymerase enzymes, with a quicker and more complete recovery of activity for RNA polymerase A, the polymerase for ribosomal RNA synthesis.

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

Rosenkranz and Klopman (1992) analyzed 1,4-dioxane using the computer automated structure evaluator (CASE) structure activity method to predict its potential genotoxicity and carcinogenicity. The CASE analysis is based on information contained in the structures of approximately 3,000 chemicals tested for endpoints related to mutagenic/genotoxic and carcinogenic potential. CASE selects descriptors (activating [biophore] or inactivating [biophobe] structural fragments) from a learning set of active and inactive molecules. Using the CASE methodology, Rosenkranz and Klopman (1992) predicted that 1,4-dioxane would be inactive for mutagenicity in several in vitro systems, including Salmonella, induction of chromosomal aberrations in CHO cells, and unscheduled DNA synthesis in rat hepatocytes. 1,4-Dioxane was predicted to induce SCE in cultured CHO cells, micronuclei formation in rat bone marrow, and carcinogenicity in rodents.

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

Table 4-23 Genotoxicity studies of 1,4-dioxane; in vitro

Test system	Endpoint	Test conditions	Results[a] Without activation	With activation	Dose[b]	Source
Prokaryotic organisms in vitro						
S. typhimurium strains TA98, TA100, TA1535, TA1537	Reverse mutation	Plate incorporation assay	–	–	10,000 µg/plate	Haworth et al. (1983)
S. typhimurium strains TA98, TA100, TA1530, TA1535, TA1537	Reverse mutation	Plate incorporation assay	–	–	ND	Khudoley et al. (1987)
S. typhimurium strains TA98, TA100, TA1535, TA1537	Reverse mutation	Plate incorporation and preincubation assays	–	–	5,000 µg/plate	Morita and Hayashi (1998)
S. typhimurium strains TA100, TA1535	Reverse mutation	Preincubation assay	–	–	103 mg	Nestmann et al. (1984a)
S. typhimurium strains TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	Plate incorporation assay	–	–	103 mg	Stott et al. (1981)
E. coli K-12 uvrB/recA	DNA repair	Host mediated assay	–	–	1,150 mmol/L	Hellmer and Bolcsfoldi (1992)
E. coli WP2/WP2uvrA	Reverse mutation	Plate incorporation and preincubation assays	–	–	5,000 µg/plate	Morita and Hayashi (1998)
P. phosphoreum M169	Mutagenicity, DNA damage	Mutatox assay	–	ND	ND	Kwan et al. (1990)
Nonmammalian eukaryotic organisms in vitro						
S. cerevisiae D61.M	Aneuploidy	Standard 16-hour incubation or cold-interruption regimen	–T	ND	4.75%	Zimmerman et al. (1985a)
D. melanogaster	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)
D. melanogaster	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. (1991a)
L5178Y mouse lymphoma cells	Forward mutation assay	Thymidine kinase mutagenicity assay (trifluorothymidine resistance)	–	–T	5,000 µg/mL	Morita and Hayashi (1998)

Test system	Endpoint	Test conditions	Results[a] Without activation	With activation	Dose[b]	Source
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)
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. (1987a)
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. (1987a)
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. (1977c)

[a]+ = positive, ± = equivocal or weak positive, – = negative, T = toxicity, ND = no data. Endogenous metabolic activation is not applicable for in vivo studies.
[b]Lowest effective dose for positive results/highest dose tested for negative results; ND = no data.
[c]A dose-related decrease in viability was observed with 0, 2.4, 8.1, 51.7, and 82.8% mortality at concentrations of 1, 1.5, 2, 3, and 3.5%, respectively. In mature oocytes, meiotic nondisjunction was decreased at 2, 3, and 3.5%; however, a dose-response trend was not evident.
[d]Exogenous metabolic activation not used for most tests of fungi and many mammalian cell types in vitro, or in vivo studies in mammals, due to endogenous metabolic ability in many of these systems.
[e]Cell viability was 98, 57, 54, 31, and 34% of control at concentrations 0, 0.03, 0.3, 10, and 30 mM. DNA damage was observed at 0.3, 3, 10, and 30 mM; however, no dose-response trend was observed for the extent of DNA damage (severity score related to the elution rate).
[f]For the 13-day exposure, relative survival was 92, 85, 92, and 61% of control for concentrations of 0.25, 0.5, 1, and 2 mg/mL, respectively. A significant increase in transformation frequency was observed at the highest dose level (2 mg/mL). Similar results were observed for the 48-hour exposure, with increased transformation frequency seen at concentrations of 2, 3, and 4 mg/mL. Concentrations >2 mg/mL also caused a significant decrease in cell survival (relative survival ranged between 6 and 52% of control).
[g]The highest concentration tested (10,520 µg/L) produced a 27% increase in the number of SCE/cell in the absence of S9mix. No effect was seen at lower doses (1050 and 3500 µg/L) in the absence of S9 mix or at any concentration level (1050, 3500, 10,500 µg/L) tested in the presence of S9.

1

Table 4-24 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 (1990a)
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 (1994a)
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. (2005a)
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.(2005a)
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)
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)

Test system	Endpoint	Test Conditions	Results[a]	Dose[b]	Source
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, ND = no data. Endogenous metabolic activation is not applicable for in vivo studies.
[b]Lowest effective dose for positive results/highest dose tested for negative results; ND = no data.
[c]Rats 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).
[d]A 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.
[e]A 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.
[f]Significant 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).
[g]A 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.
[h]No 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.
[i]A similar pattern of RNA polymerase inhibition was observed at doses of 10 and 100 mg/rat. Inhibition was more pronounced at the higher dose.
[j]Hepatocyte 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).
[k]Replicative 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%.
[l]Replicative 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 processes
2 in the rat ovary and brain. Female rats (6–9/group, unspecified strain) were exposed to 0, 10, or

100 mg/m^3 of 1,4-dioxane vapor for 4 hours/day, 5 days/week, for 1 month. Rats were sacrificed during the morning or evening following exposure and the ovaries and brain cortex were removed and frozen. Tissue preparations were analyzed for catalase activity, glutathione peroxidase activity, and protein peroxidation. Inhalation of 100 mg/m^3 of 1,4-dioxane resulted in a significant increase ($p < 0.05$) in glutathione peroxidase activity, and activation of free radical processes were apparent in both the rat ovary and brain cortex. No change in catalase activity or protein peroxidation was observed at either concentration. A circadian rhythm for glutathione peroxidase activity was absent in control rats, but occurred in rat brain and ovary following 1,4-dioxane exposure.

4.5.2.2 Induction of Metabolism

The metabolism of 1,4-dioxane is discussed in detail in Section 3.3. 1,4-Dioxane has been shown to induce its own metabolism (Young et al., 1978b; 1978a). Nannelli et al. (2005a) (study details provided in Section 3.3) characterized the CYP450 isozymes that were induced by 1,4-dioxane in the liver, kidney, and nasal mucosa of the rat. In the liver, the activities of several CYP450 isozymes were increased (i.e., CYP2B1/2, CYP2E1, CYPC11); however, only CYP2E1 was inducible in the kidney and nasal mucosa. CYP2E1 mRNA was increased approximately two- to threefold in the kidney and nasal mucosa, but mRNA levels were not increased in the liver, suggesting that regulation of CYP2E1 is organ-specific. Induction of hepatic CYPB1/2 and CYP2E1 levels by phenobarbital or fasting did not increase the liver toxicity of 1,4-dioxane, as measured by hepatic glutathione content or serum ALT activity. This result suggested that highly reactive and toxic intermediates did not play a large role in the liver toxicity of 1,4-dioxane, even under conditions where metabolism was enhanced. This finding is similar to an earlier conclusion by Kociba et al. (1975a) who evaluated toxicity from a chronic drinking water study alongside data providing a pharmacokinetic profile for 1,4-dioxane. Kociba et al. (1975a) concluded that liver toxicity and eventual tumor formation occurred only at doses where clearance pathways were saturated and elimination of 1,4-dioxane from the blood was reduced. Nannelli et al. (2005a) further suggested that a sustained induction of CYP2E1 may lead to generation of reactive oxygen species contributing to target organ toxicity and regenerative cell proliferation; however, no data were provided to support this hypothesis.

4.5.2.3 Mechanisms of Tumor Induction

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

and liver to BW ratios were also evaluated in rats from acute gavage or repeated dose drinking water experiments.

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

Goldsworthy et al. (1991) evaluated potential mechanisms for the nasal and liver carcinogenicity of 1,4-dioxane in the rat. DNA repair activity was evaluated as a measure of DNA reactivity and DNA synthesis was measured as an indicator of cell proliferation or promotional activity. In vitro DNA repair was evaluated in primary hepatocyte cultures from control and 1,4-dioxane-treated rats (1 or 2% in the drinking water for 1 week). DNA repair and DNA synthesis were also measured in vivo following a single gavage dose of 1,000 mg/kg, a drinking water exposure of 1% (1,500 mg/kg-day) for 1 week, or a drinking water exposure of 2% (3,000 mg/kg-day) for 2 weeks. Liver to BW ratios and palmitoyl CoA oxidase activity were measured in the rat liver to determine whether peroxisome proliferation played a role in the liver carcinogenesis of 1,4-dioxane. In vivo DNA repair was evaluated in rat nasal epithelial cells derived from either the nasoturbinate or the maxilloturbinate of 1,4-dioxane-treated rats. These rats received 1% 1,4-dioxane (1,500 mg/kg-day) in the drinking water for 8 days, followed by a single gavage dose of 10, 100, or 1,000 mg/kg 12 hours prior to sacrifice. Archived tissues from the NCI (1978) bioassay were reexamined to determine the primary sites for tumor formation in the nasal cavity following chronic exposure in rats. Histopathology and cell proliferation were determined for specific sites in the nasal cavity that were related to tumor formation. This evaluation was performed in rats that were exposed to drinking water containing 1% 1,4-dioxane (1,500 mg/kg-day) for 2 weeks.

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

Treatment of rats with 1% (1,500 mg/kg-day) 1,4-dioxane for 8 days did not alter DNA repair in nasal epithelial cells (Goldsworthy et al., 1991). The addition of a single gavage dose of up to

1,000 mg/kg 12 hours prior to sacrifice also did not induce DNA repair. Reexamination of tissue sections from the NCI (1978) bioassay suggested that the majority of nasal tumors were located in the dorsal nasal septum or the nasoturbinate of the anterior portion of the dorsal meatus (Goldsworthy et al., 1991). No histopathological lesions were observed in nasal section of rats exposed to drinking water containing 1% 1,4-dioxane (1,500 mg/kg-day) for 2 weeks and no increase was observed in cell proliferation at the sites of highest tumor formation in the nasal cavity.

Female Sprague Dawley rats (three to nine per group) were given 0, 168, 840, 2,550, or 4,200 mg/kg 1,4-dioxane (99% purity) by corn oil gavage in two doses at 21 and 4 hours prior to sacrifice (Kitchin and Brown, 1990a). DNA damage (single-strand breaks measured by alkaline elution), ODC activity, reduced glutathione content, and CYP450 content were measured in the liver. Serum ALT activity and liver histopathology were also evaluated. No changes were observed in hepatic reduced glutathione content or ALT activity. Light microscopy revealed minimal to mild vacuolar degeneration in the cytoplasm of hepatocytes from three of five rats from the 2,550 mg/kg dose group. No histopathological lesions were seen in any other dose group, including rats given a higher dose of 4,200 mg/kg. 1,4-Dioxane caused 43 and 50% increases in DNA single-strand breaks at dose levels of 2,550 and 4,200 mg/kg, respectively. CYP450 content was also increased at the two highest dose levels (25 and 66% respectively). ODC activity was increased approximately two-, five-, and eightfold above control values at doses of 840, 2,550, and 4,200 mg/kg, respectively. The results of this study demonstrated that hepatic DNA damage can occur in the absence of significant cytotoxicity. Parameters associated with tumor promotion (i.e., ODC activity, CYP450 content) were also elevated, suggesting that promotion may play a role in the carcinogenesis of 1,4-dioxane.

4.6 Synthesis of Major Noncancer Effects

Liver, kidney, and nasal toxicity were the primary noncancer health effects associated with exposure to 1,4-dioxane. In humans, several fatal cases of hemorrhagic nephritis and centrilobular necrosis of the liver were related to occupational exposure (i.e., inhalation and dermal contact) to 1,4-dioxane (Johnstone, 1959; Barber, 1934). Neurological changes were also reported in one case; including, headache, elevation in blood pressure, agitation and restlessness, and coma (Johnstone, 1959). Perivascular widening was observed in the brain of this worker, with small foci of demyelination in several regions (e.g., cortex, basal nuclei). In laboratory animals, following oral and inhalation exposure to 1,4-dioxane, liver and kidney degeneration and necrosis were observed(JBRC, 1998; Drew et al., 1978; David, 1964; Kesten et al., 1939; Laug et al., 1939; Schrenk and Yant, 1936; de Navasquez, 1935; Fairley et al., 1934a), in addition to changes in the nasal epithelium (Kano et al., 2009; Kasai et al., 2009; Kano et al., 2008; Kasai et al., 2008; JBRC, 1998). The results of subchronic and chronic studies are discussed below.

4.6.1 Oral

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

Table 4-25 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. (1934a)
Male Sprague Dawley Rat (4–6/group)	Rats 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)	Rats 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)	Mice 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)	Rats 0 or 640 mg/kg-day for 63 weeks	NA	640	Hepatocytes with enlarged hyperchromic nuclei; glomerulonephritis	Argus et al. (1965a)
Male Sprague Dawley Rat (30/group)	Rats 0, 430, 574, 803, or 1,032 mg/kg-day for 13 months	NA	430	Hepatocytomegaly; glomerulonephritis	Argus et al. (1973a)
Sherman Rat (60/sex/dose group)	Rats 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. (1974a)
Osborne-Mendel Rat (35/sex/dose level)	Rats 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)	Mice 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)	Rats 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)

Species	Dose/duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effect	Reference
F344/DuCrj Rat (50/sex/dose level)	Rats Males 0, 11, 55, or 274 mg/kg-day; Females 0, 18, 83, or 429 mg/kg-day for 2 years	11	55	Mixed cell liver foci	JBRC (1998); Kano et al. (2009)
F344/DuCrj Rat (50/sex/dose level)	Rats 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)	Mice 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)	Mice 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)	Rats Pregnant dams 0, 250, 500, or 1,000 mg/kg-day on gestation days 6–15	500	1,000	Delayed ossification of the sternebrae and reduced fetal BWs	Giavini et al. (1985a)

Liver effects included degeneration and necrosis, hepatocyte swelling, cells with hyperchromic nuclei, spongiosis hepatis, hyperplasia, and clear and mixed cell foci of the liver (Kano et al., 2008; NCI, 1978; Kociba et al., 1974a; Argus et al., 1973a; Argus et al., 1965a; Fairley et al., 1934a). Hepatocellular degeneration and necrosis were seen at high doses in a subchronic study (1,900 mg/kg-day in rats) (Fairley et al., 1934a) and at lower doses in a chronic study (94 mg/kg-day, male rats) (Kociba et al., 1974a). Argus et al. (1973a) described a progression of preneoplastic effects in the liver of rats exposed to a dose of 575 mg/kg-day. Early changes (8 months exposure) were described as an increased nuclear size of hepatocytes, disorganization of the rough endoplasmic reticulum, an increase in smooth endoplasmic reticulum, a decrease in glycogen, an increase in lipid droplets in hepatocytes, and formation of liver nodules. Spongiosis hepatis and clear and mixed-cell foci were also observed in the liver of rats (doses >55 mg/kg-day in male rats) (Kano et al., 2009; JBRC, 1998). Clear and mixed-cell foci are commonly considered preneoplastic changes and would not be considered evidence of noncancer toxicity when observed in conjunction with tumor formation. If exposure to 1,4-dioxane had not resulted in tumor formation, these lesions could represent potential noncancer toxicity. The nature of spongiosis hepatis as a preneoplastic change is less well understood (Bannasch, 2003; Karbe and Kerlin, 2002a; Stroebel et al., 1995). Spongiosis hepatis is a cyst-like lesion that arises from the perisinusoidal Ito cells of the liver. This change is sometimes associated with hepatocellular hypertrophy and liver toxicity (Karbe and Kerlin, 2002a), but may also occur in combination with preneoplastic foci, or hepatocellular adenoma or carcinoma (Bannasch, 2003; Stroebel et al., 1995). In the case of the JBRC (1998) study, spongiosis

hepatis was associated with other preneoplastic changes in the liver (clear and mixed-cell foci). No other lesions indicative of liver toxicity were seen in this study; therefore, spongiosis hepatis was not considered indicative of noncancer effects. The activity of serum enzymes (i.e., AST, ALT, LDH, and ALP) was increased in rats and mice exposed to 1,4-dioxane, although only in groups with high incidence of liver tumors. Blood samples were collected only at the end of the 2-year study, so altered serum chemistry may be associated with the tumorigenic changes in the liver.

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

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

4.6.2 Inhalation

Two subchronic (Kasai et al., 2008; Fairley et al., 1934a) and two chronic inhalation studies (Kasai et al., 2009; Torkelson et al., 1974a) were identified. Nasal, liver, and kidney toxicity were the primary noncancer health effects of inhalation exposure to 1,4-dioxane in rodents. Table 4-26 presents a summary of the noncancer results for the subchronic and chronic inhalation studies of 1,4-dioxane toxicity in laboratory animals.

Of the inhalation studies, nasal tissue was only evaluated in rat studies conducted by Kasai et al. (2009; 2008). Adverse effects in nasal tissue were observed frequently in these studies, and statistically significant changes were noted at vapor after concentrations as low as 50 ppm. Nasal effects included deformity of the nose and histopathological changes characterized by enlarged epithelial nuclei (respiratory epithelium, olfactory epithelium, trachea, and bronchus), atrophy (olfactory epithelium), vacuolic change (olfactory epithelium and bronchial epithelium), squamous cell metaplasia and hyperplasia (respiratory epithelium), respiratory metaplasia (olfactory epithelium), inflammation (respiratory and olfactory epithelium), hydropic change (lamina propria), and sclerosis (lamina propria). In both studies, a concentration-dependent, statistically significant incidence of enlarged nuclei of the respiratory epithelium were reported by the study authors; however, the toxicological significance of nuclear enlargement is uncertain.

At high doses, liver damage was characterized by hepatocellular degeneration which varied from swelling (Kasai et al., 2008; Fairley et al., 1934a) to necrosis (Kasai et al., 2009; Kasai et al., 2008; Fairley et al., 1934a), spongiosis hepatis (Kasai et al., 2009), nuclear enlargement of centrilobular cells (Kasai et al., 2009) and basophilic and acidophilic cell foci (Kasai et al., 2009). GST-P positive cell foci are commonly considered preneoplastic changes and would not be considered evidence of noncancer toxicity when observed in conjunction with liver tumor formation (Bannasch et al., 1982a). Since exposure to 1,4-dioxane resulted in tumor formation in the liver, these lesions are not considered as potential noncancer toxicity.

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

The lowest vapor concentration reported to produce liver lesions after 2 years of exposure was 1,250 ppm. The lesions were characterized by necrosis of centrilobular cells, spongiosis hepatis, and nuclear enlargement in the Kasai et al. (2009) study. However, as previously stated, the toxicological significance of nuclear enlargement is uncertain.

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., 1934a), hydropic change of proximal tubules (Kasai et al., 2009; Kasai et al., 2008), and as nuclear enlargement in proximal tubule cells (Kasai et al., 2009). Changes in serum chemistry and urinalysis indices 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 greater susceptibility of female rats to renal damage following inhalation of 1,4-dioxane.

Other noted noncancer effects in laboratory animals included acute vascular congestion of the lungs (Fairley et al., 1934a); 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 than male rats (Kasai et al., 2008). 1,4-Dioxane was measured in plasma along with systemic effects following subchronic inhalation exposure to 1,4-dioxane in rats (Kasai et al., 2008).

Table 4-26 Inhalation toxicity studies (noncancer effects) for 1,4-dioxane

Species	Dose/duration	NOAEL (ppm)	LOAEL (ppm)	Effect	Reference
Subchronic studies					
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. (1934a)
F344/DuCrj rat (10/sex/group)	0, 100, 200, 400, 800, 1,600, 3,200, or 6,400 ppm 6 hours/day 5 days/wk, for 13 wk	NA	100	Respiratory epithelium: nuclear enlargement of epithelial cells	Kasai et al. (2008)
Chronic studies					
Wistar rat (288/sex)	111 ppm for 7hours/day, 5days/wk, 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. (1974a)
F344/DuCrj male rat (50/group)	0, 50, 250, or 1,250 ppm for 6 hours/day, 5 days/wk for 2 years	N/A	50	Respiratory epithelium: nuclear enlargement of epithelial cells, atrophy, and metaplasia	Kasai et al. (2009)

4.6.2.1 Mode of Action Information

The metabolism of 1,4-dioxane in humans was extensive at low doses (<50 ppm). The linear elimination of 1,4-dioxane in both plasma and urine indicated that 1,4-dioxane metabolism was a nonsaturated, first-order process at this exposure level (Young et al., 1977a; 1976a). Like humans, rats extensively metabolized a single 50 ppm inhalation exposure to 1,4-dioxane; however, plasma data from rats given single i.v. doses of 3, 10, 30, 100, or 1,000 mg [^{14}C]-1,4-dioxane/kg demonstrated a dose-related shift from linear, first-order to nonlinear, saturable metabolism of 1,4-dioxane (Young et al., 1978b; 1978a). Using the Young et al. (1978b; 1978a) rat kinetic model, the metabolism of 1,4-dioxane in

rats that were exposed to 400, 800, 1,600, and 3,200 ppm via inhalation for 13 weeks could not be accurately predicted due to a lack of knowledge on needed model parameters and biological processes (See Section 3.5.3 and Appendix B). It appears, following prolonged inhalation exposure to 1,4-dioxane at concentrations up to 3,200 ppm, that metabolism is induced (Appendix B).

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

Nannelli et al. (2005a) investigated the role of CYP450 isozymes in the liver toxicity of 1,4-dioxane. Hepatic CYP2B1/2 and CYP2E1 levels were induced by phenobarbital or fasting and liver toxicity was measured as hepatic glutathione content or serum ALT activity. No increase in glutathione content or ALT activity was observed, suggesting that highly reactive and oxidative intermediates did not play a large role in the liver toxicity of 1,4-dioxane, even under conditions where metabolism was enhanced. Pretreatment with inducers of mixed-function oxidases also did not significantly change the extent of covalent binding in subcellular fractions (Woo et al., 1977c). Covalent binding was measured in liver, kidney, spleen, lung, colon, and skeletal muscle 1–12 hours after i.p. dosing with 1,4-dioxane. Covalent binding was highest in liver, spleen, and colon. Within hepatocytes, 1,4-dioxane distribution was greatest in the cytosolic fraction, followed by the microsomal, mitochondrial, and nuclear fractions.

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

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

A MOA hypothesis involving sustained proliferation of spontaneously transformed liver cells has some support from data indicating that 1,4-dioxane acts as a tumor promoter in mouse skin and rat liver bioassays (Lundberg et al., 1987; King et al., 1973a). Dose-response and temporal data support the occurrence of cell proliferation prior to the development of liver tumors (JBRC, 1998; Kociba et al., 1974a) in the rat model. However, the dose-response relationship for induction of hepatic cell proliferation has not been characterized, and it is unknown if it would reflect the dose-response relationship for liver tumors in the 2-year rat and mouse studies. Conflicting data from rat and mouse bioassays (JBRC, 1998; Kociba et al., 1974a) suggest that cytotoxicity may not be a required precursor event for 1,4-dioxane-induced cell proliferation. Data regarding a plausible dose response and temporal progression (see Table 4-21) from cytotoxicity and cell proliferation to eventual liver tumor formation are not available. Also, Kociba et al. (1974b) reported renal degeneration, necrosis, and regenerative proliferation in exposed rats, but no increase in the incidence of kidney tumors, which does not support a cytotoxicity/cell proliferation MOA.

For nasal tumors, there is a hypothesized MOA that includes metabolic induction, cytotoxicity, and regenerative cell proliferation (Kasai et al., 2009). The induction of CYP450 has some support from data illustrating that following acute oral administration of 1,4-dioxane by gavage or drinking water, CYP2E1 was inducible in nasal mucosa (Nannelli et al., 2005a). CYP2E1 mRNA was increased approximately two- to threefold in nasal mucosa (and in the kidney, see section 3.3) in the Nannelli et al. (2005a) study. While cell proliferation was observed following 1,4-dioxane exposure in both a 2-year inhalation study in male rats (1,250 ppm) (Kasai et al., 2009) and a 2-year drinking water study in male

(274 mg/kg-day) and female rats (429 mg/kg-day), no evidence of cytotoxicity in the nasal cavity was observed (Kasai et al., 2009); therefore, cytotoxicity, as a key event, is not supported. Nasal lesions, including inflammation, hyperplasia, and metaplasia, were frequently seen in inhalation studies conducted by the NTP with no evidence of nasal carcinogenicity (Haseman and Hailey, 1997b; Ward et al., 1993). Following a 13-week inhalation study in rats, a concentration-dependent increase of 1,4-dioxane in the blood was observed (Kasai et al., 2008). Studies have shown that water-soluble, gaseous irritants cause nasal injuries such as squamous cell carcinomas (Morgan et al., 1986). Similarly, 1,4-dioxane, which has been reported as a miscible compound (Hawley and Lewis, 2001), also caused nasal injuries that were concentration-dependent, including nasal tumors (Kasai et al., 2009). Additionally, it has been suggested that in vivo genotoxicity may contribute to the carcinogenic MOA for 1,4-dioxane (Kasai et al., 2009) (see Section 4.7.3.6 for further discussion). Collectively, these data are insufficient to support the hypothesized MOAs.

There are no data available regarding any hypothesized MOA by which 1,4-dioxane produces kidney, lung, peritoneal (mesotheliomas), mammary gland, Zymbal gland, and subcutis tumors.

U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) indicate that for tumors occurring at a site other than the initial point of contact, the weight of evidence for carcinogenic potential may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there is convincing information (e.g., toxicokinetic data) that absorption does not occur by other routes. Information available on the carcinogenic effects of 1,4-dioxane via the oral route demonstrates that tumors occur in tissues remote from the site of absorption. In addition, information on the carcinogenic effects of 1,4-dioxane via the inhalation route in animals also demonstrates that tumors occur at tissue sites distant from the portal of entry. Information on the carcinogenic effects of 1,4-dioxane via the inhalation and dermal routes in humans and via the dermal route in animals is absent. If sufficient external dose is applied, it is assumed that an internal dose will be achieved regardless of the route of exposure. Therefore, based on the observance of systemic tumors following oral and inhalation exposure, 1,4-dioxane is "likely to be carcinogenic to humans" by all routes of exposure.

4.7.2 Synthesis of Human, Animal, and Other Supporting Evidence

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

Several carcinogenicity bioassays have been conducted for 1,4-dioxane in mice, rats, and guinea pigs (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998; Yamazaki et al., 1994a; NCI, 1978; Kociba et al., 1974a; Torkelson et al., 1974a; Argus et al., 1973a; Hoch-Ligeti and Argus, 1970a; Hoch-Ligeti et al., 1970a; Argus et al., 1965a). Liver tumors have been observed following drinking water exposure in male Wistar rats (Argus et al., 1965a), male guinea pigs (Hoch-Ligeti and Argus, 1970a), male Sprague Dawley rats (Argus et al., 1973a; Hoch-Ligeti et al., 1970a), male and female Sherman rats (Kociba et al., 1974a), female Osborne-Mendel rats (NCI, 1978), male and female F344/DuCrj rats (Kano et al., 2009;

JBRC, 1998; Yamazaki et al., 1994a), male and female B6C3F$_1$ mice (NCI, 1978), and male and female Crj:BDF1 mice (Kano et al., 2009; JBRC, 1998; Yamazaki et al., 1994a); and following inhalation exposure in male F344 rats (Kasai et al., 2009). In the earliest cancer bioassays, the liver tumors were described as hepatomas (Argus et al., 1973a; Hoch-Ligeti and Argus, 1970a; Hoch-Ligeti et al., 1970a; Argus et al., 1965a); however, later studies made a distinction between hepatocellular carcinoma and hepatocellular adenoma (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998; Yamazaki et al., 1994a; NCI, 1978; Kociba et al., 1974a). Both tumor types have been seen in rats and mice exposed to 1,4-dioxane via drinking water and inhalation.

Kociba et al. (1974a) noted evidence of liver toxicity at or below the dose levels that produced liver tumors but did not report incidence data for these effects. Hepatocellular degeneration and necrosis were observed in the mid- and high-dose groups of male and female Sherman rats exposed to 1,4-dioxane, while tumors were only observed at the highest dose. Hepatic regeneration was indicated in the mid- and high-dose groups by the formation of hepatocellular hyperplastic nodules. Kasai et al. (2009) noted evidence of liver toxicity and tumor incidences (i.e. hepatocellular adenoma) in male F344/DuCrj rats following inhalation exposures to 1,250 ppm. Increased liver toxicities included hepatocellular necrosis, spongiosis hepatis, and acidophilic and basophilic cell foci.

Nasal cavity tumors were also observed in Sprague Dawley rats (Argus et al., 1973a; Hoch-Ligeti et al., 1970a), Osborne-Mendel rats (NCI, 1978), Sherman rats (Kociba et al., 1974a), and F344/DuCrj rats (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998; Yamazaki et al., 1994a). Most tumors were characterized as squamous cell carcinomas. Nasal tumors were not elevated in B6C3F$_1$ or Crj:BDF1 mice. Kano et al. (2009) and Kasai et al. (2009) were the only studies that evaluated nonneoplastic changes in nasal cavity tissue following prolonged exposure to 1,4-dioxane via oral and inhalation routes, respectively.

Histopathological lesions in female F344/DuCrj rats following oral exposure to 1,4-dioxane were suggestive of toxicity and regeneration in nasal tissue (i.e., atrophy, adhesion, inflammation, nuclear enlargement, and hyperplasia and metaplasia of respiratory and olfactory epithelium). Some of these effects occurred at a lower dose (83 mg/kg-day) than that shown to produce nasal cavity tumors (429 mg/kg-day) in female rats. Re-examination of tissue sections from the NCI (1978) bioassay suggested that the majority of nasal tumors were located in the dorsal nasal septum or the nasoturbinate of the anterior portion of the dorsal meatus.

Histopathological lesions in male F344/DuCrj rats following exposure to 1,4-dioxane via inhalation were also suggestive of toxicity and regeneration in nasal tissue (i.e. atrophy, inflammation, nuclear enlargement, hyperplasia and metaplasia of the respiratory and olfactory epithelium, and inflammation). Some of these effects occurred at lower concentrations (50 ppm and 250 ppm) than those shown to produce nasal cavity tumors (1,250 ppm) in male rats. Nasal squamous cell carcinomas were observed in the dorsal area of levels 1-3 of the nasal cavity and were characterized as well-differentiated and keratinized. In two cases, invasive growth into adjacent tissue was noted, marked by carcinoma growth out of the nose and through a destroyed nasal bone.

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

4.7.3 Mode of Action Information

The hypothesized MOAs for 1,4-dioxane carcinogenicity are discussed below within the context of the modified Hill criteria of causality as recommended in the most recent Agency guidelines (U.S. EPA, 2005a). MOA analyses were not conducted for kidney, peritoneal, mammary gland, Zymbal gland, or subcutis tumors due to the absence of any chemical specific information for these tumor types.

4.7.3.1 Identification of Key Events for Carcinogenicity

4.7.3.1.1 **Liver.** A key event in this MOA hypothesis is sustained proliferation of spontaneously transformed liver cells, resulting in the eventual formation of liver tumors. Precursor events in which 1,4-dioxane may promote proliferation of transformed liver cells are uncertain. One study suggests that induced liver cytotoxicity may be a key precursor event to cell proliferation leading to the formation of liver tumors (Kociba et al., 1974a), however, this study did not report incidence data for these effects. Other studies suggest that cell proliferation can occur in the absence of liver cytotoxicity. Liver tumors were observed in female rats and female mice in the absence of lesions indicative of cytotoxicity (Kano et al., 2008; JBRC, 1998; NCI, 1978). Figure 4-1 presents a schematic representation of possible key events in the MOA for 1,4-dioxane liver carcinogenicity. These include: (1) oxidation by CYP2E1 and CYP2B1/2 (i.e., detoxification pathway for 1,4-dioxane), (2) saturation of metabolism/clearance leading to accumulation of the parent 1,4-dioxane, (3) liver damage followed by regenerative cell proliferation, or (4) cell proliferation in the absence of cytotoxicity (i.e., mitogenesis), (5) hyperplasia, and (6) tumor formation. It is suggested that liver toxicity is related to the accumulation of the parent compound following metabolic saturation at high doses (Kociba et al., 1975a); however, since no in vivo or in vitro assays have identified the toxic moiety resulting from 1,4-dioxane exposure, liver toxicity due to metabolites cannot be ruled out. Therefore, this hypothesis is not supported. Nannelli et al. (2005a) demonstrated that an increase in the oxidative metabolism of 1,4-dioxane via CYP450 induction using phenobarbital or fasting does not result in an increase in liver toxicity. This result suggested that the highly reactive intermediates did not play a large role in the liver toxicity of 1,4-dioxane, even under conditions where metabolism was enhanced. Alternative metabolic pathways (e.g., not CYP450 mediated) may be present at high doses of 1,4-dioxane; although the available studies have not characterized these pathways nor identified any possible reactive intermediates. Tumor promotion studies in mouse skin and rat liver suggest that 1,4-dioxane may enhance the growth of previously initiated cells (Lundberg et al., 1987; King et al., 1973a). This is consistent with the increase in rat hepatocyte cell proliferation observed in several studies (Miyagawa et al., 1999; Uno et al., 1994; Goldsworthy et al., 1991; Stott et al., 1981). No studies have been conducted that specifically examine mouse liver, thus precluding any determination on whether 1,4-dioxane acts as a tumor promoter in the mouse liver. These mechanistic studies provide evidence of cell proliferation but do not indicate whether mitogenesis or cytotoxicity is responsible for increased cell turnover.

The doses in the hepatotoxicity studies where cytotoxicity and cell proliferation were observed are not equivalent to the doses used in the cancer bioassays. Although Kociba et al. (1974) noted evidence of liver toxicity at or below the dose levels that produced liver tumors, they did not report incidence data for these effects. Thus, a dose-response relationship is unable to be established using the available studies linking cytotoxicity and cell proliferation observations with tumorigenesis. Additionally, conflicting data from rat and mouse bioassays suggest that cytotoxicity may not be a required precursor event for 1,4-dioxane-induced cell proliferation.

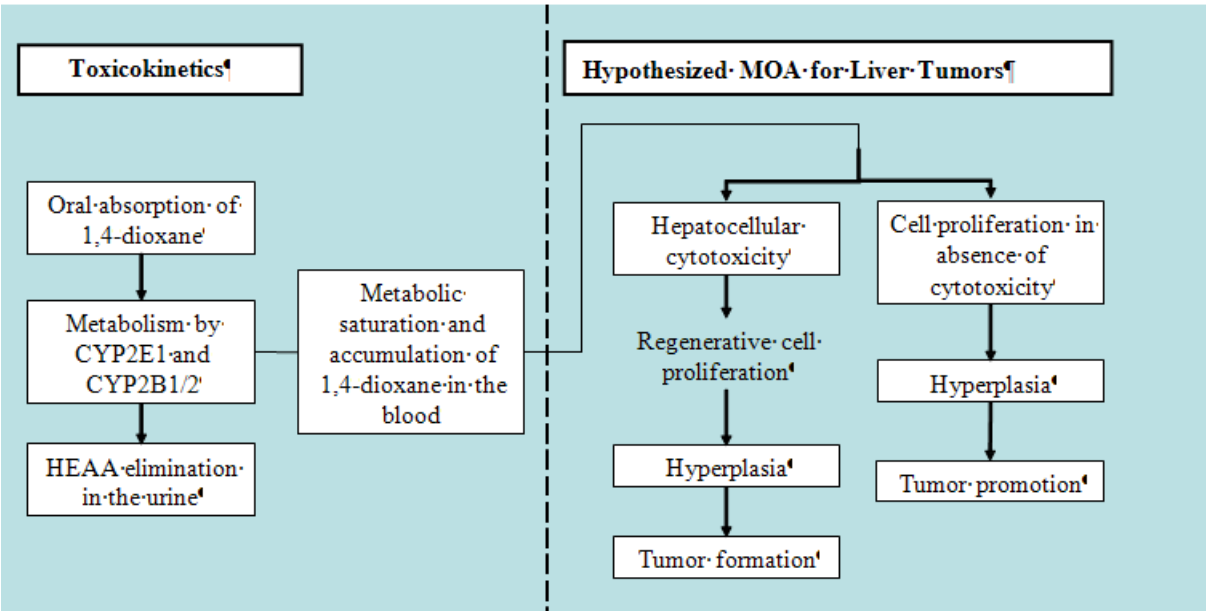


Figure 4-1 A schematic representation of the possible key events in the delivery of 1,4-dioxane to the liver and the hypothesized MOA(s) for liver carcinogenicity

4.7.3.1.2 **Nasal cavity.** A possible key event in the MOA hypothesis for nasal tumors is sustained proliferation of spontaneously transformed nasal epithelial cells, resulting in the eventual formation of nasal cavity tumors (Kasai et al., 2009). Cell proliferation was observed following 1,4-dioxane exposure in both a 2-year inhalation study in male rats (1,250 ppm) (Kasai et al., 2009) and a 2-year drinking water study in male (274 mg/kg-day) and female rats (429 mg/kg-day) (Kano et al., 2009). However, neither study reported evidence of cytotoxicity in the nasal cavity (Kasai et al., 2009) therefore, cytotoxicity as a key event is not supported. Nasal lesions, including inflammation, hyperplasia, and metaplasia, were frequently seen in inhalation studies conducted by the NTP with no evidence of nasal carcinogenicity (Haseman and Hailey, 1997b; Ward et al., 1993). Kasai et al. (2009; 2008) suggest that nasal toxicity is related to the accumulation of the parent compound following metabolic induction at high doses up to 3,200 ppm; however, since no in vivo or in vitro assays have examined the toxic moiety resulting from 1,4-dioxane exposure, nasal toxicity due to metabolites cannot be ruled out. Nannelli et al. (2005a) demonstrated that CYP2E1 was inducible in nasal mucosa following acute oral administration of 1,4-dioxane by gavage and drinking water, which could potentially lead to an increase in the oxidative metabolism of 1,4-dioxane and nasal toxicity. However, Nannelli et al. (2005a) neither characterized this pathway nor identified possible reactive intermediates or nasal toxicities.

4.7.3.2 Strength, Consistency, Specificity of Association

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

4.7.3.2.2 Nasal cavity. Nasal cavity tumors have been demonstrated in several rat strains (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998; Yamazaki et al., 1994a; NCI, 1978; Kociba et al., 1974a), but were not elevated in two strains of mice (Kano et al., 2009; JBRC, 1998; Yamazaki et al., 1994a; NCI, 1978). Irritation of the nasal cavity of rats was indicated in studies by the observation of inflammation (2009; Kasai et al., 2008) and also rhinitis (JBRC, 1998). The Kasai et al. (2009; 2008) studies also showed atrophy of the nasal epithelium in rats, and the JRBC (1998) study also observed atrophy of the nasal epithelium as well as adhesion in rats. Regeneration of the nasal epithelium is demonstrated by metaplasia and hyperplasia observed in rats exposed to 1,4-dioxane (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998; Yamazaki et al., 1994a). Oxidation of 1,4-dioxane metabolism by CYP450s is not supported as a key event in the MOA hypothesis of nasal tumors. Although Nannelli et al. (2005a) demonstrated that CYP2E1 was inducible in nasal mucosa following acute oral administration of 1,4-dioxane by gavage and drinking water, the study lacked details regarding the toxic moiety (e.g. parent compound or reactive intermediate) and resulting nasal toxicity. Accumulation of 1,4-dioxane in blood, as a precursor event of nasal tumor formation is also not supported because the parent compound 1,4-dioxane was only measured in one subchronic study (Kasai et al., 2008) and in this study no evidence of nasal cytotoxicity, cell proliferation, or incidence of nasal tumors were reported.

4.7.3.3 Dose-Response Relationship

4.7.3.3.1 Liver. Table 4-27 presents the temporal sequence (i.e., the table columns in sequential order from 1,4-dioxane metabolism, to liver damage, cell proliferation, hyperplasia, and the formation of adenomas and/or carcinomas) and dose-response relationship for possible key events in the liver carcinogenesis of 1,4-dioxane. Dose-response information provides some support for enhanced cell proliferation as a key event in the liver tumorigenesis of 1,4-dioxane; however, the role of cytotoxicity as a required precursor event is not supported by data from more than one study. Kociba et al. (1974a) demonstrated that liver toxicity and hepatocellular regeneration occurred at a lower dose level than tumor formation. Hepatocellular degeneration and necrosis were observed in the mid- and high-dose groups of Sherman rats exposed to 1,4-dioxane, although it is not possible to discern whether this effect was observed in both genders due to the lack of incidence data (Kociba et al., 1974a). Hepatic tumors were only observed at the highest dose (Kociba et al., 1974a). Hepatic regeneration was indicated in the mid- and high-dose group by the formation of hepatocellular hyperplastic nodules. Liver hyperplasia was also reported in rats from the JBRC (1998) study, at or below the dose level that resulted in tumor formation (Kano et al., 2009); however, hepatocellular degeneration and necrosis were not reported. The liver hyperplasia reported in JBRC (1998) was later reclassified to hepatocellular adenoma or altered hepatocellular foci (Kano et al., 2009). These results suggest that hepatic cell proliferation may occur in the absence of significant cytotoxicity. Liver angiectasis (i.e., dilation of blood or lymphatic vessels) was observed in male mice at the same dose that produced liver tumors; however, the relationship between this vascular abnormality and tumor formation is unclear.

Table 4-27 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., (1974a)—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)—male Osborne-Mendel rats					
0 mg/kg-day	—[a]	—[a]	—[a]	—[a]	—[a]
240 mg/kg-day	+[b]	—[a]	—[a]	—[a]	—[a]
530 mg/kg-day	+[b]	—[a]	—[a]	—[a]	—[a]
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 B6C3F$_1$ 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 B6C3F$_1$ mice					
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]	—[a]	—[a]
274 mg/kg-day	+[b]	+[c,d]	—[a]	—[a]	+[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]	—[a]	+[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]
Kasai et al., (2009)—male F344 rats					
0 ppm	—[a]	—[a]	—[a]	—[a]	—[a]
50 ppm	—[a]	—[a]	—[a]	—[a]	—[a]
250 ppm	—[a]	—[a]	—[a]	—[a]	—[a]
1,250 ppm	—[a]	+[h]	—[a]	—[a]	+[h]

[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] Statistically significant increase noted.
[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.
[f]+ Kasai et al. (2008) reported significant incidence rates for single cell necrosis in female rats only (3,200 ppm) following a 2 year bioassay.
[g]All rats died during the first week of the 13-week bioassay (Kasai et al., 2008).
[h]Kasai et al. (2009) reported incidence rates for centrilobular necrosis and hepatocellular adenomas in male rats (1,250 ppm).

4.7.3.3.2 Nasal cavity.

Table 4-28 presents the temporal sequence (i.e., the table columns in sequential order from 1,4-dioxane metabolism, to liver damage, cell proliferation, hyperplasia, and the formation of adenomas and/or carcinomas) and dose-response relationship for possible key events in the nasal tissue carcinogenesis of 1,4-dioxane. Toxicity and regeneration in nasal epithelium (i.e., atrophy, adhesion, inflammation, and hyperplasia and metaplasia of respiratory and olfactory epithelium) was evident in one study at the same dose levels that produced nasal cavity tumors (Kano et al., 2009; JBRC, 1998). In another study, dose-response information provided some support for nasal toxicity and regeneration in nasal epithelium occurring before tumor development (Kasai et al., 2009). However, the role of cytotoxicity as a required precursor event is not supported by data from any of the reviewed studies. The accumulation of parent 1,4-dioxane as a key event has some support since concentration-dependent increases were noted for 1,4-dioxane in plasma concurrent with toxicities observed that are possible precursor events (i.e., regeneration in nasal epithelium) (Kasai et al., 2008). In a subsequent study by Kasai et al. (2009) some of these same possible precursor events were observed at 50, 250, and 1,250 ppm with evidence of nasal tumors at the highest concentration (1,250 ppm).

Table 4-28 Temporal sequence and dose-response relationship for possible key events and nasal tumors in rats and mice

Dose (mg/kg-day) or Exposure (ppm)	Key event (time →)				
	Metabolism 1,4-dioxane	Nasal cytotoxicity	Cell proliferation	Hyperplasia	Adenomas and/or carcinomas
Kociba et al., (1974a)—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]	—[a]	—[a]	—[a]	—[a]
1,307 mg/kg-day	+[b]	—[a]	—[a]	—[a]	—[a]
NCI, (1978)—female Osborne-Mendel rats					
0 mg/kg-day	—[a]	—[a]	—[a]	—[a]	—[a]
350 mg/kg-day	+[b]	—[a]	—[a]	—[a]	—[a]
640 mg/kg-day	+[b]	—[a]	—[a]	—[a]	—[a]
NCI, (1978)—male B6C3F$_1$ mice					
0 mg/kg-day	—[a]	—[a]	—[a]	—[a]	—[a]
720 mg/kg-day	+[b]	—[a]	—[a]	—[a]	—[a]
830 mg/kg-day	+[b]	—[a]	—[a]	—[a]	—[a]
NCI, (1978)—female B6C3F$_1$ mice					
0 mg/kg-day	—[a]	—[a]	—[a]	—[a]	—[a]
380 mg/kg-day	+[b]	—[a]	—[a]	—[a]	—[a]
860 mg/kg-day	+[b]	—[a]	—[a]	—[a]	—[a]
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]	—[a]	—[a]
274 mg/kg-day	+[b]	—[a]	—[a]	+[c,d]	+[c,d]
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,d]	+[c,d]
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]	—[a]
191 mg/kg-day	+[b]	—[a]	—[a]	—[a]	—[a]
677 mg/kg-day	+[b]	—[a]	—[a]	—[a]	—[a]
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]	—[a]
278 mg/kg-day	+[b]	—[a]	—[a]	—[a]	—[a]
964 mg/kg-day	+[b]	—[a]	—[a]	—[a]	—[a]
Kasai et al. (2008)—F344 rats (male and female combined)					
0 ppm	—[a]	—[a]	—[a]	—[a]	—[a]
100 ppm	+[b]	—[a]	—[a]	—[a]	—[a]
200 ppm	+[b]	—[a]	—[a]	—[a]	—[a]
400 ppm	+[c]	—[a]	—[a]	—[a]	—[a]
800 ppm	+[c]	—[a]	—[a]	—[a]	—[a]
1,600 ppm	+[c]	—[a]	—[a]	—[a]	—[a]
3,200 ppm	+[c]	—[a]	—[a]	—[a]	—[a]
6,400 ppm	+[a,b,f]	—[a,f]	—[a,f]	—[a,f]	—[a,f]
Kasai et al. (2009)—male F344 rats					
0 ppm	—[a]	—[a]	—[a]	—[a]	—[a]
50 ppm	+[b]	—[a]	—[a]	—[a]	—[a]
250 ppm	+[b]	—[a]	—[a]	—[a]	—[a]

1,250 ppm	+[b]	—[a]	—[c]	+[e]	+[c]

[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

4.7.3.4.1 Liver. Available information regarding temporal relationships between the key event (sustained proliferation of spontaneously transformed liver cells) and the eventual formation of liver tumors is limited. A comparison of 13-week and 2-year studies conducted in F344/DuCrj rats and Crj:BDF1 mice at the same laboratory revealed that tumorigenic doses of 1,4-dioxane produced liver toxicity by 13 weeks of exposure (Kano et al., 2009; Kano et al., 2008; JBRC, 1998). Hepatocyte swelling of the centrilobular area of the liver, vacuolar changes in the liver, granular changes in the liver, and single cell necrosis in the liver were observed in mice and rats given 1,4-dioxane in the drinking water for 13 weeks. Sustained liver damage may lead to regenerative cell proliferation and tumor formation following chronic exposure. As discussed above, histopathological evidence of regenerative cell proliferation has been seen following long-term exposure to 1,4-dioxane (JBRC, 1998; Kociba et al., 1974a). Tumors occurred earlier at high doses in both mice and rats from this study (Yamazaki, 2006); however, temporal information regarding hyperplasia or other possible key events was not available (i.e., interim blood samples not collected, interim sacrifices were not performed). Argus et al. (1973a) studied the progression of tumorigenesis by electron microscopy of liver tissues obtained following interim sacrifices at 8 and 13 months of exposure (five rats/group, 574 mg/kg-day). The first change observed was an increase in the size of the nuclei of the hepatocytes, mostly in the periportal area. Precancerous changes were characterized by disorganization of the rough endoplasmic reticulum, increase in smooth endoplasmic reticulum, and decrease in glycogen and increase in lipid droplets in hepatocytes. These changes increased in severity in the hepatocellular carcinomas in rats exposed to 1,4-dioxane for 13 months.

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

4.7.3.4.2 Nasal cavity. No information was available regarding the temporal relationship between toxicity in the nasal epithelium and the formation of nasal cavity tumors. Sustained nasal damage may lead to regenerative cell proliferation and tumor formation following chronic exposure. As discussed above (Section 4.2.2.2.1), no evidence of cytotoxicity has been observed following exposure to 1,4-dioxane, despite histopathological evidence of regenerative cell proliferation and nasal tumors at the highest exposure concentration (Kano et al., 2009; Kasai et al., 2009) (See Table 4-28). Other incidences of nasal damage may have occurred before tumor formation; however, temporal information regarding these events was not available (i.e., interim sacrifices were not performed).

4.7.3.5 Biological Plausibility and Coherence

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

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

The National Toxicological Program (NTP) database identified 12 chemicals from approximately 500 bioassays as nasal carcinogens and 1,4-dioxane was the only identified nasal carcinogen that showed little evidence of genotoxicity (Haseman and Hailey, 1997a). Nasal tumors were not observed in an inhalation study in Wistar rats exposed to 111 ppm for 5 days/week for 2 years (Torkelson et al., 1974a), but were observed in an inhalation study in F344 rats exposed to 1,250 ppm for 5 days/week for 2 years. Two human studies of occupational exposure, ranging from 0.06 ppm to 75 ppm for 1month up to 41 years, reported negative findings regarding increased tumor risk (Buffler et al., 1978a; Thiess et al., 1976a). It is important to note, neither nasal tumors in the human studies nor genotoxicity in human or animal studies were evaluated following inhalation exposure to 1,4-dioxane

While there is no known MOA for 1,4-dioxane and the human studies are inconclusive regarding tumor risk, the noted nasal tumors in rats are considered biologically plausible and relevant to humans, since similar cell types considered to be at risk are prevalent throughout the respiratory tract of rats and humans. In general, rats may be more susceptible to nasal lesions than humans due to differences in the

anatomy and geometry of the upper respiratory tract (e.g., larger fraction of inspired air ventilates rat nasal cavity compared to the human) and resulting differences in absorption (e.g., rat nasal cavity is more efficient at scrubbing gases than human) or in local respiratory system effects; however, there is not as much known about other respiratory tract lesions (e.g., trachea or lower respiratory tract) (U.S. EPA, 2012b, 2009b). Species differences in absorption and respiratory tract uptake for 1,4- dioxane have not been studied, thus it still represents an area of uncertainty for this compound.

4.7.3.6 Other Possible Modes of Action

An alternate MOA could be hypothesized that 1,4-dioxane alters DNA, either directly or indirectly (Kasai et al., 2009), which causes mutations in critical genes for tumor initiation, such as oncogenes or tumor suppressor genes. Following these events, tumor growth may be promoted by a number of molecular processes leading to enhanced cell proliferation or inhibition of programmed cell death. The results from in vitro and in vivo assays do not provide overwhelming support for the hypothesis of a genotoxic MOA for 1,4-dioxane carcinogenicity. The genotoxicity data for 1,4-dioxane were reviewed in Section 4.5.1 and were summarized in Table 4-23. Negative findings were reported for mutagenicity in *Salmonella typhimurium*, *Escherichia coli*, and *Photobacterium phosphoreum* (Mutatox assay) (Morita and Hayashi, 1998; Hellmér and Bolcsfoldi, 1992; Kwan et al., 1990; Khudoley et al., 1987; Nestmann et al., 1984a; Haworth et al., 1983; Stott et al., 1981). Negative results were also indicated for the induction of aneuploidy in yeast (*Saccharomyces cerevisiae*) and the sex-linked recessive lethal test in *Drosophila melanogaster* (Zimmermann et al., 1985a). In contrast, positive results were reported in assays for sister chromatid exchange (Galloway et al., 1987a), DNA damage (Kitchin and Brown, 1990a), and in in vivo micronucleus formation in bone marrow (Roy et al., 2005a; Mirkova, 1994a), and liver (Roy et al., 2005a; Morita and Hayashi, 1998). Lastly, in the presence of toxicity, positive results were reported for meiotic nondisjunction in drosophila (Munoz and Barnett, 2002), DNA damage (Sina et al., 1983), and cell transformation (Sheu et al., 1988).

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

4.7.3.7 Conclusions About the Hypothesized Mode of Action

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

4.7.3.7.2 Nasal cavity. The MOA for the formation of nasal cavity tumors is unknown, and evidence in support of any hypothetical mode of carcinogenic action for 1,4-dioxane is inconclusive. Nasal carcinogens are generally characterized as potent genotoxins (Ashby, 1994); however, other MOAs have been proposed for nasal carcinogens that induce effects through other mechanisms (Kasper et al., 2007; Green et al., 2000). In the human studies evidence of nasal tumors were not assessed, nor genotoxicity in human or animal studies following inhalation exposure to 1,4-dioxane, so the role of genotoxicity cannot be ruled out. A MOA hypothesis involving nasal damage, cell proliferation, and hyperplasia is possible, but data are not available to support this hypothesis. In studies that examined nasal effects after exposure to 1,4-dioxane, at least one of these events is missing. More specifically, nasal cavity tumors have been reported by Kasai et al. (2009) in the absence of cytotoxicity and in Kano et al. (2009) in the absence of hyperplasia. Therefore, as per EPA's Cancer Guidelines (U.S. EPA, 2005a), there is insufficient biological support for potential key events and to have reasonable confidence in the sequence of events and how they relate to the development of nasal tumors following exposure to 1,4-dioxane. Using the modified Hill criteria, exposure-response and temporal relationships have not been established in support of any hypothetical mode of carcinogenic action for 1,4-dioxane.

4.7.3.8 Relevance of the Mode of Action to Humans

Several hypothesized MOAs for 1,4-dioxane induced tumors in laboratory animals have been discussed along with the supporting evidence for each. Some mechanistic information is available to inform the MOA of the liver and nasal tumors but no information exists to inform the MOA of the other tumor types (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998; Yamazaki et al., 1994b). Human relevancy is assumed unless information indicates otherwise (U.S. EPA, 2005b).

4.8 Susceptible Populations and Life Stages

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

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

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

Liver and kidney toxicity were the primary noncancer health effects associated with exposure to 1,4-dioxane in humans and laboratory animals. Occupational exposure to 1,4-dioxane has resulted in hemorrhagic nephritis and centrilobular necrosis of the liver (Johnstone, 1959; Barber, 1934). In animals, liver and kidney degeneration and necrosis were observed frequently in acute oral and inhalation studies (JBRC, 1998; Drew et al., 1978; David, 1964; Kesten et al., 1939; Laug et al., 1939; Schrenk and Yant, 1936; de Navasquez, 1935; Fairley et al., 1934a). Liver and kidney effects were also observed following chronic oral exposure to 1,4-dioxane in animals (Kano et al., 2009; JBRC, 1998; Yamazaki et al., 1994a; NCI, 1978; Kociba et al., 1974a; Argus et al., 1973a; Argus et al., 1965a) (see Table 4-25).

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

Kidney damage in chronic toxicity studies was characterized by degeneration of the cortical tubule cells, necrosis with hemorrhage, and glomerulonephritis (NCI, 1978; Kociba et al., 1974a; Argus et al., 1973a; Argus et al., 1965a; Fairley et al., 1934a). Kociba et al. (1974a) described renal tubule epithelial cell degeneration and necrosis at doses of 94 mg/kg-day (LOAEL in male rats) or greater, with a NOAEL of 9.6 mg/kg-day. No quantitative incidence data were provided in this study (Kociba et al.,

1974a). Doses of ≥ 430 mg/kg-day 1,4-dioxane induced marked kidney alterations (Argus et al., 1973a). The observed changes included glomerulonephritis and pyelonephritis, with characteristic epithelial proliferation of Bowman's capsule, periglomerular fibrosis, and distension of tubules. Quantitative incidence data were not provided in this study. In the NCI (1978) study, kidney lesions in rats consisted of vacuolar degeneration and/or focal tubular epithelial regeneration in the proximal cortical tubules and occasional hyaline casts. Kidney toxicity was not seen in rats from the JBRC (1998) study at any dose level (highest dose was 274 mg/kg-day in male rats and 429 mg/kg-day in female rats).

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

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

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

Available human PBPK models were evaluated to determine if an adequate fit of the model to the empirical model output or experimental observations could be attained using biologically plausible values for the model parameters. The re-calibrated model predictions for blood 1,4-dioxane levels did not adequately fit the experimental values (See Appendix B). The model structure is insufficient to capture the apparent species difference in the blood 1,4-dioxane V_d between rats and humans. Differences in the ability of rat and human blood to bind 1,4-dioxane may contribute to the difference in V_d. However, this is expected to be evident in very different values for rat and human blood:air partition coefficients, which is not the case (Table B-1). Additionally, the models do not account for induction in metabolism, which may be present in animals exposed repeatedly to 1,4-dioxane. Therefore, some other modification(s) to the Reitz et al. (1990b) PBPK model structure would be necessary to correct the PBPK models for use in derivation of toxicity values (See Appendix B for more details).

Kociba et al. (1974a) did not provide quantitative incidence or severity data for liver and kidney degeneration and necrosis. Therefore, benchmark dose (BMD) modeling could not be performed for this

study, and thus the NOAEL for liver and kidney degeneration (9.6 mg/kg-day in male rats) was used as the point of departure (POD) in deriving the RfD for 1,4-dioxane.

An alternative POD was derived using incidence data reported for cortical tubule degeneration in the kidneys in male and female rats (NCI, 1978). The incidence data for cortical tubule cell degeneration in male and female rats exposed to 1,4-dioxane in the drinking water for 2 years are presented in Table 5-1. Details of the BMD analysis of these data are presented in Appendix C. Male rats were more sensitive to the kidney effects of 1,4-dioxane than females, and the male rat data provided the lowest POD based on cortical tubule degeneration in the NCI (1978) study ($BMDL_{10}$ of 22.3 mg/kg-day) (Table 5-2). The $BMDL_{10}$ value of 22.3 mg/kg-day from the NCI (1978) study is about double the NOAEL (9.6 mg/kg-day) observed by Kociba et al. (1974a).

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]

[a]Statistically significant trend for increased incidence by Cochran-Armitage test ($p < 0.05$) performed for this review.
[b]Incidence 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 the incidence 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).

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

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

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

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

A default interspecies UF of 10 (UF_A) was used to account for pharmacokinetic and pharmacodynamic differences between rats and humans. Existing PBPK models could not be used to derive an oral RfD for 1,4-dioxane (Appendix B).

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

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

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

An UF to extrapolate from a LOAEL to a NOAEL (UF_L) was not necessary because the RfD was based on a NOAEL. Kociba et al. (1974a) was a well-conducted, chronic drinking water study with an adequate number of animals. Histopathological examination was performed for many organs and tissues, but clinical chemistry analysis was not performed. NOAEL and LOAEL values were derived by the study authors based on liver and kidney toxicity; however, quantitative incidence data were not reported. Several additional oral studies (of acute/short-term, subchronic, and chronic durations) were available that support liver and kidney toxicity as the critical effect (Kano et al., 2008; JBRC, 1998; NCI, 1978; Argus et al., 1973a) (Table 4-15 and Table 4-17). Although degenerative liver and kidney toxicity was not observed in rats from the JBRC (1998) study at doses at or below the LOAEL in the Kociba et al. (1974a) study, other endpoints such as metaplasia and hyperplasia of the nasal epithelium, nuclear enlargement, and hematological effects, were noted.

5.1.4 RfD Comparison Information

PODs and candidate oral RfDs based on selected studies included in Table 4-18 are arrayed in Figure 5-1 to Figure 5-3, and provide perspective on the RfD supported by Kociba et al. (1974a). These figures should be interpreted with caution because the PODs across studies are not necessarily comparable, nor is the confidence in the data sets from which the PODs were derived the same. PODs in these figures may be based on a NOAEL, LOAEL, or BMDL (as indicated), and the nature, severity, and

incidence of effects occurring at a LOAEL are likely to vary. To some extent, the confidence associated with the resulting candidate RfD is reflected in the magnitude of the total UF applied to the POD (i.e., the size of the bar); however, the text of Sections 5.1.1 and 5.1.2 should be consulted for a more complete understanding of the issues associated with each data set and the rationale for the selection of the critical effect and principal study used to derive the candidate RfD.

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

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

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

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

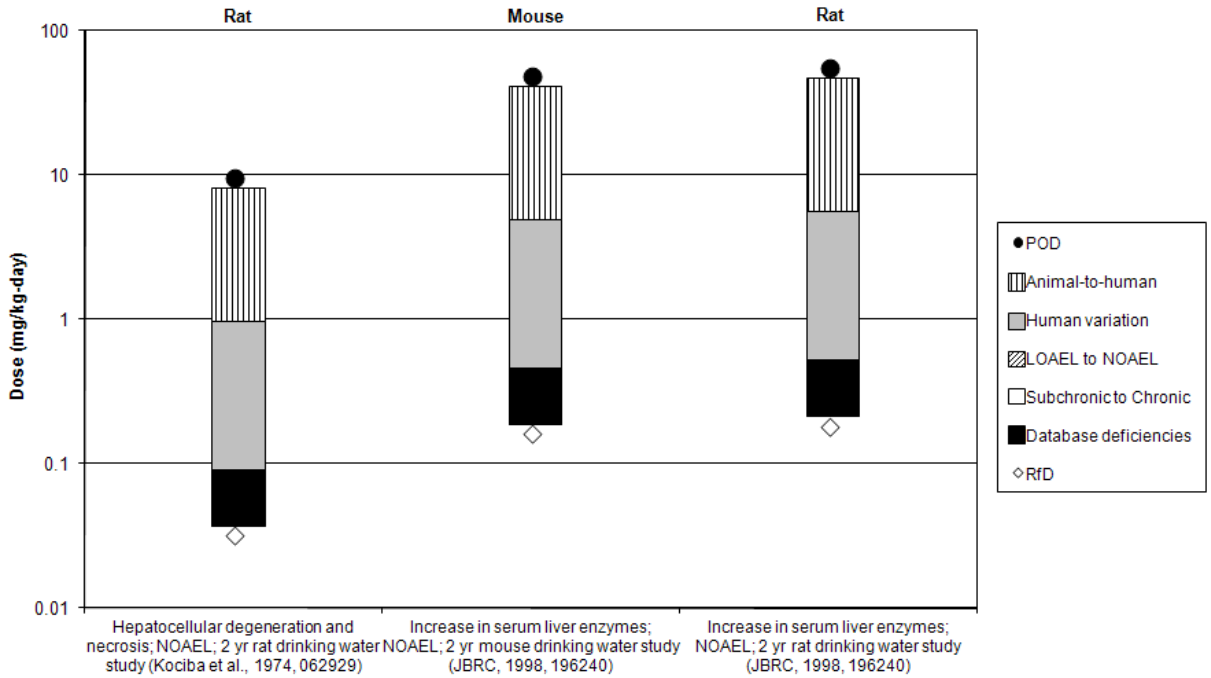


Figure 5-1 Potential points of departure (POD) based on liver toxicity with corresponding applied uncertainty factors and derived candidate RfDs following chronic oral exposure to 1,4-dioxane.

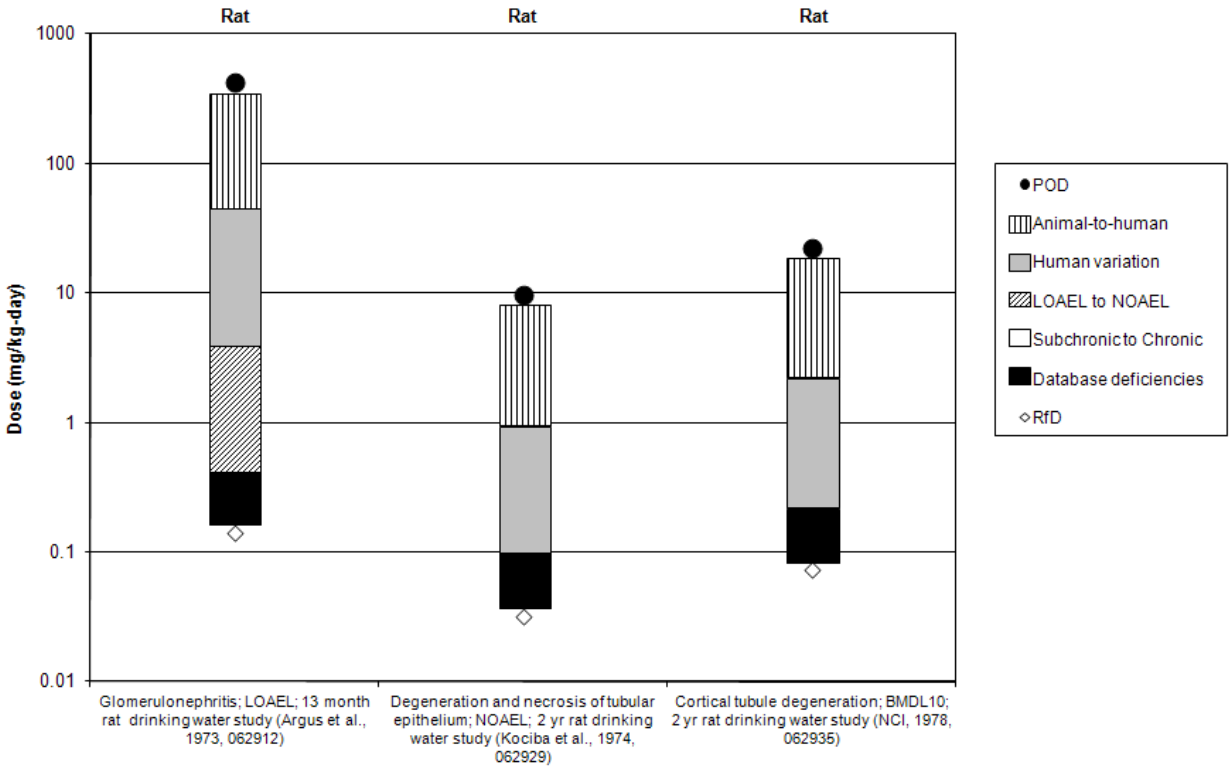


Figure 5-2 Potential points of departure (POD) based on kidney toxicity with corresponding applied uncertainty factors and derived candidate RfDs following chronic oral exposure to 1,4-dioxane.

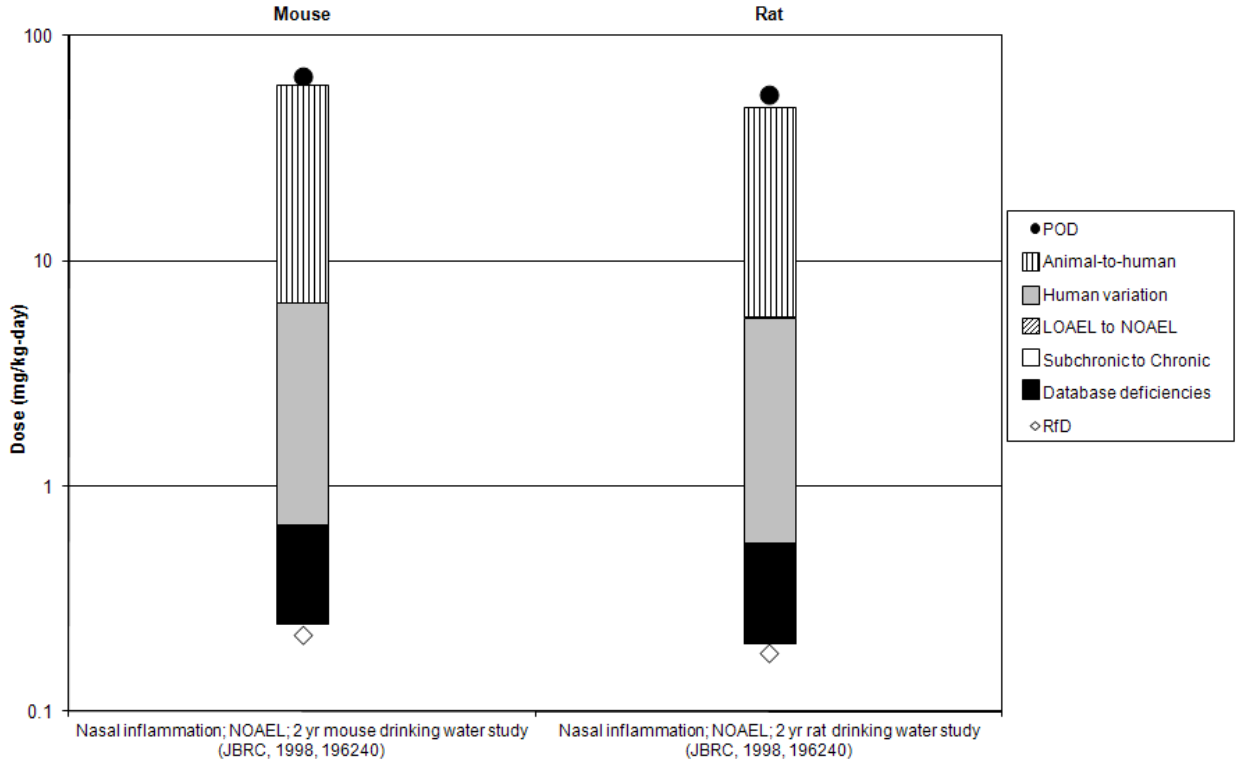


Figure 5-3 Potential points of departure (POD) based on nasal inflammation with corresponding applied uncertainty factors and derived candidate RfDs following chronic oral exposure to 1,4-dioxane.

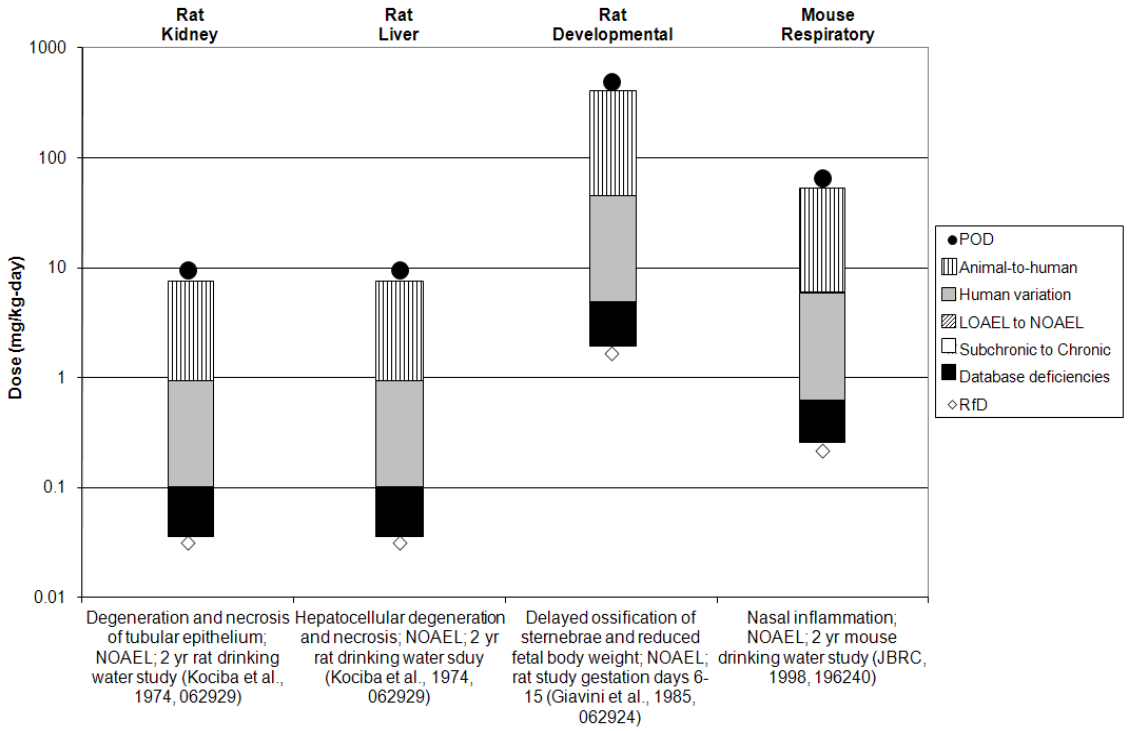


Figure 5-4 Potential points of departure (POD) based on organ-specific toxicity endpoints with corresponding applied uncertainty factors and derived candidate RfDs following chronic oral exposure to 1,4-dioxane.

5.1.5 Previous RfD Assessment

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

5.2 Inhalation Reference Concentration (RfC)

5.2.1 Choice of Principal Study and Candidate Critical Effect(s) with Rationale and Justification

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

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

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

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

similar (i.e., slight) at the four lowest tested vapor levels (i.e., 100, 200, 400 and 800 ppm) in male and female rats; with only a moderate observation of severity noted at the two highest tested vapor levels (i.e., 1,600 and 3,200 ppm). Furthermore, nuclear enlargement has only been reported in studies conducted by JBRC (Kano et al., 2009; Kasai et al., 2009; Kano et al., 2008; Kasai et al., 2008), and there is a lack of information concerning the nature, severity, and significance of this observation elsewhere in the literature. Thus, the toxicological significance of nuclear enlargement is largely unknown.

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

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

Whether spongiosis hepatis/cystic degeneration represents a preneoplastic change or a nonneoplastic change has been the subject of scientific controversy (Karbe and Kerlin, 2002b; Stroebel et al., 1995; Bannasch et al., 1982b). Spongiosis hepatis is commonly seen in aging rats, but has been shown to increase in incidence following exposure to hepatocarcinogens. Spongiosis hepatis can be seen in combination with preneoplastic foci in the liver or with hepatocellular adenoma or carcinoma and has been considered a preneoplastic lesion (Bannasch, 2003; Stroebel et al., 1995). In contrast, it can also be associated with hepatocellular hypertrophy and liver toxicity and has been regarded as a secondary effect of some liver carcinogens (Karbe and Kerlin, 2002a). Following inhalation of 1,4-dioxane, spongiosis hepatis was associated with other preneoplastic (e.g., liver foci) and nonneoplastic (e.g., centrilobular necrosis) changes in the liver (Kasai et al., 2009). However, the incidence rates of spongiosis hepatis and liver tumors were highly correlated; therefore, spongiosis hepatis was considered a preneoplastic lesion following inhalation exposure and not considered further in the noncancer analysis.

The Fairley et al. (1934a) study was inadequate to characterize the inhalation risks of 1,4-dioxane because control animals were not used, thus limiting the ability to perform statistical analysis; additionally, no data for low-dose exposure were reported. Because Torkelson et al. (1974a) identified a free-standing NOAEL only, this study was also deemed inadequate to characterize the inhalation risks of

1,4-dioxane. A route-to-route extrapolation from the oral toxicity data was not performed because 1,4-dioxane inhalation causes direct effects on the respiratory tract (i.e., respiratory irritation in humans, pulmonary congestion in animals) (Wirth and Klimmer, 1936; Fairley et al., 1934a; Yant et al., 1930), which would not be accounted for in a cross-route extrapolation. In addition, available kinetic models are not suitable for this purpose (Appendix B).

Therefore, the chronic Kasai et al. (2009) study was selected as the principal study for the derivation of the RfC. The Kasai et al. (2009) 2-year bioassay utilized 50 animals per exposure group, a range of exposure concentrations which were based on the results of the subchronic study (Kasai et al., 2008), and thoroughly examined toxicity of 1,4-dioxane in multiple organ systems. Based on the noncancer database for 1,4-dioxane, this study demonstrated exposure concentration-related effects for histopathological lesions at a lower concentration (50 ppm) compared to the subchronic Kasai et al. study (2008). The 2-year bioassay (Kasai et al., 2009) did not observe effects in both sexes, but the use of only male rats was proposed by the study authors as justified because of data illustrating the absence of induced mesotheliomas in female rats following exposure to 1,4-dioxane in drinking water (Yamazaki et al., 1994a). Additionally, a similar pattern of effects was observed after oral exposure to 1,4-dioxane (Kano et al., 2009; JBRC, 1998) as was observed in the Kasai et al. (2009) 2-year inhalation study.

Incidences of nonneoplastic lesions from the Kasai et al. (2009) study that were statistically significantly increased as compared to control were considered candidates for the critical effect. These candidate endpoints included centrilobular necrosis of the liver, squamous cell metaplasia of the nasal respiratory epithelium, squamous cell hyperplasia of the nasal respiratory epithelium, respiratory metaplasia of the nasal olfactory epithelium, sclerosis in the lamina propria of the nasal cavity, and two degenerative nasal lesions, that is, atrophy of the nasal olfactory epithelium and hydropic change in the lamina propria (Table 5-). Despite statistically significant increases at the low- and mid-exposure concentrations (50 and 250 ppm, respectively), incidences of nuclear enlargement of the respiratory epithelium (nasal cavity), olfactory epithelium (nasal cavity), and proximal tubule (kidney) were not considered candidates for the critical effect given that the toxicological significance of nuclear enlargement is uncertain, as discussed previously (See Section 4.6.2 and Table 4-22).

Table 5-3 Incidences of nonneoplastic lesions resulting from chronic exposure (ppm) to 1,4-dioxane considered for identification of a critical effect.

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

[a]$p \leq 0.01$ by χ^2 test.
[b]$p \leq 0.05$ by χ^2 test.

Source: Kasai et al. (2009).

5.2.2 Methods of Analysis

Benchmark dose (BMD) modeling (U.S. EPA, 2012a) was used to analyze the candidate endpoints identified for 1,4-dioxane. Use of BMD methods involves fitting mathematical models to the observed dose-response data and provides a BMD and its 95% lower confidence limit (BMDL) associated with a predetermined benchmark response (BMR). For 1,4-dioxane, the selected datasets in Table 5- were considered as candidate critical effects and analyzed using BMD modeling to determine potential PODs. Information regarding the degree of change in the selected endpoints that is considered biologically significant was not available. Therefore, a BMR of 10% extra risk was selected under the assumption that it represents a minimally biologically significant response level (U.S. EPA, 2012a).

The estimated BMDs and BMDLs based on incidences of centrilobular necrosis, squamous cell metaplasia and hyperplasia of the respiratory epithelium, and hydropic change of lamina propria are presented in Table 5-. Due to lack of fit or substantial model uncertainty, BMD modeling results were deemed inadequate for the following endpoints: atrophy (olfactory epithelium), respiratory metaplasia (olfactory epithelium), and sclerosis (lamina propria). Consequently, for these last three endpoints, the NOAEL/LOAEL approach was used to determine potential PODs. The detailed results of the BMD analysis are provided in Appendix F.

5.2.3 Exposure Duration and Dosimetric Adjustments

Because an RfC assumes continuous human exposure over a lifetime, data derived from inhalation studies in animals need to be adjusted to account for the noncontinuous exposure protocols used in these studies. In the Kasai et al. (2009) study, rats were exposed to 1,4-dioxane for 6 hours/day, 5

days/week for 2 years. Therefore, the duration-adjusted PODs for liver and nasal lesions in rats were calculated as follows:

$$\mathrm{POD_{ADJ}\ (ppm) = POD\ (ppm)\ x\ \frac{hours\ exposed\ per\ day}{24\ hours}\ x\ \frac{days\ exposed\ per\ week}{7\ days}}$$

RfCs are typically expressed in units of mg/m^3; so POD_{ADJ} (ppm) values were converted using the chemical specific conversion factor of 1 ppm = 3.6 mg/m^3 for 1,4-dioxane (Table 2-1). The following calculation was used:

$$\mathrm{POD_{ADJ}\ (mg/m^3) = POD_{ADJ}\ (ppm)\ x\ \frac{3.6\ mg/m^3}{1\ ppm}}$$

The calculated POD_{ADJ} (mg/m^3) values for all considered endpoints are presented in the last column of Table 5-4.

Table 5-4 Duration adjusted POD estimates for BMDLs (from best fitting BMDS models) or NOAELs/LOAELs from chronic exposure to 1,4-dioxane

Endpoint	NOAEL[a] (ppm)	LOAEL[b] (ppm)	Model	BMR (%)	BMD (ppm)	BMDL (ppm)	POD_{ADJ} (ppm)	POD_{ADJ} (mg/m^3)
Liver Effects								
Centrilobular necrosis; Liver	--	--	Dichotomous-Hill	10	220	60	10.7	38.6
Nasal Effects								
Squamous cell metaplasia; respiratory epithelium	--	--	Log-probit	10	218	160	28.6	103
Squamous cell hyperplasia; respiratory epithelium	--	--	Log-probit	10	756	561	100	361
Respiratory metaplasia; olfactory epithelium	--	50	--[c]	--	--	--	8.9	32.2
Atrophy; olfactory epithelium	--	50	--[c]	--	--	--	8.9	32.2
Hydropic change; lamina propria	--	--	Log-logistic	10	69	47	8.4	30.2
Sclerosis; lamina propria	50	250	--[c]	--	--	--	8.9	32.2[d]

[a]NOAEL is identified in this assessment as the highest tested exposure dose at which there is no statistically significant effect in the exposed group as compared to control.
[b]LOAEL is identified in this assessment as the lowest tested exposure dose at which there is a statistically significant effect in the exposed group as compared to control.
[c]BMD modeling results are inadequate for use in deriving a POD. Therefore, the NOAEL/LOAEL approach is used to determine a POD for these endpoints. BMD analysis for these endpoints is described in Appendix F.
[d]Based on the NOAEL of 50 ppm.

Based on a review of the data in Table 5-4, hepatic centrilobular necrosis was shown to be less sensitive than the nasal effects and was not considered further as a candidate critical effect. Similarly, the squamous cell metaplasia and hyperplasia of the respiratory epithelium yielded potential PODs that were at least 3-fold higher than the remaining nasal effects; thus, these two effects were not considered further as candidate critical effects. The PODs (adjusted for continuous exposure) for sclerosis of the lamina

propria, atrophy of the olfactory epithelium, and respiratory metaplasia of the olfactory epithelium were identical (32.2 mg/m^3) and similar to the POD_{ADJ} for hydropic change of the lamina propria (30.2 mg/m^3). Although the POD_{ADJ} estimates for these four endpoints were either identical or similar, the magnitude of response (i.e., increased incidence of effect) at each POD_{ADJ} for these effects varied (i.e., 0% for sclerosis, 10% for hydropic change, 59% for respiratory metaplasia, 80% for atrophy).

As shown in Table 5-3, atrophy and respiratory metaplasia of the olfactory epithelium were the most sensitive effects based on responses of 80 and 59% at their respective PODs of 50 ppm (LOAELs). Increased incidences of the other nasal effects, as well as liver effects (i.e., centrilobular necrosis), were observed at exposures of 50 ppm or greater and the magnitude of the responses at these exposures were lower than those observed for atrophy and respiratory metaplasia of the olfactory epithelium. Typically, chemically-induced nasal effects include atrophy and/or necrosis, cell proliferation/hyperplasia, and metaplasia depending on the nature of the tissue damage and level of exposure (Harkema et al., 2006; Boorman et al., 1990; Gaskell, 1990). However, the pathological progression of these events is uncertain and often accompanied by an inflammatory response. Since the data do not support a continuum of pathological events associated with respiratory tract effects, both atrophy and respiratory metaplasia of the olfactory epithelium were selected as co-critical effects in this assessment. Additionally, these effects were the most sensitive non-cancer effects considered following inhalation of 1,4-dioxane.

For the derivation of a RfC based upon an animal study, the selected POD must be adjusted to reflect the human equivalent concentration (HEC). The HEC was calculated by the application of a dosimetric adjustment factor (DAF), in accordance with the U.S. EPA *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (hereafter referred to as the RfC methodology) (U.S. EPA, 1994a). DAFs are ratios of animal and human physiologic parameters, and are dependent on the nature of the contaminant (particle or gas) and the target site (e.g., respiratory tract or remote to the portal-of-entry) (U.S. EPA, 1994b).

1,4-Dioxane is miscible with water and has a high blood:air partition coefficient. Typically, highly water-soluble and directly reactive chemicals (i.e. Category 1 gases) partition predominantly into the upper respiratory tract, induce portal-of-entry effects, and do not accumulate significantly in the blood. 1,4-Dioxane induces effects at the portal-of-entry (i.e., respiratory tract), liver, and kidneys, and it has been measured in the blood after inhalation exposure (Kasai et al., 2009; Kasai et al., 2008). The observations of systemic (i.e., nonrespiratory) effects and measured blood levels resulting from 1,4-dioxane exposure indicate that this compound is absorbed into the bloodstream and distributed throughout the body. Thus, 1,4-dioxane might be best described as a water-soluble and non-directly reactive gas. Gases such as these are readily taken up into respiratory tract tissues and can also diffuse into the blood (Medinsky and Bond, 2001). The effects observed in the olfactory epithelium may be the result of the metabolism of 1,4-dioxane to an acid metabolite; however, for the reasons stated above, it is unclear whether or not these effects are solely the result of portal-of-entry or systemic delivery. A similar pattern of effects was observed after oral exposure to 1,4-dioxane (Kano et al., 2009; JBRC, 1998).

In consideration of the evidence described above, the human equivalent concentration (HEC) for 1,4-dioxane was calculated by the application of the appropriate dosimetric adjustment factor (DAF) for systemic acting gases, in accordance with the U.S. EPA RfC methodology (U.S. EPA, 1994a).

The calculation of the HEC used in this assessment is as follows:

$$DAF = (Hb/g)A/(Hb/g)H$$

$$DAF = 1{,}861/1{,}666$$

$$DAF = 1.12$$

where:

$(Hb/g)_A$ = the animal blood:air partition coefficient =1,861 (Sweeney et al., 2008a)

$(Hb/g)_H$ = the human blood:air partition coefficient =1,666 (Sweeney et al., 2008a)

Given that the animal blood:air partition coefficient is higher than the human value resulting in a DAF>1, a default value of 1 is substituted in accordance with the U.S. EPA RfC methodology (U.S. EPA, 1994a). Analysis of the existing inhalation dosimetry modeling database supports the application of a DAF of 1 for a systemic acting gas (U.S. EPA, 2012b, 2009c, b). In addition, a robust computational fluid dynamic (CFD) and PBPK modeling database supports the scientific rationale to apply of DAF of 1 for both portal of entry and systemic effects irrespective of "gas categorization" (U.S. EPA, 2012b). Application of these models to gases that have similar physicochemical properties and induce similar nasal effects as 1,4-dioxane yield estimated DAFs ≥ 1.

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

$$\begin{aligned} POD_{HEC}\ (mg/m^3) &= POD_{ADJ}\ (mg/m^3) \times DAF \\ &= POD_{ADJ}\ (mg/m^3) \times 1.0 \\ &= 32.2\ mg/m^3 \times 1.0 \\ &= 32.2\ mg/m^3 \end{aligned}$$

Therefore, the POD_{HEC} of 32.2 mg/m^3 for the co-critical effects of atrophy and respiratory metaplasia of the olfactory epithelium is used for the derivation of a RfC for 1,4-dioxane.

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

The RfC of 3×10^{-2} mg/m^3 is based on atrophy and respiratory metaplasia of the olfactory epithelium in male rats exposed to 1,4-dioxane via inhalation for 2 years (Kasai et al., 2009). The RfC for 1,4-dioxane is derived by dividing the POD_{HEC} by a composite UF of 1,000.

$$RfC = POD_{HEC} / UF$$
$$= 32.2\ mg/m^3 / 1{,}000$$
$$= 0.0322 \text{ or } 3 \times 10^{-2}\ mg/m^3 \text{ (rounded to 1 significant figure)}$$

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

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

An UF to extrapolate from a subchronic to a chronic (UF_S) exposure duration was not necessary (e.g., UFS = 1) because the RfC was derived from a study using a chronic exposure protocol.

An UF of 10 (UF_L) was used to extrapolate from a LOAEL to a NOAEL because a LOAEL was used as the POD. A NOAEL for atrophy and respiratory metaplasia of the olfactory epithelium was not identified in the study by Kasai et al. (2009).

An UF of 3 for database deficiencies (UF_D) was applied due to the lack of a multigeneration reproductive toxicity study. The oral toxicity database included a single prenatal developmental study that indicated the developing fetus may be a target of toxicity (Giavini et al., 1985a). Giavini et al. (1985b) administered 1,4-dioxane by gavage in water to pregnant rats. The authors found statistically significant changes in fetal body weight at the highest dose group and reduced ossification of the sternebrae; however, the lack of a multigenerational reproductive study in which animals were exposed to 1,4-dioxane via oral or inhalation routes warrants the use of a 3 for UF_D.

5.2.5 RfC Comparison Information

Figure 5-5 presents PODs, applied UFs, and derived candidate RfCs based on each of the endpoints from the chronic inhalation study by Kasai et al. (2009) in male rats. The PODs are based on the $BMDL_{10}$, NOAEL, or LOAEL, and appropriate unit conversions, duration, and dosimetric adjustments were applied before applications of UFs. The predominant noncancer effects of chronic inhalation exposure to 1,4-dioxane include nasal and liver effects. Figure 5-5 provides a graphical display of these effects that were observed in the Kasai et al. (2009) study. The nasal effects involving the olfactory epithelium represent the most sensitive effects.

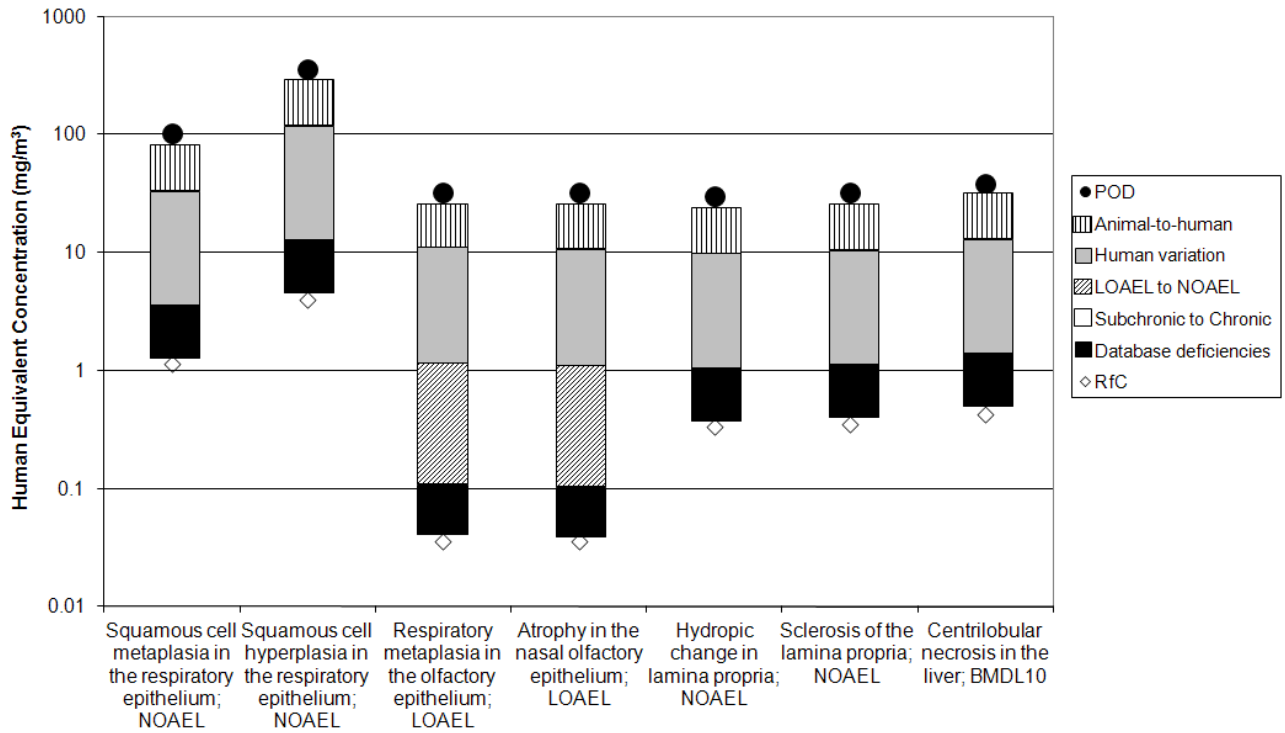


Figure 5-5 Potential points of departure (POD) for candidate endpoints with corresponding applied uncertainty factors and derived candidate RfCs following chronic inhalation exposure of F344 male rats to 1,4-dioxane.

Source: Kasai et al. (2009)

5.2.6 Previous RfC Assessment

An RfC for 1,4-dioxane was not previously available on the IRIS database.

5.3 Uncertainties in the Oral Reference Dose and Inhalation Reference Concentration

The following discussion identifies the uncertainties associated with deriving the RfD and RfC for 1,4-dioxane. As presented earlier in 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 uncertainty factor approach (U.S. EPA, 2002b, 1994a) was used to derive the RfD and RfC for 1,4-dioxane. Using this approach, the POD was divided by a set of factors to account for uncertainties associated with a number of steps in the analysis, including extrapolation from LOAEL to NOAEL, extrapolation from animals to humans, a diverse population of varying susceptibilities, and to account for database deficiencies. Because information specific to 1,4-dioxane was unavailable to fully inform these extrapolations, default factors were generally applied.

An adequate range of animal toxicology data are available for the hazard assessment of 1,4-dioxane, as described throughout the previous section (Section 4). The database of oral toxicity

studies includes chronic drinking water studies in rats and mice, multiple subchronic drinking water studies conducted in rats and mice, and a developmental study in rats. Toxicity associated with oral exposure to 1,4-dioxane is observed predominately in the liver and kidney. The database of inhalation toxicity studies in animals includes two subchronic bioassays in rabbits, guinea pigs, mice, and rats, and two chronic inhalation bioassays in rats. Toxicity associated with inhalation exposure to 1,4-dioxane was observed predominately in the liver and nasal cavity. In addition to oral and inhalation data, there are PBPK models and genotoxicity studies of 1,4-dioxane. Critical data gaps have been identified and uncertainties associated with data deficiencies of 1,4-dioxane are more fully discussed below.

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

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

The RfC was derived by applying UFs to a LOAEL for atrophy and respiratory metaplasia of the olfactory epithelium. The incidence data for the observed effects were not amenable to BMD modeling (see Appendix F). The LOAEL for these effects was less than or equal to the LOAEL or NOAEL for other effects observed in the Kasai et al. (2009) study.

Extrapolating from animals to humans embodies further issues and uncertainties. The effect and the magnitude associated with the dose at the POD in rodents are extrapolated to human response. Pharmacokinetic models are useful to examine species differences in pharmacokinetic processing; however, it was determined that dosimetric adjustment using pharmacokinetic modeling to reduce uncertainty following oral exposure to 1,4-dioxane was not supported. Insufficient information was available to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans, so a 10-fold UF was used to account for uncertainty in extrapolating from laboratory animals to humans in the derivation of the RfD. A DAF was used to account for pharmacokinetic differences between rodents and humans in the derivation of the RfC; however, there was no information to inform pharmacodynamic differences between species, so an UF of 3 was used in derivation of the RfC to account for these uncertainties.

Heterogeneity among humans is another uncertainty associated with extrapolating doses from animals to humans. Uncertainty related to human variation needs consideration. In the absence of 1,4-dioxane-specific data on human variation, a factor of 10 was used to account for uncertainty associated with human variation in the derivation of the RfD and RfC. Human variation may be larger or

smaller; however, 1,4-dioxane-specific data to examine the potential magnitude of over- or under-estimation are unavailable.

Uncertainties in the assessment of the health hazards of 1,4-dioxane are associated with deficiencies in reproductive toxicity information. The oral and inhalation databases lack a multigeneration reproductive toxicity study. A single oral prenatal developmental toxicity study in rats was available for 1,4-dioxane (Giavini et al., 1985a). This developmental study indicates that the developing fetus may be a target of toxicity. No developmental studies are available following inhalation to 1,4-dioxane.

5.4 Cancer Assessment

5.4.1 Choice of Study/Data – with Rationale and Justification

5.4.1.1 Oral Study/Data

Three chronic drinking water bioassays provided incidence data for liver tumors in rats and mice, and nasal cavity, peritoneal, and mammary gland tumors in rats only (Kano et al., 2009; JBRC, 1998; Yamazaki et al., 1994a; NCI, 1978; Kociba et al., 1974a). The dose-response data from each of these studies are summarized in Table 5-5. With the exception of the NCI (1978) study, the incidence of nasal cavity tumors was generally lower than the incidence of liver tumors in exposed rats. The Kano et al. (2009) drinking water study was chosen as the principal study for derivation of an oral cancer slope factor (CSF) for 1,4-dioxane. This study used three dose groups in addition to controls and characterized the dose-response relationship at lower exposure levels, as compared to the high doses employed in the NCI (1978) bioassay (Table 5-5). The Kociba et al. (1974a) study also used three dose groups and low exposures; however, the study authors only reported the incidence of hepatocellular carcinomas, which may underestimate the combined incidence of rats with adenomas or carcinomas. In addition to increased incidence of liver tumors, chosen as the most sensitive target organ for tumor formation, the Kano et al. (2009) study also noted increased incidence of peritoneal and mammary gland tumors, and nasal cavity tumors were also seen in high-dose male and female rats.

In a personal communication, Dr. Yamazaki (2006) provided data that showed that the survival of mice in the Kano et al. (2009) study was low in all male groups (31/50, 33/50, 25/50 and 26/50 in control, low-, mid-, and high-dose groups, respectively) and particularly low in high-dose females (29/50, 29/50, 17/50, and 5/50 in control, low-, mid-, and high-dose groups, respectively). These deaths occurred primarily during the second year of the study. Survival at 12 months in male mice was 50/50, 48/50, 50/50, and 48/50 in control, low-, mid-, and high-dose groups, respectively. Female mouse survival at 12 months was 50/50, 50/50, 48/50, and 48/50 in control, low-, mid-, and high-dose groups, respectively (Yamazaki, 2006). Furthermore, these deaths were primarily tumor related. Liver tumors were listed as

the cause of death for 31 of the 45 pretermination deaths in high-dose female Crj:BDF1 mice (Yamazaki, 2006).

Table 5-5 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. (1974a)	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[j,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[j,k]	8/50[j,k]	0/50	18/50[j,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[j,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[j,k]	1/50	NA	NA

[a]Incidence of hepatocellular carcinoma.
[b]Incidence of nasal squamous cell carcinoma.
[c]Incidence of hepatocellular adenoma.
[d]Incidence of hepatocellular adenoma or carcinoma.
[e]Incidence (sum) of all nasal tumors including squamous cell carcinoma, sarcoma, rhabdomyosarcoma, and esthesioneuroepithelioma.
[f]Incidence of peritoneal tumors (mesothelioma).
[g]Incidence of mammary gland tumors (fibroadenoma or adenoma)
[h]$p < 0.05$; positive dose-related trend (Cochran-Armitage or Peto's test).
[i]Significantly different from control at $p < 0.05$ by Fisher's Exact test.
[j]Significantly 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

5.4.1.2 Inhalation Study/Data

Epidemiological studies of populations exposed to 1,4-dioxane via inhalation are not adequate for dose-response analysis and thus derivation of an inhalation unit risk (IUR). However, two chronic inhalation studies in animals are available and were evaluated for the potential to estimate an IUR (Table 5-6). The chronic inhalation study conducted by Torkelson et al. (1974a) in rats did not find any treatment-related tumors; however, only a single exposure concentration was used (111 ppm 1,4-dioxane vapor for 7 hours/day, 5 days/week for 2 years). A chronic bioassay of 1,4-dioxane by the inhalation route reported by Kasai et al. (2009) provides data adequate for dose-response modeling and was subsequently chosen as the study for the derivation of an IUR for 1,4-dioxane. In this bioassay, groups of 50 male F344 rats were exposed to either 0, 50, 250 or 1,250 ppm 1,4-dioxane, 6 hours/day, 5 days/week, for 2 years (104-weeks). In male F344 rats, 1,4-dioxane produced a statistically significant increase in incidence and/or a statistically significant dose-response trend for the following tumor types: hepatomas, nasal squamous cell carcinomas, renal cell carcinomas, peritoneal mesotheliomas, mammary gland fibroadenomas, Zymbal gland adenomas, and subcutis fibromas (Kasai et al., 2009). The incidence of adenomas and carcinomas were combined in this assessment in accordance with EPA's *Guidelines on Carcinogen Risk Assessment* which notes that etiologically similar tumor types, i.e., benign and malignant tumors of the same cell type, can be combined due to the possibility that benign tumors could progress to the malignant form (U.S. EPA, 2005a; McConnell et al., 1986). Consistent with the oral cancer assessment (Appendix D), the incidence of hepatic adenomas and carcinomas (combined) was used to calculate an IUR (See Table 5-6).

Table 5-6 Incidence of liver, nasal cavity, kidney, peritoneal, and mammary gland, Zymbal gland, and subcutis tumors in rats exposed to 1,4-dioxane via inhalation 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. (1974a)[a]	Male Wistar rats	0	0/150	0/150	0/150[i]	NA	NA	NA	0/150
		111	0/206	0/206	1/206[i]	NA	NA	NA	2/206
	Female Wistar rats	0	0/139	0/139	1/139[j]	NA	11/139[k]	NA	0/139
		111	0/217	0/217	0/217[j]	NA	29/217[k]	NA	0/217
Kasai et al. (2009)[b]	Male F344 rats	0	1/50	0/50	0/50	2/50	1/50[l]	0/50	1/50
		50	2/50	0/50	0/50	4/50	2/50[l]	0/50	4/50
		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

[a]Incidence reported based on survival to 9 months.
[b]Incidence reported based on survival to 12 months.
[c]Incidence 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 5-8.
[d]Incidence of nasal squamous cell carcinoma.
[e]Incidence of renal cell carcinoma.
[f]Incidence of peritoneal mesothelioma.
[g]Incidence of Zymbal gland adenoma.
[h]Incidence of subcutis fibroma.
[i]Incidence of kidney fibroma.
[j]Incidence of kidney adenocarcinoma
[k]Incidence of mammary gland adenoma.
[l]Incidence of mammary gland fibroadenoma.
[m]Tumor incidence significantly elevated compared with that in controls by Fisher's exact test ($p \leq 0.05$).
[n]Tumor 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

Table 5-7 summarizes the incidence of hepatocellular adenoma or carcinoma in rats and mice from the Kano et al. (2009) 2-year drinking water study. There were statistically significant increasing trends in tumorigenic response for males and females of both species. The dose-response curve for female mice is steep, with 70% incidence of liver tumors occurring in the low-dose group (66 mg/kg-day). Exposure to 1,4-dioxane increased the incidence of these tumors in a dose-related manner.

A statistically significant increase in the incidence of peritoneal mesotheliomas was observed in high-dose male rats only (28/50 rats, Table 5-5). The incidence of peritoneal mesotheliomas was lower than the observed incidence of hepatocellular adenomas or carcinomas in male rats (Table 5-7); therefore, the incidence of hepatocellular adenomas or carcinomas was used to derive an oral CSF for 1,4-dioxane.

Table 5-7 Incidence of hepatocellular adenomas or carcinomas 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]

[a]Incidence of either hepatocellular adenomas or carcinomas.
[b]$p < 0.05$; positive dose-related trend (Peto's test).
[c]Significantly different from control at $p < 0.01$ by Fisher's Exact test.
[d]Significantly different from control at $p < 0.01$ by Fisher's Exact test.

Source: Reprinted with permission of Elsevier, Ltd., Kano et al. (2009).

5.4.2.2 Inhalation Data

Multi-tumor dose-response modeling was performed for all tumor responses from the Kasai et al. (2009) bioassay. Kasai et al. (2009) reported tumor incidence data for male F344 rats exposed via inhalation to 0, 50, 250, or 1,250 ppm 1,4-dioxane for 6 hours/day, 5 days/week, for 2 years (104-weeks). Statistically significant positive dose-response trends were observed for nasal cavity squamous cell carcinomas, hepatomas, renal cell carcinomas, peritoneal mesotheliomas, mammary gland fibroadenomas, and Zymbal gland adenomas. Following 250 ppm 1,4-dioxane exposure, statistically significantly elevated tumor incidences were found in two tissue types (i.e., peritoneal mesothelioma and subcutis fibroma) compared to controls. It is important to note, for observations of subcutis fibroma, the incidence was increased compared to controls at all concentrations, but a decrease in incidence, compared to the mid-concentration, was noted at the highest concentration (1,250 ppm). However, a statistically significantly decreased survival rate was noted in this exposure group by the study authors. Interim sacrifices were not performed. Tumor incidences following 1,250 ppm inhalation exposure to 1,4-dioxane were statistically elevated compared to controls in three tissues (i.e., nasal cavity squamous cell carcinoma, hepatomas, and peritoneal mesothelioma). Incidence data for the tumor types reported by Kasai et al. (2009) are summarized in Table 5-8.

Table 5-8 Incidence of tumors in F344 male rats exposed to 1,4-dioxane via inhalation for 104 weeks (6 hours/day, 5 days/week)

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

[a]Statistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.01$).
[b]Tumor incidence significantly elevated compared with that in controls by Fisher's exact test ($p \leq 0.05$).
[c]Tumor incidence significantly elevated compared with that in controls by Fisher's exact test ($p \leq 0.01$).
[d]Statistically significant trend for increased tumor incidence by Peto's test ($p \leq 0.05$).
[e]Provided 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

Human equivalent doses (HEDs) were calculated from the administered animal doses using a BW scaling factor ($BW^{0.75}$) (U.S. EPA, 2011). This was accomplished using the following equation:

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

For all calculations, a human BW of 70 kg was used. HEDs for the principal study (Kano et al., 2009) are given in Table 5-9. HEDs were also calculated for supporting studies (NCI, 1978; Kociba et al., 1974a) and are also shown in Table 5-9.

Table 5-9 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. (1974a)	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.
[b]TWA BWs were determined from BW curve provided for control animals.
[c]BWs 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).
[d]HEDs are calculated as HED = (animal dose) × (animal BW / human BW)$^{0.25}$.

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

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

However, several of the external peer review panel members for the oral assessment (see Appendix A: Summary of External Peer Review and Public Comments and Disposition) recommended that the mode of action data support the use of a nonlinear extrapolation approach to estimate human carcinogenic risk associated with exposure to 1,4-dioxane and that such an approach should be presented in the Toxicological Review. As discussed in Section 4.5.1, numerous short-term in vitro and a few in vivo tests were nonpositive for 1,4-dioxane-induced genotoxicity. Results from two-stage mouse skin tumor bioassays demonstrated that 1,4-dioxane does not initiate mouse skin tumors, but it is a promoter of skin tumors initiated by DMBA (King et al., 1973a). These data suggest that a potential mode of action for 1,4-dioxane-induced tumors may involve proliferation of cells initiated spontaneously, or by some

other agent, to become tumors (Miyagawa et al., 1999; Uno et al., 1994; Goldsworthy et al., 1991; Lundberg et al., 1987; Bull et al., 1986; Stott et al., 1981; King et al., 1973a). However, key events related to the promotion of tumor formation by 1,4-dioxane are unknown. Therefore, under the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), EPA concluded that the available information does not establish a plausible mode of action for 1,4-dioxane and data are insufficient to establish significant biological support for a nonlinear approach. EPA determined that there are no data available to inform the low-dose region of the dose response, and thus, a nonlinear approach was not included.

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

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

The oral CSF was calculated using the following equation:

$$CSF = BMR / BMDL_{HED}$$

5.4.3.2 Inhalation

In accordance with the U.S. EPA (1994a) RfC methodology, the HEC values were calculated by the application of DAFs. As discussed in Section 5.2.3. since 1,4-dioxane is miscible with water, has a high partition coefficient, and induces effects throughout the body of the rat , this substance was considered to be a systemic acting gas and a DAF of 1.0 was applied. The lifetime continuous inhalation risk for humans is defined as the slope of the line drawn from the POD through the origin, with the POD defined as the lower 95% bound on the exposure associated with a level of extra risk near the low end of the data range.

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

Given the multiplicity of tumor sites observed in animals, basing the IUR on one tumor site may underestimate the carcinogenic potential of 1,4-dioxane via inhalation. Also, simply pooling the counts of animals with one or more tumors (i.e., counts of tumor bearing animals) would tend to underestimate the overall risk for tumors observed at independent sites and ignores potential differences in the dose-response relationships across the sites (NRC, 1994; Bogen, 1990). NRC (1994) has also noted that

the assumption of independence across tumor types is not likely to produce substantial error in the risk estimates unless tumors across multiple sites are known to be biologically dependent.

The U.S. EPA's BMDS (v2.2 beta) MS_Combo program was utilized as a computational approach to calculating the dose associated with a specified composite risk under the assumption of independence of tumors. The best fitting BMDS multistage model was determined for each individual tumor type as shown in Section 5.4.4.2 and APPENDIX G. These models account for spontaneous tumor generation in controls. The *Guidelines for Carcinogen Risk Assessment* recommend calculation of an upper bound to account for uncertainty in the estimate (U.S. EPA, 2005a). Complete details of this analysis are included in Appendix G. In addition, Bayesian MCMC computations were conducted as described by Kopylev et al. (2009) using WinBugs (Spiegelhalter et al., 2003). For uncertainty characterization, MCMC methods have the advantage of providing information about the full distribution of risk and/or BMDs, which can be used in generating a confidence bound. This MCMC approach, which builds on the re-sampling approach recommended by Bogen (1990), also provides a distribution of the combined potency across sites. This supporting analysis was completed in addition to the MS_Combo analysis and additional details are included in Appendix G.

Several hypothesized MOA(s) have been proposed for liver and nasal tumors, although these MOA(s) are not supported by the available data (see Sections 4.7.3.3 and 4.7.3.4). Specifically, tumors occur in rodent models in the absence of data to identify hypothesized key events (e.g., cytotoxicity). Also, studies evaluating the kinetics of 1,4-dioxane suggest that liver carcinogenicity is related to the accumulation of the parent compound following metabolic saturation; however, data are not available to determine the toxic moiety (i.e., parent compound and/or metabolite(s)) (see Section 3.3 and 4.7.3.1.1). For kidney, lung, peritoneal (mesotheliomas), mammary gland, Zymbal gland, and subcutis tumors, there are no available data regarding any hypothesized carcinogenic MOA(s) for 1,4-dioxane.

The EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), recommend that the method used to characterize and quantify cancer risk from a chemical is determined by what is known about the MOA of the carcinogen and the shape of the cancer dose-response curve. The linear extrapolation approach is used as a default option if the mode of carcinogenic action is not identified. A nonlinear extrapolation approach can be used for cases with sufficient data to ascertain the mode of action and to conclude that it is not linear at low doses. Also, nonlinear extrapolation having significant biological support may be presented in addition to a linear approach when the available data and weight of evidence support a nonlinear approach. In the case of 1,4-dioxane, there is insufficient biological support to identify key events and to have reasonable confidence in the sequence of events and how they relate to the development of tumors following exposure to 1,4-dioxane; thus, the data are not strong enough to ascertain the mode of action applying the Agency's mode of action framework (U.S. EPA, 2005a). Therefore, EPA concluded that a default linear extrapolation should be utilized to estimate the cancer risk estimates for inhalation and oral exposure to 1,4-dioxane.

IUR estimates were calculated using the following equation:

$$IUR = BMR / BMCL_{HEC}$$

5.4.4 Oral Slope Factor and Inhalation Unit Risk

5.4.4.1 Oral Slope Factor

The dichotomous models available in the Benchmark Dose Software (BMDS, version 2.1.1) were fit to the incidence data for "either hepatocellular carcinoma or adenoma" in rats and mice, as well as mammary and peritoneal tumors in rats exposed to 1,4-dioxane in drinking water (Kano et al., 2009; NCI, 1978; Kociba et al., 1974a) (Table 5-5). Animal doses were used for BMD modeling, and then HED BMD and BMDL values were calculated using $BW^{3/4}$ scaling employing animal TWA body weights (Table 5-10) and a human BW of 70 kg. For all models, a BMR of 10% extra risk was employed. BMDs and BMDLs from all models are reported, and the model outputs and plots corresponding to the best-fitting models are shown (Appendix D). When the best-fitting model is not a multistage model, the multistage model output and plot are also provided (Appendix D). A summary of the BMD modeling results for the Kano et al. (2009), NCI (1978), and Kociba et al. (1974a) studies is shown in Table 5-10.

Table 5-10 BMD_{HED} and $BMDL_{HED}$ values from best-fit 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 $(mg/kg\text{-}day)^{-1}$
Kano et al. (2009)	Male F344/DuCrj rats[b]	Hepatocellular adenoma or carcinoma	17.43	14.33	7.0×10^{-3}
	Female F344/DuCrj rats[c]		19.84	14.43	6.9×10^{-3}
	Male Crj:BDF1 mice[d]		5.63	2.68	3.7×10^{-2}
	Female Crj:BDF1 mice[d]		0.83	0.55	1.8×10^{-1}
	Female Crj:BDF1 mice[d, e]		3.22[e]	2.12[e]	1.4×10^{-1}
	Female Crj:BDF1 mice[d, f]		7.51[f]	4.95[f]	1.0×10^{-1}
	Female F344/DuCrj rats[g]	Nasal squamous cell carcinoma	94.84	70.23	1.4×10^{-3}
	Male F344/DuCrj rats[g]		91.97	68.85	1.5×10^{-3}
	Male F344/DuCrj rats[b]	Peritoneal mesothelioma	26.09	21.39	4.7×10^{-3}
	Female F344/DuCrj rats[d]	Mammary gland adenoma	40.01	20.35	4.9×10^{-3}
Kociba et al. (1974a)	Male and female (combined) Sherman rats[g]	Nasal squamous cell carcinomas	448.24	340.99	2.9×10^{-4}
	Male and female (combined) Sherman rats[b]	Hepatocellular carcinoma	290.78	240.31	4.2×10^{-4}
NCI (1978)	Male Osborne Mendel rats[d]	Nasal squamous cell carcinomas	16.10	10.66	9.4×10^{-3}
	Female Osborne Mendel rats[d]		40.07	25.82	3.9×10^{-3}
	Female Osborne Mendel rats[d]	Hepatocellular adenoma	28.75	18.68	5.4×10^{-3}
	Female $B6C3F_1$ mice[c]	Hepatocellular adenoma or carcinoma	23.12	9.75	1.0×10^{-2}
	Male $B6C3F_1$ mice[h]		87.98	35.67	2.8×10^{-3}

[a]Values associated with a BMR of 10% unless otherwise noted.
[b]Probit model, slope parameter not restricted.
[c]Multistage model, degree of polynomial = 2.
[d]Log-logistic model, slope restricted ≥ 1.
[e]Values associated with a BMR of 30%.
[f]Values associated with a BMR of 50%.
[g]Multistage model, degree of polynomial =3.
[h]Gamma model.

The multistage model did not provide an adequate fit (as determined by p-value < 0.1, and χ^2 p > |0.1|) to the data for the incidence of hepatocellular adenoma or carcinoma in female mice (Appendix D). The high dose was dropped for the female mouse liver tumor dataset in an attempt to achieve an adequate fit; however, an adequate fit was still not achieved. Because the female mice were clearly the most sensitive group tested, other BMD models were applied to the female mouse liver tumor dataset to achieve an adequate fit. The log-logistic model was the only model that provided adequate fit for this data set due to the steep rise in the dose-response curve (70% incidence at the low dose) followed by a plateau at near maximal tumor incidence in the mid- and high-dose regions (82 and 92% incidence, respectively). The predicted BMD_{10} and $BMDL_{10}$ for the female mouse data are presented in Table 5-10, as well as BMD_{HED} and $BMDL_{HED}$ values associated with BMRs of 30 and 50% .

The multistage model also did not provide an adequate fit to mammary tumor incidence data for the female rat or male rat peritoneal tumors. The predicted BMD_{10} and $BMDL_{10}$ for female rat mammary

tumors and male peritoneal tumors obtained from the log-logistic and probit models, respectively, are presented in Table 5-10.

A comparison of the BMD and BMDL estimates derived for rats and mice from the Kano et al. (2009), NCI (1978), and Kociba et al. (1974a) studies (Table 5-10) indicates that female mice are more sensitive to liver carcinogenicity induced by 1,4-dioxane compared to other species or tumor types. Therefore, the $BMDL_{50\ HED}$ for the female mouse data was chosen as the POD and the CSF of 0.10 $(mg/kg\text{-}day)^{-1}$ was calculated as follows:

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

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

5.4.4.2 Inhalation Unit Risk

As stated in Section 5.4.2.2, multiple tumor types have been observed in rats following inhalation exposure to 1,4-dioxane. These data have been used to develop IUR estimates for 1,4-dioxane. The multistage cancer models available in the BMDS (version 2.1.1) were fit to the incidence data for each tumor type observed in rats exposed to 1,4-dioxane via inhalation (Kasai et al., 2009) to determine the degree (e.g., 1st, 2nd, or 3rd) of the multistage model that best fit the data (details in Appendix G). In contrast to the oral slope factor analysis, suitable multistage model fits were obtained for all of the datasets included in the inhalation unit risk analysis. Then, the best fitting models for each endpoint were used in the BMDS (version 2.2Beta) MS_Combo program to estimate a total tumor BMC and $BMCL_{10}$. A Bayesian MCMC analysis was also performed using WinBUGS to calculate the total tumor risk and it yielded similar results (See Appendix G). A summary of the BMDS model predictions for the Kasai et al. (2009) study is shown in Table 5-11. Experimental exposure concentrations were used for BMD modeling and then continuous human equivalent exposures were calculated by adjusting for duration of exposure (Table 5-11) and applying an appropriate DAF (see Section 5.2.3). In accordance with the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (2005a), the $BMCL_{10}$ (lower bound on the concentration estimated to produce a 10% increase in tumor incidence over background) was estimated for the dichotomous incidence data and the results of the model that best characterized the cancer incidences were selected. BMCs and BMCLs from all models are reported, and the output and plots corresponding to the best-fitting model are shown (Appendix G).

The IUR estimates are provided in Table 5-11. Human equivalent risks estimated from the individual rat tumor sites ranged from 2×10^{-7} to 2×10^{-6} $(\mu g/m^3)^{-1}$ (rounded to one significant figure). The highest IUR (2×10^{-6} $(\mu g/m^3)^{-1}$) corresponded to peritoneal mesotheliomas in male rats, and the lowest IUR (2×10^{-7} $(\mu g/m^3)^{-1}$) corresponded to renal cell carcinoma and Zymbal gland adenomas in male rats. The MS_Combo analysis yielded an IUR estimate of 5×10^{-6} $(\mu g/m^3)^{-1}$.

Table 5-11 Dose-response modeling summary results for male rat tumors associated with inhalation exposure to 1,4-dioxane for 2 years

Tumor Type[a]	Multistage Model Degree[b]	Point of Departure[c]				IUR Estimate[e] $(\mu g/m^3)^{-1}$
		Bioassay Exposure Concentration (ppm)		HEC (mg/m^3)[d]		
		BMC_{10}	$BMCL_{10}$	BMC_{10}	$BMCL_{10}$	
Nasal cavity squamous cell carcinoma	1	1107	629.9	712.3	405.3	2.5×10^{-7}
Hepatocellular adenoma or carcinoma	1	252.8	182.3	162.7	117.3	8.5×10^{-7}
Renal cell carcinoma	3	1355	1016	872	653.7	1.5×10^{-7}
Peritoneal mesothelioma	1	82.21	64.38	52.89	41.42	2.4×10^{-6}
Mammary gland fibroadenoma	1	1635	703.0	1052	452.4	2.2×10^{-7}
Zymbal gland adenoma	3	1355	1016	872	653.7	1.5×10^{-7}
Subcutis fibroma	1	141.8	81.91	91.21	52.70	1.9×10^{-6}
BMDS MS_Combo Total Tumor Analysis[f]		40.4	30.3	26.0	19.5	5.0×10^{-6}

[a]Tumor incidence data from Kasai et al. (2009).
[b]Best-fitting multistage model degree (p>0.1, lowest AIC). See Appendix G for modeling details.
[c]BMC = Concentration at specified extra risk (benchmark dose); BMCL = 95% lower bound on concentration at specified extra risk.
[d]Human continuous equivalent estimated by multiplying exposures by [(6 hours)/(24 hours) × (5 days)/(7 days) × molecular weight of 1,4-dioxane]/ 24.45.
[e]The inhalation unit risk $(\mu g/m^3)^{-1}$ was derived from the $BMCL_{10}$, the 95% lower bound on the concentration associated with a 10% extra cancer risk. Specifically, by dividing the BMR (0.10) by the $BMCL_{10}$. Thus, representing an upper bound, continuous lifetime exposure estimate of cancer potency.
[f]Results in this table are from the BMDS MS_Combo model. Additionally, Bayesian analysis using WinBUGS was performed and yielded similar results (See Appendix G).

Given the multiplicity of tumor sites, basing the inhalation unit risk on one tumor site may underestimate the carcinogenic potential of 1,4-dioxane. Consistent with recommendations of the NRC (1994) and the EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), the total risk and upper bound risk for all tumor sites in male F344 rats was estimated. This estimate of total risk describes the risk of developing any combination of the tumor types considered. As shown in Table 5-11, the resulting inhalation unit risk for all tumor types in male F344 rats was 5×10^{-6} $(\mu g/m^3)^{-1}$. Consideration of all tumor sites approximately doubled the unit risk compared to the highest unit risk associated with any individual tumor type, 2×10^{-6} $(\mu g/m^3)^{-1}$ for male peritoneal mesotheliomas.

The HEC $BMCL_{10}$ for the combined tumor estimate in male rats was chosen as the POD and the IUR of 5×10^{-6} $(\mu g/m^3)^{-1}$ was calculated as follows:

$$\text{IUR } (\text{mg/m}^3)^{-1} = \frac{0.10}{19.5 \text{ mg/m}^3} = 0.005 \ (\text{mg/m}^3)^{-1}$$

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

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

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

5.4.5 Previous Cancer Assessment

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

5.5 Uncertainties in Cancer Risk Values

In this assessment, extrapolation of high-dose data from laboratory animals to estimate potential risks to human populations from low-dose exposure to 1,4-dioxane has engendered some uncertainty in the results. Several types of uncertainty may be considered quantitatively, but other important uncertainties can only be considered qualitatively. Thus, an overall integrated quantitative uncertainty analysis is not presented. However, the sources of uncertainty and their potential impacts on the assessment are described below and in Table 5-12.

5.5.1 Sources of Uncertainty

5.5.1.1 Choice of Low-Dose Extrapolation Approach

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

It is not possible to predict how additional MOA information would impact the dose-response assessment for 1,4-dioxane because of the variety of tumors observed and the lack of data on how

1,4-dioxane or its metabolite interacts with cells initiating the progression to the observed tumors. In general, the Agency has preferred to use the multistage model for analyses of tumor incidence and related endpoints because this model has a generic biological motivation based on long-established biologically-based mathematical models such as the Moolgavkar-Venzon-Knudsen (MVK) model. The MVK model does not necessarily characterize all modes of tumor formation, but it is a starting point for most investigations and, much more often than not, has provided at least an adequate description of tumor incidence data.

The multistage cancer model provided adequate fits for the tumor incidence data following a 2-year inhalation exposure to 1,4-dioxane by male rats (Kasai et al., 2009). In the studies evaluated for the oral cancer assessment (Kano et al., 2009; NCI, 1978; Kociba et al., 1974a), the multistage model provided good descriptions of the incidence of a few tumor types in male (nasal cavity) and female (hepatocellular and nasal cavity) rats and in male mice (hepatocellular) exposed to 1,4-dioxane via ingestion (Appendix D for details). The multistage model did not provide an adequate fit for the female mouse liver tumor dataset based upon the following (U.S. EPA, 2012a):

- Goodness-of-fit *p*-value was less than 0.10 indicating statistically significant lack of fit;
- Akaike's Information Criterion (AIC) was larger than other acceptable models;
- Observed data deviated substantially from the fitted model, as measured by their standardized χ^2 residuals (i.e., residuals with values greater than an absolute value of one).

By default, the BMDS software imposes constraints on the values of certain parameters of the models. When these constraints were imposed, the multistage model and most other models did not fit the incidence data for female mouse liver adenomas or carcinomas, even after dropping the highest dose group.

The log-logistic model was selected because it was the only model that provided an adequate fit to the female mouse liver tumor data (Kano et al., 2009). A BMR of 50% was used because it is proximate to the response at the lowest dose tested, and the $BMDL_{50\ HED}$ was estimated by applying appropriate parameter constraints to the selected model, consistent with the BMD Technical Guidance Document (U.S. EPA, 2012a).

The human equivalent oral CSFs estimated from tumor datasets with statistically significant increases ranged from 4.2×10^{-4} to 1.0×10^{-1} per mg/kg-day (Table 5-10), a range of about three orders of magnitude, with the upper and lower extremes coming from the combined male and female rat data for hepatocellular carcinomas (Kociba et al., 1974a) and the female mouse combined liver adenoma and carcinomas (Kano et al., 2009).

5.5.1.2 Dose Metric

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

5.5.1.3 Cross-Species Scaling

For the oral cancer assessment, an adjustment for cross-species scaling ($BW^{0.75}$) was applied(U.S. EPA, 2011) to address toxicological equivalence of internal doses between each rodent species and humans, consistent with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a). It is assumed that equal risks result from equivalent constant lifetime exposures.

Differences in the anatomy of the upper respiratory tract and resulting differences in absorption or in local respiratory system effects are sources of uncertainty in the inhalation cancer assessment. However, since similar cell types are prevalent throughout the respiratory tract of both rats and humans, the tumors are considered biologically plausible and relevant to humans.

5.5.1.4 Statistical Uncertainty at the POD

Parameter uncertainty can be assessed through confidence intervals. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. For the log-logistic model applied to the female mouse data following oral exposure, there is a reasonably small degree of uncertainty at the 50% excess incidence level (the POD for linear low-dose extrapolation), as indicated by the proximity of the $BMDL_{HED}$ (4.95 mg/kg-day) to the BMD_{HED} (7.51 mg/kg-day). For the multistage model applied for the male rat inhalation dataset, there is a reasonably small degree of uncertainty at the 10% extra risk level (the POD for linear low-dose extrapolation).

5.5.1.5 Bioassay Selection

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

The study by Kasai et al. (2009) was used for derivation of an inhalation unit risk. This was a well-designed study, conducted in male rats with a sufficient number (N=50) of animals per dose group. Three dose levels plus an untreated control group were examined following exposure to 1,4-dioxane via inhalation for 2 years.

5.5.1.6 Choice of Species/Gender

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

A personal communication from Dr. Yamazaki (2006) provided that the survival of mice in the Kano et al. (2009) study was particularly low in high-dose females (29/50, 29/50, 17/50, and 5/50 in control, low-, mid-, and high-dose groups, respectively). These deaths occurred primarily during the second year of the study. Female mouse survival at 12 months was 50/50, 50/50, 48/50, and 48/50 in control, low-, mid-, and high-dose groups, respectively (Yamazaki, 2006). Furthermore, these deaths were primarily tumor related. Liver tumors were listed as the cause of death for 1/21, 2/21, 8/33, and 31/45 of the pretermination deaths in control, low-, mid- and, high-dose female Crj:BDF1 mice (Yamazaki, 2006). Therefore, because a number of the deaths in female mice were attributed to liver tumors, this endpoint and species was still considered to be relevant for this analysis; however, the high mortality rate does contribute uncertainty. Additionally, the OSF may actually be larger if the survival adjusted tumor data were available.

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

Male F344 rat data were used to estimate risk following inhalation of 1,4-dioxane. Kano et al. (2009) showed that male rats were more sensitive than female rats to the effects of 1,4-dioxane following oral administration; therefore, male rats were chosen to be studies in the 2-year bioassay conducted by the same laboratory (Kasai et al., 2009). The sensitivity and tumorigenic response of female rats or male or female mice following inhalation of 1,4-dioxane is unknown. Since female mice were the most sensitive

gender and species examined in the Kano et al. (2009) oral study, female mice may also be more sensitive to the inhalation of 1,4-dioxane, which would result in a greater risk.

5.5.1.7 Relevance to Humans

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

The derivation of the inhalation unit risk is based on the tumor incidence at multiple sites in male rats. There is no information on 1,4-dioxane to indicate that the observed rodent tumors are not relevant to humans. Further, no data exist to guide quantitative adjustment for differences in sensitivity among rodents and humans. In the absence of information to indicate otherwise and considering similar cell types are prevalent throughout the respiratory tract of rats and humans, the nasal, liver, renal, peritoneal, mammary gland, Zymbal gland and subcutis tumors were considered relevant to humans.

5.5.1.8 Human Population Variability

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

Table 5-12 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 *Guidelines for Carcinogen Risk Assessment* POD paradigm, if justified, could ↓ or ↑ unit risk an unknown extent	Log-logistic model to determine POD, for CSF; Combined tumor 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	CSF (Kano et al., 2009); IUR (Kasai et al., 2009)	Alternative bioassays were available and considered for derivation of oral CSF and inhalation IUR.
Species /gender combination	Human risk could ↓ or ↑, depending on relative sensitivity	Female mouse (CSF); male rat (IUR)	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. No female mouse data were available for derivation of the IUR.
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 CSF). 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	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

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

Liver and kidney toxicity were the primary noncancer health effects of subchronic and chronic oral exposure to 1,4-dioxane in animals. Hepatocellular degeneration and necrosis were observed (Kociba et al., 1974a) and preneoplastic changes were noted in the liver following chronic administration of 1,4-dioxane in drinking water (Kano et al., 2008; JBRC, 1998; NCI, 1978; Argus et al., 1973a). Liver and kidney toxicity appear to be related to saturation of clearance pathways and an increase in the 1,4-dioxane concentration in the blood (Kociba et al., 1974b). Kidney damage was characterized by degeneration of the cortical tubule cells, necrosis with hemorrhage, and glomerulonephritis (NCI, 1978; Kociba et al., 1974b; Argus et al., 1973b; Argus et al., 1965b; Fairley et al., 1934b). In chronic inhalation studies conducted in rats, nasal and liver toxicity were the primary noncancer health effects. Degeneration of nasal tissue (i.e. metaplasia, hyperplasia, atrophy, hydropic change, and vacuolic change) and preneoplastic cell proliferation were observed in the nasal cavity following inhalation exposure to 1,4-dioxane for 2 years (Kasai et al., 2009). Liver toxicity was described as necrosis of the centrilobular region and preneoplastic changes were noted as well.

Several carcinogenicity bioassays have been conducted for 1,4-dioxane in mice, rats, and guinea pigs (Kano et al., 2009; Kasai et al., 2009; JBRC, 1998; NCI, 1978; Kociba et al., 1974b; Torkelson et al., 1974b; Argus et al., 1973b; Hoch-Ligeti and Argus, 1970b; Hoch-Ligeti et al., 1970b; Argus et al., 1965b). Liver tumors (hepatocellular adenomas and carcinomas) have been observed following drinking water exposure in several species and strains of rats, mice, and guinea pigs and following inhalation exposure in rats. Nasal (squamous cell carcinomas), peritoneal, mammary, Zymbal gland, and subcutaneous tumors were also observed in rats, but were not seen in mice. With the exception of the NCI

(1978) study, the incidence of nasal cavity tumors was generally lower than that of tumors observed in other tissues of the same study population.

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

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

6.2 DOSE RESPONSE

6.2.1 Noncancer/Oral

The RfD of 3×10^{-2} mg/kg-day was derived based on liver and kidney toxicity in rats exposed to 1,4-dioxane in the drinking water for 2 years (Kociba et al., 1974b). This study was chosen as the principal study because it provides the most sensitive measure of adverse effects by 1,4-dioxane. The incidence of liver and kidney lesions was not reported for each dose group. Therefore, BMD modeling could not be used to derive a POD. Instead, the RfD is derived by dividing the NOAEL of 9.6 mg/kg-day by a composite UF of 300 (factors of 10 for animal-to-human extrapolation and interindividual variability, and an UF of 3 for database deficiencies). Information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans and the potential

variability in human susceptibility; thus, the interspecies and intraspecies uncertainty factors of 10 were applied. In addition, a threefold database uncertainty factor was applied due to the lack of information addressing the potential reproductive toxicity associated with 1,4-dioxane.

The overall confidence in the RfD is medium. Confidence in the principal study (Kociba et al., 1974b) is medium. Confidence in the database is medium due to the lack of a multigeneration reproductive toxicity study. Reflecting medium confidence in the principal study and medium confidence in the database, confidence in the RfD is medium.

6.2.2 Noncancer/Inhalation

The RfC of 3×10^{-2} mg/m^3 was derived based on co-critical effects of olfactory epithelium atrophy and respiratory metaplasia in rats exposed for 2 years to 1,4-dioxane via inhalation (Kasai et al., 2009). This study was chosen as the principal study because it provides an adequate study design and the most sensitive measure of adverse effects by 1,4-dioxane. The POD was derived using the LOAEL for olfactory epithelium atrophy and respiratory metaplasia in male rats (Kasai et al., 2009). A composite UF of 1,000 was applied, consisting of factors of 10 for a LOAEL-to NOAEL extrapolation, 10 for interindividual variability, 3 for animal-to-human extrapolation, and 3 for database deficiencies.

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

6.2.3 Cancer

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

6.2.3.1 Oral

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

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

6.2.3.2 Inhalation

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

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

6.2.3.3 Choice of Low-Dose Extrapolation Approach

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

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

The multistage cancer model provided adequate fits for the tumor incidence data following a 2-year inhalation exposure to 1,4-dioxane by male rats (Kasai et al., 2009). However, in the studies evaluated for the oral cancer assessment (Kano et al., 2009; NCI, 1978; Kociba et al., 1974b) the multistage model provided good descriptions of the incidence of a few tumor types in male (nasal cavity) and female (hepatocellular and nasal cavity) rats and in male mice (hepatocellular) exposed to

1,4-dioxane via ingestion (see Appendix D for details). However, the multistage model did not provide an adequate fit for female mouse liver tumor dataset based upon the following (U.S. EPA, 2012a):

- Goodness-of-fit *p*-value was less than 0.10 indicating statistically significant lack of fit;
- AIC was larger than other acceptable models;
- Observed data deviated substantially from the fitted model, as measured by their standardized χ^2 residuals (i.e., residuals with values greater than an absolute value of one).

By default, the BMDS software imposes constraints on the values of certain parameters of the models. When these constraints were imposed, the multistage model and most other models did not fit the incidence data for female mouse liver adenomas or carcinomas, even after dropping the highest dose group.

The log-logistic model was selected because it was the only model that provided an adequate fit to the female mouse liver tumor data (Kano et al., 2009). A BMR of 50% was used because it is proximate to the response at the lowest dose tested and the $BMDL_{50}$ was derived by applying appropriate parameter constraints, consistent with recommended use of BMDS in the BMD Technical Guidance Document (U.S. EPA, 2012a).

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

6.2.3.4 Dose Metric

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

6.2.3.5 Cross-Species Scaling

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

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

6.2.3.6 Statistical Uncertainty at the POD

Parameter uncertainty can be assessed through confidence intervals. Each description of parameter uncertainty assumes that the underlying model and associated assumptions are valid. For the log-logistic model applied to the female mouse data following oral exposure, there is a reasonably small degree of uncertainty at the 50% excess incidence level (the POD for linear low-dose extrapolation), as indicated by the proximity of the $BMDL_{HED}$ (4.95 mg/kg-day) to the BMD_{HED} (7.51 mg/kg-day) . For the multistage model applied for the male rat inhalation dataset, there is a reasonably small degree of uncertainty at the 10% extra risk level (the POD for linear low-dose extrapolation).

6.2.3.7 Bioassay Selection

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

The study by Kasai et al. (2009) was used for derivation of an inhalation unit risk. This was a well-designed study, conducted in male rats with a sufficient number (N=50) of animals per dose group. Three dose levels plus an untreated control group were examined following exposure to 1,4-dioxane via inhalation for 2 years.

6.2.3.8 Choice of Species/Gender

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

Male F344 rat data were used to estimate risk following inhalation of 1,4-dioxane. Kano et al. (2009) showed that male rats were more sensitive than female rats to the effects of 1,4-dioxane following oral administration; therefore, male rats were studied in the 2-year bioassay conducted by the same laboratory (Kasai et al., 2009). The sensitivity and tumorigenic response of female rats or male or female mice following inhalation of 1,4-dioxane is unknown. Since female mice were the most sensitive gender and species examined in the Kano et al. (2009) study, female mice may also be more sensitive to the inhalation of 1,4-dioxane which would result in a greater risk.

6.2.3.9 Relevance to Humans

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

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

6.2.3.10 Human Population Variability

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

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

The *Toxicological Review of 1,4-Dioxane* has undergone two formal external peer reviews performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2006a, 2000b). The first peer review focused on the toxicity following oral exposure to 1,4-dioxane. For completeness, the inhalation data were added to the assessment and the combined document was submitted for a second peer review and public comment – with a request for reviewers to focus on the inhalation portion of the assessment.

The external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. A summary of significant comments made by the external reviewers and EPA's responses to these comments arranged by charge question follow for both the oral assessment and inhalation update. In many cases the comments of the individual reviewers have been synthesized and paraphrased for development of Appendix A. The majority of the specific observations (in addition to EPA's charge questions) made by the peer reviewers were incorporated into the document and are not discussed further in this Appendix. Public comments that were received are summarized and addressed following the peer-reviewers' comments and disposition for both the oral assessment and inhalation update.

A.1 External Peer Review Panel Comments -- Oral Assessment

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

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

A.1.1 General Charge Questions

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

   Comment: All reviewers found the *Toxicological Review* to be logical, clear, and concise. One reviewer remarked that it was an accurate, open-minded and balanced analysis of the literature. Most reviewers found that the scientific evidence was presented objectively and transparently; however, one reviewer suggested two things to improve the objectivity and transparency (1) provide a clear description of the mode of action and how it feeds into the choice of the

extrapolation for the cancer endpoint and (2) provide a presentation of the outcome if internal dose was used in the cancer and noncancer assessments.

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

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

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

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

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

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

b. Kasai T; Kano Y; Umeda T; et al. (2009) Two-year inhalation study of carcinogenicity and chronic toxicity of 1,4-dioxane in male rats. Inhal Toxicol *in press*.

Other references suggested by reviewers include:

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

d. National Research Council (2009) Science and Decisions: Advancing Risk Assessment. Committee on Improving Risk Analysis Approaches Used by the U.S. EPA. Washington, D.C., National Academy Press.

e. ATSDR (2007) Toxicological Profile for 1,4-dioxane. Agency for Toxic Substances and Disease Registry. Atlanta, GA.

f. Stickney JA; Sager SL; Clarkson JR; et al. (2003) An updated evaluation of the carcinogenic potential of 1,4-dioxane. Regul Toxicol Pharmacol 38: 183-195.

g. Yamamoto S; Ohsawa M; Nishizawa T; et al. (2000) Long-term toxicology study of 1,4-dioxane in R344 rats by multiple-route exposure (drinking water and inhalation). J Toxicol Sci 25: 347.

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

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

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

Comment: All reviewers provided suggestions for additional research that would strengthen the assessment and reduce uncertainty in several areas. The following is a brief list of questions that were identified that could benefit from further research. What are the mechanisms responsible for the acute and chronic nephrotoxicity? Is the acute kidney injury (AKI) multifactorial? Are there both tubular and glomerular/vascular toxicities that result in cortical tubule degeneration and evidence for glomerulonephritis? What are the functional correlates of the histologic changes in terms of assessment of renal function? What is the exposure in utero and risk to the fetus and newborn? What are the concentrations in breast milk following maternal exposure to 1,4-dioxane? What is the risk for use of contaminated drinking water to reconstitute infant formula? What are the exposures during early human development? What is the pharmacokinetic and metabolic profile of 1,4-dioxane during development? What are the susceptible populations (e.g., individuals with decreased renal function or chronic renal disease, obese individuals, gender, age)?

Additional suggestions for future research include: evaluation of potential epigenetic mechanisms of carcinogenicity, additional information on sources of exposure and biological concentrations as well as human toxicokinetic data for derivation of parameter to refine PBPK model, studies to

determine toxic moiety, focused studies to inform mode of action, additional inhalation studies and a multigeneration reproductive toxicity study.

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

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

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

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

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

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

Clarification regarding the uncertainty surrounding the identification of the toxic moiety was added to Section 4.6.2.1 stating that the mechanism by which 1,4-dioxane induces tissue damage is not known, nor is it known whether the toxic moiety is 1,4-dioxane or a metabolite of 1,4-dioxane. Additional text was added to Section 4.7.3 clarifying that available data also do not clearly identify whether 1,4-dioxane or one of its metabolites is responsible for the observed

effects. The impact of the lack of evidence to clearly identify a toxic moiety related to 1,4-dioxane exposure was summarized in Sections 5.5.1.2 and 6.2.3.2.

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

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

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

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

   Response: The reviewer is correct that Kociba et al. (1974a) did not provide incidence data; however, Kociba et al. (1974a) identified a NOAEL (9.6 mg/kg-day) and LOAEL (94 mg/kg-day) within the text of the manuscript. Kociba et al. (1974a) was a well conducted chronic bioassay (four dose levels, including controls, with 60 rats/sex/group) and seven of the peer reviewers found this study to be appropriate as the basis for the RfD. Further support for the selection of the Kociba et al. (1974a) as the principal study comes from comparison of the liver and kidney toxicity data reported by JBRC (1998) and NCI (1978), which was presented in Section 5.1. The effects reported by JBRC (1998) and NCI (1978) were consistent with what was observed by Kociba et al. (1974a) and within a similar dose range. Derivation of an RfD from these datasets resulted in a similar value (Section 5.1.).

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

   Comment: Five of the reviewers agreed with the selection of liver and kidney effects as the critical effect. One of these reviewers suggested analyzing all datasets following dose adjustment

(e.g., body weight scaling or PBPK model based) to provide a better rationale for selection of a critical effect.

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

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

Response: The liver and kidney effects from Kociba et al. (1974a) was supported as the critical effect by most of the reviewers. PBPK model adjustment was not performed because the PBPK model was found to be inadequate for use in the assessment. EPA acknowledges that neither the mechanisms of injury nor the clinical correlates to histologic data exist for 1,4-dioxane. This type of information could improve future health assessments of 1,4-dioxane.

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

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

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

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

Response: The suggestion to "semi-qualitatively represent the histopathological observations to facilitate a quantitative analysis" was not incorporated into the document because it is unclear how this would be conducted since Kociba et al. (1974a) did not provide incidence data and the reviewer did not illustrate their suggested approach. See responses to B1 and B2 regarding the NOAEL and LOAEL approach. The Agency agrees that a Benchmark Dose approach is preferred

over the use of a NOAEL or LOAEL for the POD if suitable data (e.g., reflecting the most sensitive sex, species, and endpoint identified) are available for modeling and, if suitable data are not available, then NOAEL and LOAEL values are utilized. In this case, the data were not suitable for BMD modeling and the LOAEL or NOAEL approach was used.

4. EPA evaluated the PBPK and empirical models available to describe kinetics following inhalation of 1,4-dioxane (Reitz et al., 1990a; Young et al., 1978a; Young et al., 1978b; Young et al., 1977a). EPA concluded that the use of existing, revised, and recalibrated PBPK models for 1,4-dioxane were not superior to default approaches for the dose-extrapolation between species. Please comment on whether EPA's rationale regarding the decision to not utilize existing or revised PBPK models has been adequately and transparently described and is supported by the available data. Please identify and provide the rationale for any alternative approaches that should be considered or preferred to the approach presented in the toxicological review.

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

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

a. In the document, KLC is defined as a first-order rate constant and is scaled by $BW^{0.7}$. This is inconsistent when multiplied by concentration does not result in units of mg/hr. However, if the parameter is actually considered a clearance constant (zero-order rate constant) then the scaling rule used, as well as the interpretations provided, would be acceptable.

b. It is unclear as to why AM is calculated on the basis of RAM and not RMEX. RMEX seems to represent the amount metabolized per unit time.

Response: The U.S. EPA performed a rigorous evaluation of the PBPK models available for 1,4-dioxane. This effort was extensively described in Section 3.5 and in Appendix B. In short, several procedures were applied to the human PBPK model to determine if an adequate fit of the model to the empirical model output or experimental observations could be attained using

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

The results of U.S. EPA model evaluation were confirmed by other investigators (Sweeney et al., 2008a). Sweeney et al. (2008a) concluded that the available PBPK model with refinements resulted in an under-prediction of human blood levels for 1,4-dioxane by six- to seven fold. It is anticipated that the high uncertainty in predictions of the PBPK model for 1,4-dioxane would not result in a more accurate derivation of human health toxicity values.

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

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

KLC was implemented by the U.S. EPA during the evaluation of the model and should have been described as a clearance constant (first-order rate constant) with units of $L/hr/kg^{0.70}$. These corrections have been made in the document; however, this does not impact the model predictions because it was in reference to the terminology used to describe this constant.

The reviewer is correct that RMEX is the rate of metabolism of 1,4-dioxane per unit time; however an amount of 1,4-dioxane metabolized was not calculated in the Reitz et al. (1990a)

model code. Thus, AM is the amount of the metabolite (i.e., HEAA) in the body rather than the amount metabolized of 1,4-dioxane. RAM was published by Reitz et al. (1990a) as equation 2 for the change in the amount of metabolite in the body per unit time. AMEX is the amount of the metabolite excreted in the urine. While the variables used are confusing, the code describes the metabolism of 1,4-dioxane as published in the manuscripts. The comments in the model code were updated to make this description more clear (Appendix B).

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

- An interspecies uncertainty factor of 10 was used to account for uncertainties in extrapolating from laboratory animals to humans because a PBPK model to support interspecies extrapolation was not suitable.

- An intraspecies (human variability) uncertainty factor of 10 was applied in deriving the RfD because the available information on the variability in human response to 1,4-dioxane is considered insufficient to move away from the default uncertainty factor of 10.

- A database uncertainty factor of 3 was used to account for lack of adequate reproductive toxicity data for 1,4-dioxane, and in particular absence of a multigeneration reproductive toxicity study. Has the rationale for the selection of these uncertainty factors been transparently and objectively described in the document? Please comment on whether the application of these uncertainty factors has been scientifically justified.

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

- Five reviewers agreed that the use of an uncertainty factor of 10 for the interspecies extrapolation is fully supportable. One reviewer suggested using $BW^{3/4}$ scaling rather than an uncertainty factor of 10 for animal to human extrapolation. Along the same lines, one reviewer suggested a steady-state quantitative analysis to determine the importance of pulmonary clearance and hepatic clearance and stated that if hepatic clearance scales to body surface and pulmonary clearance is negligible, then an adjusted uncertainty factor based on body surface scaling would be more appropriate.

- Seven reviewers stated that the uncertainty factor of 10 for interindividual variability (intraspecies) is fully supportable.

- Six reviewers commented that the uncertainty factor of 3 for database deficiencies is fully justifiable. One reviewer suggested adding text to clearly articulate the science policy for the use of a factor of 3 for database deficiencies.

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

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

A.1.3 Carcinogenicity of 1,4-dioxane and derivation of an oral slope factor

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

Comment: All reviewers agreed with the Agency's conclusion that 1,4-dioxane is "likely to be carcinogenic to humans". However, two reviewers also thought 1,4-dioxane could be categorized as a potential human carcinogen, since low-dose environmental exposures would be unlikely to result in cancer. One reviewer also suggested providing a brief recapitulation of the guidance provided by the 2005 *Guidelines for Carcinogen Risk Assessment* regarding classification of a compound as likely to be carcinogenic to humans and how a chemical falls into this category.

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

2. Evidence indicating the mode of action of carcinogenicity of 1,4-dioxane was considered. Several hypothesized MOAs were evaluated within the Toxicological Review and EPA reached the conclusion that a MOA(s) could not be supported for any tumor types observed in animal models. Please comment on whether the weight of the scientific evidence supports this conclusion. Please comment on whether the rationale for this conclusion has been transparently and objectively

described. Please comment on data available for 1,4-dioxane that may provide significant biological support for a MOA beyond what has been described in the Toxicological Review. Considerations should include the scientific support regarding the plausibility for the hypothesized MOA(s), and the characterization of uncertainty regarding the MOA(s).

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

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

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

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

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

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

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

Additional text has been added to Section 5.4.3.2 to relay the fact that several reviewers recommended that the MOA data support the use of a nonlinear extrapolation approach to

estimate human carcinogenic risk associated with exposure to 1,4-dioxane and that such an approach should be presented in the Toxicological Review. Additional text has also been added to the summary statement in Section 6.2.3 stating that the weight of evidence is inadequate to establish a MOA(s) by which 1,4-dioxane induces peritoneal, mammary, or nasal tumors in rats and liver tumors in rats and mice (see Section 4.7.3 for a more detailed discussion of 1,4-dioxane's hypothesized MOAs).

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

Comment: Seven reviewers agreed with the choice of the JBRC (1998) study as the principal study for the development of an OSF. However, two reviewers that agreed with the choice of JBRC (1998) also commented on the description and evaluation of the study. One reviewer commented the evaluation of the study should be separated from the evaluation/selection of endpoints within the study. The other reviewer suggested that details on the following aspects should be added to improve transparency of the study: (1) rationale for selection of doses; (2) temporal information on body weight for individual treatment groups; (3) temporal information on mortality rates; and (4) dosing details.

One reviewer thought that the complete rationale for selection of the JBRC (1998) study was not provided because there was no indication of whether the study was conducted under GLP conditions, and the study was not peer reviewed or published. This reviewer noted the NCI (1978) study was not appropriate for use, but that the Kociba et al. (1974a) study may have resulted in a lower POD had they employed both sexes of mice and combined benign and malignant tumors.

Response: Since the External Peer Review draft of the *Toxicological Review of 1,4-Dioxane* was released (U.S. EPA, 2009a), the cancer portion of the study conducted by the JBRC laboratory was published in the peer-reviewed literature as Kano et al. (2009). This manuscript was reviewed by EPA. EPA determined that the data published by Kano et al. (2009) should be included in the assessment of 1,4-dioxane for several reasons: (1) while the JBRC (1998) was a detailed laboratory report, it was not peer-reviewed; (2) the JBRC improved the diagnosis of pre- and neoplastic lesions in the liver according to the current diagnostic criteria and submitted the manuscript based on this updated data; (3) the Kano et al. (2009) peer-reviewed manuscript included additional information such as body weight growth curves and means and standard deviations of estimated dose for both rats and mice of both sexes. Thus, the Toxicological Review was updated to reflect the inclusion of the data from Kano et al. (2009), and Appendix E was added for a clear and transparent display of the data included in the multiple reports.

In response to the peer reviewers, dose information was updated throughout the assessment and are also provided in detail in Section 4.2.1.2.6, along with temporal information on body weights and mortality. Text was also added to Section 4.2.1.2.6 regarding the choice of high dose selection as included in the Kano et al. (2009) manuscript. Additional discussion regarding the

mortality rates was also added to Section 5.4.1 in selection of the critical study for the oral cancer assessment. Documentation that the study was conducted in accordance with Organization for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practice (GLP) is provided in the manuscript (Kano et al., 2009) and this was also added to the text in Section 4.2.1.2.6.

4. Combined liver tumors (adenomas and carcinomas) in female Cjr:BDF1 mice from the JBRC (1998) study were chosen as the most sensitive species and gender for the derivation of the final OSF. Please comment on the appropriateness of the selections of species and gender. Please comment on whether the rationale for these selections is scientifically justified. Has the rationale for these choices been transparently and objectively described?

Comment: Six reviewers agreed the female Cjr:BDF1 mice should be used for the derivation of the OSF. Five of these reviewers agreed with the rationale for the selection of the female Cjr:BDF1 mouse as the most sensitive gender and species. However, one reviewer suggested that the specific rationale (i.e., that the final OSF is determined by selecting the gender/species that gives the greatest OSF value) be stated clearly in a paragraph separate from the other considerations of study selection.

One reviewer was unsure of both the scientific justification for combining benign and malignant liver tumors, as well as the background incidence of the observed liver tumors in historical control Cjr:BDF1 male and female mice.

One reviewer commented that the scientific basis for the selection of female Cjr:BDF1 mice was unclear. This reviewer thought that the rationale for the choice of this strain/sex compared to all others was not clearly articulated.

Response: Using the approach described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) studies were first evaluated based on their quality and suitability for inclusion in the assessment. Once the studies were found to be of sufficient quality for inclusion in the assessment, the dose-response analysis was performed with the goal of determining the most appropriate endpoint and species for use in the derivation of an OSF. These topics are discussed in detail in Section 4.7 and 5.4.

Benign and malignant tumors that arise from the same cell type (e.g., hepatocellular) may be combined to more clearly identify the weight of evidence for a chemical. This is in accordance with the U.S. EPA 2005 *Guidelines for Carcinogen Risk Assessment* as referenced in the Toxicological Review. In the absence of a MOA (MOA analysis described in detail in Section 4.7.) for 1,4-dioxane carcinogenicity, it is not possible to determine which species may more closely resemble humans. Text in Section 5.4.4 indicates that the calculation of an OSF for 1,4-dioxane is based upon the dose-response data for the most sensitive species and gender.

5. Has the scientific justification for deriving a quantitative cancer assessment been transparently and objectively described? Regarding liver cancer, a linear low-dose extrapolation approach was utilized

to derive the OSF. Please provide detailed comments on whether this approach to dose-response assessment is scientifically sound, appropriately conducted, and objectively and transparently described in the document. Please identify and provide the rationale for any alternative approaches for the determination of the OSF and discuss whether such approaches are preferred to EPA's approach.

Comment: Four reviewers agreed with the approach for the dose-response assessment. One reviewer commented that even if a nongenotoxic MOA were identified for 1,4-dioxane it may not be best evaluated by threshold modeling. One reviewer commented the use of the female mouse data provided an appropriate health protective and scientifically valid approach.

One reviewer commented that the basic adjustments and extrapolation method for derivation of the OSF were clearly and adequately described, but disagreed with the linear low-dose extrapolation. This reviewer suggested that the lack of certainty regarding the MOA was not a sufficient cause to default to a linear extrapolation. Another reviewer commented that the rationale for a linear low-dose extrapolation to derive the OSF was not clear, but may be in accordance with current Agency policy in the absence of a known MOA. This reviewer also commented that 1,4-dioxane appears to be non-genotoxic and nonlinear models should be tested on the available data to determine if they provide a better fit and are more appropriate.

One reviewer thought that the justification for a linear extrapolation was not clearly provided and that a disconnect between the MOA summary and the choice of a linear extrapolation model existed. In addition, this reviewer commented that the pharmacokinetic information did not support the use of a linear extrapolation approach, but rather use of animal PBPK models to extrapolate from high to low dose that would result in a mixture of linear and nonlinear extrapolation models was warranted.

One reviewer suggested consideration of an integrated assessment of the cancer and noncancer endpoints; however, if linear low-dose extrapolation remains the approach of choice by the Agency, then the effect of choosing BMRs other than 10% was recommended to at least be included in the uncertainty discussion. Using BMRs lower than 10% may allow for the identification of a risk level for which the low-dose slope is 'best' estimated.

Response: The EPA conducted a cancer MOA analysis evaluating all of the available data for 1,4-dioxane. Application of the framework in the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (2005a) demonstrates that the available evidence to support any hypothesized MOA for 1,4-dioxane-induced tumors does not exist. In the absence of a MOA, the U.S. EPA *Guidelines for Carcinogen Risk Assessment* (2005a) indicate that a low dose linear extrapolation should be utilized for dose response analysis (see Section 5.4). Some of the potential uncertainty associated with this conclusion was characterized in Section 5.5. Note that there is no scientific basis to indicate that in the absence of evidence for genotoxicity a nonlinear low-dose extrapolation should be used. As concluded in the Toxicological Review, the application of a nonlinear low-dose extrapolation approach was not supported.

With regards to the PBPK model available for 1,4-dioxane, it is clear that there currently exist deficiencies within the model and as such, the model was not utilized for interspecies extrapolation. Given the deficiencies and uncertainty in the 1,4-dioxane model it also does not provide support for a MOA.

Lastly, in the absence of a MOA for 1,4-dioxane carcinogenicity it is not possible to harmonize the cancer and noncancer effects to assess the risk of health effects due to exposure. However, the choice of the $BMDL_{10}$,which was more than 15-fold lower than the response at the lowest dose (66 mg/kg-day), was reconsidered in response to a public comment. BMDs and BMDLs were calculated using a BMR of 30 and 50% extra risk (BMD_{30}, $BMDL_{30}$, BMD_{50}, and $BMDL_{50}$). A BMR of 50% was used as it resulted in a BMDL closest to the response level at the lowest dose tested in the bioassay.

A.2 Public Comments – Oral Assessment

Comments on the *Toxicological Review of 1,4-Dioxane* submitted by the public for the external peer review of the oral toxicity values are summarized below in the following categories: Oral reference dose for 1,4-dioxane, carcinogenicity of 1,4-dioxane, PBPK modeling, and other comments.

A.2.1 Oral reference dose (RfD) for 1,4-dioxane

Comment: An UF for database deficiencies is not necessary because of considerable evidence showing no reproductive or developmental effects from 1,4-dioxane exposure.

Response: Due to the lack of a multigenerational reproductive study for 1,4-dioxane an UF of 3 was retained for database deficiencies. Without clear evidence showing a lack of reproductive or developmental effects in a multigenerational reproductive study, there is still uncertainty in this area.

A.2.2 Carcinogenicity of 1,4-dioxane

Comment: Using liver tumors as the basis for the oral CSF is more appropriate than nasal tumors (1988 IRIS assessment of 1,4-dioxane); however, the use of mouse liver tumor data is inappropriate because it is inconsistent with other liver models both quantitatively and in the dose-response pattern. High mortality rates in the study are also a limitation. Liver tumor data from rats should be used instead, which represents a better animal model for 1,4-dioxane carcinogenicity assessment.

Response: Even though the dose-response is different for mice and rats, the female mice were considered to be appropriate for the carcinogenicity assessment for several reasons. The female mouse liver tumors from the Kano et al. (2009) report were found to be the most sensitive species and endpoint. Section 4.2.1.2.6 was updated to include additional information on mortality rates. The majority of the animals lived past 52 weeks (only 4 females died prior to 52 weeks, 2 in each the mid- and high-dose groups). The cause of death in the female mice that died between 1 and 2 years was attributed to liver tumors.

Comment: The OSF was based on the most sensitive group, Crj:BDF1 mice; however BDF1 mice have a high background rate of liver tumors. The incidence of liver tumors in historical controls for this gender/species should be considered in the assessment. Sensitivity of the test species/gender as well as other criteria should be considered in the selection of the appropriate study, including internal and external validity as outlined in Lewandowski and Rhomberg (2005). The female Crj:BDF1 mice had a low survival rate that should be considered in the selection of the animal model for 1,4-dioxane carcinogenicity.

Response: Katagiri et al. (1998) summarized the incidence of hepatocellular adenomas and carcinomas in control male and female BDF1 mice from ten 2-year bioassays at the JBRC. For female mice, out of 499 control mice, the incidence rates were 4.4% for hepatocellular adenomas and 2.0% for hepatocellular carcinomas. Kano et al. (2009) reported a 10% incidence rate for hepatocellular adenomas and a 0% incidence rate for hepatocellular carcinomas in control female BDF1. These incidence rates are near the historical control values and thus are appropriate for consideration in this assessment. Additional text regarding these historical controls was added to the study description in Section 4.2.1.2.6.

Comment: Low-dose linear extrapolation for the oral CSF is not appropriate nor justified by the data. The weight of evidence supports a threshold (nonlinear) MOA when metabolic pathway is saturated at high doses. Nonlinear extrapolations should be evaluated and presented for 1,4-dioxane. Oral CSFs should be derived and presented using both the $BW^{3/4}$ scaling as well as available PBPK models to extrapolate across species.

Response: The absence of evidence for genotoxicity/mutagenicity does not indicate the use of nonlinear low-dose extrapolation. For 1,4-dioxane, a MOA to explain the induction of tumors does not exist so the nature of the low-dose region of the dose-response is unknown. The oral CSF for 1,4-dioxane was derived using $BW^{3/4}$ scaling for interspecies extrapolation. The PBPK and empirical models available for 1,4-dioxane were evaluated and found not to be adequate for use in this assessment, described in detail in Appendix B.

Comment: The POD for the BDF1 female mouse is 15-fold lower than the lowest dose in the bioassay, thus the POD is far below the lower limit of the data and does not follow the U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

Response: The comment is correct that the animal $BMDL_{10}$ was more than 15-fold lower than the response at the lowest dose (66 mg/kg-day) in the bioassay. BMDs and BMDLs were calculated

using a BMR of 30 and 50% extra risk (BMD_{30}, $BMDL_{30}$, BMD_{50}, and $BMDL_{50}$). A BMR of 50% was chosen as it resulted in a BMDL closest to the response level at the lowest dose tested in the bioassay.

Comment: The geometric mean of the oral cancer slope factors (as done with B[a]P & DDT) should have been used instead of relying on the female BDF1 mouse data, since a MOA could not be determined for 1,4-dioxane.

Response: In accordance with the BMD Technical Guidance Document (U.S. EPA, 2012a) averaging tumor incidence is not a standard or default approach. Averaging the tumor incidence response diminishes the effect seen in the sensitive species/gender.

Comment: EPA should critically reexamine the choice of JBRC (1998) as the principal study since it has not been published or peer-reviewed. A transcript of e-mail correspondence should be provided.

Response: JBRC (1998) was published as conference proceedings as Yamazaki et al. (1994a) and recently in the peer-reviewed literature as Kano et al. (2009). Additional study information was also gathered from the authors (Yamazaki, 2006) and is available upon request from the IRIS Hotline. The peer-reviewed and published data from Kano et al. (2009) was incorporated into the final version of the *Toxicological Review of 1,4-Dioxane.*

Comment: The WOE does not support a cancer descriptor of *likely to be carcinogenic to humans* determination, but rather *suggestive human carcinogen at the high dose levels used in rodent studies* seems more appropriate for the following reasons: 1) lack of conclusive human epidemiological data; 2) 1,4-dioxane is not mutagenic; and 3) evidence at high doses it would act via cell proliferation MOA.

Response: A cancer classification of "*likely,"* based on evidence of liver carcinogenicity in several two-year bioassays conducted in three strains of rats, two strains of mice, and in guinea pigs was chosen. Also, mesotheliomas of the peritoneum, mammary, and nasal tumors have been observed in rats. The Agency agrees that human epidemiological studies are inconclusive. The evidence at any dose is insufficient to determine a MOA.

A.2.3 PBPK Modeling

Comment: EPA should have used and considered PBPK models to derive the oral toxicity values (rat to human extrapolation) rather than relying on a default method. The draft did not consider the Sweeney et al. (2008a) model. The PBPK model should be used for both noncancer and cancer dose extrapolation.

Response: The Agency evaluated the Sweeney et al. (2008a) publication and this was included in Appendix B of the document. Text was added to the main document in Section 3.5.2.4 and 3.5.3 regarding the evaluation of Sweeney et al. (2008a). This model was determined not to be

appropriate for interspecies extrapolation. Additionally, see response to the external peer review panel comment B4.

Comment: EPA should use the modified inhalation inputs used in the Reitz et al. (1990a) model and the updated input parameters provided in Sweeney et al. (2008a) and add a compartment for the kidney

Response: See response to previous comment regarding evaluation of Sweeney et al. (2008a). Modification of the model to add a kidney compartment is not within the scope of this assessment.

A.2.4 Other Comments

Comment: EPA should consider the Kasai et al. (2009; 2008) studies for inhalation and MOA relevance.

Response: The 13 week and 2-year inhalation studies by Kasai et al. (2009; 2008) were published late in the development stage of this assessment. The IRIS Program will evaluate these recently published 1,4-dioxane inhalation data for the potential to derive an RfC in a separate assessment.

***Comment*:** 1,4-Dioxane is not intentionally added to cosmetics and personal care products – correct sentence on page 4.

***Response*:** This oversight was corrected in the document.

A.3 External Peer Review Panel Comments -- Inhalation Update

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

In addition, the external peer reviewers commented on decisions and analyses in the *Toxicological Review of 1,4-Dioxane* under multiple charge questions, and these comments were organized and summarized under the most appropriate charge question. In cases where comments were made regarding the oral assessment for 1,4-dioxane, those comments are noted, considered, and changes were made to the oral assessment as appropriate; however this was not intended to be a second peer review of the oral assessment finalized in 2010 (U.S. EPA, 2010a).

A.3.1 General Charge Questions

1. Is the Toxicological Review logical, clear and concise? Has EPA clearly presented and synthesized the scientific evidence for noncancer and cancer health effects from exposure to 1,4-dioxane viainhalation?

Comment: Four reviewers agreed that the Toxicological Review of 1,4-dioxane was logical, clear, and concise. Two reviewers commented that the majority of the Toxicological Review was logical, clear, and concise, but provided several recommendations to improve the document. The specific recommendations included: (1) documentation of literature search terms, (2) description of the severity of the lesions observed by Kasai et al. (2008) should be included in the main body of the text, (3) clarification of the toxicological significance of nuclear enlargement with clear differentiation between study author and EPA's conclusions regarding this endpoint, (4) improvement of Tables 4-27 and 4-28 as they do not readily demonstrate temporal relationships of interest, (5) removal of repetitive text, (6) reduction of unnecessary text in the mode of action analysis, (7) correction of inconsistencies between oral and inhalation approaches to derive the reference values, (8) the addition of information on ambient exposures to 1,4-dioxane, and (9) improve the writing of the text of section 4.6.2 and expand Section 4.6.2.1 to focus on the possibility that the parent compound is the toxic moiety.

Additionally, one reviewer made reference to a public comment noting an error in the PBPK model code in the description of the slowly perfused tissue. This reviewer suggested the code be corrected and provided in the assessment. However, the reviewer did agree with the conclusion that the existing PBPK models are inadequate to perform route-to-route and cross-species extrapolation of animal studies.

Response: (1) Additional information was provided in Section 1.1 regarding the literature search strategy employed for 1,4-dioxane. (2) The severity of the nasal lesions observed by Kasai et al. (2008) was included in Table 4-17; no additional language was added to the text as the data is presented clearly in tabular format. (3) With regards to nuclear enlargement, consultation with an Agency pathologist and a search of published literature did not provide any information concerning the nature, severity, and significance of this observed response. Reports of nuclear enlargement have only been found in JBRC published studies and have not been reported elsewhere in literature. Thus, consideration and selection of this response as a critical endpoint would add unnecessary uncertainty to the assessment of the effects induced by 1,4-dioxane. Clarifying text was added to the document regarding the uncertainty surrounding this reported observation to Sections 4.2.1.1.3, 4.2.1.2.6, 4.2.2.1.2, 4.2.2.2.2, and 5.2.1. (4) Tables 4-27 and 4-28 were described in more depth in their accompanying sections to describe their content and the temporal nature. (5)/(6) The Agency continues to evaluate and incorporate recommendations made by the NAS that should streamline (i.e. reduce redundancy), strengthen and improve transparency within the IRIS documents. (7) There are necessary differences in the derivation of oral and inhalation reference values, discussed in Section 5.4.4, and clarified in Section 5.4.4.2. For instance, the oral slope factor derivation does not use the multistage model, whereas the

inhalation unit risk derivation does. This is due to a lack of a suitable multistage model being identified for the female mouse liver tumor data used to derive the oral slope factor, whereas appropriate multistage model fits were obtained for the tumor data used to derive the inhalation unit risk. This departure resulted in a necessary and significant difference in approaches. (8) While it is important for risk assessors to understand ambient exposure levels in utilization of IRIS reference values, ambient exposure levels are dependent upon location and media and thus are not included in IRIS assessments. In the context of the overall risk assessment paradigm, IRIS documents provide the hazard identification information and the dose-response analysis in support of the derivation of reference values for the chemical of interest. (9) The suggestions made by the reviewer to improve the writing and summaries in 4.6.2 were all incorporated. The mechanism by which 1,4-dioxane induces tissue damage is not known, nor is it known whether the toxic moiety is 1,4-dioxane or a transient or terminal metabolite. As the reviewer notes, and is already stated in the toxicological review, it is possible that the parent compound is the toxic moiety; however, the section was not rewritten with a focus on the parent compound. Regarding the PBPK model, the code errors identified by a public commenter and referenced by a member of the peer review panel were corrected (discussed further in response to public comments, below). Additionally, the model equations have been available in Appendix B of previous version of the toxicological review released. In this final version, however, the model code is not provided in the text, but is available electronically via HERO, along with the executable .m script files (provide HERO link).

2. Please identify any additional peer-reviewed studies from the primary literature that should be considered in the assessment of noncancer and cancer health effects from exposure to 1,4-dioxane via inhalation.

Comment: Four reviewers stated they were unaware of any additional studies available to add to the inhalation toxicity evaluation of 1,4-dioxane. One reviewer provided additional general references pertaining to dose extrapolation for the derivation of the RfC specifically regarding the default values used for the human extrathoracic surface area and minute ventilation. Another reviewer provided some general references related to evaluation of tumors and mode of action, along with a few 1,4-dioxane specific papers. The 1,4-dioxane specific papers suggested for consideration were:

a. Takano, T, Murayama, N, Horiuchi, K, Kitajima, M, Shono, F. (2010). Blood concentrations of 1,4-dioxane in humans after oral administration extrapolated from in vivo rat pharmacokinetics, in vitro human metabolism, and physiologically based pharmacokinetic modeling. *J Health Sci* 56: 557-565. (*Note*: The reviewer noted that this paper is not likely to be useful in the assessment; however, a short summary should be added to the appropriate section in the toxicological review)

b. U.S. Army Public Health Command (2010). Studies on Metabolism of 1,4-Dioxane, Toxicology Report No. 87-08 WR-09, Aberdeen Proving Ground, MD.

c. WHO (World Health Organization). (2005). 1,4-Dioxane in Drinking Water, WHO/SDE/WSH/05.08/120, Geneva.

Response: Reference (a) above was evaluated for the utility of the described PBPK model in predicting toxicokinetics of 1,4-dioxane in rats and humans. A summary of Takano et al. (2010) and an evaluation of the model was added to Section 3.5.2.5. Reference (b) above is a report that has not undergone formal peer-review and thus, is generally not considered in the development of an IRIS assessment. Reference (c) listed is a report produced by an organization other than the U.S. EPA and was considered during development of this assessment; however, the Agency performed an independent analysis of the scientific informa available for 1,4-dioxane and did not cite this document.

The additional general references pertaining to dose extrapolation for the derivation of the RfC specifically regarding the default values used for the human extrathoracic surface area and minute ventilation were related to the inclusion of the alternative RfC calculation in Appendix G. This appendix was removed following external peer review. See response to charge question 4, below, relating to the RfC for more details.

A.3.2 Inhalation reference concentration (RfC) for 1,4-dioxane

1. A 2-year inhalation bioassay in male rats (Kasai et al., 2009) was selected as the basis for the derivation of the RfC. Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the RfC, please identify this study and provide scientific support for this choice.

Comment: Four reviewers agreed that the selection of the 2-year bioassay in male rats (Kasai et al. 2009) as the critical study used for the derivation of the RfC was scientifically justified. Two reviewers also agreed with the aforementioned, but stated that decision not to collect female rat data for the 2-year bioassay was not scientifically supported by the study authors (Kasai et al., 2009), especially given that the 13-week bioassay (Kasai et al., 2008) showed female rats more responsive than male rats following inhalation exposure. More specifically, the two reviewers highlighted that one of the selected critical effects (atrophy of the olfactory epithelium) was observed in female rats and not male rats following 13 weeks of exposure to 1,4-dioxane vapors, thus making the female rat more responsive to 1,4-dioxane following inhalation exposure.

Response: The Agency did not conclude that the available data supports the female rats as definitively more responsive than male rats following 13 weeks of exposure to 1,4-dioxane vapors. BMD analysis of the incidence of olfactory atrophy in female rats from the Kasai et al. (2008) study provides a $BMCL_{10}$ of 65 ppm (fit with the Dichotomous Hill model). Application of a total UF of 1,000 would yield an RfC of 0.065 ppm compared to an RfC of 0.05 ppm calculated from the 2 year bioassay. A review of the pathological observations also does not indicate that females are definitively more responsive to 1,4-dioxane exposure. Of the lesions

noted, most were considered to be of the lowest severity grade. Of these lesions, equivalent responses were observed between males and females and in some cases greater in females and in others greater in males. Thus, information to suggest that females are more responsive than males is currently lacking. Additionally, in accordance with the weight-of-evidence framework described in the *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994a), the selection of the 2-year bioassay in male rats as the critical study is justified. Furthermore, an uncertainty factor of 3 for an incomplete database was applied. This uncertainty factor is intended to account for the inability of any single laboratory animal study to adequately address all possible adverse outcomes in humans. Therefore, in consideration of the data presented in each of the studies as well as the difference in the study durations (13 vs. 104 wks), the selection of the 2-year bioassay in male rats as the critical study is justified.

2. Atrophy and respiratory metaplasia of the olfactory epithelium in male rats were concluded by EPA to be adverse effects and were selected as co-critical effects for the derivation of the RfC. Please comment on whether the selection of these co-critical effects and their characterization is scientifically supported and clearly described. If a different health endpoint is recommended as the critical effect for deriving the RfC, please identify this effect and provide scientific support for this choice.

Comment: Four reviewers agreed with the selection of co-critical effects in the derivation of the RfC and stated that the selection was scientifically supported and clearly described. The remaining two reviewers also agreed with the selection of co-critical effects in the derivations of the RfC; however, they provided suggestions on how to strengthen the justification for EPA's decision or improve clarity. These reviewers suggested EPA (1) provide further justification for why nuclear enlargement was not considered as a critical effect and (2) clearly state the criteria for selection of the critical effect. One reviewer also noted inconsistency between the oral and inhalation assessments regarding the consideration of spongiosis hepatis as a nonneoplastic lesion and potential critical effect.

Response: In response to reviewer comments, EPA further investigated nuclear enlargement. As stated in response to inhalation assessment general charge question 1 (Section A.3.1), a search of the literature demonstrated a lack of clear information concerning the nature, severity, and significance of nuclear enlargement. Thus, the etiology of nuclear enlargement or its downstream effects have not been elucidated. Therefore, consideration and selection of this response as a critical endpoint would not be supported by the available scientific information. Clarifying text was added to the document regarding nuclear enlargement as noted in response to charge question A1, and specifically in Section 5.2.1 as to why it was not considered as a critical effect.

Additional clarifying text was added to Section 5.2.3 regarding the use of respiratory metaplasia and atrophy of the olfactory epithelium as co-critical effects, noting that they were the most sensitive effects considered following inhalation of exposure to 1,4-dioxane. EPA agrees there was inconsistency in way spongiosis hepatis was considered between the oral and inhalation

assessments. Spongiosis hepatis was removed from the list of candidate critical effects in the inhalation assessment. However, whether spongiosis hepatis/cystic degeneration represents a preneoplastic change or a nonneoplastic change has been the subject of scientific controversy (Karbe and Kerlin, 2002b; Stroebel et al., 1995; Bannasch et al., 1982b). Spongiosis hepatis is commonly seen in aging rats, but has been shown to increase in incidence following exposure to hepatocarcinogens. Spongiosis hepatis can be seen in combination with preneoplastic foci in the liver or with hepatocellular adenoma or carcinoma and has been considered a preneoplastic lesion (Bannasch, 2003; Stroebel et al., 1995). In contrast, it can also be associated with hepatocellular hypertrophy and liver toxicity and has been regarded as a secondary effect of some liver carcinogens (Karbe and Kerlin, 2002a). Following inhalation of 1,4-dioxane, spongiosis hepatis was associated with other preneoplastic (e.g., liver foci) and nonneoplastic (e.g., centrilobular necrosis) changes in the liver (Kasai et al., 2009). Additionally, the incidence rates of spongiosis hepatis and liver tumors were highly correlated; therefore, spongiosis hepatis was considered a preneoplastic lesion following inhalation exposure and not considered further in the noncancer analysis. This justification was added to the document in Section 5.2.1.

3. Benchmark dose (BMD) modeling methodology (U.S. EPA, 2000d) was used to analyze the candidate endpoints identified for 1,4-dioxane. However, due to poor fit or substantial model uncertainty, BMD model results were inadequate for the following nasal lesions: atrophy (olfactory epithelium), respiratory metaplasia (olfactory epithelium), and sclerosis (lamina propria). Consequently, the NOAEL/LOAEL approach was used to identify the POD for derivation of the RfC. Please comment on whether this approach is scientifically supported and clearly described.

   Comment: Six reviewers agreed that the use of the NOAEL/LOAEL approach in the derivation of the RfC is scientifically supported and clearly described.

   Response: EPA agrees with the reviewers regarding the use of the NOAEL/LOAEL approach in the derivation of the RfC, no changes were made to the document.

4. The human equivalent concentration (HEC) for 1,4-dioxane was calculated by the application of the dosimetric adjustment factor (DAF) for systemic acting gases (i.e. Category 3 gases), in accordance with the U.S. EPA RfC methodology (U.S. EPA, 1994b). This conclusion was based upon a number of factors, including the low reactivity of 1,4-dioxane, and the occurrence of systemic effects following oral and inhalation exposure to 1,4-dioxane. However, since 1.4-dioxane is water soluble and induces effects in portal-of-entry tissues, an alternative calculation of the HEC for 1,4-dioxnae based on the application of the corresponding DAF for the portal-of entry acting gases (i.e., Categrory 1) is provided in Appendix G. Please comment on EPA's conclusion that 1.4-dioxane is a Category 3 gas, and the resulting application of the corresponding dosimetric adjustment factor (DAF) in deriving the RfC. If a different approach is recommended in the derivation of the RfC, please identify this approach and provide scientific support for the proposed changes.

   Comment: All of the reviewers thought the approach used in the main body of the document was reasonable and consistent with the Agency's current definitions and approaches, as well as the

effects observed. Two reviewers thought the inclusions of an alternative approach in Appendix G was reasonable. Two other reviewers noted problems with the outcome of the default calculation used in the alternative approach. Two reviewers thought the lesions seen in the inhalation study may represent portal-of-entry responses; one of these reviewers thought additional text should be added to the document.

Response: Since the reviewers were in agreement with the extrapolation approach employed and described in the main body of the document, Appendix G in the external peer review draft that demonstrated the application of the Agency's default method for deriving an RfC for category 1 gases was removed. The alternative approach used default ratios of ventilation rate and surface areas cited and often used in accordance with the Agency's RfC Methods (U.S. EPA, 1994b), which are also supported by several sources including ICRP (2002), Guilmette et al. (1997), and Liu et al. (2009).

5. The text corresponding to the dosimetric extrapolation approach applied for 1,4-dixoane has been revised for clarity and transparency; however, no changes to the quantitative approach were made. EPA agrees that 1,4-dioxane induces portal of entry effects. 1,4-Dioxane is miscible with water and has a high blood:air partition coefficient. Unlike typical highly water soluble and reactive portal-of-entry acting gases, 1,4-dioxane also induces lower respiratory tract and systemic effects and has been measured in the blood after inhalation exposure. Thus, it is difficult to determine what contribution circulating 1,4-doxane makes to the portal-of-entry effects observed. Therefore, for the purposes of dosimetric extrapolation, 1,4-dioxane was treated as a systemic acting gas and a DAF of 1 was applied. In addition, a robust CFD and PBPK modeling database supports the scientific rationale to apply of DAF of 1 for both portal of entry and systemic effects irrespective of "gas categorization" (U.S. EPA, 2012b). Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. Are the UFs appropriate based on *A Review of the Reference Dose and Reference Concentration Processes* ((U.S. EPA, 2002c); Section 4.4.5; www.epa.gov/iris/backgrd.html) and clearly described? If changes to the selected UFs are proposed, please identify and provide scientific support for the proposed changes.

Comment: Four reviewers agreed with the selection and justification of the UFs applied to the POD for the derivation of the RfC. One of these reviewers, however, suggested that it be noted that the reproductive toxicity and teratogenicity indices monitored in rats by Giavini et al. (1985b) were unremarkable. Two reviewers agreed with the selection of the UFs but requested clarification of the justification for the database uncertainty factor. One reviewer further questioned the reliability of the UF of 10 to extrapolate to a NOAEL given the lack of an exposure group below 50 ppm where one of the critical effects was noted with an incidence rate of 80% (olfactory epithelium), and the lack of female rats exposure in the 2 year bioassay despite evidence of increased responsiveness to 1,4-dioxane vapors following inhalation as compared to the male rat in a 13 week bioassay. Additionally, one reviewer debated the application of the UF of 10 for individual differences among human subjects given that dosimetric differences for particles among human subjects is often 1.3 rather than 3.

Response: In accordance with U.S. EPA (2002c), the database was characterized and applied to the derivation of the RfC. The EPA has added clarification to Section 5.2.4 regarding the strengths and limitations of the data which support an uncertainty factor of 3 for the database. Giavini et al. (1985b) administered 1,4-dioxane by gavage in water to pregnant rats. The authors found statistically significant changes in fetal body weight at the highest dose group and reduced ossification of the sternebrae; however, the lack of a multigenerational reproductive study warrants the use of a 3 for UF_D. As outlined in detail in response to the inhalation assessment charge question B1, the available data do not support female rats as definitively more responsive than male rats following 13 weeks of exposure to 1,4-dioxane vapors. A recent modeling study by Valcke and Krishnan (2011) assessed the impact of exposure duration and concentration on the human kinetic adjustment factor and estimated the neonate vs. adult 1,4-dioxane blood concentration ratio to be 3.2.

A.3.3 Carcinogenicity of 1,4-dioxane and derivation of an inhalation unit risk

1. Under EPA's *Guidelines for Carcinogen Risk Assessment* ((U.S. EPA, 2005b); Section 2.5; www.epa.gov/iris/backgrd.html), the draft IRIS assessment characterizes 1,4-dioxane as "likely to be carcinogenic to humans" by all routes of exposure. Please comment on whether this characterization of the human cancer potential of 1,4-dioxane is scientifically supported and clearly described.

Comment: Five out of six reviewers agreed with the characterization that 1,4-dioxane is "likely to be carcinogenic to humans." However, one of these reviewers suggested a more transparent application of the criteria to the inhalation cancer data to classify the compound as "likely" would be beneficial. One reviewer disagreed with the cancer classification of "likely to be carcinogenic to humans" and suggested that it should be classified as a "possible human carcinogen". This reviewer provided several arguments as a basis for a different classification: 1) no evidence of increased cancer incidence in humans exposed to 1,4-dioxane in the limited number of epidemiology studies, 2) negative in vivo and in vitro genotoxicity experiments suggesting that 1,4-dioxane is, at most, a weak genotoxicant, 3) data demonstrating observed tumors in rodents occur following high chronic exposures, 4) the parent compound is the proximate irritant, cytotoxicant, and carcinogenic moiety, and 5) conclusions and classifications by other organizations (i.e., German Commission for the Health Hazards of Chemical Compounds in the Work Area, ACGIH, IARC and WHO).

Response: Five of the six reviewers agreed with the characterization of "likely to be carcinogenic to humans" and no change was made to this conclusion in the final Toxicological Review. With respect to the one reviewer who suggested applying the criteria more transparently to the inhalation data alone; when considering the characterization of the carcinogenic potential for a compound, the available data across all exposure routes is first considered. If , for example, portal of entry effects are observed for one route of exposure and not the other, or there is evidence that a chemical is not absorbed from a particular route of exposure, then separate cancer

descriptors may be used to describe the cancer potential. In the case of 1,4-dioxane, the tumors that were observed in animals were systemic and independent of the route of exposure.

The one reviewer that disagreed with the classification provided a suggested classification that appears to be based on earlier 1986 U.S. EPA cancer classification terminology. As summarized in Section 4.7.1, the available human studies with small cohorts and limited number of reported cases are inconclusive. The Agency agrees with the reviewer that the majority of the genotoxicity studies are negative, suggesting 1,4-dioxane is not genotoxic (Section 4.5.1), and that tumors have been observed in rodents following chronic exposure (summarized in Section 4.7.2). A lack of data to determine the toxic moiety (e.g., parent compound, intermediate, or terminal metabolite), does not impact the Agency's cancer classification.

2. The draft assessment concludes that there is insufficient information to identify the mode(s) of carcinogenic action for 1,4-dioxane. Please comment on whether this determination is appropriate and clearly described. If it is judged that a mode of action can be established for 1,4-dioxane, please identify the mode of action and its scientific support (i.e., studies that support the key events, and specific data available to inform the shape of the exposure-response curve at low doses).

Comment: Five out of six reviewers agreed with EPA's conclusion that there is insufficient scientific information to establish the mode(s) of carcinogenic action for 1,4-dioxane. However, one of these reviewers suggested integrating the sequence of events for a possible mode of action described in a public comment into the body of the Toxicological Review. Another one of these five reviewers provided several examples of places in the toxicological review that could use clarification of study limitations and consideration of pertinent data: impact of 1,4-dioxane volatility on in vitro and skin/paint study results; mechanistic section needs more discussion and analysis of a potential genotoxic mode of action; critical deficiencies in the database should be noted in the discussion of cytotoxicity/cell proliferation mode of action; examine dose-response relationships for effects seen in the 13-week studies and how they may predict tumor incidence; the lack of mouse liver initiation-promotion studies should be noted; and data do not support statements regarding metabolic saturation and subsequent toxicity. One of the six reviewers disagreed with EPA's conclusion that there is insufficient information to identify a MOA for 1,4-dioxane. This reviewer commented that data clearly support a cytotoxicity/inflammation/ regenerative hyperplasia MOA with a dose threshold, citing the Kociba et al. (1974b), Kano et al. (2008), and Kasai et al. (2009; 2008) studies.

Response: The Agency agrees with five of the six reviewers that there is insufficient evidence to establish a carcinogenic MOA for 1,4-dioxane. As seen in responses to the public comments regarding the carcinogenicity of 1,4-dioxane (Section A.4.2.), the sequence of events proposed by the public commenter are not supported by the available data. These key events for the hypothesized MOA are visualized in Figure 4-1 of the Toxicological Review.
The available data do not clearly support a cytotoxic/inflammation/regenerative hyperplasia MOA (Section 4.7.3). Specifically, the studies referenced by the reviewer (Kasai et al., 2009; Kano et al., 2008; 2008; Kociba et al., 1974b) do not examine cytotoxicity or regenerative cell

proliferation in the nasal cavity. Further, the existing data examine a small number of exposures and timepoints. Kasai et al. (2009) suggests either genotoxic or cytotoxic MOA for 1,4-dioxane, but their data do not provide sufficient evidence to conclude one way or the other. Furthermore, there is no evidence of cytotoxicity in the nasal cavity in the Kasai et al. (2009; 2008) studies. Additionally, evidence of cytotoxicity in one tissue type, does not dictate that cytotoxicity will be present in all tissues at the same dose. Thus, the database does not provide evidence for each stage of a regenerative hyperplasia MOA.

A number of changes were made as a result of the specific comments made regarding clarity and study limitations. Regarding the volatility of 1,4-dioxane and reliability of the negative in vitro studies and skin paint studies, text was added to Section 4.5.1 noting the four negative in vitro studies that reported using closed systems and to Section 4.2.3 regarding the reliability of the data from unoccluded versus occluded skin paint initiation/promotion studies. Text was revised in Section 4.5.1 to state clearly that half of the studies showed 1,4-dioxane was not genotoxic; however, data are not sufficient to support a genotoxic MOA and no additional discussion regarding this MOA was added to the document. Text was added to Section 4.7.3 noting deficiencies in the database surrounding a cytotoxicity/cell proliferation MOA. As a result of the peer review comment, the noncancer effects were reexamined in detail and how they may relate to the cancer effects seen. An attempt was made to create new tables showing the noncancer and cancer effects across the dose and time; however, these tables were found to introduce more confusion. Therefore, only clarifying text was added (Sections 4.7.1, 4.7.3.1.2, and 4.7.3.3) regarding the noncancer effects and their relation to the cancer effects and the temporal sequence of events, as well as clarifying the. In response to another comment from the reviewer, a statement was added to Section 4.7.3.1.1 to clearly state that no studies have been conducted to specifically examine the mouse liver, thus precluding any determination on whether 1,4-dioxane acts as a tumor promoter in the mouse liver. A thorough review of statements in the document pertaining to metabolic saturation and its relation to toxicity was performed in response to the reviewers comment. Several changes were made throughout the document (e.g., Section 3.5.1, 4.6.2.1, and 4.7.3.7.1) clarifying relationships observed (or not) between metabolic saturation and toxicity. In general metabolic saturation was observed in single dose studies (Young et al., 1978b; 1978a). We agree with the reviewer that a single dose study does not provide adequate information to support metabolic saturation following repeated long-term exposures, and that since 1,4-dioxane induces P450 enzymes it is likely to enhance metabolic elimination in long term exposure scenarios. Additional kinetic information is needed to determine if metabolic saturation is a precursor to a toxic effect. Kociba et al. (Kociba et al., 1975b) that stated toxicity was only observed after metabolism was saturated did not present data for repeated doses to support this conclusion.

3. A two-year inhalation cancer bioassay in male rats (Kasai et al., 2009) was selected as the basis for the derivation of the inhalation unit risk (IUR). Please comment on whether the selection of this study is scientifically supported and clearly described. If a different study is recommended as the basis for the IUR, please indentify this study and provide scientific support for this choice.

Comment: Five of the six reviewers agreed that the use of the two year inhalation cancer bioassay in male rats Kasai et al. (2009) is the most appropriate study to use for the derivation of the IUR. Five of the six reviewers also stated the selection was clearly described and justified or supported within the toxicological review. The other reviewer neither disagreed or agreed with the selection of the study; however, the reviewer noted that the Kasai et al. (2009) study is the only comprehensive inhalation study available for this chemical, because the other study by Torkelson et al. (1974a) used only one dose and did not perform histology on the nasal tissues.

Response: No dissenting opinions or comments warranting additional justification were provided by the external review panel regarding selection of the principal study for derivation of the IUR. Thus, no changes were made to the assessment related to the selection and justification of the Kasai et al. (2009) study for derivation of the IUR.

4. The incidence of hepatocellular adenomas and carcinomas, nasal cavity squamous cell carcinoma, renal cell carcinoma, peritoneal mesothelioma, mammary gland fibroadenoma, Zymbal gland adenoma, and subcutis fibroma were selected to serve as the basis for the derivation of the IUR. Please comment on whether this selection is scientifically supported and clearly described. If a different health endpoint is recommended for deriving the IUR, please identify this endpoint and provide scientific support for this choice.

Comment: Five of the six reviewers agreed with EPA's choice to combine these tumor types for derivation of the IUR, noting the statistically significant tumor incidence rates and the dose related increase in tumors. One of the five reviewers that agreed with the approach questioned if data are available to fully justify the pooling of certain tumor types. One of these five reviewers noted that the mice were more sensitive than rats to the hepatocarcinogenic effects of 1,4-dioxane following drinking water exposure. Thus, since mice were not included in a 2-year inhalation cancer bioassay, the IUR may be underestimated and this should be noted as a source of uncertainty qualitatively and a quantitatively. This reviewer suggested a quantitative adjustment to the IUR by multiplying the IUR by the ratio of hepatocellular neoplasms in male rats: female mice from the oral study. The sixth reviewer disagreed with combining all of these tumor types, arguing that Zymbal gland tumors are limited to male rats; and peritoneal mesothelioma, subcutis fibroma, and mammary fibroadenoma are typical spontaneous tumors in F344 rats (Haseman et al., 1998; Hall, 1990).

Response: In agreement with five of the six reviewers, the Agency retained the combination of the tumor types with statistically significant incidence rates different from control or a statistically determined dose-related trend in the combined tumor analysis for the derivation of the IUR. Data were not available to establish whether the tumor types were biologically dependent, thus independence was assumed and is not expected to produce substantial error in the risk estimates (!!! INVALID CITATION !!!). It is acknowledged that Zymbal gland tumors do not occur in humans due to the lack of a Zymbal gland; however, site concordance is not always assumed for animals and humans (U.S. EPA, 2005a) because events leading to Zymbal gland tumors may occur at other sites in humans. Additional text was added to section 5.5.1.6 and

6.2.3.8 to address the possible underestimation of the carcinogenic inhalation potential of 1,4-dioxane since female mice were the most sensitive following oral administration and were not included in the 2-year inhalation cancer bioassay. While the uncertainties were noted qualitatively, a quantitative adjustment was not performed on the IUR as this is not a standard approach conducted by the agency. The sixth reviewer raised objections to using peritoneal mesothelioma, subcutis fibroma, and mammary fibroadenoma as the reviewer characterized them as "very commonly observed, spontaneous tumors in control F344 rats." The study authors used untreated, clean air exposed rats as an experimental control to account for any possible spontaneous tumors that may arise. Furthermore, the Agency accounts for the background rate in controls when using the multistage cancer model.

5. The IUR was derived based on multiple carcinogenic effects observed in rats exposed to 1,4-dioxane via inhalation. A Bayesian approach was used to estimate a $BMDL_{10}$ associated with the occurrence of these multiple tumors, and then a linear low-dose extrapolation from this POD was performed to derive the IUR. Additionally, for comparative purposes only, a total tumor analysis was performed with the draft BMDS (version 2.2Beta) MSCombo model that yielded similar results (See Appendix H). Please comment on whether these approaches for deriving the IUR have been clearly described and appropriately conducted?

Comment: Two reviewers commented that the approaches were clearly described and appropriately conducted; however, the methods to quantitate cancer risk are outside of their areas of expertise. Four of the reviewers commented that both methods, Bayesian and BMDS, are clearly described and appear appropriately conducted since both methods yielded similar results. However, one of these four reviewers noted that additional information to reproduce the Bayesian analysis should be provided. Another of these four reviewers noted that IUR estimates may actually be larger since survival was significantly reduced in the high exposure group and that the cancer dose-response modeling did not use survival adjusted data. One reviewer commented that the limitations and assumptions related to the risk of developing any combination of the tumor types is not well documented in the toxicological review. Additionally, one reviewer noted that the total tumor approach was not utilized in the derivation of the oral CSF and recommended a total tumor analysis for male and female rats exposed to 1,4-dioxane in drinking water. One reviewer did not support the Agency's default use of Haber's Law to make adjustments for the exposure duration in the derivation of the IUR (or RfC). This reviewer suggested additional examination of the 1,4-dioxane data to gain insights into α and β, if possible to further describe uncertainties associated with this duration adjustment.

Response: Overall, the reviewers were in support of the quantitative approaches to the multitumor analysis for the derivation of the IUR. As a result of the public comments regarding the documentation and reproducibility of the Bayesian WinBUGS approach, and the fact that the BMDS MS_Combo model has completed peer review since the draft of this assessment was released, the transparent, reproducible MS_Combo approach is now considered the primary approach for derivation of the IUR and the Bayesian WinBUGS approach is a supporting analysis with details in Appendix G (formerly Appendix H). Additional details on the WinBUGS analysis

was added to the appendix and the model code was made available via HERO (EPA, 2013). Using MS_Combo approach as the primary approach did not result in any quantitative changes to the IUR.

As stated in response to general charge question 1, similar methods to analyze the total tumor risk were not available at the time of the completion of the oral assessment. Additionally, the multistage model was not the best fitting model for female mouse liver tumors and was not used in derivation of the oral slope factor, whereas the inhalation unit risk derivation does utilize the multistage model. However, in response to the reviewer's comment, the male and female rat data were analyzed using the BMDS MS_Combo model. $BMDL_{HEC}$ values for male rat and female rat combined tumors were determined to be 7.59 and 11.26 mg/kg-day, respectively. Using a BMR of 0.1 oral CSFs of 0.013 and 0.0088 $(mg/kg\text{-}day)^{-1}$ were calculated for the male and female rat data, respectively. Thus the combined tumor analysis for the oral assessment does not impact the selection of the gender/species or overall oral CSF for 1,4-dioxane.The Agency concurs with the reviewer who states that the IUR estimates may actually be larger if survival adjusted data were used and this was noted in Section 5.5.1.6. However, day of death data were not available in the Kasai (2009) study, thus this analysis cannot be performed.

Data are not available to move away from the default value of 1 for α and β in the C x T duration adjustment approach for inhalation exposure. Two, 13-week subchronic studies in laboratory animals (Kasai et al., 2008; Fairley et al., 1934a) and two, 2-year chronic studies in rats (Kasai et al., 2009; Torkelson et al., 1974a) were identified; however, these data did not report the severity of the lesions for multiple timepoints.

A.4 Public Comments – Inhalation Update

The *Toxicological Review of 1,4-Dioxane (with Inhalation Update)* was released for a 60-day public comment period in September 2011. A listening session was scheduled in October 2011; however, no participants registered to speak, so the listening session was cancelled. EPA received written public comments on the draft assessment from Toxicology Excellence for Risk Assessment (TERA) and joint comments from the National Association of Manufacturers (NAM) the Aerospace Industries Association (AIA) provided by ARCADIS. The major comments received have been synthesized and paraphrased below. EPA's responses to the comments and information regarding how the assessment has been revised, where applicable, are included.

A.4.1 Inhalation reference concentration (RfC) for 1,4-dioxane

Comment: The use of 3 for the database uncertainty factor (UF_D) based on the lack of a multigenerational reproductive study is not warranted. Statistically significant changes in fetal weight and ossified sternebrae reported by Giavini et al. (1985b) are not toxicologically

significant. No effects were seen on reproductive organs in the oral or inhalation subchronic and chronic studies (Kano et al., 2009; Kasai et al., 2009; Kano et al., 2008; Kasai et al., 2008; NCI, 1978; Kociba et al., 1974b; Torkelson et al., 1974b). For these reasons the UF_D should be reconsidered in the derivation of the RfC.

Response: Giavini et al. (1985b) administered 1,4-dioxane by gavage in water to pregnant rats. The authors found statistically significant changes in fetal body weight at the highest dose group and reduced ossification of the sternebrae. The other studies were not designed to examine reproductive or developmental outcomes, and thus cannot be used to infer the reproductive/developmental toxicity of 1,4-dioxane. While Torkelson et al. (1974b) did examine the testes and uterus for gross histopathological changes (e.g., tumor) and did not find increased incidence of tumors, this does not indicate that 1,4-dioxane may not be a developmental toxicant. The study of reproductive organs in subchronic and chronic studies is not a replacement for a multigeneration reproductive/developmental study. A UF_D of 3 was used for the oral assessment and was retained for the inhalation assessment.

A.4.2 Carcinogenicity of 1,4-dioxane

Comment: Low dose linearity should not have been assumed to derive the proposed IUR since sufficient data exist to support a cytotoxic-proliferative mode of action (MOA) based generally on the following arguments: 1,4-dioxane is neither mutagenic nor an initiator, but it can act as a promoter, "literature indicates that 1,4-dioxane is a weak genotoxic carcinogen", Kasai et al. (2009) characterized the MOA as "cytotoxic-proliferative". Additionally, the Agency's statement that there is insufficient evidence to support any hypothesized MOA is not supported by the "open literature and the data summarized and interpreted in the draft TR". Histopathology results for the nasal cavity/olfactory epithelium, liver, and kidney from Kasai et al. (2009) clearly indicate that cytotoxicity precedes tumor development.

Response: The Kasai et al. (2009) study does not provide evidence of cytotoxicity in the nasal cavity. Kasai et al. (2009) suggest either a genotoxic or cytotoxic MOA for 1,4-dioxane, but their data do not provide sufficient evidence for one hypothesis over the other. There is no evidence of cytotoxicity in the Kasai et al. (2009; 2008) study. For instance, inflammation by itself is not direct evidence of cytotoxicity. For the liver and kidney, Kasai et al. (2009) provide direct evidence of cytotoxicity including clinical pathology (liver) and histopathology (liver and kidney) data. Additionally, evidence of cytotoxicity in one tissue type, does not dictate that cytotoxicity will be present in all tissues at the same dose.

Due to a lack of information to inform the MOA, the Agency used the default linear extrapolation approach per the EPA *Guidelines for Carcinogen Risk Assessment* (2005b). Specifically, the Guidelines state that "nonlinear approaches generally should not be used in cases where the mode of action has not been ascertained" and that linear extrapolation will be used as the default in these cases.

It is important to note that five of the six members on the independent expert peer review panel for this draft assessment agreed with EPA's conclusions regarding the weight of evidence in support of a linear approach to derive the IUR, and all reviewers, including the public commenters, supported EPA's decision to use the Kasai et al. (2009) study as the basis for determining the IUR.

Comment: 1,4-Dioxane dose not cause mutagenicity, initiation, or DNA repair. 1,4-Dioxane dose cause promotion and DNA replication. Occurrence of respiratory tumors in rodents may be caused by 1,4-dioxane exceeding the metabolic capacity of the tissue. 1,4-Dioxane does cause liver tumors and liver toxicity precedes tumors in time in both sexes of rats and mice, and precedes tumors in dose in both sexes of rats. Liver toxicity indicated by biochemical measures does occur at similar tumorigenic doses in mice; however histopathological indication of liver toxicity does not appear to precede tumors in either sex of mice. EPA needs to show the liver hyperplasia noted in Kano et al. (2009) in Appendix E of the draft toxicological review. 1,4-Dioxane does cause dose-dependent nasal toxicity as indicated in the histological analyses at all time points in both sexes of rats and mice and this toxicity precedes tumors in time and dose. It is hypothesized that 1,4-dioxane causes liver and nasal tumors in rats and mice through a regenerative hyperplasia MOA, which demonstrates a threshold. The applicability of this MOA to other tumor types is unknown, so a separate, default linear extrapolation may be appropriate for those tumor types.

Response: The Agency's determination that the MOA has not been established is supported by five of the six external peer reviewers. The samples associated with liver hyperplasia for rats and mice in Yamazaki et al. (1994a) and JBRC (1998) were re-examined according to updated criteria for liver lesions and were afterwards classified as either hepatocellular adenoma or altered hepatocellular foci in Kano et al. (2009), therefore there are no liver hyperplasia incidence data from Kano et al. (2009) to report in Appendix E as the commenter suggests.

Due to a lack of information to substantiate the MOA, the Agency used the default linear extrapolation approach per the EPA *Guidelines for Carcinogen Risk Assessment* (2005b). Specifically, the Guidelines state that "nonlinear approaches generally should not be used in cases where the mode of action has not been ascertained" and that linear extrapolation will be used as the default in these cases.

Comment: Peritoneal mesotheliomas found in male rats, but not female counterparts, is likely due to the occurrence of tunica vaginalis mesotheliomas in male rats. Rats are much more sensitive to developing mesotheliomas from the tunica vaginalis than humans.

Response: The etiology and origin of the peritoneal mesotheliomas reported in Kano et al. (2009) and Kasai et al. (2009) are unknown. The commenter indicated a range of considerations including human sensitivity and / or relevance for the peritoneal mesotheliomas observed in male rats(Kano et al., 2009; Kasai et al., 2009). The EPA *Guidelines for Carcinogen Risk Assessment* (2005b) state that all tumor types are to be analyzed in a dose-response assessment followed by a synthesis that considers, among other things, human relevance of each tumor type. In the absence

of scientific information to evaluate the human relevance of peritoneal mesotheliomas observed in male rats exposed to 1,4-dioxane EPA is required to implement the approaches from the guidance (U.S. EPA, 2005b). EPA concluded there continues to be uncertainty as to the etiology, origin, and species sensitivity of the peritoneal mesotheliomas found in the rats, and the tumor is relevant to humans and evaluated in the cancer assessment.

Comment: EPA should document a complete MOA evaluation for each relevant tumor type by including a discussion on what is known about the key events in each tissue.

Response: MOA information available for tumors associated with exposure to 1,4-dioxane was evaluated in the Toxicological Review (Section 4.7.3). The MOA by which 1,4-dioxane produces liver, nasal, kidney, peritoneal (mesotheliomas), mammary gland, Zymbal gland, and subcutis tumors is unknown, and the available data do not support any hypothesized mode of carcinogenic action for 1,4-dioxane. Available data also do not identify whether 1,4-dioxane or one of its metabolites is responsible for the observed effects. Thus, it is not possible to document a complete MOA in any tissue. This conclusion is supported by five of the six external reviewers.

Comment: The parameters necessary to reproduce the total tumor analysis using the Bayesian method (WinBUGS) are not provided; the analysis is poorly documented; and the rationale for application of the analysis is incomplete.

Response: The BMDS (version 2.2Beta) MS_Combo approach for total tumor analysis that was also included in support of the WinBUGS approach in the draft toxicological review, is now highlighted as the main approach in the body of the document. The MS_Combo approach uses the U.S. EPA's Benchmark Dose Software and is a transparent, reproducible approach that provided similar to the output from the complex WinBUGS analysis. The WinBUGS analysis is still included in this toxicological review as a supporting analysis in Appendix G. Additional details on the WinBUGS analysis was included in Appendix G and the model code made available via HERO (EPA, 2013). Using MS_Combo approach as the primary approach did not result in any quantitative changes to the IUR.

Comment: The requirements for scientific data to support a MOA appear too stringent. EPA should provide guidance on what would be considered sufficient scientific evidence to determine a MOA.

Response: It is not feasible to describe the exact data that would be necessary to conclude that a particular MOA was operating to induce the tumors observed following 1,4-dioxane exposure. The data would fit the criteria described in the U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a).

Comment: The attribution of some tumor types to exposure to 1,4-dioxane is questionable based on statistics, including subcutis fibromas and Zymbal Gland adenomas. There is also uncertainty surrounding the origin of the tumors reported in the Kasai et al. (2009) study (e.g., may be the result of metastatic deposition), and hence the assumption of biological independence among the

tumor types included in the total tumor analysis is not supported. Thus, the pooling of tumor types for derivation of the IUR in the draft TR leads to overestimation of the actual carcinogenicity, and only tumor types with statistically significant differences in incidence rate compared to control animals should be used. Additionally, the highest dose used in the Kasai et al. (2009) study exceeds the maximum tolerated dose (MTD) and should be excluded from the dose-response analysis to derive the IUR.

Response: The commenter suggested that Zymbal Gland adenomas should not be considered related to 1,4-dioxane exposure because the incidence rate at the highest dose group was not statistically different from control; however, the Peto test did find a statistically significant increasing trend. Tumor types were included in the analysis if they showed a statistical difference from control or a statistically significant trend was evident. Zymbal Gland adenomas were included in the analysis because the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) do not require site concordance and a statistically significant dose-response trend was observed for these tumors. Similarly, subcutis fibromas were included in the total tumor analysis because a statistically significant difference was seen in the mid dose group. The rationale for inclusion of tumors in the multitumor analysis is described in Section 5.4.4.2. Additional scientific information would be required to evaluate the hypothesis that the tumors "may be the result of metastatic deposition."

The Kasai et al. (2008) study demonstrates that the high dose used in determining the IUR is below the MTD for 1,4-dioxane. Kasai et al. (2008) state that the MTD is likely higher than the 111 ppm reported by Torkelson et al. The 3,200 ppm high dose for 13wks in Kasai et al. (2008) is higher than the 1,250 ppm dose used in Kasai et al. (2009), and no overt toxicity was reported at the 3,200 ppm.

A.4.3 PBPK modeling

***Comment*:** PBPK models of sufficient quality are available and should have been used to reduce uncertainty in both the oral and inhalation assessments. Technical errors were identified in the PBPK analysis that should be addressed and the use of the models should be reevaluated for both the oral and inhalation assessment.

Response: The model code errors noted in the public comments were addressed as noted below; however, the changes did not significantly impact model predictions nor the overall decision on model use in the assessment.

Comment: The permeation constant to describe the slowly perfused (diffusion-limited) tissue compartment was improperly used in the PBPK model.

Response: If one assumes that the exiting venous concentration is at equilibrium with the tissue, then the diffusion-limited tissue mass balance could be described as was shown in the model code. It does slowly transport in/out of the tissue while having the property that the tissue moves

toward equilibrium with the blood, so it is empirically correct, though it is acknowledged that this was not the most common way to code this compartment. Therefore, to be up-to-date with current modeling practices, the blood flow to the slowly perfused tissues (QS) was used instead of the diffusion limited constant (SPDC) change was made to the model code; however, this had very minimal quantitative impact on model output. Additionally, the fraction of fat and slowly perfused tissue compartments was updated to be more similar to the values used in the values used in the published models (see Table B-1).

Comment: The metabolism of 1,4-dioxane in misused a zero order rate constant in the equation.

Response: The metabolic constant was correctly used in the model code as a first order rate constant; however, it was incorrectly described in the text and code comments as zero-order. The description of the rate constant was corrected in the text and the model code to be clear it is a first-order rate constant.

Comment: The model description for the urinary excretion of HEAA is not adjusted to the ratio of the molecular weights, thus under predicting the concentration of HEAA in urine.

Response: The reviewer is correct that the molecular weight was not accounted for, and since the model mass units are in milligrams, the urinary excretion was corrected to account for the mass conversion to HEAA. The corrected model predicts the human urinary HEAA early time points well and over predicts the latter time points (694 mg vs. 621 mg) – See Appendix B. Following all updates to the model, metabolic parameters were re-optimized and the plots and predictions updated in Appendix B. These changes improved the model fits, but the model predictions of blood 1,4-dioxane were still 4- to 7-fold lower than the data.

Comment: Complete model code (including all .m and .csl files) should be included for the public and reviewers to use. It should be clear what model code was used to generate each figure in the appendix.

Response: New practice within NCEA for transparency is to make the model code accessible via the Health and Environmental Research Online (HERO) database. The model code is now available via the online database and has been removed from the appendix (EPA, 2013).

Comment: Although the Young et al. (1977b) paper does have value in the model development process, there are issues with the study design and exposure estimation, so it should not be used to dismiss the use of the PBPK model for the assessment.

Response: In the absence of evidence to the contrary, the Agency cannot discount the human blood kinetic data published by Young et al. (1977a). As the commenter noted, the liquids likely absorbed some 1,4-dioxane; however, if the volume of air they extract is much less than the volume inhaled by a subject in an hour, then they won't contribute much to the overall absorption. Thus, this reason presented by the commenter is not sufficient for the Agency to discount the data for model validation.

A.4.4 Other comments

Comment: There are other relevant data that are missing from this assessment. Reports that should be referenced include: Takano et al. (2010) *J Health Sci* 56(5): 557-565 and Department of the Army (2010) Toxicology Report No., 87-XE-08WR-09, Studies on Metabolism of 1,4-dioxane.

Response: These same references were mentioned by a member of the independent external peer review panel – refer to the response to the inhalation assessment update general charge question #2 above. Briefly, Takano et al. (2010) was evaluated and added to the assessment in Section 3.5.2.5. The Army study is a report that has not undergone formal peer-review and thus, is generally not considered in the development of an IRIS assessment.

APPENDIX B. EVALUATION OF EXISTING PHARMACOKINETIC MODELS FOR 1,4-DIOXANE

B.1 Background

Several pharmacokinetic models have been developed to predict the absorption, distribution, metabolism, and elimination of 1,4-dioxane in rats and humans. Single compartment, empirical models for rats (Young et al., 1978b; 1978a) and humans (Young et al., 1977a) were developed to predict blood levels of 1,4-dioxane and urine levels of the primary metabolite, β-hydroxyethoxy acetic acid (HEAA). Physiologically based pharmacokinetic (PBPK) models that describe the kinetics of 1,4-dioxane using biologically realistic flow rates, tissue volumes and affinities, metabolic processes, and elimination behaviors, were also developed (Takano et al., 2010; Fisher et al., 1997; Leung and Paustenbach, 1990b; Reitz et al., 1990b).

In developing toxicity values for 1,4-dioxane, the available PBPK models were evaluated for their ability to predict observations made in experimental studies of rat and human exposures to 1,4-dioxane. The model of Reitz et al. (1990a) was identified for further consideration to assist in the derivation of toxicity values. Issues related to the biological plausibility of parameter values in the Reitz et al. (1990a) human model were identified. The model was able to predict the only available human inhalation data set (Young et al., 1977a) by increasing (i.e., doubling) parameter values for human alveolar ventilation, cardiac output, and the blood:air partition coefficient above the measured values. Furthermore, the measured value for the slowly perfused tissue:air partition coefficient (i.e., muscle) was replaced with the measured liver value to improve the fit. Analysis of the Young et al. (1977a) human data suggested that the apparent volume of distribution (V_d) for 1,4-dioxane was approximately 10-fold higher in rats than humans, presumably due to species differences in tissue partitioning or other process not represented in the model. Subsequent exercising of the model demonstrated that selecting a human slowly perfused tissue:air partition coefficient much lower than the measured rat value resulted in better agreement between model predictions of 1,4-dioxane in blood and experimental observations. Based upon these observations, several model parameters (e.g., metabolism/elimination parameters) were re-calibrated using biologically plausible values for flow rates and tissue:air partition coefficients.

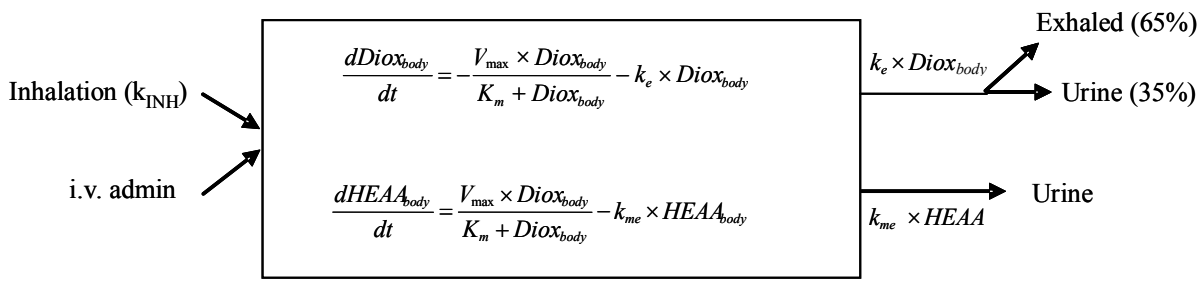
This appendix describes activities conducted in the evaluation of the empirical models (1978c, d; Young et al., 1977b) and re-calibration and exercising of the Reitz et al. (1990a) PBPK model using parameter values identified by Leung and Paustenbach (1990b) and Sweeney et al. (2008b), as well as optimized values, to determine the potential utility of the models for 1,4-dioxane for interspecies and route-to-route extrapolation.

B.2 Implementation of the Empirical Models in acslX

The scope of this effort consisted of implementation of the Young et al. (1978c, d; 1977b) empirical rat and human models using acslX, version 3.0.2.1 (Aegis Technologies, Huntsville, AL). Using the model descriptions and equations given in Young et al. (1978c, d; 1977b), model code was developed for the empirical models and executed, simulating the reported experimental conditions. The model output was then compared with the model output reported in Young et al. (1978c, d; 1977b). All model files are available electronically via HERO (U.S. EPA, 2013).

B.2.1 Model Descriptions

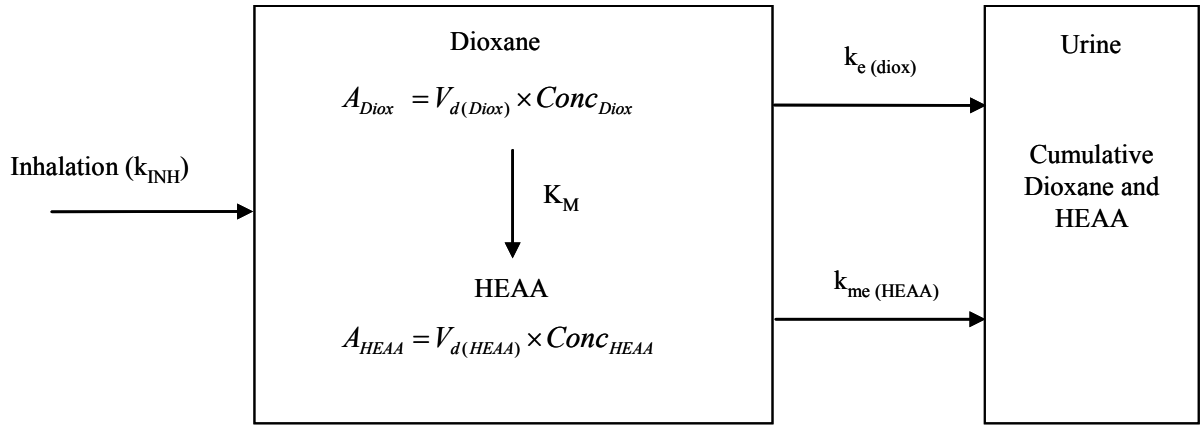
The empirical model of Young et al. (1978b; 1978a) for 1,4-dioxane in rats is shown in Figure B-1. This is a single-compartment model that describes the absorption and metabolism kinetics of 1,4-dioxane in blood and urine. Pulmonary absorption is described by a first-order rate constant (k_{INH}). The metabolism of 1,4-dioxane and subsequent appearance of HEAA is described by Michaelis-Menten kinetics governed by a maximum rate (V_{max}, mg/hour and affinity constant (K_m, mg). The elimination of both 1,4-dioxane and HEAA were described with first-order elimination rate constants, k_e and k_{me}, respectively (hour^{-1}) by which 35% of 1,4-dioxane and 100% of HEAA appear in the urine, while 65% of 1,4-dioxane is exhaled. Blood concentration of 1,4-dioxane was determined by dividing the amount of 1,4-dioxane in blood by a volume of distribution (V_d) of 0.301 L, which was the average V_d determined from the i.v. dose studies.



Source: Reprinted with permission of Taylor & Francis, Young et al. (1978b; 1978a).

Figure B-1 Schematic representation of empirical model for 1,4-dioxane in rats.

Figure B-2 illustrates the Young et al. (1977b) human empirical model for 1,4-dioxane. Like the rat model, the human model predicts blood 1,4-dioxane and urinary 1,4-dioxane and HEAA levels using a single-compartment structure. However, the metabolism of 1,4-dioxane to HEAA in humans is modeled as a first-order process governed by a rate constant, K_M (hour^{-1}). Urinary deposition of 1,4-dioxane and HEAA is described using the first order rate constants, $k_{e\,(diox)}$ and $k_{me\,(HEAA)}$, respectively. Pulmonary absorption is described similar to the approach used in the rat empirical model. Blood concentrations of 1,4-dioxane and HEAA are calculated as instantaneous amount (mg) divided by volume of distribution (V_d): $V_{d(diox)}$ or $V_{d(HEAA)}$ (104 and 480 mL/kg BW, respectively [calculated by Young et al. (1977b)]).



Source: Reprinted with permission of Taylor & Francis, Young et al. (1977a).

Figure B-2 Schematic representation of empirical model for 1,4-dioxane in humans.

B.2.2 Modifications to the Empirical Models

Several modifications were made to the empirical models. The need for the modifications arose in some cases from incomplete reporting of the Young et al. (1978b; 1978a; 1977a) studies and in other cases from the desire to add capabilities to the models to assist in the derivation of toxicity values.

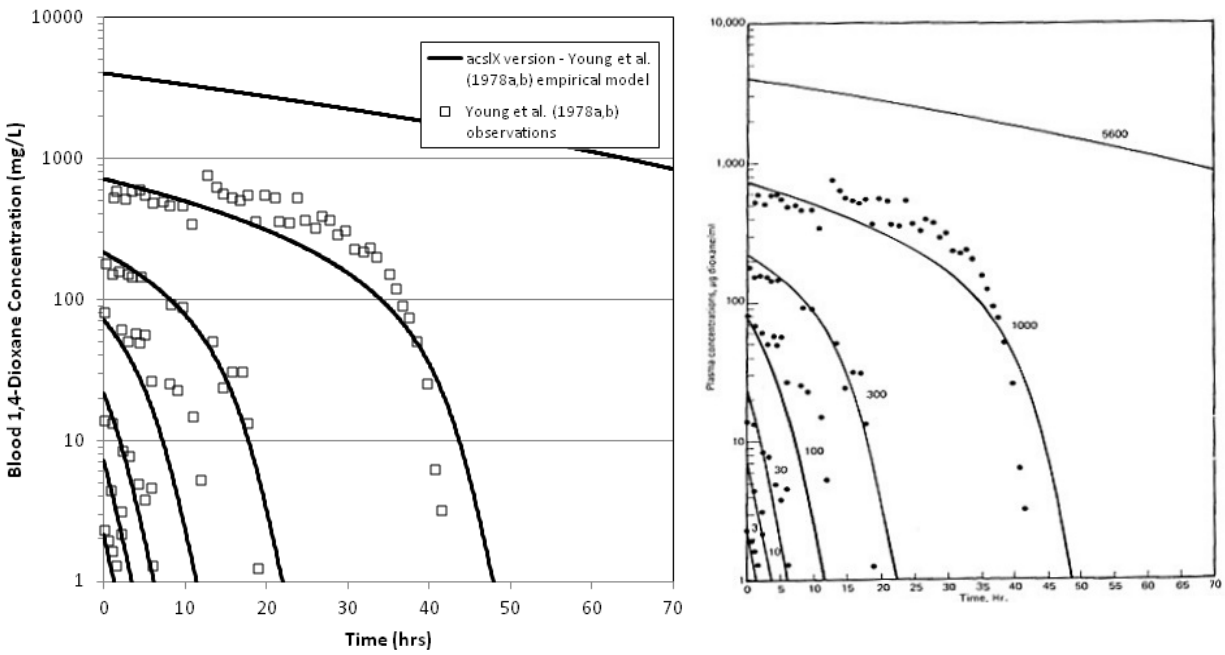
For the rat model, no information was given by Young et al. (1978b; 1978a) regarding the parameterization of pulmonary absorption (or exhalation) or i.v. administration of 1,4-dioxane. Therefore, additional parameters were added to simulate these processes in the simplest form. To replicate 1,4-dioxane inhalation, a first-order rate constant, k_{INH} ($hour^{-1}$), was introduced. k_{INH} was multiplied by the inhalation concentration and the respiratory minute volume of 0.238 L/minute (Young et al., 1978b; 1978a). The value for k_{INH} (0.43 $hour^{-1}$) was estimated by optimization against the blood time course data of Young et al. (1978b; 1978a). Intravenous (i.v.) administration was modeled as instantaneous appearance of the full dose at the start of the simulation. Rat urinary HEAA data were reported by Young et al. (1978b; 1978a) in units of concentration. To simulate urinary HEAA concentration, an estimate of urine volume was required. Since observed urinary volumes were not reported by Young et al. (1978b; 1978a), a standard rat urine production rate of 0.00145 L/hour was used.

For humans, Young et al. (1977a) used a fixed 1,4-dioxane inhalation uptake rate of 76.1 mg/hour, which corresponded to observations during a 50 ppm exposure. In order to facilitate user-specified inhalation concentrations, pulmonary absorption was modeled similar to the rat model addition (e.g., using k_{INH}, 1.06 $hour^{-1}$) but using a human minute volume of 7.5 L/minute. Urinary HEAA data were reported by Young et al. (1977a) as a cumulative amount (mg) of HEAA. Cumulative amount of HEAA in the urine is readily calculated from the rate of transfer of HEAA from plasma to urine, so no modification was necessary to simulate this dose metric for humans.

Neither empirical model of Young et al. (1978b; 1978a; 1977a) described oral uptake of 1,4-dioxane. Adequate data to estimate oral absorption parameters are not available for either rats or humans; therefore, neither empirical model was modified to include oral uptake.

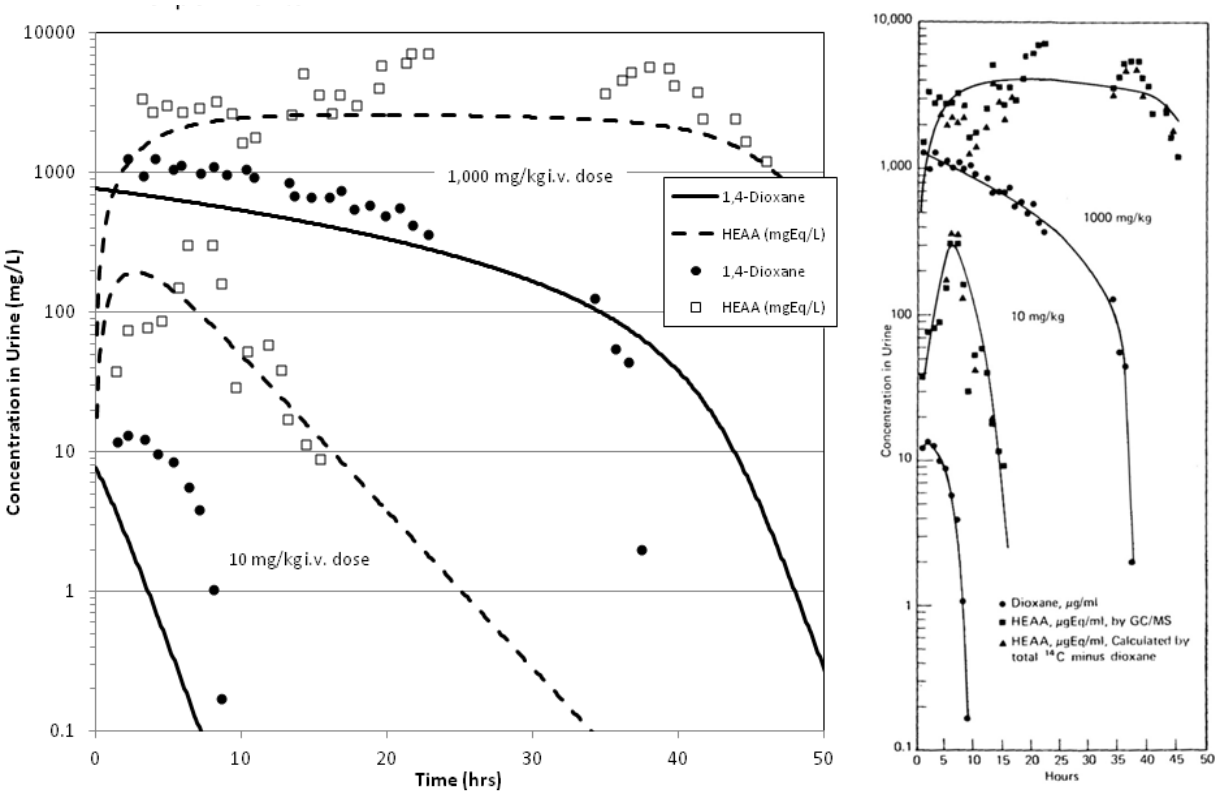
B.2.3 Results

The acslX implementation of the Young et al. (1978b; 1978a) rat empirical model is in good agreement with the 1,4-dioxane blood levels from the i.v. experiments and the model output reported in the published paper (Figure B-3). However, the acslX version predicts urinary HEAA following i.v. dose to reach a maximum sooner than the measured and predicted levels reported in the paper (Figure B-4). These discrepancies may be due, at least in part, to the reliance in the acslX implementation on a constant, standard, urine volume rather than experimental measurements, which may have been different from the assumed value and may have varied over time. Unreported model parameters (e.g., lag times for appearance of excreted HEAA in bladder urine) may also contribute to the discrepancy.



Source: Reprinted with permission of Taylor & Francis, Young et al. (1978b; 1978a).

Figure B-3 Output of 1,4-dioxane blood level data from the acslX implementation (left) and published (right) empirical rat model simulations of i.v. administration experiments.



Source: Reprinted with permission of Taylor & Francis, Young et al. (1978b; 1978a).

Figure B-4 Output of HEAA urine level data from acslXtreme implementation of the empirical rat model (left) and published (right)data following i.v. administration experiments. The lines in the figure from Young et al. (1978c, d) are best fit lines, and do not represent empirical model simulations.

The Young et al. (1978b; 1978a) report did not provide model predictions for the 50-ppm inhalation experiment. However, the acslX implementation produces blood 1,4-dioxane predictions that are similar to the reported observations (Figure B-5). As with the urine data from the i.v. experiment, the amount of HEAA in urine predicted using the acslX implementation was approximately threefold lower than the observations However, this prediction is the amount of HEAA excreted over time and does not rely on an estimate of urine volume to calculate, thus the reason for the discrepancy is likely due unreported model parameters (e.g., lag times for appearance of excreted HEAA in bladder urine) or to more complex kinetics than described using this simple model structure.

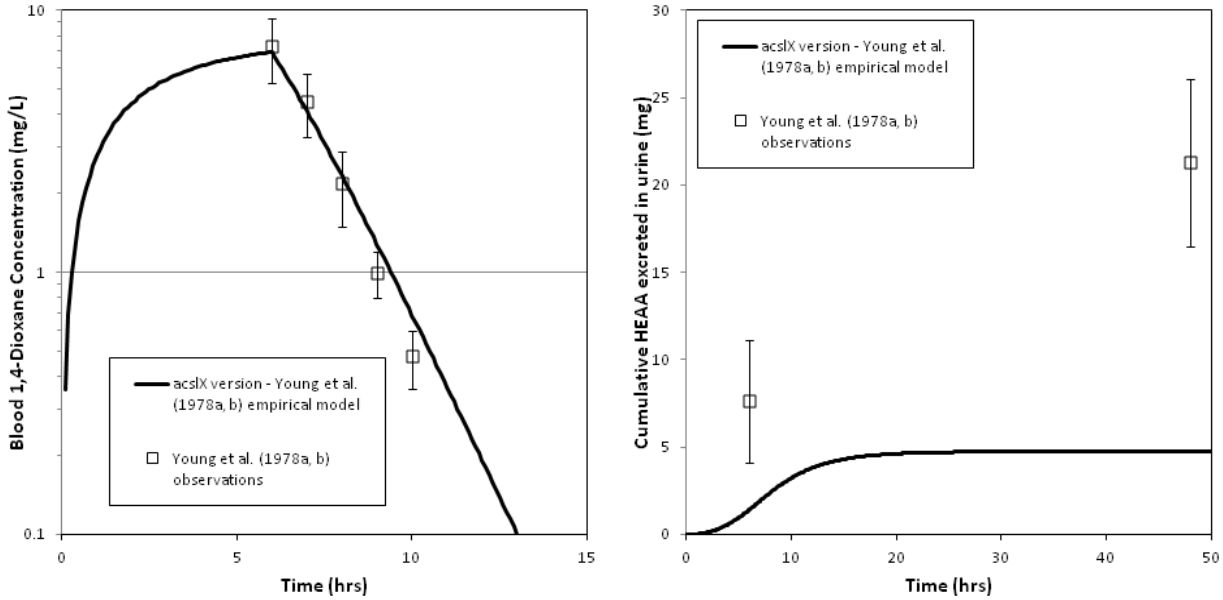


Figure B-5 acslX empirical rat model predictions of blood 1,4-dioxane concentration and total amount of HEAA levels in the urine for a 6-hour, 50-ppm 1,4-dioxane inhalation exposure.

Further evaluation of the Young et al. (1978c, d) empirical model was conducted against subchronic inhalation exposure data reported by Kasai et al. (2008). In the experimental study, male and female F344 rats were exposed to 0, 100, 200, 400, 800, 1,600, 3,200, or 6,400ppm 1,4-dioxane in a 13-week inhalation study. With the exception of the 6,400ppm dose, the Kasai et al. (2008) doses were within the range of the doses modeled by Young et al. (1978c, d); however, the model was unable to fit the measured 1,4-dioxane plasma levels reported by Kasai et al. (2008) (Figure B-6). This is could be due to a difference in metabolism of 1,4-dioxane following the single exposure (Young et al., 1978c, d) compared to the 13-week repeated exposure (Kasai et al., 2008).

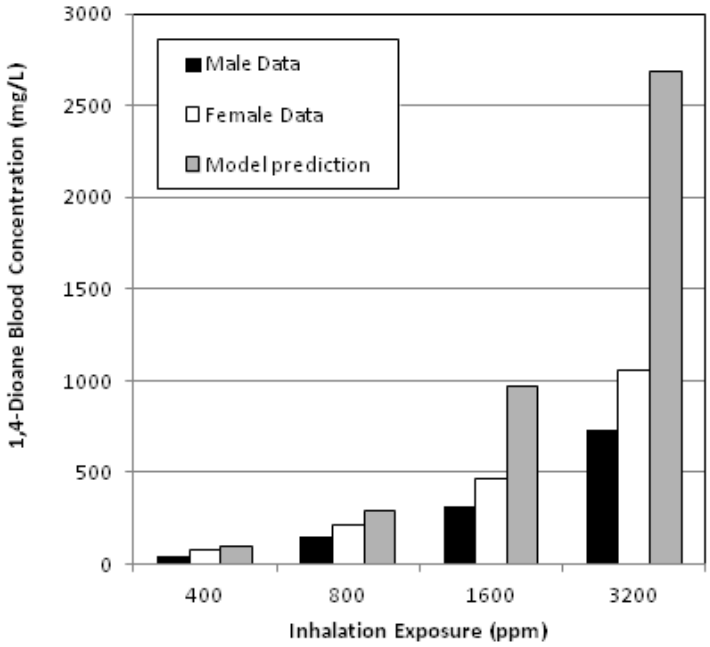
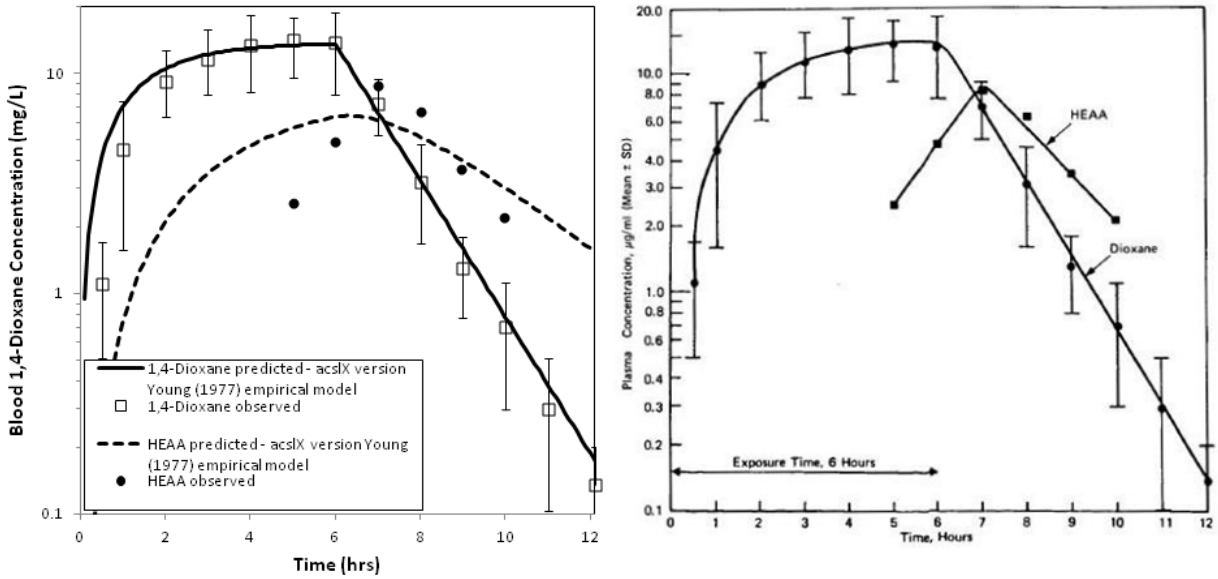


Figure B-6 acslX predictions of blood 1,4-dioxane levels using the Young et al. (1978c, d) model compared with data from Kasai et al. (2008).

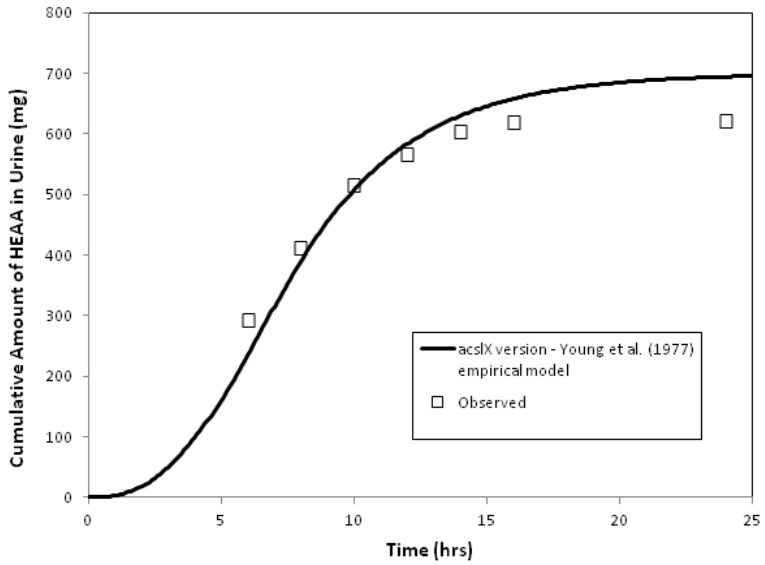
Inhalation data for a single exposure level (50 ppm) are available for humans. The acslX predictions of the blood 1,4-dioxane observations are similar to the predictions reported in Young et al. (1977a) (Figure B-). Limited blood HEAA data were reported (n = 2-3 animals), and the specimen analysis was highly problematic (e.g., an analytical interference was sometimes present from which HEAA could not be separated). For this reason, Young et al. (1977a) did not compare predictions of the blood HEAA data to observations in their manuscript. Young et al. (1977a) only compared model simulations to blood 1,4-dioxane in their report.

Data for cumulative urinary HEAA amounts are provided in Young et al. (1977b), and no analytical problems associated with these data were reported. The acslX prediction of the HEAA kinetics profile is similar to the observations (Figure B-8). Unlike urinary HEAA observations in the rat, human observations were reported as cumulative amount produced, negating the need for urine volume data. Therefore, discrepancies between model predictions and experimental observations were reduced.



Source: Reprinted with permission of Taylor & Francis, Young et al. (1978b; 1978a).

Figure B-7 Output of 1,4-dioxane and HEAA blood concentrations from the acslX implementation (left) and published (right) data of a 6-hour, 50-ppm inhalation exposure.



Source: Reprinted with permission of Taylor & Francis, Young et al. (1977a).

B.2.4 Figure B-8 Observations and acslX predictions of the cumulative amount of HEAA in human urine following a 6-hour, 50-ppm inhalation exposure.Conclusions for Empirical Model Implementation

The empirical models described by Young et al. (1978b; 1978a; 1977a) for rats and humans were implemented using acslX. The models were modified to allow for user-defined inhalation exposures by addition of a first-order rate constant for pulmonary uptake of 1,4-dioxane, fitted to the inhalation data. No modifications were made to describe oral absorption as adequate data are not available for parameter estimation. The acslX predictions of 1,4-dioxane in the blood are similar to the published data and simulations of 6-hour, 50-ppm inhalation exposures in rats (Figure B-5) and humans (Figure B-7) and 3 to 1,000 mg/kg i.v. doses in rats (Figure B-3). However, the acslX version predicts lower urinary HEAA amounts and concentrations in rats appearing earlier than either the Young et al. (1978b; 1978a) model predictions or the experimental observations (Figures B-4 and B-5). The lower predicted urinary HEAA concentrations in the acslXtreme implementation for rats are likely due to use of default values for urine volume in the absence of measured volumes. The reason for the differences in time-to-peak levels or amount of HEAA in urine is unknown, but may be the result of an unreported adjustment by Young et al. (1978b; 1978a) in model parameter values or more complex kinetics than can be described with this model structure. Additionally, the acslX implementation of the Young et al. (1978c, d) model failed to provide adequate fit to blood data reported following subchronic inhalation of 1,4-dioxane in rats at the two high doses (Kasai et al., 2008).

For humans, Young et al. (1977a) did not report model predictions of urinary HEAA levels. The urinary HEAA levels predicted by acslX approximated the observations reasonably well (Figure B-8), while the blood HEAA did not (Figure B-7). However, unlike the situation in rats, these urine data are not dependent on urine volumes (observations were reported as cumulative HEAA amount rather than HEAA concentration). Presently, there is no explanation for the lack of fit of the empirical model to the blood HEAA data. Since no blood HEAA model fits were shown in Young et al. , it is unclear if the discrepancy is in the original model or only in the acslX implementation.

B.3 Initial Evaluation of the PBPK Models

The PBPK model of Reitz et al. (1990b) was selected for further evaluation of its potential application in this assessment. The model was not sufficient as published, and thus was re-calibrated using measured values for cardiac and alveolar flow rates and tissue:air partition coefficients (Sweeney et al., 2008b; Leung and Paustenbach, 1990b). The predictions of blood and urine levels of 1,4-dioxane and HEAA, respectively, from the re-calibrated model were compared with the empirical model predictions of the same dosimeters to determine whether the re-calibrated PBPK model could perform similarly to the empirical model. As part of the PBPK model evaluation, EPA performed a sensitivity analysis to identify the model parameters having the greatest influence on the primary dosimeter of interest, the blood level of 1,4-dioxane. Variability data for the experimental measurements of the tissue:air partition coefficients were incorporated to determine a range of model outputs bounded by biologically plausible values for

these parameters. Additionally, the models were tested using first-order metabolism (instead of Michaelis-Menten saturable metabolism) to determine if better model predictions could be generated.

B.3.1 Initial Recalibration of the Reitz et al. PBPK Model

Concern regarding adjustments made to some of the parameter values in Reitz et al. (1990a) prompted a re-calibration of the Reitz et al. (1990a) human PBPK model using more biologically plausible values for all measured parameter values. Reitz et al. (1990a) doubled the measured physiological flows and blood:air partition coefficient and substituted the slowly-perfused tissue:air partition coefficient with the liver:air value in order to attain an adequate fit to the observations. This approach increases uncertainty in these parameter values, and in the utilization of the model for extrapolation. Therefore, the model was re-calibrated using parameter values that are more biologically plausible to determine whether an adequate fit of the model to the available data can be attained.

B.3.2 Flow Rates

The cardiac output of 30 L/hour/kg$^{0.74}$ (Table B-1) reported by Reitz et al. (Reitz et al., 1990a) is approximately double the mean resting value of 14 L/hour/kg$^{0.74}$ reported in the widely accepted compendium of Brown et al. (1997). Resting cardiac output was reported to be 5.2 L/minute (or 14 L/hour/kg$^{0.74}$), while strenuous exercise resulted in a flow of 9.9 L/minute (or 26 L/hour/kg$^{0.74}$) (Brown et al., 1997). Brown et al. (1997) also cite the ICRP (1975) as having a mean respiratory minute volume of 7.5 L/minute, which results in an alveolar ventilation rate of 6.86 L/minute (assuming 8.5% lung dead space, (Overton et al., 2001)), or 17.7 L/minute/kg$^{0.74}$. Again, this is roughly half the value of 30 L/hour/kg$^{0.74}$ employed for this parameter by Reitz et al. (1990a). Young et al. (1977a) reported that the human subjects exposed to 50 ppm for 6 hours were resting inside a walk-in exposure chamber. Thus, use of cardiac output and alveolar ventilation rates of 30 L/hour/kg$^{0.74}$ is not consistent with the experimental conditions being simulated.

A minute volume of 7.5 L/minute (or 17 L/hour/kg$^{0.74}$) was used in the acslX implementation of the Young et al. (1977b) model for volunteers having a mean BW of 84 kg and fit the blood 1,4-dioxane data reasonably well. Based on these findings, the cardiac output and alveolar ventilation rates of 17.0 and 17.7 L/hour/kg$^{0.74}$ were biologically plausible for the experimental subjects. These rate estimates are based on calculations made using empirical data and are consistent with standard human values and the experimental conditions (i.e., subject exertion level) reported by Young et al. (1977b). Therefore, these flow values were chosen for the model re-calibration.

Table B-1 Human PBPK model parameter values published in literature and values used by EPA in this assessment for 1,4-dioxane

Parameter (Abbreviation)	Reitz et al. (1990b)	Leung and Paustenbach (1990b)	Sweeney et al. (2008b)	EPA[b]
Body weight (BW)	70	84.1	70	84.1
Cardiac output (QCC)[a]	30	15	13	17.0
Alveolar ventilation (QPC)[a]	30	15	13	17.7
Fractional Blood Flows				
Liver (QLC)	0.25	0.25	0.227	0.25
Fat (QFC)	0.05	0.05	0.052	0.05
Richly perfused (QRC)	0.52	0.51	0.472[p]	0.52[p]
Slowly perfused (QSC)	0.18	0.19	0.249	0.18
Fractional Tissue Volumes				
Liver (VLC)	0.031	0.04	0.033	0.04
Fat (VFC)	0.231	0.20	0.214	0.20
Richly perfused (VRC)	0.037	0.05	0.166[q]	0.05[q]
Slowly perfused (VSC)	0.561	0.62	0.437	0.57
Blood (VBC)	0.05	--	0.079	0.05
Unperfused tissue (VUC)	--	--	0.071	0.09
Partition Coefficients (PCs)				
Blood:air (PB)	3,650[c]	1,825 ± 94[d] (*n*=14)	1,666 ± 287 (*n*=36)	1,825
Fat:air (PFA)	851	851 ± 118[d] (*n*=8)	865[e]	851
Liver:air (PLA)	1,557	1,557 ± 114[d] (*n*=4)	1,862 ± 739[f] (*n*=14)	1,557
Rapidly perfused tissue:air (PRA)	1,557	1,557[g]	560 ± 175[h] (*n*=7)	1,557
Slowly perfused tissue:air (PSA)	1,557[i]	997 ± 254[d] (*n*=6)	1,348 ± 290[f] (*n*=7)	260[j,m]
Metabolic Constants				
Maximum rate for 1,4-dioxane metabolism (V_{maxC}; mg/hr-kg $BW^{0.7}$)	12.5[n]	13.3[o]	54, 75, or 192[k]	5.8[j]
Metabolic affinity constant (K_m; mg/L)	3.00	15	29, 32, or 147[l]	5.3[j]
HEAA urinary elimination rate constant (k_{me}, hour-1)	0.56	--	0.35	0.30[j]

[a]L/hour/kg $BW^{0.74}$
[b]Values utilized by EPA in this assessment. Body weight was mean weight reported by Young et al. (1977b).
[c]Doubled from experimental value (1825) to obtain better fit to human data (Reitz et al., 1990b).
[d]Leung as Paustenbach (1990b) did not state if the values were reported ± standard deviation or standard error.
[e]Average of Reitz et al. (1990b) rat value and mouse value determined by Sweeney et al. (2008b).
[f]Assumed equal to the measurement for rat tissue determined by Sweeney et al. (2008b).
[g]Assumed equal to liver:air partition coefficient.
[h]Assumed equal to mouse kidney determined by Sweeney et al. (2008b).
[i]Authors reported poor fits to the venous blood data for rats and humans when the experimentally determined muscle:air partition coefficient was used (value not reported) and had improved fits of the data when the partition coefficient for liver:air was used.
[j]Obtained by model optimization.
[k]Used parallelogram scaling approach based on scaled in vitro data to give a range of values referred to by the authors as "minimum, representative, and maximum."
[l]Scaled rat in vitro data according to in vitro human:rat ratios to give a similar range as Vmax, referred to by the authors as "minimum, representative, and maximum."
[m]Value used in Figure B-11, estimated 4-fold lower value than Leung as Paustenbach (1990b) because recalibrated model was predictions were 4- to 7-fold lower than the data; however, this parameter value is not considered "biologically plausible."
[n]Reported in manuscript as 6.55 mg/hr-kg $BW^{0.86}$. Converted to mg/hr-kg $BW^{0.7}$ for consistency.
[o]Reported in manuscript as 6.55 mg/hr-kg $BW^{0.86}$. Converted to mg/hr-kg $BW^{0.7}$ for consistency.
[p]Calculated from QRC=1-(QFC+QSC+QLC)
[q]Calculated from VRC=1-(VLC+VFC+VSC+VBC+VUC)

B.3.3 Partition Coefficients

Two data sources are available for the tissue:air equilibrium partition coefficients for 1,4-dioxane: Leung and Paustenbach (1990a) and Sweeney et al. (2008a). Both investigators used vial equilibration techniques for experimental determinations. The values reported in Leung and Paustenbach (1990b) were also used, at least as starting points, by Reitz et al. (1990b). Leung and Paustenbach (1990b) reported mean values and an indication of variance (it was not clear if the values were standard deviations or standard errors) for human blood:air, rat blood:air, rat liver:air, rat muscle:air (e.g., slowly perfused tissue:air), and rat fat:air (Table B-1). They assumed the rapidly perfused tissue:air partition coefficient was equal to the value for the liver and that all human tissue partition coefficients were equivalent to the rat, except where the separate determination was made for human blood:air partition coefficient.

Sweeney et al. (2008b) experimentally determined partition coefficients for blood:air (mouse, rat, and human), liver:air (mouse and rat), fat:air (mouse), richly perfused tissue:air (mouse), and slowly perfused tissue:air (mouse). Values for human tissue:air partition coefficients for the model were estimated as averages of rat and mouse values (liver:air, fat:air, and slowly perfused tissue:air) or set equal to the mouse value (richly perfused:air set equal to mouse kidney:air partition coefficient) (Sweeney et al., 2008b). For example, the human fat:air partition coefficient, used an average (851) of the Reitz et al. (1990b) rat value (851) and their experimentally determined mouse value (879) (Sweeney et al., 2008b).

For the PBPK model implementation, tissue:blood partition coefficients for each compartment were determined by dividing the tissue:air partition coefficients by the blood:air partition coefficient.

B.3.4 Calibration Method

The PBPK model was re-calibrated three times using the physiological values selected by EPA (current assessment, Table B-1) and the (1) partition coefficients of Leung and Paustenbach (1990a), (2) Sweeney et al. (2008a), and (3) biologically plausible values based on these two publications, separately. For each calibration, the metabolic parameters V_{maxC} and K_m, were simultaneously fit (using the parameter estimation tool provided in the acslX software) to the output of 1,4-dioxane blood concentrations generated by the acslX implementation of the Young et al. (1977a) empirical human model for a 6 hour, 50 ppm inhalation exposure. Subsequently, the HEAA urinary elimination rate constant, k_{me}, was fitted to the urine HEAA predictions from the empirical model. The empirical model predictions that were validated against the experimental observations were used to provide a more robust data set for model fitting, since the empirical model simulation provided 240 data points (one prediction every 0.1 hour) compared with hourly experimental observations, and to avoid introducing error by calibrating the model to data digitally captured from Young et al. (1977a).

B.3.5 Results

Results of the model re-calibration are provided in Table B-2. The re-calibrated values for V_{maxC} and k_{me} associated with the Leung and Paustenbach (1990a) or Sweeney et al. (2008a) tissue:air partition coefficients are very similar. Plots of predicted and experimentally observed blood 1,4-dioxane and urinary HEAA levels are shown in Figure B-6 and B-10 for Leung and Paustenbach (1990b) and Sweeney et al. (2008b) partition coefficients. Neither re-calibration resulted in an adequate fit to the blood 1,4-dioxane data from the empirical model output or the experimental observations. Re-calibration using either the Leung and Paustenbach (1990b) or Sweeney et al. (2008b) partition coefficients resulted in blood 1,4-dioxane predictions that were 4- to 7-fold lower than empirical model predictions or observations.

The refitted values for k_{me} resulted in HEAA levels in urine that were very similar to the empirical model output (compare Figure B-7, Figure B-6, and Figure B-7), which was not surprising, given the fitting of a single parameter to the data.

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 (1990a)	Sweeney et al. (2008a)	EPA
Maximum rate for 1,4-dioxane metabolism (V_{maxC})[b]	4.9	4.0	5.8
Metabolic affinity constant (K_m)[c]	1.8	0.78	5.3
HEAA urinary elimination rate constant (k_{me})[d]	0.27	0.25	0.30

[a]Cardiac output = 17.0 L/hour/kg $BW^{0.74}$, alveolar ventilation = 17.7 L/hour/kg $BW^{0.74}$
[b]mg/hour/kg $BW^{0.7}$
[c]mg/L
[d]$hour^{-1}$

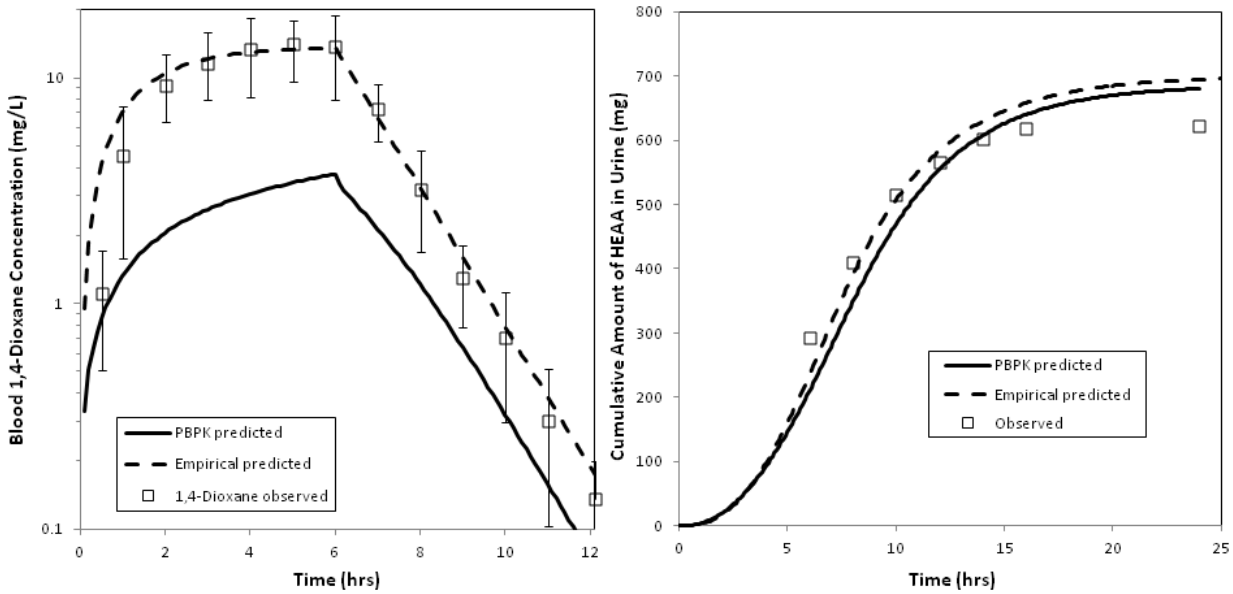


Figure B-6 Human predicted and observed blood 1,4-dioxane concentrations (left) and urinary HEAA levels (right) following a 6-hour, 50 ppm 1,4-dioxane exposure and re-calibration of the PBPK model with tissue:air partition coefficient values from Leung and Paustenbach (1990b).

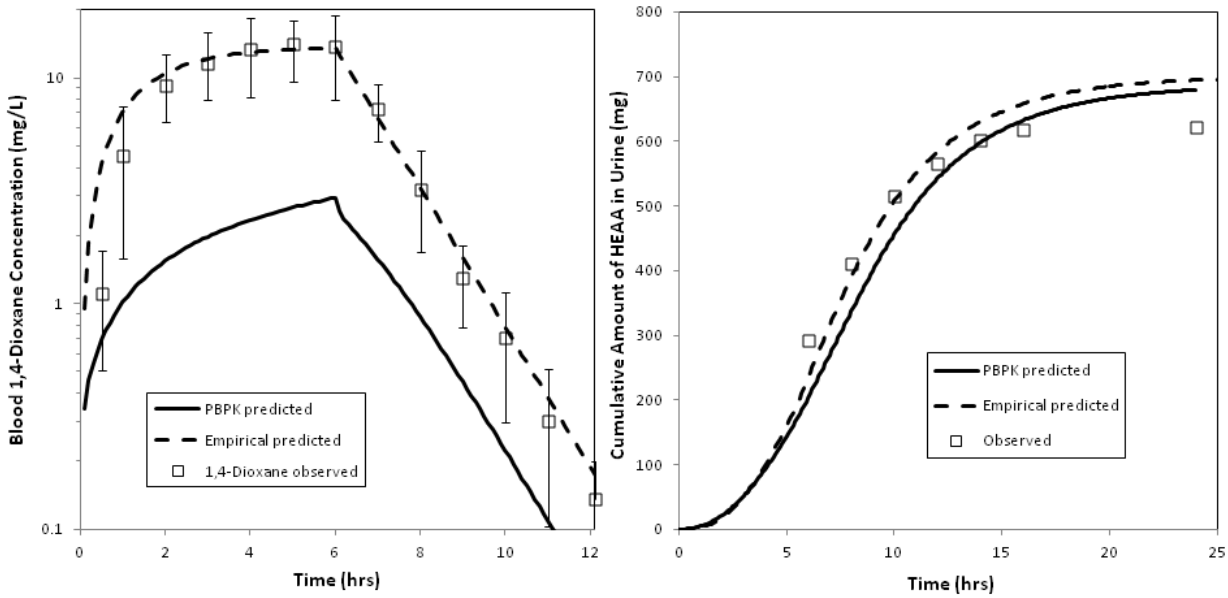


Figure B-7 Human predicted and observed blood 1,4-dioxane concentrations (left) and urinary HEAA levels (right) following a 6-hour, 50 ppm 1,4-dioxane exposure and re-calibration of the PBPK model with tissue:air partition coefficient valuesfrom Sweeney et al. (2008b).

Model outputs of the blood 1,4-dioxane and urinary HEAA levels using the EPA suggested (Table B-2) parameters are shown in Figure B-8. To obtain these improved fits, a very low value for the slowly perfused tissue:air partition coefficient (22) was used. The value was 4- to 6-fold lower than the measured values reported in Leung and Paustenbach (1990a) and Sweeney et al. (2008a), and 7-fold lower than the value used by Reitz et al. (1990a). While the predicted maximum blood 1,4-dioxane levels

are much closer to the observations (e.g., 2- to 3-fold lower than the observations), the value used for the slowly perfused tissue partition coefficient is not supported by laboratory data.

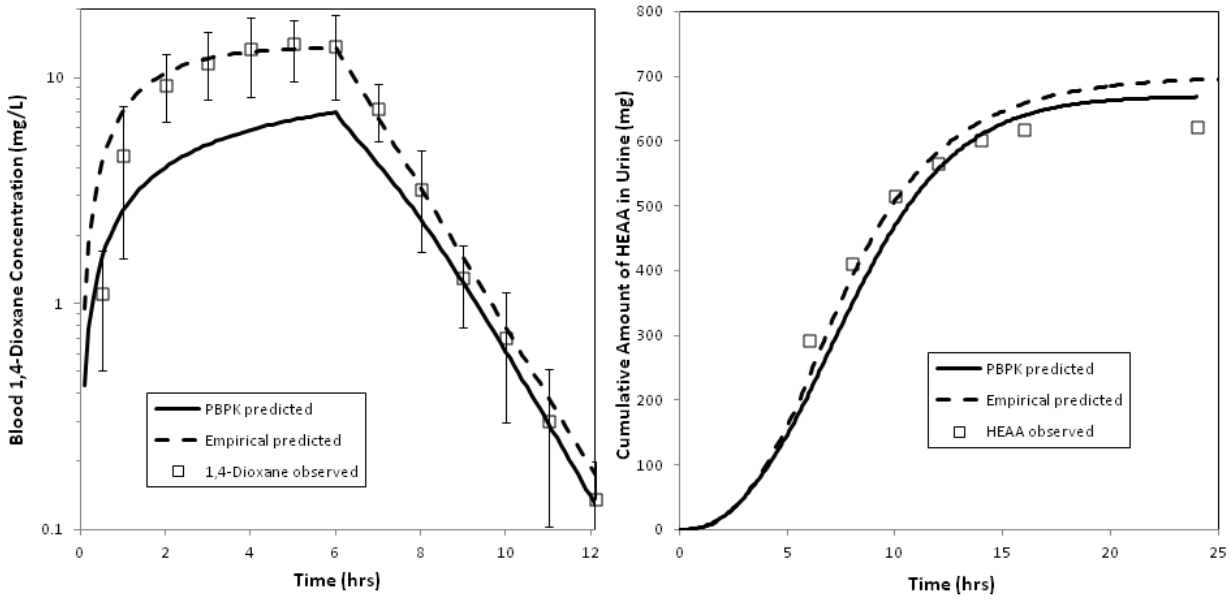


Figure B-8 Human predicted and observed blood 1,4-dioxane concentrations (left) and urinary HEAA levels (right) following a 6-hour, 50 ppm 1,4-dioxane exposure using EPA estimated biologically plausible parameters (Table B-1).

B.3.6 Conclusions for PBPK Model Implementation

Re-calibration of the human PBPK model was performed using experiment-specific values for cardiac output and alveolar ventilation (Young et al., 1977a) and measured mean tissue:air 1,4-dioxane partition coefficients reported by Leung and Paustenbach (1990a) or Sweeney et al. (2008a). The resulting predictions of 1,4-dioxane in blood following a 6-hour, 50-ppm inhalation exposure were 4- to 7-fold lower than either the observations or the empirical model predictions, while the predictions of urinary HEAA by the PBPK and empirical models were similar to each other (Figure B-6 and Figure B-7). Output from the model using biologically plausible physiological parameter values (Table B-1), Figure B-8 shows that application of a value for the slowly perfused tissue:air partition coefficient, which is 6-fold lower than the measured value reported by Leung and Paustenbach (1990a), results in closer agreement of the predictions to observations. Thus, model re-calibration using experiment-specific flow rates and mean *measured* partition coefficients does not result in an adequate fit of the PBPK model to the available data.

The Sweeney et al. (2008a) PBPK model consisted of compartments for fat, liver, slowly perfused, and other well perfused tissues. Lung and stomach compartments were used to describe the route of exposure, and an overall volume of distribution compartment was used for calculation of urinary excretion levels of 1,4-dioxane and its metabolite, HEAA. Metabolic constants (V_{maxC} and K_m) for the rat

PBPK model were derived by optimization data from an i.v. exposure of 1,000 mg/kg data (Young et al., 1978b; 1978a) for induced metabolism. For uninduced metabolism data generated by i.v. exposures to 3, 10, 30, and 100 mg/kg were used (Young et al., 1978b; 1978a). Data generated from the 300 mg/kg i.v. exposure were not used to estimate V_{maxC} and K_m. The best fitting values for V_{maxC} to estimate the blood data from the Young et al. (1978b; 1978a) study using the Sweeney et al. (2008a) model resulted in V_{maxC} values of 12.7, 10.8, 7.4 mg/kg-hr$^{0.7}$; suggesting a gradual dose dependent increase in metabolic rate with dose. These estimates were for a range of doses between 3 and 1,000 mg/kg i.v. dose. Although the Sweeney et al. (2008a) model utilized two values for V_{maxC} (induced and uninduced), the PBPK model does not include dose-dependent function description of the change of Vmax for i.v. doses between 100 and 1,000 mg/kg. PBPK model outputs were compared with other data not used in fitting model parameters by visual inspection. The model predictions gave adequate match to the 1,4-dioxane exhalation data after a 1,000 mg/kg i.v. dose. 1,4-Dioxane exhalation was overpredicted by a factor of about 3 for the 10 mg/kg i.v. dose. Similarly, the simulations of exhaled 1,4-dioxane after oral dosing were adequate at 1,000 mg/kg, and 100 mg/kg (within 50%), but poor at 10 mg/kg (model overpredicted by a factor of five). The fit of the model to the human data (Young et al., 1977a) was also problematic (Sweeney et al., 2008a). Using physiological parameters of Brown et al. (1997) and measured partitioning parameters (Sweeney et al., 2008a; Leung and Paustenbach, 1990a) with no metabolism, measured blood 1,4-dioxane concentrations reported by Young et al. (1977a) could not be achieved using the reported exposure concentrations. Inclusion of any metabolism further decreased predicted blood concentrations. If estimated metabolism rates were used with the reported exposure concentration, urinary metabolite (HEAA) excretion was underpredicted (Sweeney et al., 2008a). Thus, the models were inadequate to use for rat to human extrapolation.

B.3.7 Sensitivity Analysis

A sensitivity analysis of the Reitz et al. (1990a) model was performed, using the EPA values listed in Table B-1, to determine which PBPK model parameters exert the greatest influence on the outcome of dosimeters of interest—in this case, the concentration of 1,4-dioxane in blood. Knowledge of model sensitivity is useful for guiding the choice of parameter values to minimize model uncertainty.

B.3.8 Method

A univariate sensitivity analysis was performed on all of the model parameters for two endpoints: blood 1,4-dioxane concentrations after 1 and 4 hours of exposure. These time points were chosen to assess sensitivity during periods of rapid uptake (1 hour) and as the model approached steady state (4 hours) for blood 1,4-dioxane. Model parameters were perturbated 1% above and below nominal values and sensitivity coefficients were calculated as follows:

$$f'(x) \approx \frac{f(x+\Delta x)-f(x)}{\Delta x} \cdot \frac{x}{f(x)}$$

where × is the model parameter, f(×) is the output variable, Δx is the perturbation of the parameter from the nominal value, and f'(×) is the sensitivity coefficient. The sensitivity coefficients were scaled to the nominal value of × and f(×) to eliminate the potential effect of units of expression. As a result, the sensitivity coefficient is a measure of the proportional change in the blood 1,4-dioxane concentration produced by a proportional change in the parameter value, with a maximum value of 1.

B.3.9 Results

The sensitivity coefficients for the seven most influential model parameters at 1 and 4 hours of exposure are shown in Figure B-9. The three parameters with the highest sensitivity coefficients in descending order are alveolar ventilation (QPC), the blood:air partition coefficient (PB), and the slowly perfused tissue:air partition coefficient (PSA). Not surprisingly, these were the parameters that were doubled or given surrogate values in the Reitz et al. (1990a) model in order to achieve an adequate fit to the data. Because of the large influence of these parameters on the model, it is important to assign values to these parameters in which high confidence is placed, in order to reduce model uncertainty.

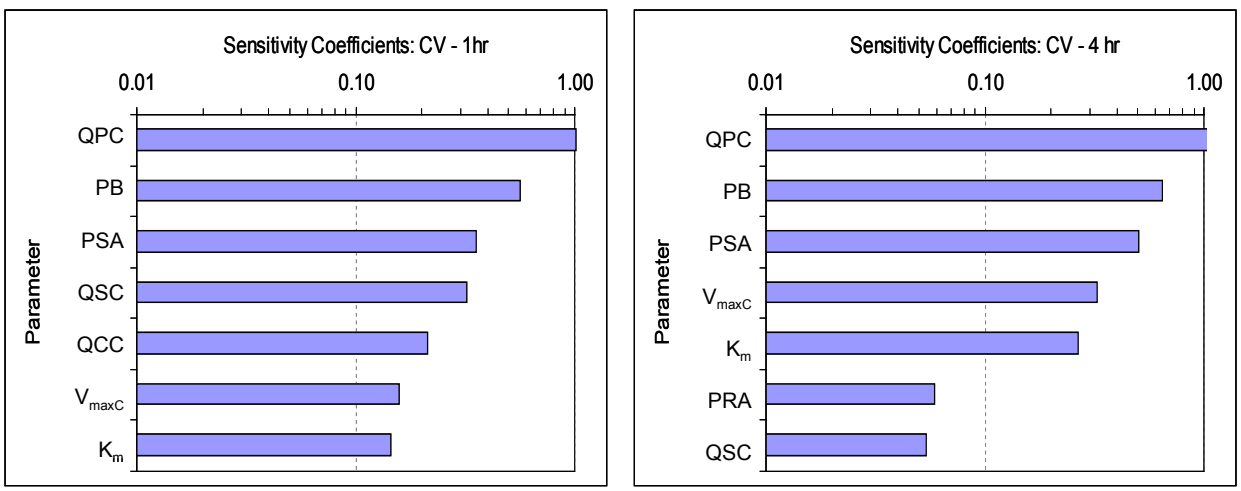


Figure B-9 The highest seven sensitivity coefficients (and associated parameters) for blood 1,4-dioxane concentrations (CV) at 1 (left) and 4 (right) hours of a 50-ppm inhalation exposure.

B.4 PBPK Model Exercises Using Biologically Plausible Parameter Boundaries

The PBPK model includes numerous physiological parameters whose values are typically taken from experimental observations. In particular, values for the flow rates (cardiac output and alveolar

ventilation) and tissue:air partition coefficients (i.e., mean and standard deviations) are available from multiple sources as means and variances. The PBPK model was exercised by varying the partition coefficients over the range of biological plausibility (parameter mean ± 2 standard deviations), re-calibrating the metabolism and elimination parameters, and exploring the resulting range of blood 1,4-dioxane concentration time course predictions. Cardiac output and alveolar ventilation were not varied because the experiment-specific values used did not include any measure of inter-individual variation.

B.4.1 Observations Regarding the Volume of Distribution

Young et al. (1978b; 1978a) used experimental observations to estimate a V_d for 1,4-dioxane in rats of 301 mL or 1,204 mL/kg BW. For humans, the V_d was estimated to be 104 mL/kg BW (Young et al., 1977a). It is possible that a very large volume of the slowly perfused tissues in the body of rats and humans may be a significant contributor to the estimated 10-fold difference in distribution volumes for the two species. This raises doubt regarding the appropriateness of using the measured rat slowly perfused tissue:air partition coefficient as a surrogate values for humans in the PBPK model.

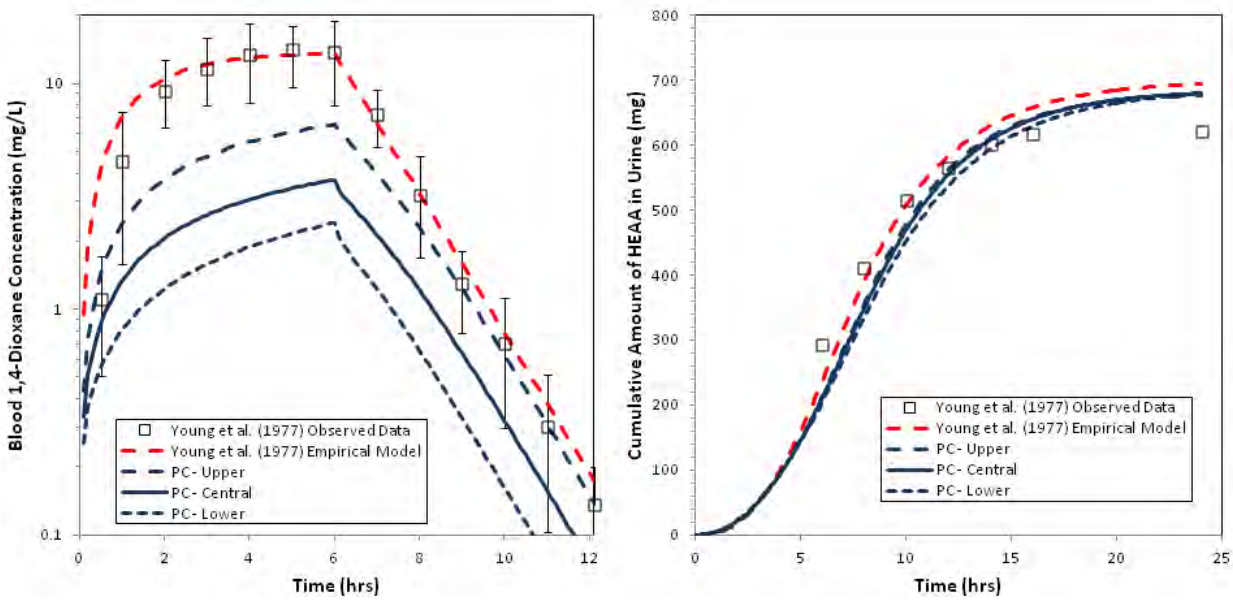
B.4.2 Defining Boundaries for Parameter Values

Given the possible 10-fold species differences in the apparent V_d for 1,4-dioxane in rats and humans, boundary values for the partition coefficients were chosen to exercise the PBPK model across its performance range to either minimize or maximize the simulated V_d. This was accomplished by defining biologically plausible values for the partition coefficients as the mean ± 2 standard deviations of the measured values. Thus, to minimize the simulated V_d for 1,4-dioxane, the selected blood:air partition coefficient was chosen to be the mean + 2 standard deviations, while all of the other tissue:air partition coefficients were chosen to be the mean – 2 standard deviations. This created conditions that would sequester 1,4-dioxane in the blood, away from other tissues. To maximize the simulated 1,4-dioxane V_d, the opposite selections were made: blood:air and other tissue:air partition coefficients were chosen as the mean – 2 standard deviations and mean + 2 standard deviations, respectively. Subsequently, V_{maxC}, K_m, and k_{me} were optimized to the empirical model output data as described in Section B.3.4. This procedure was performed for both the Leung and Paustenbach (1990a) and Sweeney et al. (2008a) partition coefficients (Table B-1). The two predicted time courses resulting from the re-calibrated model with partition coefficients chosen to minimize or maximize the 1,4-dioxane V_d represent the range of model performance as bounded by biologically plausible parameter values.

B.4.3 Results

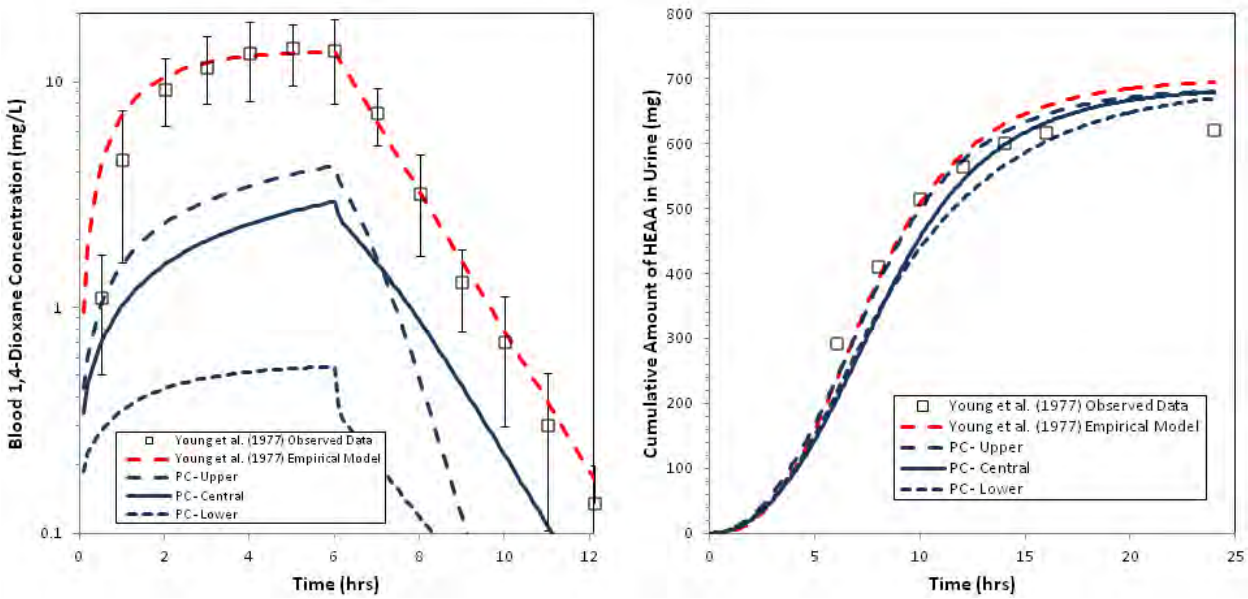
The predicted time courses for a 6-hour, 50-ppm inhalation exposure for the re-calibrated human PBPK model with mean (central tendency) and ± 2 standard deviations from the mean values for partition

coefficients are shown in Figure B-10 for the Leung and Paustenbach (1990a) values and Figure B-11 for the Sweeney et al. (2008a) values. The resulting fitted values for V_{maxC}, K_m, and k_{me}, are given in Table B-3. By bounding the tissue:air partition coefficients with upper and lower limits on biologically plausible values from Leung and Paustenbach (1990a) or Sweeney et al. (2008a), the model predictions are still at least 2- to 4-fold lower than either the empirical model output or the experimental observations. The range of possible urinary HEAA predictions approximate the prediction of the empirical model, but this agreement is not surprising, as the cumulative rate of excretion depends only on the rate of metabolism of 1,4-dioxane, and not on the apparent V_d for 1,4-dioxane. These data show that the PBPK model cannot adequately reproduce the predictions of blood 1,4-dioxane concentrations of the Young et al. (1977a) human empirical model or the experimental observations when constrained by biologically plausible values for physiological flow rates and tissue:air partition coefficients.



Source: Reprinted with permission of Elsevier, Ltd., Leung and Paustenbach (1990a)

Figure B-10 Comparisons of the range of PBPK model predictions from upper and lower boundaries on partition coefficients from Leung & Paustenbach (1990b) with empirical model predictions and experimental observations for blood 1,4-dioxane concentrations (left) and amount of HEAA in urine (right) from a 6-hour, 50-ppm inhalation exposure.



Source: Reprinted with permission of Oxford Journals, Sweeney et al. (2008a); Used with permission of Taylor & Francis, Young et al. (1977a).

Figure B-11 Comparisons of the range of PBPK model predictions from upper and lower boundaries on partition coefficients from Sweeney et al (2008b) with empirical model predictions and experimental observations for blood 1,4-dioxane concentrations (left) and amount of HEAA in urine (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 (1990a)		Sweeney et al. (2008a)	
	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]	3.63	6.2	8.7	5.3
Metabolic dissociation constant (K_m)[c]	0.41	5.6	0.000038	3.8
HEAA urinary elimination rate constant (k_{me})[d]	0.24	0.29	0.18	0.28

[a]Cardiac output = 17.0 L/hour/kg $BW^{0.74}$; alveolar ventilation = 17.7 L/hour/kg $BW^{0.74}$
[b]mg/hour/kg $BW^{0.7}$
[c]mg/L
[d]$hour^{-1}$

B.4.4 Alternative Model Parameterization

Since the PBPK model does not predict the experimental observations of Young et al. (1977a) when parameterized by biologically plausible values, an exercise was performed to explore alternative parameters and values capable of producing an adequate fit of the data. Since the metabolism of 1,4-dioxane appears to be linear in humans for a 50-ppm exposure (Young et al., 1977a), the parameters V_{maxC} and K_m were replaced by a first-order, non-saturable metabolism rate constant, k_{LC}. This rate

constant was fitted to the experimental blood 1,4-dioxane data using partition coefficient values of Sweeney et al. (2008a) to minimize the V_d (i.e., maximize the blood 1,4-dioxane levels). The resulting model predictions are shown in Figure B-12. As before, the maximum blood 1,4-dioxane levels were approximately sevenfold lower than the observed values.

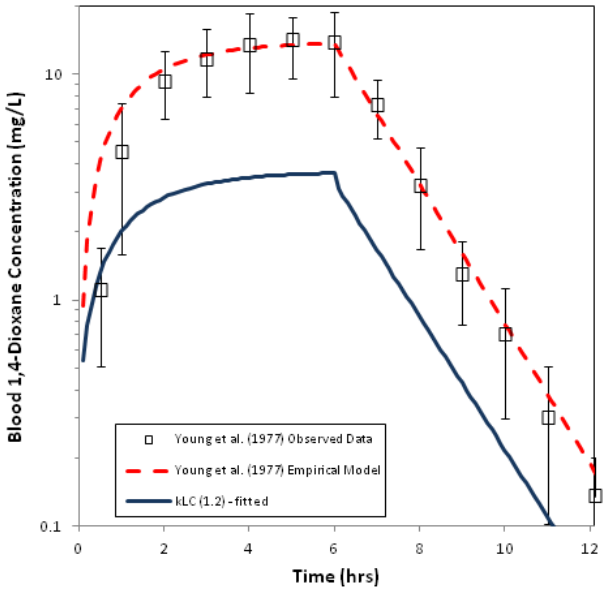


Figure B-12 Predictions of blood 1,4-dioxane concentration following calibration of a first-order metabolism rate constant, k_{LC} (1.2 $hour^{-1}$), to the experimental data.

A re-calibration was performed using only the data from the exposure phase of the experiment, such that the elimination data did not influence the initial metabolism and tissue distribution. The model predictions from this exercise are shown in Figure B-13. These predictions are more similar to the observations made during the exposure phase of the experiment; however, this is achieved at greatly reduced elimination rate and hence under predictions of urinary HEAA (compare Figure B-8 and Figure B-13).

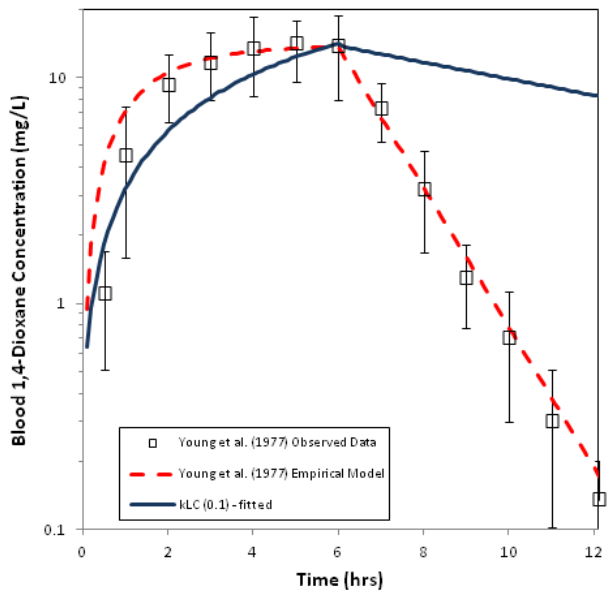


Figure B-13 Predictions of blood 1,4-dioxane concentration following calibration of a first-order metabolism rate constant, k_{LC} (0.1 hour^{-1}), to only the exposure phase of the experimental data.

Finally, the model was re-calibrated by simultaneously fitting k_{LC} and the slowly perfused tissue:air partition (PSA) coefficient to the experimental data with no bounds on possible values (except that they be non-zero). The fitted slowly perfused tissue:air partition coefficient was a very low value of 10 (compared to experimentally determined values, see Table B-1). The resulting model predictions, however, were closer to the observations (Figure B-14). These exercises show that better fits to the observed blood 1,4-dioxane kinetics are achieved only when parameter values are adjusted in a way that corresponds to a substantial decrease in apparent V_d of 1,4-dioxane in the human, relative to the rat (e.g., decreasing the slowly perfused tissue:air partition coefficient to extremely low values, relative to observations). Downward adjustment of the elimination parameters (e.g., decreasing k_{LC}) increases the predicted blood concentrations of 1,4-dioxane, achieving better agreement with observations during the exposure phase of the experiment; however, it results in unacceptably slow elimination kinetics, relative to observations following cessation of exposure and poor predictions of urinary elimination of HEAA. These observations suggest that some other process not captured in the present PBPK model structure is responsible for the species differences in 1,4-dioxane V_d and the inability to reproduce the human experimental inhalation data with biologically plausible parameter values.

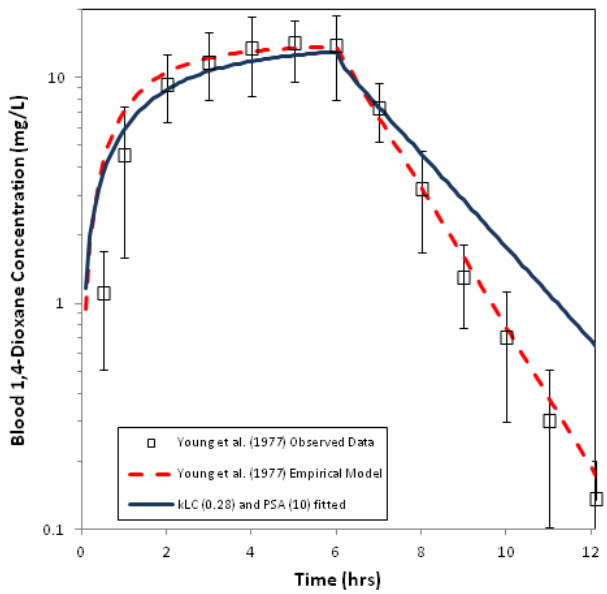


Figure B-14 Predictions of blood 1,4-dioxane concentration following simultaneous calibration of a first-order metabolism rate constant (k_{LC}) and slowly perfused tissue:air partition coefficient (PSA) to the experimental data.

B.5 Conclusions

The rat and human empirical models of Young et al. (1978b; 1978a; 1977a) were successfully implemented in acslXtreme and perform identically to the models reported in the published papers (Figures B-3, B-4, B-5, B-7, and B-8), with the exception of the lower predicted HEAA concentrations and early appearance of the peak HEAA levels in rat urine. The early appearance of peak HEAA levels cannot presently be explained, but may result from manipulations of k_{me} or other parameters by Young et al. (1978b; 1978a) that were not reported. The lower predictions of HEAA levels are likely due to reliance on a standard urine volume production rate in the absence of measured (but unreported) urine volumes. While the human urinary HEAA predictions were closer to the observed data of Young et al. (1977a), no model output was published in Young et al. (1977a) for comparison. The empirical models were modified to allow for user-defined inhalation exposure levels; however, they were not modified to describe oral exposures due to a lack of adequate human or animal data for parameterization. Additionally, the inhalation Young et al. (1977a) model did not provide adequate fits to the subchronic exposure plasma levels of 1,4-dioxane in rats using the data from the Kasai et al. (2008) study, which is likely due to the absence of a model description for metabolic induction.

Several procedures were applied to the human PBPK model to determine if an adequate fit of the model to the empirical model output or experimental observations could be attained using biologically plausible values for the model parameters. The re-calibrated model predictions for blood 1,4-dioxane did not adequately fit the experimental values using measured tissue:air partition coefficients from Leung and Paustenbach (1990a) or Sweeney et al. (2008a) (Figure B-6 and Figure B-7). Use of a slowly perfused tissue:air partition coefficient 4- to 7-fold lower than measured values produces exposure-phase predictions that are much closer to observations, but does not replicate the elimination kinetics (Figure B-

14). Re-calibration of the model with upper bounds on the tissue:air partition coefficients results in predictions that are still 2- to 4-fold lower than empirical model prediction or observations (Figure B-10 and Figure B-11). Exploration of the model space using an assumption of first-order metabolism (valid for the 50-ppm inhalation exposure) showed that an adequate fit to the exposure and elimination data can be achieved only when unrealistically low values are assumed for the slowly perfused tissue:air partition coefficient (Figure B-14). Artificially low values for the other tissue:air partition coefficients are not expected to improve the model fit, because blood 1,4-dioxane is less sensitive to these parameters than it is to V_{maxC} and K_m. This suggests that the model structure is insufficient to capture the apparent species difference in the blood 1,4-dioxane V_d between rats and humans. Differences in the ability of rat and human blood to bind 1,4-dioxane may contribute to the difference in V_d. However, this is expected to be evident in very different values for rat and human blood:air partition coefficients, which is not the case (Table B-1). Additionally, the models do not account for induction in metabolism, which may be present in animals exposed repeatedly to 1,4-dioxane. Therefore, some other modification(s) to the Reitz et al. (1990b) model structure may be necessary. Sweeney et al. (2008a) PBPK model provided an overall improvement on previous models; however, the Sweeney et al. (2008a) inhalation model predictions of animal and human data were still problematic.

B.6 acslX Model Code

The PBPK acslX model code is made available electronically through EPA's Health and Environmental Research Online (HERO) database. All model files may be downloaded in a zipped workspace from HERO (U.S. EPA, 2013).

APPENDIX C. DETAILS OF BMD ANALYSIS FOR ORAL RFD FOR 1,4-DIOXANE

C.1 Cortical Tubule Degeneration

All available dichotomous models in the Benchmark Dose Software (version 2.1.1) were fit to the incidence data shown in Table C-4, for cortical tubule degeneration in male and female Osborne-Mendel rats exposed to 1,4-dioxane in the drinking water (NCI, 1978). Doses associated with a BMR of a 10% extra risk were calculated.

Table C-4 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%)

[a]Statistically significant trend for increased incidence by Cochran-Armitage test ($p < 0.05$) performed for this review.
[b]Incidence significantly elevated compared to control by Fisher's exact test ($p < 0.05$) performed for this review.

Source: NCI (1978).

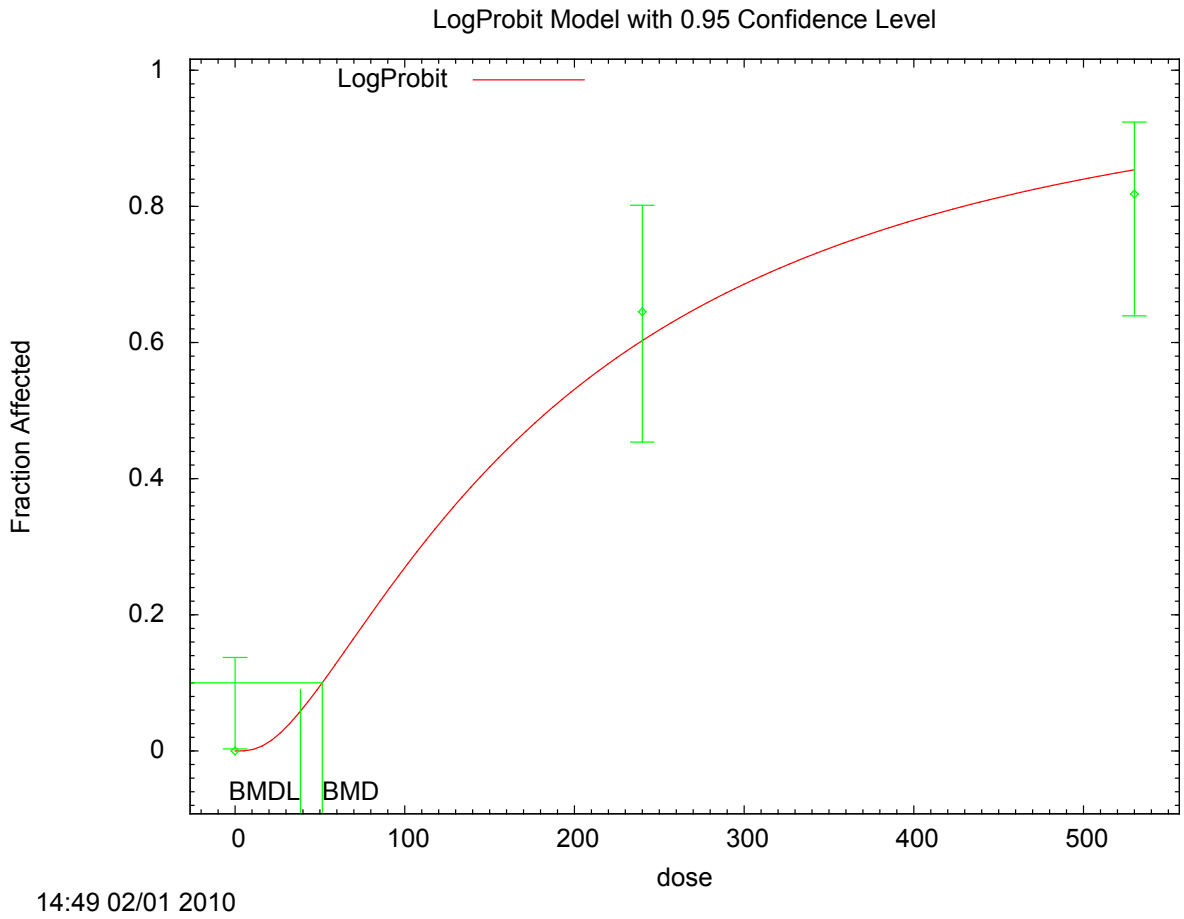
As assessed by the χ^2 goodness-of-fit test, several models in the software provided adequate fits to the data for the incidence of cortical tubule degeneration in male and female rats (χ^2 $p \geq 0.1$) (Table C-5). Comparing across models, a better fit is indicated by a lower AIC value (U.S. EPA, 2012a). As assessed by Akaike's Information Criterion (AIC), the log-probit model provided the best fit to the cortical tubule degeneration incidence data for male rats (Table C-5, Figure C-15) and could be used to derive a POD of 38.5 mg/kg-day for this endpoint. The Weibull model provided the best fit to the data for female rats (Table C-5,) and could be used to derive a POD of 452.4 mg/kg-day for this endpoint. For those models that exhibit adequate fit, models with the lower AIC values are preferred. Differences in AIC values of less than 1 are generally not considered important. BMDS modeling results for all dichotomous models are shown in Table C-5.

Table C-5 Goodness-of-fit statistics and BMD_{10} and $BMDL_{10}$ 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

Model	AIC	*p*-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.
[b]Power restricted to ≥ 1.
[c]Slope restricted to ≥ 1.
[d]Betas restricted to ≥0.

Source: NCI (1978).

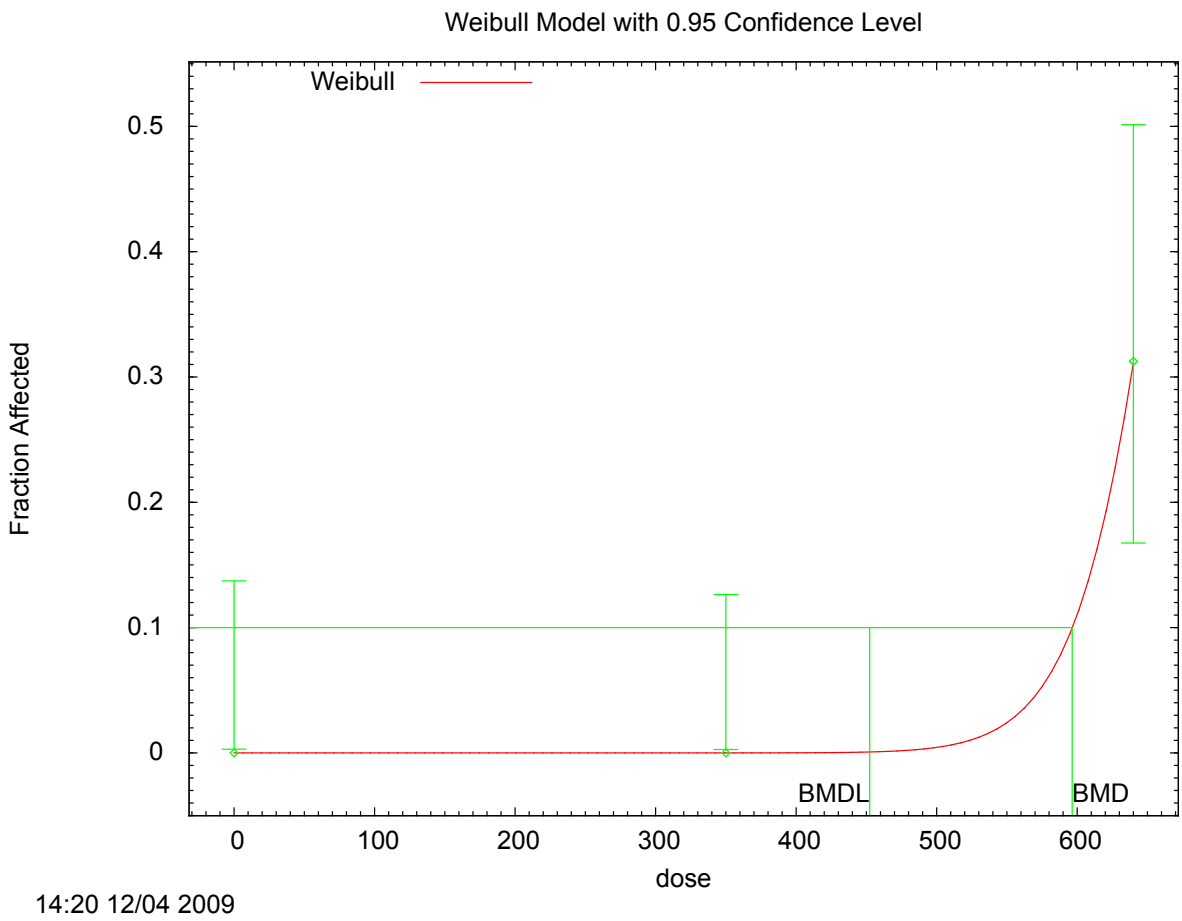


Source: NCI (1978).

Figure C-15 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

```
=====================================================================
Probit Model. (Version: 3.1; Date: 05/16/2008)
Input Data File: C:\14DBMDS\lnp_nci_mrat_cortdeg_Lnp-BMR10-restrict.(d)
Gnuplot Plotting File: C:\14DBMDS\lnp_nci_mrat_cortdeg_Lnp-BMR10-restrict.plt
                                                Mon Feb 01 14:49:17 2010
=====================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is:

 P[response] = Background + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

 where CumNorm(.) is the cumulative normal distribution function

 Dependent variable = Effect
 Independent variable = Dose
 Slope parameter is restricted as slope >= 1

 Total number of observations = 3
 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
 User has chosen the log transformed model
```

```
 Default Initial (and Specified) Parameter Values
 background = 0
 intercept = -5.14038
 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

 95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
background 0 NA
 intercept -5.22131 0.172682 -5.55976 -4.88286
 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 -35.8087 3
 Fitted model -36.084 1 0.550629 2 0.7593
 Reduced model -65.8437 1 60.07 2 <.0001

 AIC: 74.168


 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size 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

 Chi^2 = 0.57 d.f. = 2 P-value = 0.7532


 Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 51.4062
 BMDL = 38.5284
```



Source: NCI (1978).

Figure C-16 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-5.

```
1   ==================================================================
2   Weibull Model using Weibull Model (Version: 2.12; Date: 05/16/2008)
3   Input Data File: Z:\14Dioxane\BMDS\wei_nci_frat_cortdeg_Wei-BMR10-Restrict.(d)
4   Gnuplot Plotting File: Z:\14Dioxane\BMDS\wei_nci_frat_cortdeg_Wei-BMR10-Restrict.plt
5                                          Fri Dec 04 14:20:41 2009
6   ==================================================================
7    BMDS 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
25   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 -1.$

 Parameter Estimates
 95.0% Wald Confidence Interval
Variable Estimate Std. Err. 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
 Scaled
 Dose Est._Prob. Expected Observed Size 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
```

APPENDIX D. DETAILS OF BMD ANALYSIS FOR ORAL CSF FOR 1,4-DIOXANE

Dichotomous models available in the Benchmark Dose Software (BMDS) (version 2.1.1) were fit to the incidence data for hepatocellular carcinoma and/or adenoma for mice and rats, as well as nasal cavity tumors, peritoneal mesotheliomas, and mammary gland adenomas in rats exposed to 1,4-dioxane in the drinking water. Doses associated with a benchmark response (BMR) of a 10% extra risk were calculated. BMD_{10} and $BMDL_{10}$ values from the best fitting model, determined by adequate global- fit (χ^2 $p \geq 0.1$) and AIC values, are reported for each endpoint (U.S. EPA, 2012a). If the multistage cancer model is not the best fitting model for a particular endpoint, the best-fitting multistage cancer model for that endpoint is also presented as a point of comparison.

A summary of the model predictions for the Kano et al. (2009) study are shown in Table D-6. The data and BMD modeling results are presented separately for each dataset as follows:

- Hepatic adenomas and carcinomas in female F344 rats (Table D-7 and Table D-8; Figure D-17)
- Hepatic adenomas and carcinomas in male F344 rats (Table D-9 and Table D-10; Figure D-18 and Figure D-19)
- Significant tumor incidence data at sites other than the liver (i.e., nasal cavity, mammary gland, and peritoneal) in male and female F344 rats (Table D-11)
    - Nasal cavity tumors in female F344 rats (Table D-12; Figure D-20)
    - Nasal cavity tumors in male F344 rats (Table D-13; Figure D-21)
    - Mammary gland adenomas in female F344 rats (Table D-14; Figure D-22 and Figure D-23)
    - Peritoneal mesotheliomas in male F344 rats (Table D-15; Figure D-24 and Figure D-25)
- Hepatic adenomas and carcinomas in female BDF1 mice (Table D-16, Table D-17, and Table D-18; Figure D-26, Figure D-27, Figure D-28, and Figure D-29)
- Hepatic adenomas and carcinomas in male BDF1 mice (Table D-19 and Table D-20; Figure D-30 and Figure D-31)

Data and BMD modeling results from the additional chronic bioassays (NCI, 1978; Kociba et al., 1974a) were evaluated for comparison with the data from Kano et al. (2009). These results are presented as follows:

- 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 (Table D-21)

- Incidence of hepatocellular carcinoma and nasal squamous cell carcinoma in male and female Sherman rats (combined) (Kociba et al., 1974a) treated with 1,4-dioxane in the drinking water for 2 years (Table D-22)
    - BMDS dose-response modeling results for incidence of hepatocellular carcinoma in male and female Sherman rats (combined) (Kociba et al., 1974a) exposed to 1,4-dioxane in drinking water for 2 years (Table D-23; Figure D-32 and Figure D-33)
    - BMDS dose-response modeling results for incidence of nasal squamous cell carcinoma in male and female Sherman rats (combined) (Kociba et al., 1974a) exposed to 1,4-dioxane in the drinking water for 2 years (Table D-24; Figure D-34)
- 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 (Table D-25)
    - BMDS dose-response modeling results for incidence of hepatocellular adenoma in female Osborne-Mendel rats (NCI, 1978) exposed to 1,4-dioxane in the drinking water for 2 years (Table D-26; Figure D-35 and Figure D-36)
    - BMDS dose-response modeling results for 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 (Table D-27; Figure D-37 and Figure D-38)
    - BMDS dose-response modeling results for incidence of nasal cavity squamous cell carcinoma in male Osborne-Mendel rats (NCI, 1978) exposed to 1,4-dioxane in the drinking water for 2 years (Table D-28; Figure D-39 and Figure D-40)
- Incidence of hepatocellular adenoma or carcinoma in male and female $B6C3F_1$ mice (NCI, 1978) exposed to 1,4-dioxane in drinking water (Table D-29)
    - BMDS dose-response modeling results for the combined incidence of hepatocellular adenoma or carcinoma in female $B6C3F_1$ mice (NCI, 1978) exposed to 1,4-dioxane in the drinking water for 2 years (Table D-30; Figure D-41)
    - BMDS dose-response modeling results for incidence of combined hepatocellular adenoma or carcinoma in male $B6C3F_1$ mice (NCI, 1978) exposed to 1,4-dioxane in the drinking water for 2 years (Table D-31; Figure D-42 and Figure D-43).

D.1 General Issues and Approaches to BMDS Modeling

D.1.1 Combining Data on Adenomas and Carcinomas

The incidence of adenomas and the incidence of carcinomas within a dose group at a site or tissue in rodents are sometimes combined. This practice is based upon the hypothesis that adenomas may

develop into carcinomas if exposure at the same dose was continued (U.S. EPA, 2005a; McConnell et al., 1986). The incidence at high doses of both tumors in rat and mouse liver is high in the key study (Kano et al., 2009). The incidence of hepatic adenomas and carcinomas was summed without double-counting them so as to calculate the combined incidence of either a hepatic carcinoma or a hepatic adenoma in rodents.

The variable N is used to denote the total number of animals tested in the dose group. The variable Y is used here to denote the number of rodents within a dose group that have characteristic X, and the notation Y(X) is used to identify the number with a specific characteristic X. Modeling was performed on the adenomas and carcinomas separately and the following combinations of tumor types:

- Y(adenomas) = number of animals with adenomas, whether or not carcinomas are present;
- Y(carcinomas) = number of animals with carcinomas, whether or not adenomas are also present;
- Y(either adenomas or carcinomas) = number of animals with adenomas or carcinomas, not both = Y(adenomas) + Y(carcinomas) – Y(both adenomas and carcinomas);
- Y(neither adenomas nor carcinomas) = number of animals with no adenomas and no carcinomas = N - Y(either adenomas or carcinomas).

D.1.2 Model Selection Criteria

Multiple models were fit to each dataset. The model selection criteria used in the BMD Technical Guidance Document (U.S. EPA, 2012a) were applied as follows:

- *p*-value for goodness-of-fit > 0.10
- AIC smaller than other acceptable models
- χ^2 residuals as small as possible
- No systematic patterns of deviation of model from data

Additional criteria were applied to eliminate implausible dose-response functions:

- Monotonic dose-response functions, e.g. no negative coefficients of polynomials in MS models
- No infinitely steep dose-response functions near 0 (control dose), achieved by requiring the estimated parameters “power” in the Weibull and Gamma models and “slope” in the log-logistic model to have values ≥ 1.

Because no single set of criteria covers all contingencies, an extended list of preferred models are presented below in Table D-6.

D.1.3 Summary

The BMDS models recommended to calculate rodent BMD and BMDL values and corresponding human BMD_{HED} and $BMDL_{HED}$ values are summarized in Table D-6.

Table D-6 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	$BMDL_{HED}$[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 F344 Rat								
Hepatic Tumors	Lowest AIC	Probit	147.787	0.9867	62.20	51.12	17.43	14.33
Peritoneal Meso-thel ioma	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

[a]Values for BMR 10% unless otherwise noted.
[b]BMR 50%.

D.2 Female F344 Rats: Hepatic Carcinomas and Adenomas

The incidence data for hepatic carcinomas and adenomas in female F344 rats (Kano et al., 2009) are shown in Table D-7.

Table D-7 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)

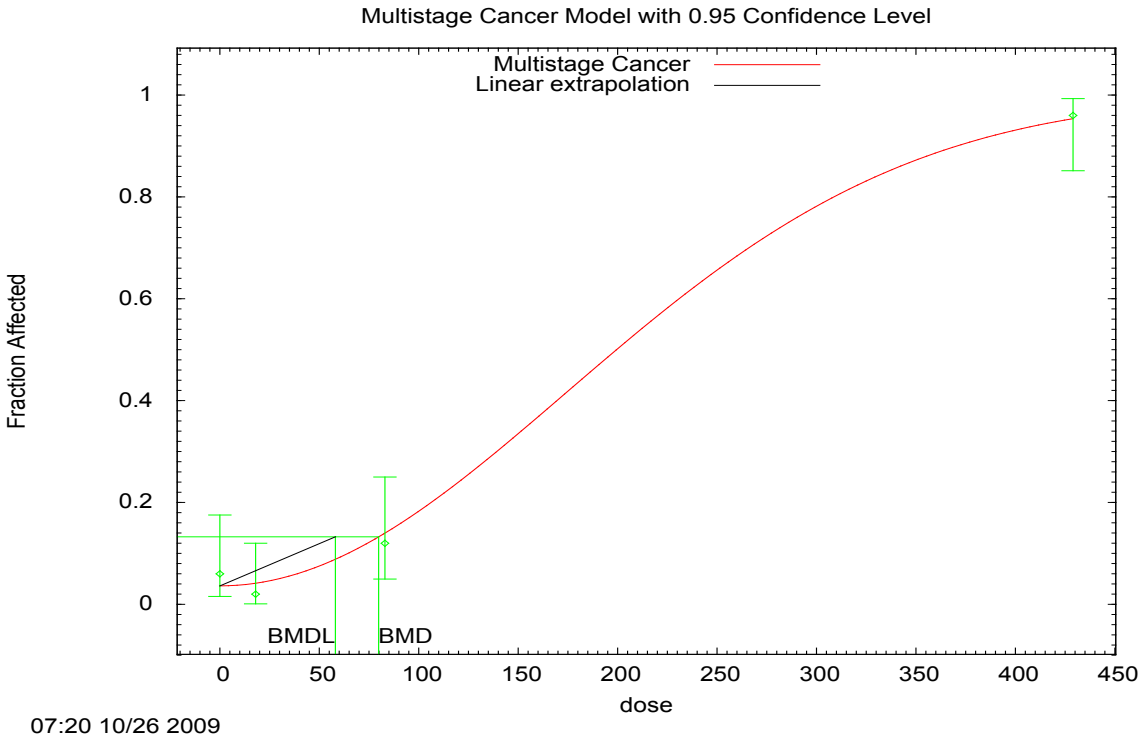
Note that the incidence of rats with adenomas, with carcinomas, and with either adenomas or carcinomas are monotone non-decreasing functions of dose except for 3 female rats in the control group. These data therefore appear to be appropriate for dose-response modeling using BMDS.

The results of the BMDS modeling for the entire suite of models are presented in Table D-8.

Table D-8 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^{2a}	$BMD_{10\ HED}$ 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

[a]Maximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.
[b]Slope restricted ≥ 1.
[c]Best-fitting model.
[d]Value unable to be calculated (NC: not calculated) by BMDS.



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

Figure D-17 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:
4   L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_frat_hepato_adcar_Msc-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
32  Beta(1) = 0
33  Beta(2) = 1.73306e-005
```

```
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 -0.2
Beta(2) -0.2 1

                                  Parameter Estimates
 95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
Background 0.0362773 * * *
Beta(1) 0 * * *
Beta(2) 1.65328e-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 -42.9938 4
 Fitted model -43.7949 2 1.60218 2 0.4488
 Reduced model -120.43 1 154.873 3 <.0001

 AIC: 91.5898

 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size 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

Chi^2 = 1.59 d.f. = 2 P-value = 0.4516

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 79.8299
 BMDL = 58.085
 BMDU = 94.0205

Taken together, (58.085 , 94.0205) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00172161
```

D.3 Male F344 Rats: Hepatic Carcinomas and Adenomas

The data for hepatic adenomas and carcinomas in male F344 rats (Kano et al., 2009) are shown in Table D-9.

Table D-9 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 Elservier, Ltd., Kano et al. (2009).

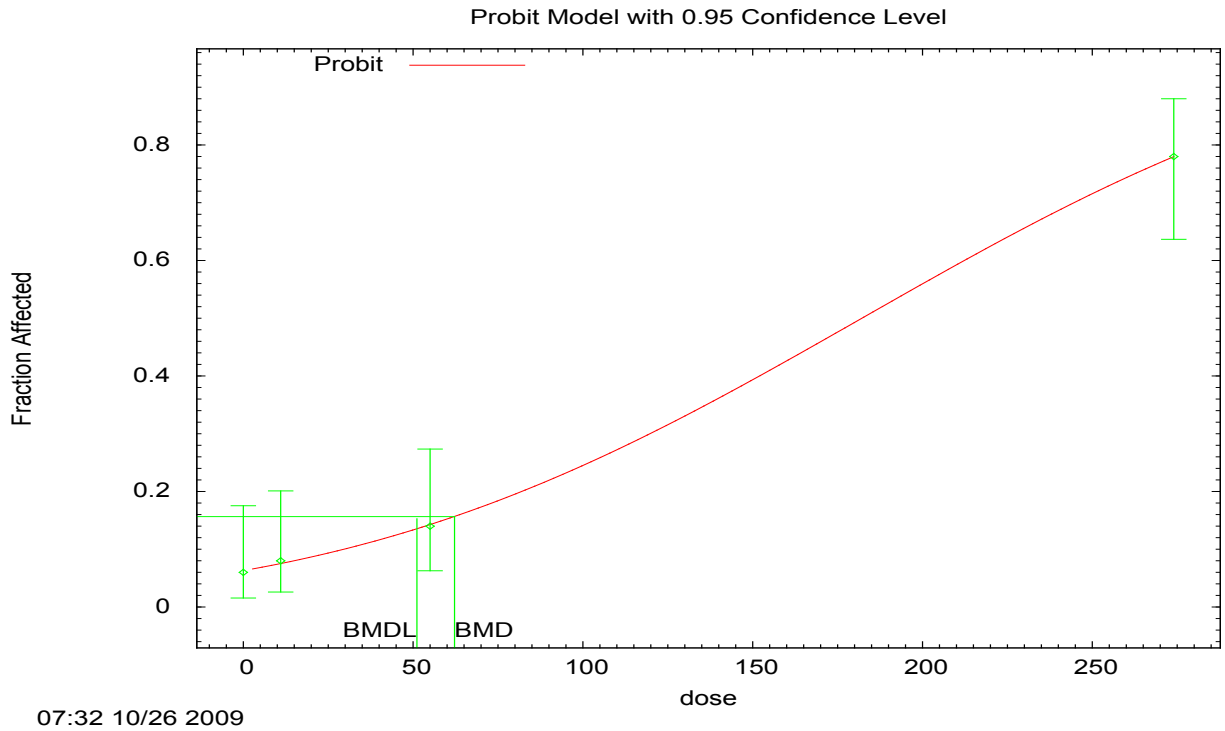
Note that the incidence of rats with hepatic adenomas, carcinomas, and with either adenomas or carcinomas are monotone non-decreasing functions of dose. These data therefore appear to be appropriate for dose-response modeling using BMDS.

The results of the BMDS modeling for the entire suite of models tested using the data for hepatic adenomas and carcinomas for male F344 rats are presented in Table D-10.

Table D-10 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^{2a}	$BMD_{10\ HED}$ 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

[a]Maximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.
[b]Slope restricted ≥ 1.
[c]Best-fitting model.
[d]Value unable to be calculated (NC: not calculated) by BMDS.

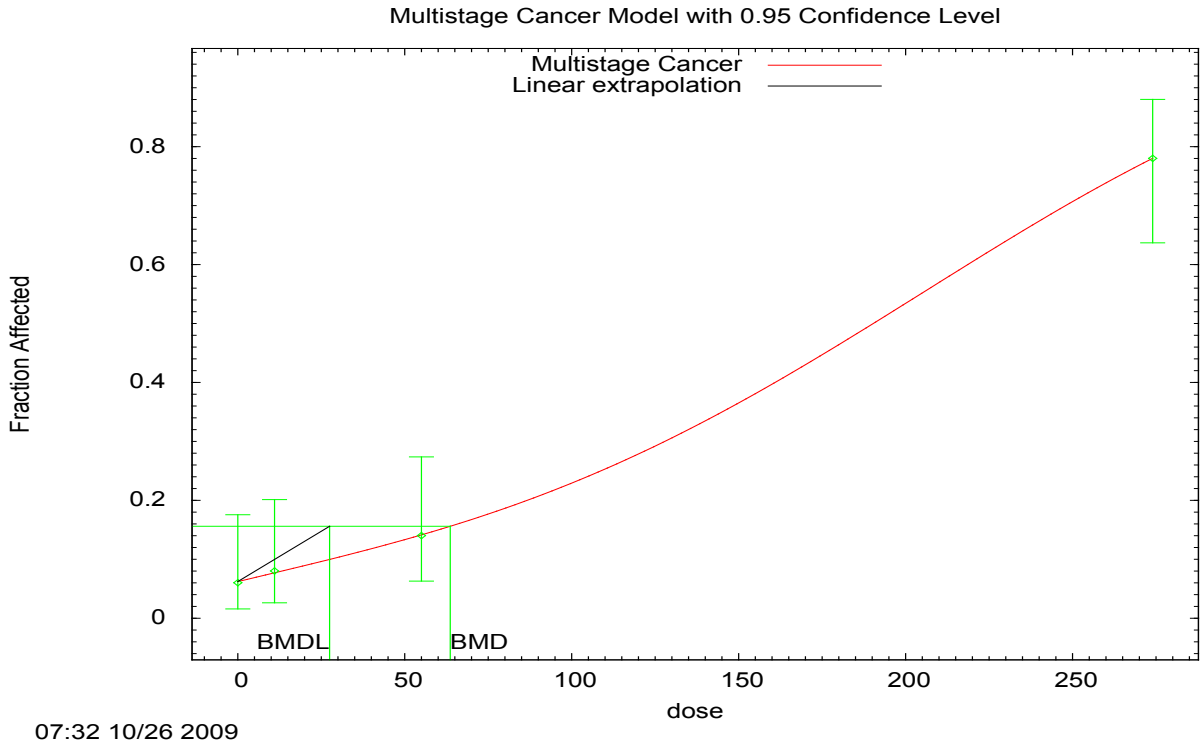


Source: Used with permission from Elservier, Ltd., Kano et al. (2009).

Figure D-18 Probit BMD model for the combined incidence of hepatic adenomas and carcinomas in male F344 rats.

```
==================================================================
Probit Model. (Version: 3.1; Date: 05/16/2008)
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\pro_kano2009_mrat_hepato_adcar_Prb-BMR10.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\pro_kano2009_mrat_hepato_adcar_Prb-BMR10.plt
Mon Oct 26 08:32:08 2009
=================================================================
BMDS Model Run
~~~
```

```
 intercept slope
intercept 1 -0.69
slope -0.69 1


                                    Parameter Estimates
 95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
intercept 1.53138 0.160195 -1.84535 -1.2174
slope 0.00840347 0.000976752 0.00648907 0.0103179


 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


 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size 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

 Chi^2 = 0.03 d.f. = 2 P-value = 0.9867

 Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 62.1952
 BMDL = 51.1158
```



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

Figure D-19 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:
4   L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mrat_hepato_adcar_Msc-BMR10-3poly.(d)
5   Gnuplot Plotting File:
6   L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mrat_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 +
14  (1-background)*[1-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
32  Background = 0.0623822
33  Beta(1) = 0.00142752
```

```
Beta(2) = 0
Beta(3) = 5.14597e-008
Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -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(1) Beta(3)
Background 1 -0.67 0.58
Beta(1) -0.67 1 -0.95
Beta(3) 0.58 -0.95 1


 Parameter Estimates

 95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
Background 0.0619918 * * *
Beta(1) 0.001449 * * *
Beta(2) 0 * * *
Beta(3) 5.11829e-008 * * *

* - Indicates that this value is not calculated.



 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


 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size 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

 Chi^2 = 0.01 d.f. = 1 P-value = 0.9171


 Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 63.6179
 BMDL = 27.4913
 BMDU = 123.443

Taken together, (27.4913, 123.443) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00363752
```

D.4 F344 Rats: Tumors at Other Sites

The data for tumors at sites other than the liver in male and female F344 rats (Kano et al., 2009) are shown in Table D-11. Note that the incidence of rats with these endpoints are monotone non-decreasing functions (except female peritoneal mesotheliomas). These data therefore appear to be appropriate for dose-response modeling using BMDS.

Table D-11 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

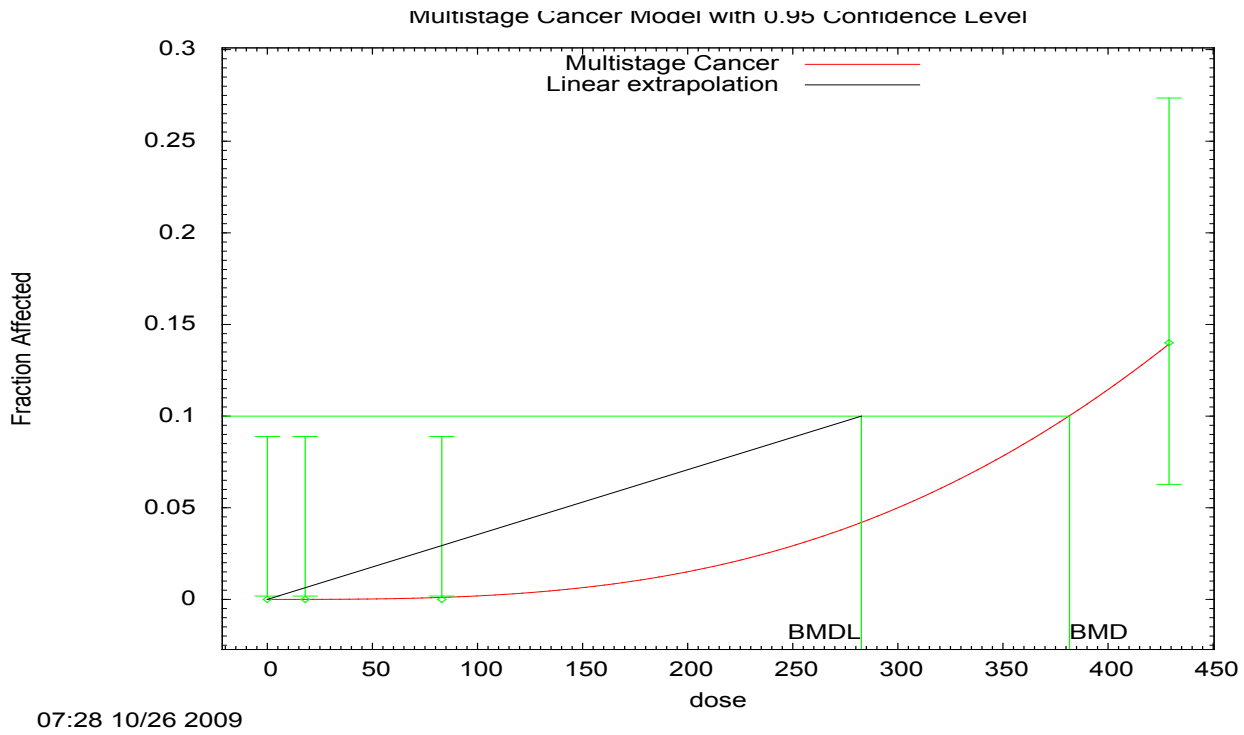
Source: Used with permission from Elsevier, Ltd., Kano et al., (2009).

The results of the BMDS modeling for the entire suite of models are presented in Table D-12 through Table D-15 for tumors in the nasal cavity, mammary gland, and peritoneal cavity.

Table D-12 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^{2b}	$BMD_{10\ HED}$ 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

[a]Nasal cavity tumors in female F344 rats include squamous cell carcinoma and esthesioneuro-epithelioma.
[b]Maximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.
[c]Slope restricted ≥ 1.
[d]Best-fitting model.



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

Figure D-20 Multistage BMD model (3 degree) for nasal cavity tumors in female F344 rats.

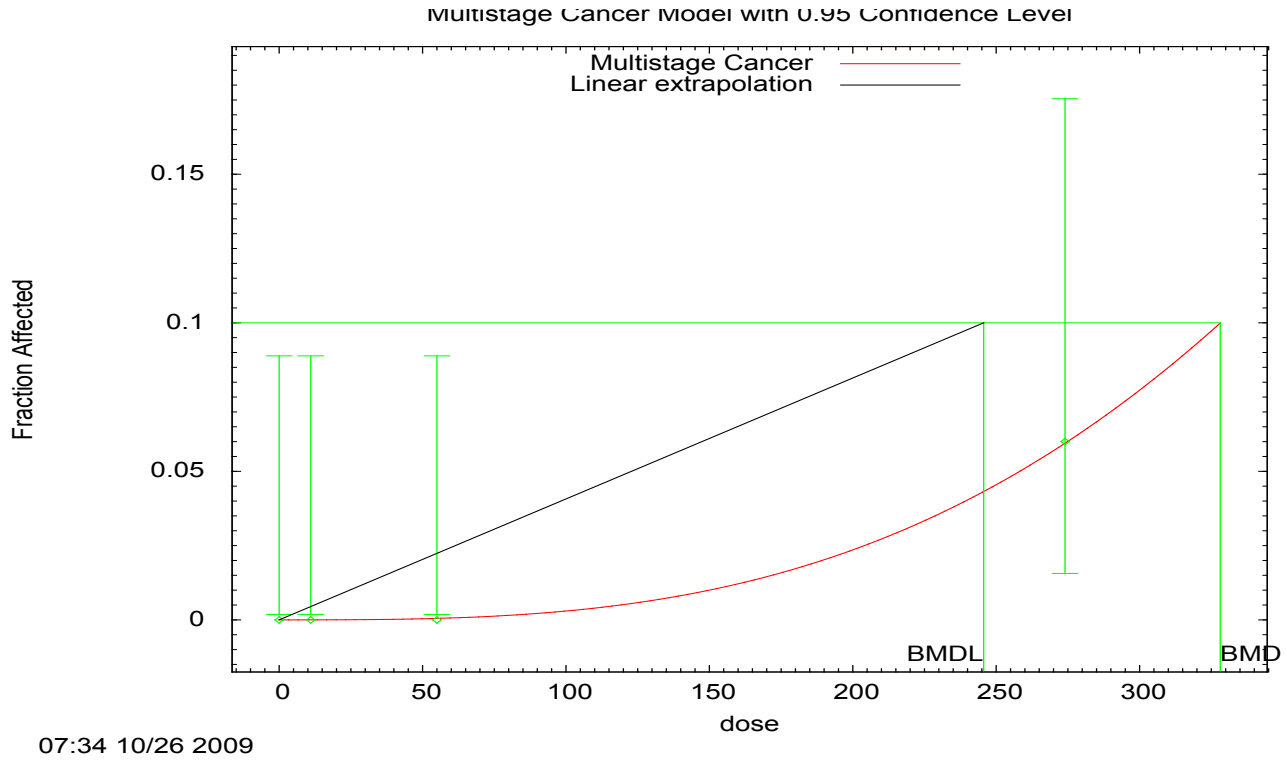
```
1   ======================================================================
2   Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
3   Input Data File:
4   L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_frat_nasal_car_Msc-BMR10-3poly.(d)
5   Gnuplot Plotting File:
6   L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_frat_nasal_car_Msc-BMR10-3poly.plt
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 +
12  (1-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
33  Asymptotic Correlation Matrix of Parameter Estimates
34  (*** The model parameter(s) -Background -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 )

 Beta(3)
 Beta(3) 1

 Parameter Estimates

 95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
Background 0 * * *
Beta(1) 0 * * *
Beta(2) 0 * * *
Beta(3) 1.89531e-009 * * *

* - Indicates that this value is not calculated.


 Analysis of Deviance Table

 Model Log(likelihood) # Param's Deviance Test d.f. P-value
 Full model -20.2482 4
 Fitted model -20.3031 1 0.109908 3 0.9906
 Reduced model -30.3429 1 20.1894 3 0.0001551

 AIC: 42.6063


 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size Residual
 ---------------------------------------------------------------------
 0.0000 0.0000 0.000 0.000 50 0.000
 18.0000 0.0000 0.001 0.000 50 -0.024
 83.0000 0.0011 0.054 0.000 50 -0.233
 429.0000 0.1390 6.949 7.000 50 0.021

 Chi^2 = 0.06 d.f. = 3 P-value = 0.9966


 Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 381.651
 BMDL = 282.609
 BMDU = 500.178

Taken together, (282.609, 500.178) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000353846
```

Table D-13 BMDS dose-response modeling results for the incidence of nasal cavity tumors in male F344 rats[a] (Kano et al., 2009)

Model	AIC	*p*-value	BMD_{10} mg/kg-day	$BMDL_{10}$ mg/kg-day	χ^{2b}	$BMD_{10\ HED}$ 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

[a]Nasal cavity tumors in male F344 rats include squamous cell carcinoma, Sarcoma: NOS, rhabdomyosarcoma, and esthesioneuro-epithelioma.
[b]Maximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.
[c]Slope restricted ≥ 1.
[d]Best-fitting model.



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

Figure D-21 Multistage BMD model (3 degree) for nasal cavity tumors in male F344 rats.

```
==================================================================
Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
```

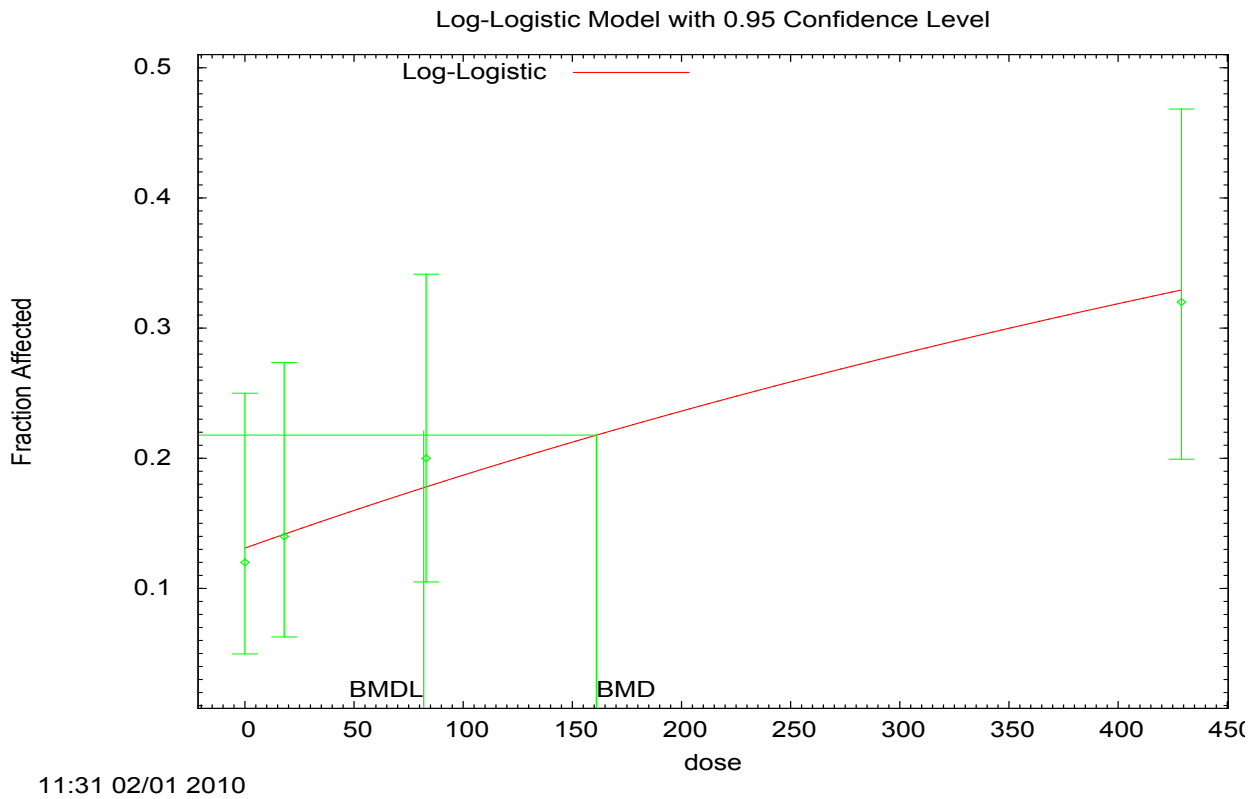
```
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mrat_nasal_car_Msc-BMR10-3poly.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mrat_nasal_car_Msc-BMR10-3poly.plt
Mon Oct 26 08:34:20 2009
=================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
The form of the probability function is: P[response] = background +
(1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2-beta3*dose^3)]

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 = 4
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) = 3.01594e-009


Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background -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 )

 Beta(3)
 Beta(3) 1


 Parameter Estimates

 95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
Background 0 * * *
Beta(1) 0 * * *
Beta(2) 0 * * *
Beta(3) 2.98283e-009 * * *

* - Indicates that this value is not calculated.



 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


 Goodness of Fit
 Scaled
```

```
 Dose Est._Prob. Expected Observed Size 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

 Chi^2 = 0.03 d.f. = 3 P-value = 0.9989


 Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 328.108
 BMDL = 245.634
 BMDU = 1268.48

Taken together, (245.634, 1268.48) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00040711
```

Table D-14 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^{2a}	$BMD_{10\ HED}$ 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

[a]Maximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.
[b]Best-fitting model.
[c]Slope restricted ≥ 1.
[d]Value unable to be calculated (NC: not calculated) by BMDS.

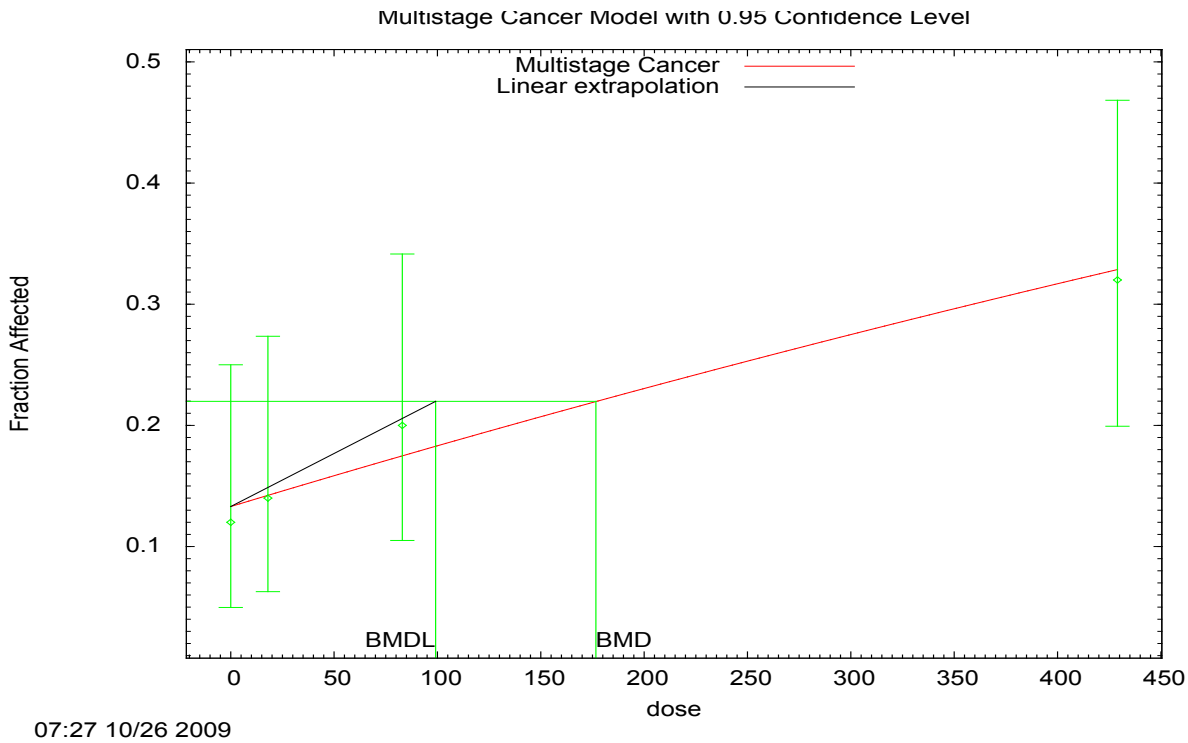


Source: Use with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-22 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
30
```

```
(*** 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

 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. 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
 Scaled
 Dose Est._Prob. Expected Observed Size 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-23 Multistage BMD model (1 degree) for mammary gland adenomas in female F344 rats.

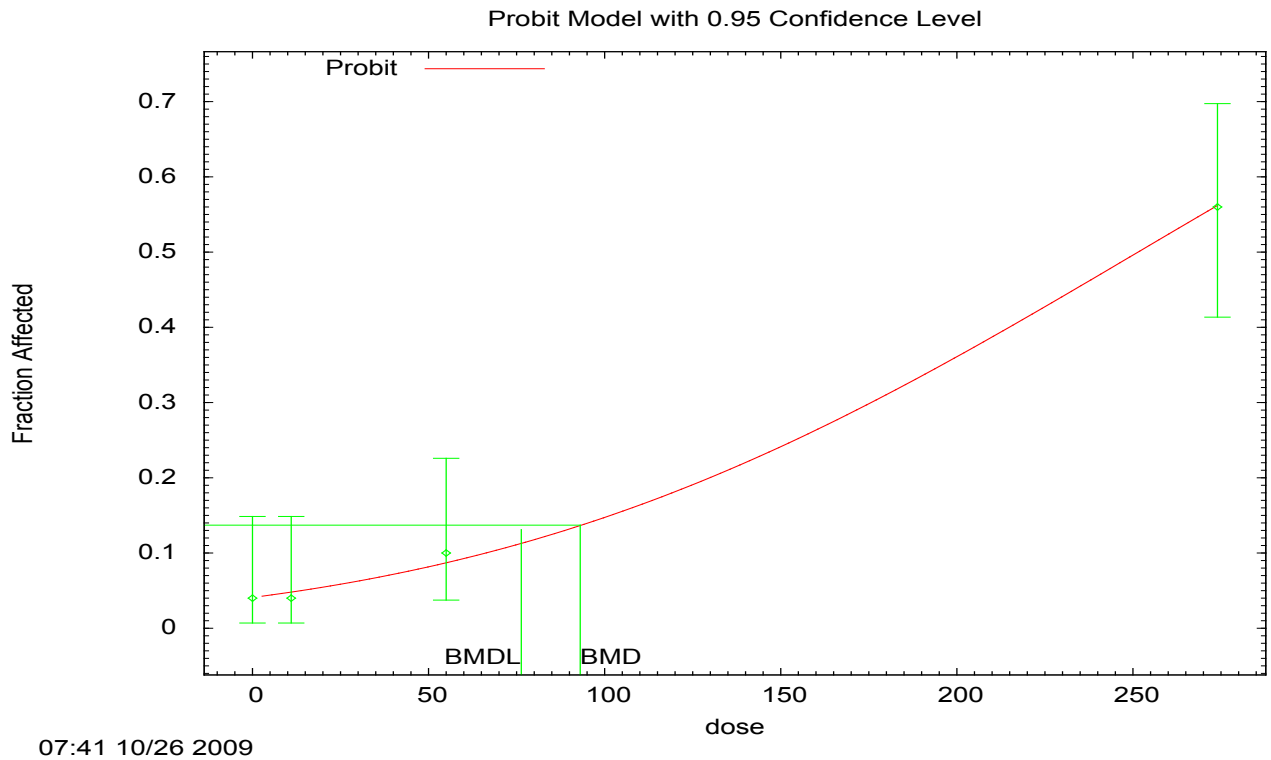
```
=================================================================
Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_frat_mamm_ad_Msc-BMR10-1poly.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_frat_mamm_ad_Msc-BMR10-1poly.plt
Mon Oct 26 08:27:02 2009
=================================================================
 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 = 0.136033
Beta(1) = 0.000570906
Asymptotic Correlation Matrix of Parameter Estimates

```

```
 Background Beta(1)
Background 1 -0.58
Beta(1) -0.58 1


Parameter Estimates

 95.0% Wald Confidence Interval Variable Estimate Std. Err. Lower Conf. Limit Upper
Conf. Limit
Background .133161 * * *
Beta(1) 0.000596394 * * *

* - 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 -95.111 2 0.305898 2 0.8582
 Reduced model -98.6785 1 7.4409 3 0.0591

 AIC: 194.222


 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size Residual
 ---------------------------------------------------------------------
 0.0000 0.1332 6.658 6.000 50 -0.274
 18.0000 0.1424 7.121 7.000 50 -0.049
 83.0000 0.1750 8.751 10.000 50 0.465
 429.0000 0.3288 16.442 16.000 50 -0.133

 Chi^2 = 0.31 d.f. = 2 P-value = 0.8559


 Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 176.663
 BMDL = 99.1337
 BMDU = 501.523

Taken together, (99.1337, 501.523) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00100874
```

Table D-15 BMDS dose-response modeling results for the incidence of peritoneal mesotheliomas in male F344 rats (Kano et al., 2009)

Model	AIC	*p*-value	BMD_{10} mg/kg-day	$BMDL_{10}$ mg/kg-day	χ^{2a}	$BMD_{10\ HED}$ 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

[a]Maximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.
[b]Slope restricted ≥ 1.
[c]Best-fitting model.
[d]Value unable to be calculated (NC: not calculated) by BMDS.

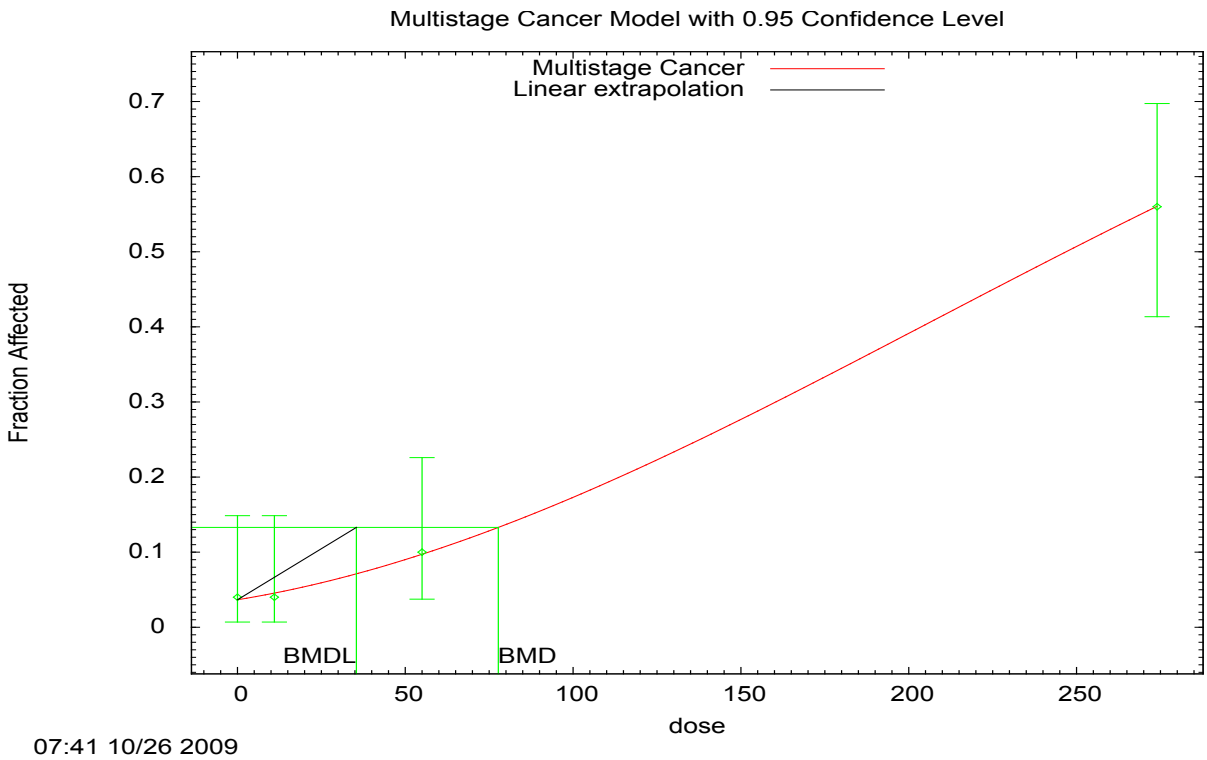


Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-24 Probit BMD model for peritoneal mesotheliomas in male F344 rats.

```
==================================================================
Probit Model. (Version: 3.1; Date: 05/16/2008)
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\pro_kano2009_mrat_peri_meso_Prb-BMR10.(d)
```

```
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\pro_kano2009_mrat_peri_meso_Prb-BMR10.plt
Mon Oct 26 08:41:29 2009
====================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the probability function is: P[response] = CumNorm(Intercept+Slope*Dose),
where CumNorm(.) is the cumulative normal distribution function

Dependent variable = Effect
Independent variable = Dose
Slope parameter is not restricted

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 (and Specified) Parameter Values
background = 0 Specified
intercept = -1.73485
slope = 0.00692801

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 -0.75
slope -0.75 1

                                  Parameter Estimates
 95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
intercept -1.73734 0.18348 -2.09695 -1.37772
slope 0.00691646 0.000974372 0.00500672 0.00882619

 Analysis of Deviance Table
 Model Log(likelihood) # Param's Deviance Test d.f. P-value
 Full model -67.3451 4
 Fitted model -67.4344 2 0.178619 2 0.9146
 Reduced model -95.7782 1 56.8663 3 <.0001
 AIC: 138.869

 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size Residual
 ---------------------------------------------------------------------
 0.0000 0.0412 2.058 2.000 50 -0.041
 11.0000 0.0483 2.417 2.000 50 -0.275
 55.0000 0.0874 4.370 5.000 50 0.315
 274.0000 0.5627 28.134 28.000 50 -0.038

 Chi^2 = 0.18 d.f. = 2 P-value = 0.9148
 Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 93.0615
 BMDL = 76.3242
```



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

Figure D-25 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:
4   L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mrat_peri_meso_Msc-BMR10-2poly.(d)
5   Gnuplot Plotting File:
6   L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mrat_peri_meso_Msc-BMR10-2poly.plt
7   Mon Oct 26 08:41:28 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-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
32  Default Initial Parameter Values
33  Background = 0.0358706
```

```
Beta(1) = 0.000816174
Beta(2) = 7.47062e-006


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

                                  Parameter Estimates
 95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
Background 0.0366063 * * *
Beta(1) 0.000757836 * * *
Beta(2) 7.6893e-006 * * *

* - Indicates that this value is not calculated.

 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


 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size 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

 Chi^2 = 0.06 d.f. = 1 P-value = 0.8135


 Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 77.7277
 BMDL = 35.4296
 BMDU = 118.349

Taken together, (35.4296, 118.349) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0028225
```

D.5 Female BDF1 Mice: Hepatic Carcinomas and Adenomas

Data for female BDF1 mouse hepatic carcinomas and adenomas are shown in Table D-16. Note that the incidence of carcinomas and the incidence of either adenomas or carcinomas are monotone non-decreasing functions of dose. These data therefore appear to be appropriate for dose-response modeling using BMDS. However, the incidence of adenomas clearly reaches a peak value at

66 mg/kg-day and then decreases sharply with increasing dose. This cannot be modeled by a multistage model using only non-negative coefficients. To some extent the incidence of "either adenomas or carcinomas" retains some of the inverted-U shaped dose-response of the adenomas, which dominate based on their high incidence at the lowest dose groups (66 and 278 mg/kg-day), thus is not well characterized by any multistage model.

Table D-16 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).

The results of the BMDS modeling for the entire suite of models for hepatic adenomas and carcinomas in female BDF1 mice are presented in Table D-17. The multistage models did not provide reasonable fits to the incidence data for hepatocellular adenoma or carcinoma in female BDF1 mice. The log-logistic model provided the best-fit to the data as indicated by the AIC and *p*-value as was chosen as the best-fitting model to carry forward in the analysis; however, this model resulted in a $BMDL_{10}$ much lower than the response level at the lowest dose in the study (Kano et al., 2009). Thus, the log-logistic model was run for BMRs of 30 and 50%. The output from these models are shown in Figures D-11 and D-12. A summary of the BMD results for BMRs of 10, 30, and 50% are shown in Table D-18. Using a higher BMR resulted in BMDLs closer to the lowest observed response data, and a BMR of 50% was chosen to carry forward in the analysis.

The graphical output from fitting these models suggested that a simpler model obtained by dropping the data point for the highest dose (964 mg/kg-day) might also be adequate. This was tested and the results did not affect the choice of the model, nor significantly affect the resulting BMDs and BMDLs.

Table D-17 BMDS dose-response modeling results for the combined incidence of hepatic adenomas and carcinomas in female BDF1 mice (Kano et al., 2009)

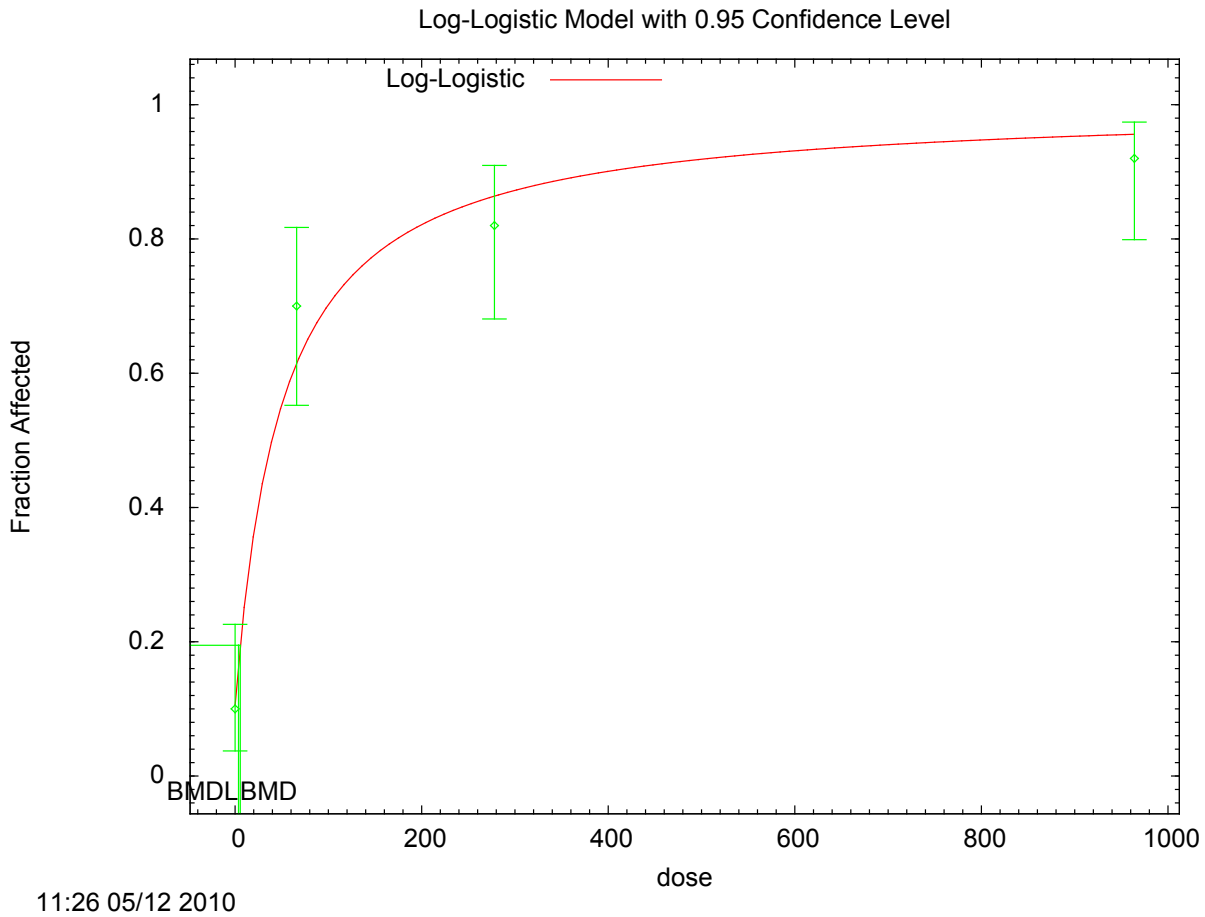
Model	AIC	*p*-value	BMD_{10} mg/kg-day	$BMDL_{10}$ mg/kg-day	χ^{2a}	$BMD_{10\ HED}$ mg/kg-day	$BMDL_{10\ HED}$ mg/kg-day
Gamma	203.331	0	26.43	19.50	-2.654	3.98	2.94
Logistic	214.951	0	58.05	44.44	3.201	8.74	6.69
LogLogistic[b]	176.214	0.1421	5.54	3.66	-0.121	0.83	0.55
LogProbit[c]	198.354	0	26.37	19.57	-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

[a]Maximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.
[b]Best-fitting model, lowest AIC value.
[c]Slope restricted ≥ 1.
[d]Value unable to be calculated (NC: not calculated) by BMDS.

Table D-18 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	*p*-value	BMD mg/kg-day	BMDL mg/kg-day	χ^{2a}	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

[a]Maximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.

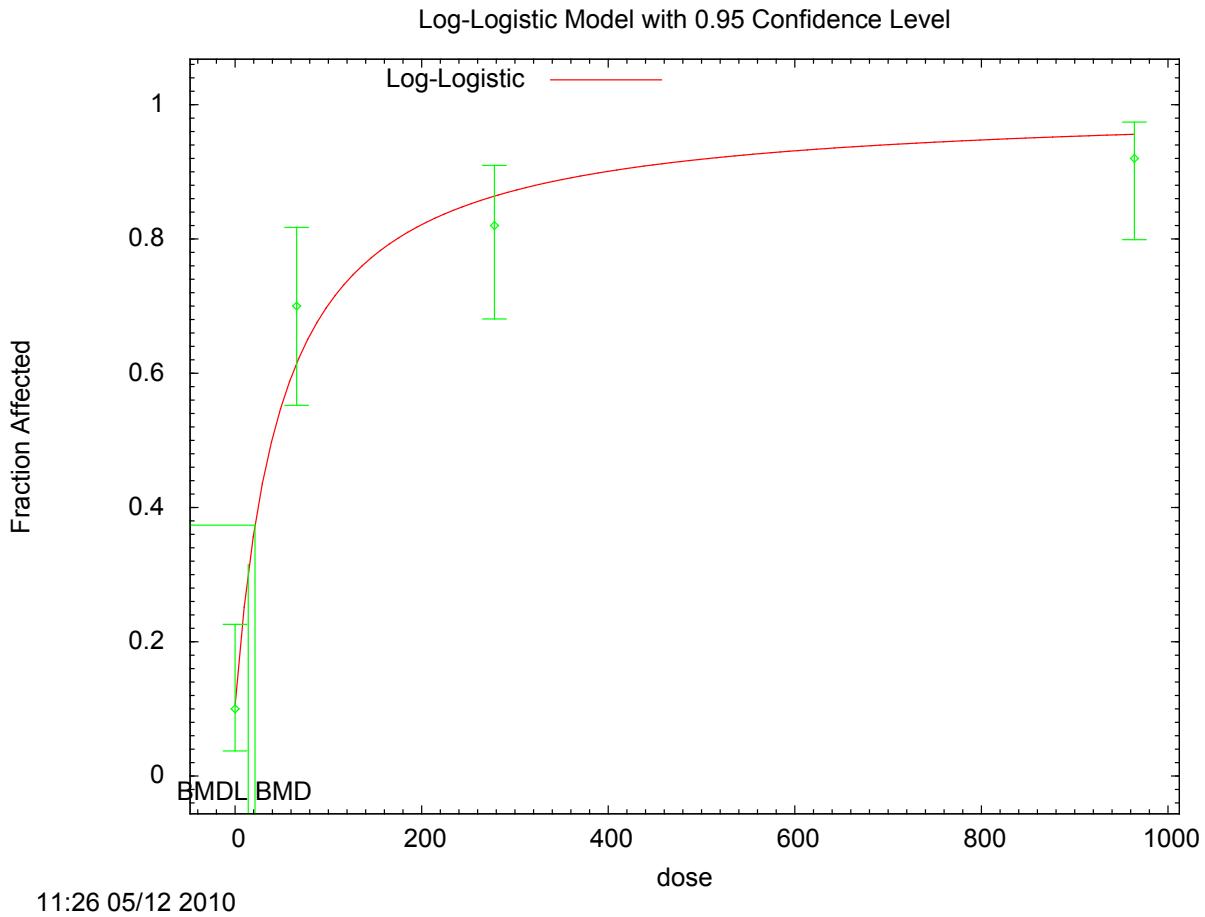


Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-26 LogLogistic BMD model for the combined incidence of hepatic adenomas and carcinomas in female BDF1 mice with a BMR of 10%.

```
==================================================================
Logistic Model. (Version: 2.12; Date: 05/16/2008)
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_fmouse_hepato_adcar_Lnl-BMR10-Restrict.(
d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_fmouse_hepato_adcar_Lnl-BMR10-Restrict.p
lt
                                                Wed May 12 11:26:35 2010
==================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 The form of the probability function is:
 P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

 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

 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

 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. 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
 Scaled
 Dose Est._Prob. Expected Observed Size 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

```

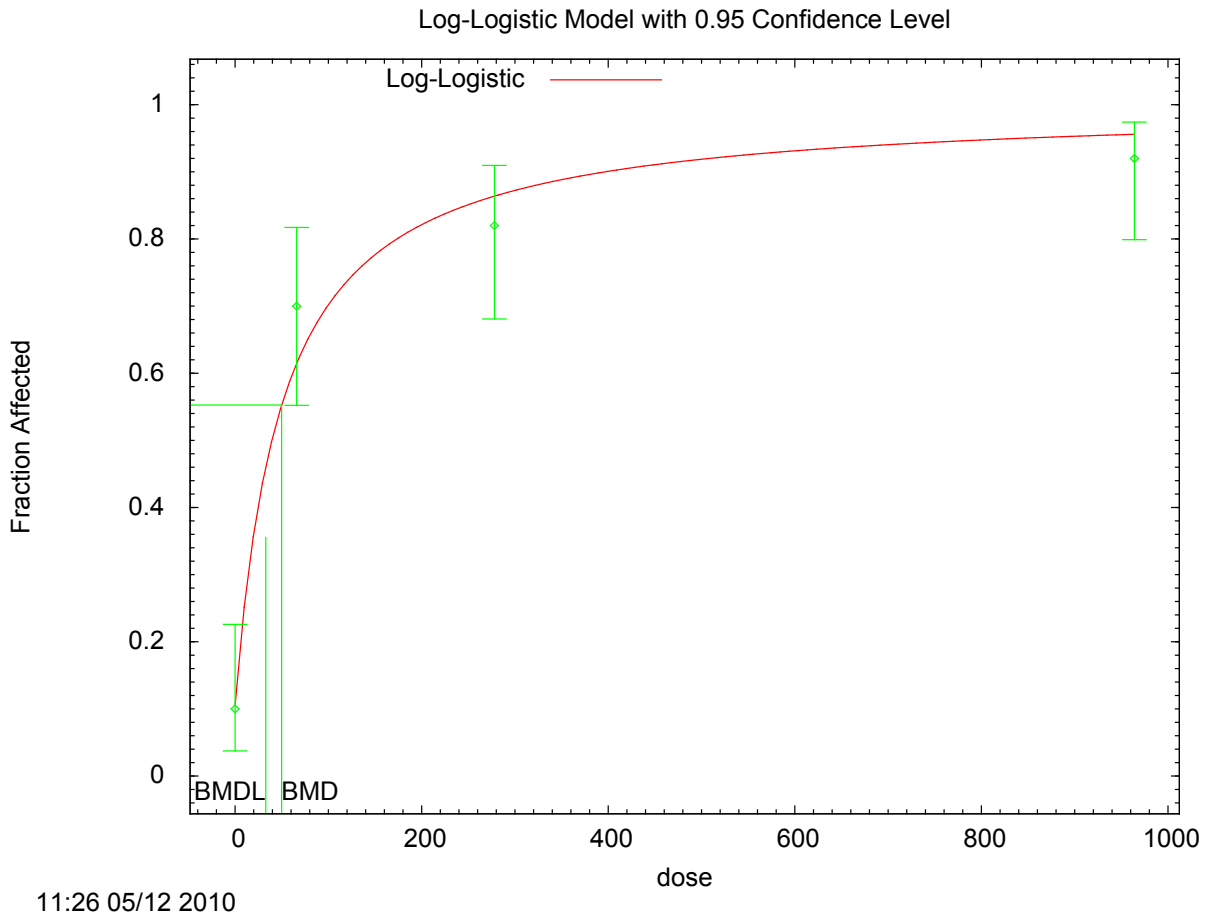


Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-27 LogLogistic BMD model for the combined incidence of hepatic adenomas and carcinomas in female BDF1 mice with a BMR of 30%.

```
==================================================================
Logistic Model. (Version: 2.12; Date: 05/16/2008)
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_fmouse_hepato_adcar_Lnl-BMR30-Restrict.(
d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_fmouse_hepato_adcar_Lnl-BMR30-Restrict.p
lt
                                                Wed May 12 11:26:36 2010
==================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 The form of the probability function is:
 P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

 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
 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

 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. 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
 Scaled
 Dose Est._Prob. Expected Observed Size 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
```

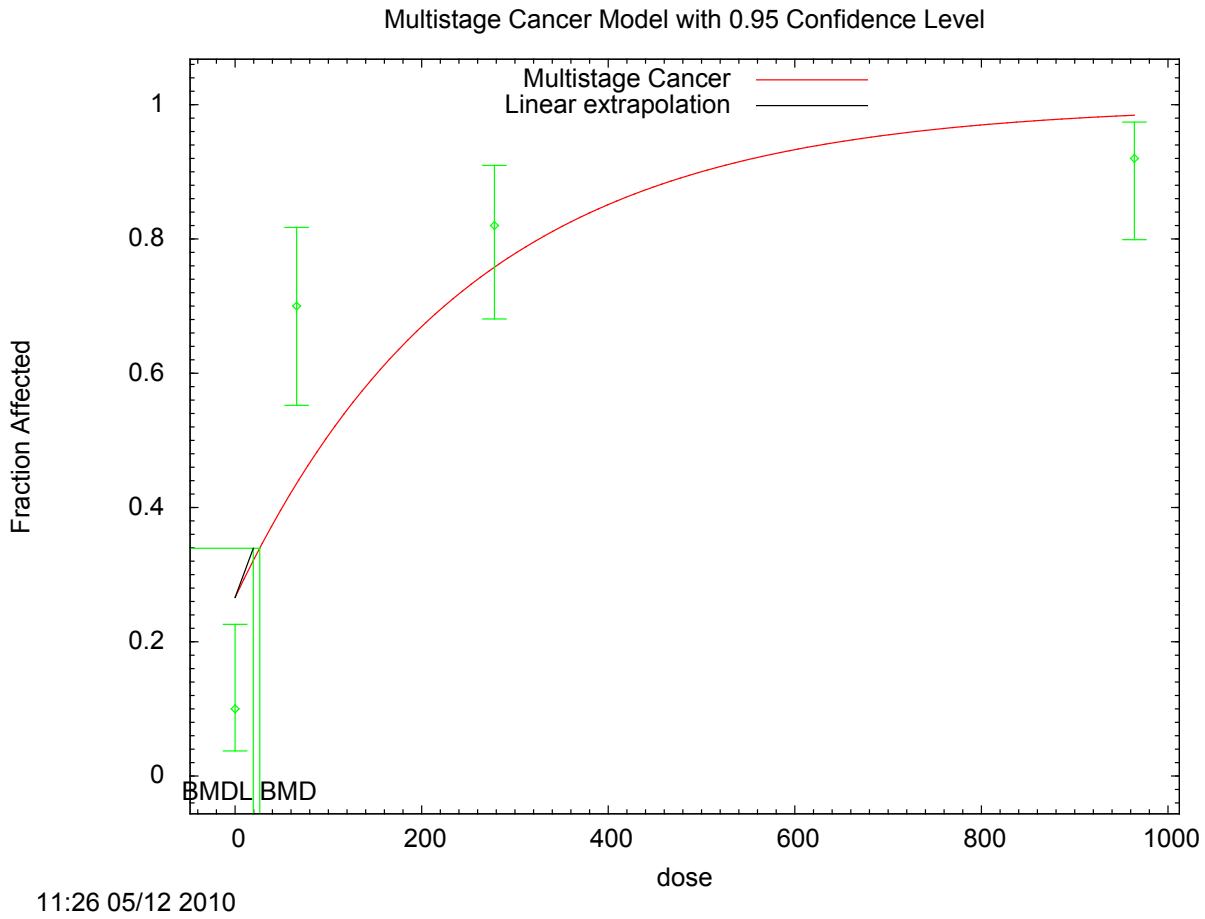


Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-28 LogLogistic BMD model for the combined incidence of hepatic adenomas and carcinomas in female BDF1 mice with a BMR of 50%.

```
==================================================================
Logistic Model. (Version: 2.12; Date: 05/16/2008)
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_fmouse_hepato_adcar_Lnl-BMR50-Restrict.(
d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_fmouse_hepato_adcar_Lnl-BMR50-Restrict.p
lt
                                                Wed May 12 11:26:36 2010
==================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 The form of the probability function is:
 P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

 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

 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

 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. 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
 Scaled
 Dose Est._Prob. Expected Observed Size 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
```



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

Figure D-29 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    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
25   Maximum number of iterations = 250
26   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

 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. 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
 Scaled
 Dose Est._Prob. Expected Observed Size 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

Data for hepatic carcinomas and adenomas in male BDF1 mice (Kano et al., 2009) are shown in Table D-19. Note that the incidence of carcinomas and the incidence of either adenomas or carcinomas are monotone non-decreasing functions of dose. These data therefore appear to be appropriate for dose-response modeling using BMDS. However, the incidence of adenomas clearly reaches a peak value

at 191 mg/kg-day and then decreases sharply with increasing dose. This cannot be modeled by a multistage model using only non-negative coefficients. To some extent the incidence of "either adenomas or carcinomas or both" retains some of the inverted-U shaped dose-response of the adenomas, which dominate based on their high incidence at the lowest dose groups (49 and 191 mg/kg-day), thus is not well characterized by any multistage model.

Table D-19 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

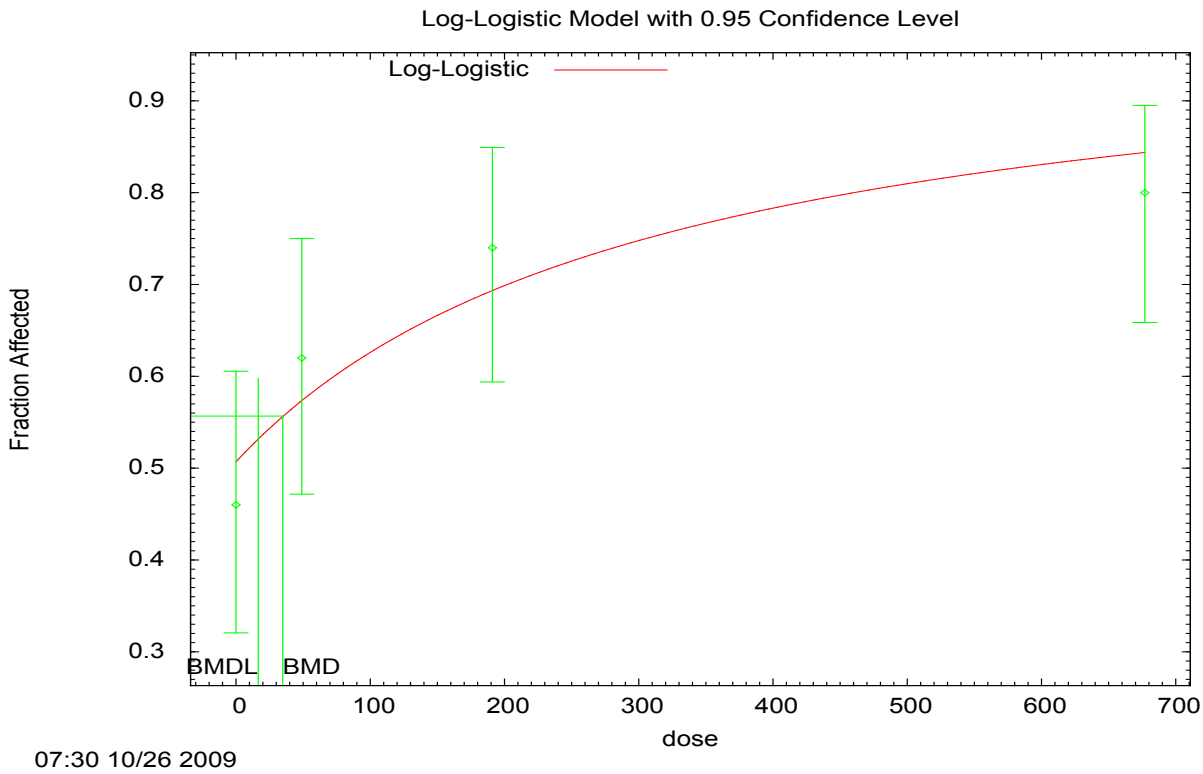
Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

The results of the BMDS modeling for the entire suite of models for hepatic adenomas and carcinomas in male BDF1 mice are presented in Table D-20.

Table D-20 BMDS dose-response modeling results for the combined incidence of hepatic adenomas and carcinomas in male BDF1 mice (Kano et al., 2009)

Model	AIC	*p*-value	BMD_{10} mg/kg-day	$BMDL_{10}$ mg/kg-day	χ^{2a}	$BMD_{10\ HED}$ 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

[a]Maximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.
[b]Best-fitting model.
[c]Slope restricted ≥ 1.
[d]Value unable to be calculated (NC: not calculated) by BMDS.

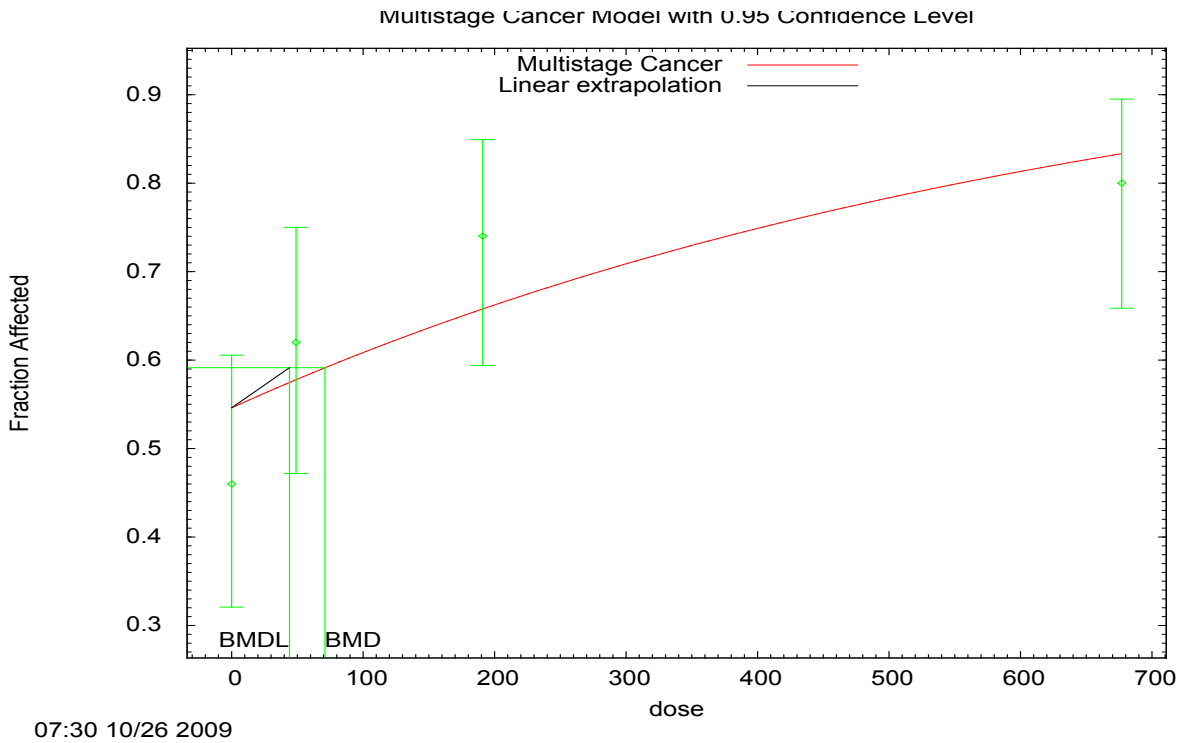


Source: Used with permission from Elsevier, Ltd., Kano et al. (2009).

Figure D-30 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-Restrict.(
5   d)
6   Gnuplot Plotting File:
7   L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_mmouse_hepato_adcar_Lnl-BMR10-Restrict.p
8   lt
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
27
28  Default Initial Parameter Values
29   background = 0.46
30   intercept = -5.58909
31   slope = 1
32   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

 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. 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
 Scaled
 Dose Est._Prob. Expected Observed Size 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
```



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

Figure D-31 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
30  Default Initial Parameter Values
31  Background = 0.573756
32  Beta(1) = 0.00123152
33
34  Asymptotic Correlation Matrix of Parameter Estimates
```

```
 Background Beta(1)
Background 1 -0.58
Beta(1) -0.58 1


Parameter Estimates

 95.0% Wald Confidence Interval
Variable Estimate Std. Err. 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
 Scaled
 Dose Est._Prob. Expected Observed Size 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

Data and BMDS modeling results for the additional chronic bioassays (NCI, 1978; Kociba et al., 1974a) were evaluated for comparison with the Kano et al. (2009) study. These results are presented in the following sections.

The BMDS dose-response modeling estimates and HEDs that resulted are presented in detail in the following sections and a summary is provided in Table D-21.

Table D-21 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., (1974a) 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

[a]Incidence of hepatocellular carcinoma.
[b]Incidence of nasal squamous cell carcinoma.
[c]Incidence of hepatocellular adenoma.
[d]Incidence of hepatocellular adenoma or carcinoma.

D.7.1 Hepatocellular Carcinoma and Nasal Squamous Cell Carcinoma (Kociba et al., 1974a)

The incidence data for hepatocellular carcinoma and nasal squamous cell carcinoma are presented in Table D-22. The predicted $BMD_{10\ HED}$ and $BMDL_{10\ HED}$ values are also presented in Table D-23 and Table D-24 for hepatocellular carcinomas and nasal squamous cell carcinomas, respectively.

Table D-22 Incidence of hepatocellular carcinoma and nasal squamous cell carcinoma in male and female Sherman rats (combined) (Kociba et al., 1974a) 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
1,307	10/66[d]	3/66[d]

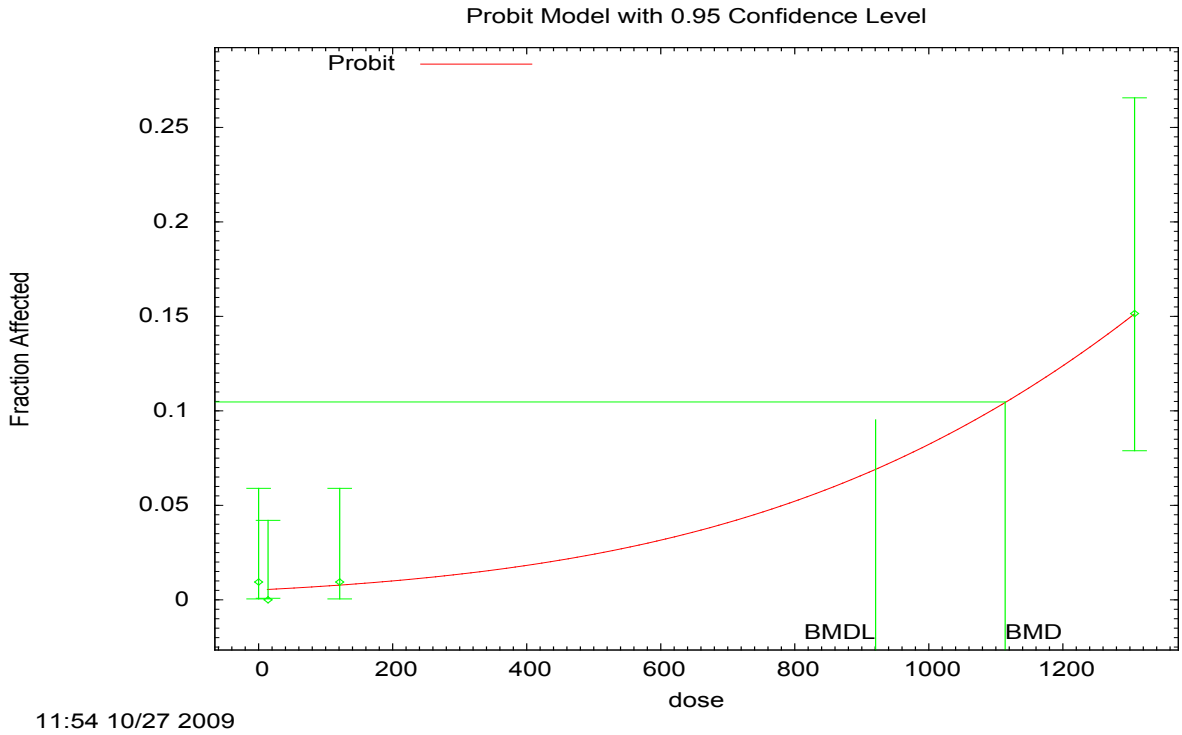
[a]Rats surviving until 12 months on study.
[b]$p < 0.001$; positive dose-related trend (Cochran-Armitage test).
[c]$p < 0.01$; positive dose-related trend (Cochran-Armitage test).
[d]$p < 0.001$; Fisher's Exact test.

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

Table D-23 BMDS dose-response modeling results for the incidence of hepatocellular carcinoma in male and female Sherman rats (combined) (Kociba et al., 1974a) exposed to 1,4-dioxane in the drinking water for 2 years

Model	AIC	*p*-value	BMD_{10} mg/kg-day	$BMDL_{10}$ mg/kg-day	χ^{2a}	$BMD_{10\ HED}$ 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

[a]Maximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.
[b]Slope restricted ≥ 1.
[c]Best-fitting model.
[d]Value unable to be calculated (NC: not calculated) by BMDS.

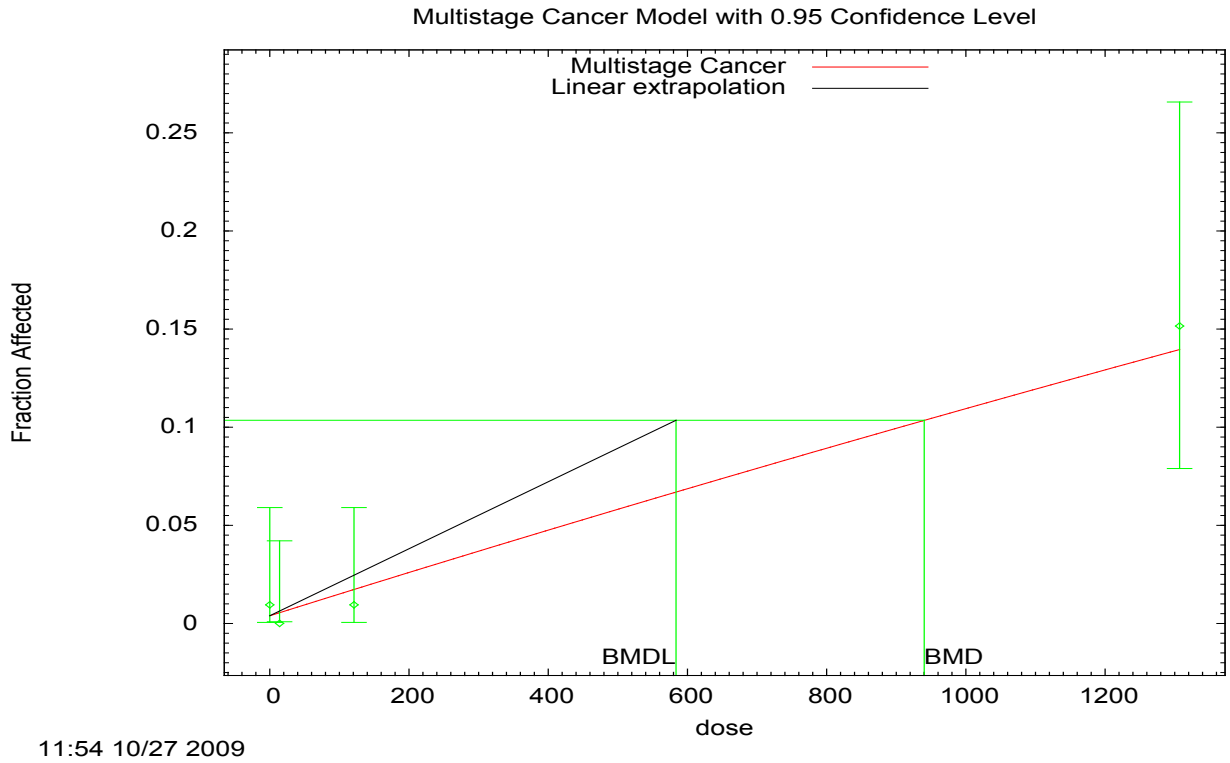


Source: Used with permission from Elsevier, Ltd., Kociba et al. (1974a).

Figure D-32 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:
4   L:\Priv\NCEA_HPAG\14Dioxane\BMDS\pro_kociba_mf_rat_hepato_car_Prb-BMR10.(d)
5   Gnuplot Plotting File:
6   L:\Priv\NCEA_HPAG\14Dioxane\BMDS\pro_kociba_mf_rat_hepato_car_Prb-BMR10.plt
7   Tue Oct 27 12:54:14 2009
8   ====================================================================
9    BMDS Model Run
10  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
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
29  slope = 0.0012323
30  Asymptotic Correlation Matrix of Parameter Estimates
31  (*** The model parameter(s) -background have been estimated at a boundary point, or
32  have been specified by the user, and do not appear in the correlation matrix )
```

```
 intercept slope
intercept 1 -0.82
slope -0.82 1


Parameter Estimates

 95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
intercept -2.55961 0.261184 -3.07152 -2.0477
slope 0.00117105 0.000249508 0.000682022 0.00166008


 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

 AIC: 84.3126


 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size 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

 Chi^2 = 1.00 d.f. = 2 P-value = 0.6060


 Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 1,113.94
 BMDL = 920.616
```



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

Figure D-33 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.

```
=====================================================================
Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kociba_mf_rat_hepato_car_Msc-BMR10-1poly.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kociba_mf_rat_hepato_car_Msc-BMR10-1poly.plt
Tue Oct 27 12:54:10 2009
=====================================================================
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 = 0.000925988
```

```
Beta(1) = 0.000124518


Asymptotic Correlation Matrix of Parameter Estimates
 Background Beta(1)
Background 1 -0.44
Beta(1) -0.44 1


Parameter Estimates

 95.0% Wald Confidence Interval
Variable Estimate Std. Err. 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
 Scaled
 Dose Est._Prob. Expected Observed Size 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 = 1,685.88

Taken together, (583.576, 1685.88) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000171357
```

Table D-24 BMDS dose-response modeling results for the incidence of nasal squamous cell carcinoma in male and female Sherman rats (combined) (Kociba et al., 1974a) exposed to 1,4-dioxane in the drinking water for 2 years

Model	AIC	*p*-value	BMD_{10} mg/kg-day	$BMDL_{10}$ mg/kg-day	χ^{2a}	$BMD_{10\ HED}$ mg/kg-day	$BMDL_{10\ HED}$ mg/kg-day
Gamma	28.4078	1	1,572.09	1,305.86	0	410.37	340.87
Logistic	28.4078	1	1,363.46	1,306.67	0	355.91	341.09
LogLogistic	28.4078	1	1,464.77	1,306.06	0	382.35	340.93
LogProbit[b]	28.4078	1	1,644.38	1,305.49	0	429.24	340.78
Multistage-Cancer (1 degree)	27.3521	0.9163	3,464.76	1,525.36	0.272	904.42	398.17
Multistage-Cancer (2 degree)	26.4929	0.9977	1,980.96	1,314.37	0.025	517.10	343.10
Multistage-Cancer (3 degree)[c]	26.4156	0.9999	1,717.16	1,306.29	0.002	448.24	340.99
Probit	28.4078	1	1,419.14	1,306.44	0	370.44	341.03
Weibull	28.4078	1	1,461.48	1,306.11	0	381.50	340.94
Quantal-Linear	27.3521	0.9163	3,464.76	1,525.35	0.272	904.42	398.17
Dichotomous-Hill	30.4078	0.9997	1,465.77	1319.19	5.53×10^{-7}	382.62	344.35

[a]Maximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.
[b]Slope restricted ≥ 1.
[c]Best-fitting model.

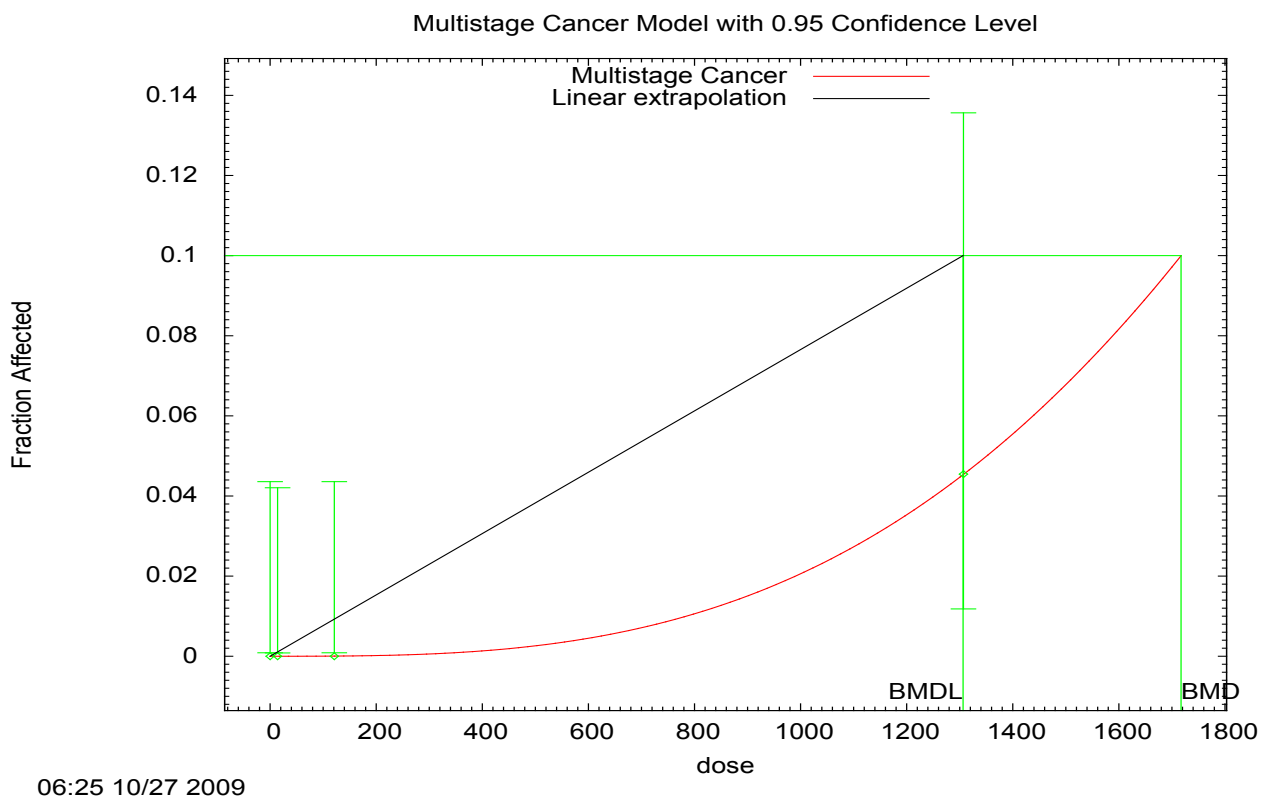


Figure D-34 Multistage BMD model (3 degree) for the incidence of nasal squamous cell carcinoma in male and female Sherman rats exposed to 1,4-dioxane in drinking water.

```
==================================================================
Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kociba_mf_rat_nasal_car_Msc-BMR10-3poly.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kociba_mf_rat_nasal_car_Msc-BMR10-3poly.plt
Tue Oct 27 07:25:02 2009
==================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the probability function is:

P[response] = background +
(1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2-beta3*dose^3)]

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 = 4
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) = 2.08414e-011


Asymptotic Correlation Matrix of Parameter Estimates

 (*** The model parameter(s) -Background -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 )

 Beta(3)
 Beta(3) 1


                                  Parameter Estimates

 95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
Background 0 * * *
Beta(1) 0 * * *
Beta(2) 0 * * *
Beta(3) 2.08088e-011 * * *

* - Indicates that this value is not calculated.



 Analysis of Deviance Table

 Model Log(likelihood) # Param's Deviance Test d.f. P-value
 Full model -12.2039 4
 Fitted model -12.2078 1 0.00783284 3 0.9998
 Reduced model -17.5756 1 10.7433 3 0.0132
```

```
 AIC: 26.4156


 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size Residual
 ----------------------------------------------------------------------
 0.0000 0.0000 0.000 0.000 106 0.000
 14.0000 0.0000 0.000 0.000 110 -0.003
 121.0000 0.0000 0.004 0.000 106 -0.063
 1307.0000 0.0454 2.996 3.000 66 0.002

 Chi^2 = 0.00 d.f. = 3 P-value = 0.9999


 Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 1,717.16
 BMDL = 1,306.29
 BMDU = 8,354.46

Taken together, (1306.29, 8354.46) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 7.65529e-005
```

D.7.2 Nasal Cavity Squamous Cell Carcinoma and Liver Hepatocellular Adenoma in Osborne-Mendel Rats (NCI, 1978)

The incidence data for hepatocellular adenoma (female rats) and nasal squamous cell carcinoma (male and female rats) are presented in Table D-25. The log-logistic model adequately fit both the male and female rat nasal squamous cell carcinoma data, as well as female hepatocellular adenoma incidence data. For all endpoints and genders evaluated in this section, compared to the multistage models, the log-logistic model had a higher *p*-value, as well as both a lower AIC and lower BMDL. The results of the BMDS modeling for the entire suite of models are presented in Table D-26 through Table D-28.

Table D-25 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]

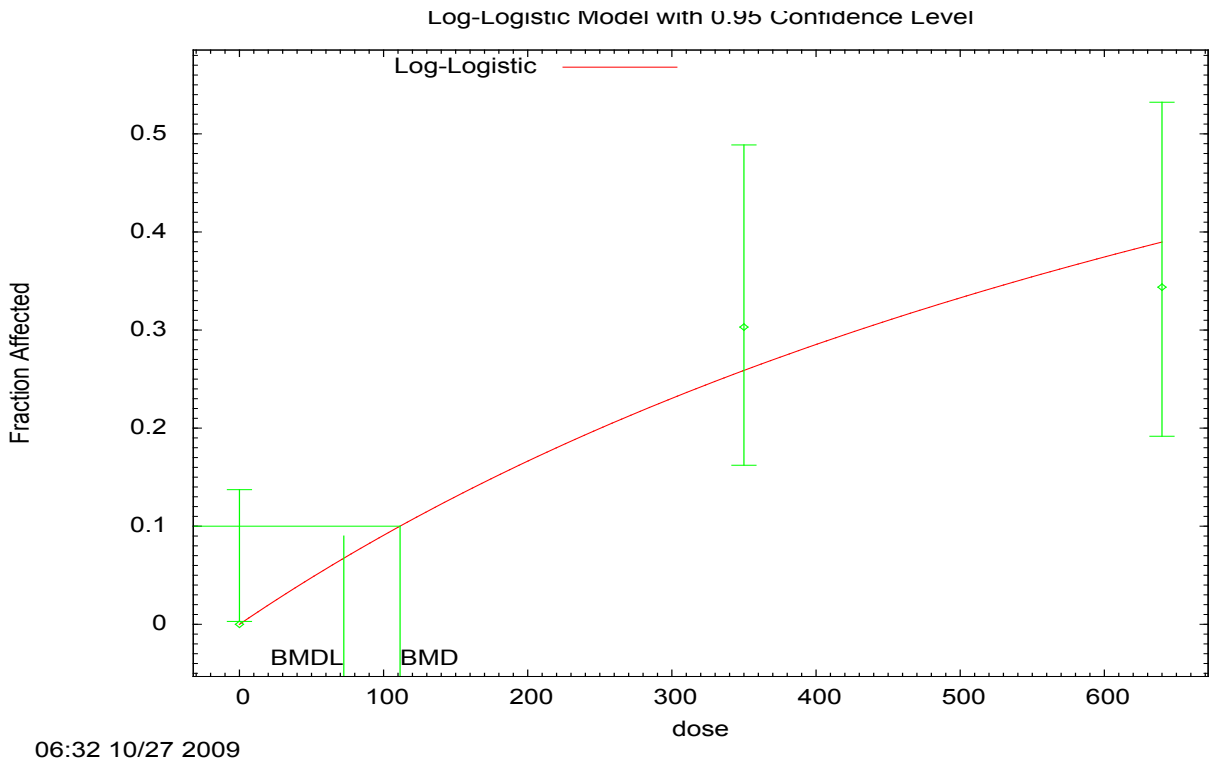
[a]Tumor incidence values were adjusted for mortality (NCI, 1978).
[b]Group not included in statistical analysis by NCI (1978) because the dose group was started a year earlier without appropriate controls.
[c]$p \leq 0.001$; positive dose-related trend (Cochran-Armitage test).
[d]$p \leq 0.001$; Fisher's Exact test.

Source: NCI (1978).

Table D-26 BMDS dose-response modeling results for the incidence of hepatocellular adenoma in female Osborne-Mendel rats (NCI, 1978) exposed to 1,4-dioxane in the drinking water for 2 years

Model	AIC	*p*-value	BMD_{10} mg/kg-day	$BMDL_{10}$ mg/kg-day	χ^{2a}	$BMD_{10\ HED}$ 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

[a]Maximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.
[b]Best-fitting model.

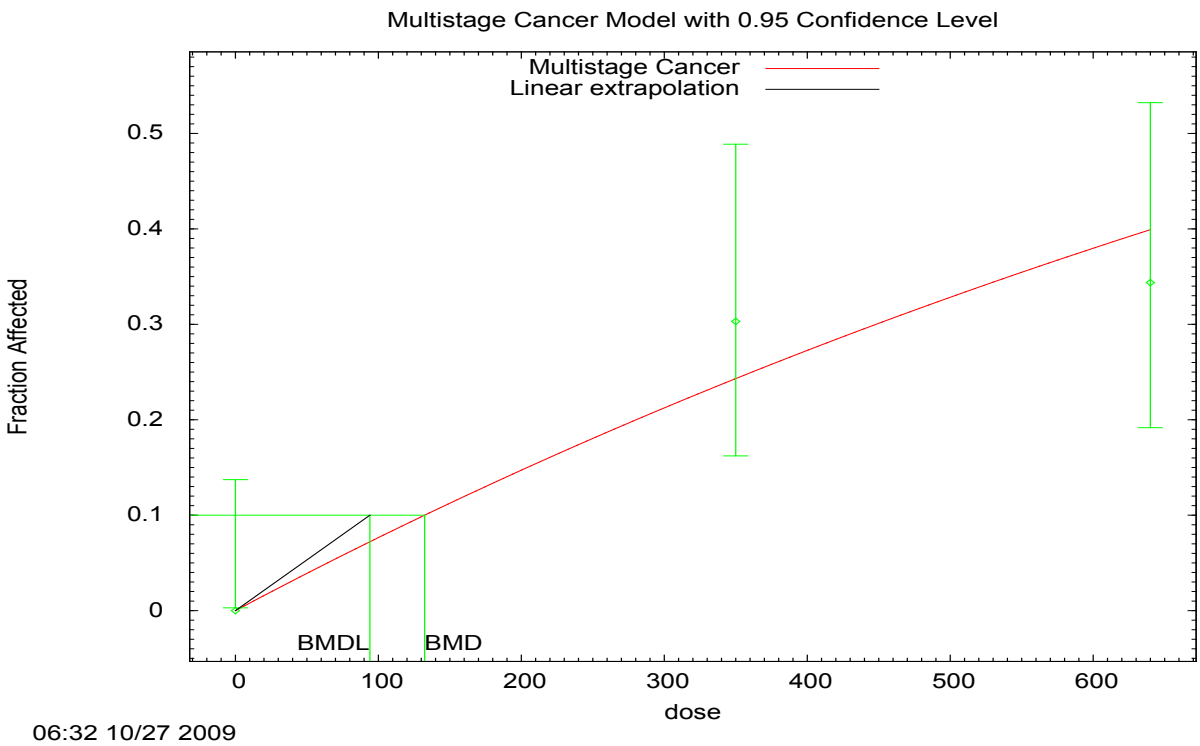


Source: NCI (1978).

Figure D-35 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:
4   L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_nci_frat_hepato_ad_Lnl-BMR10-Restrict.(d)
5   Gnuplot Plotting File:
6   L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_nci_frat_hepato_ad_Lnl-BMR10-Restrict.plt
7   Tue Oct 27 07:32:13 2009
8   ==================================================================
9    BMDS 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
30
31  Asymptotic Correlation Matrix of Parameter Estimates
32
```

```
(*** 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

 95.0% Wald Confidence Interval
Variable Estimate Std. Err. 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
 Scaled
 Dose Est._Prob. Expected Observed Size 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
```



Source: NCI (1978).

Figure D-36 Multistage BMD model (1 degree) for the incidence of hepatocellular adenoma in female Osborne-Mendel rats exposed to 1,4-dioxane in drinking water.

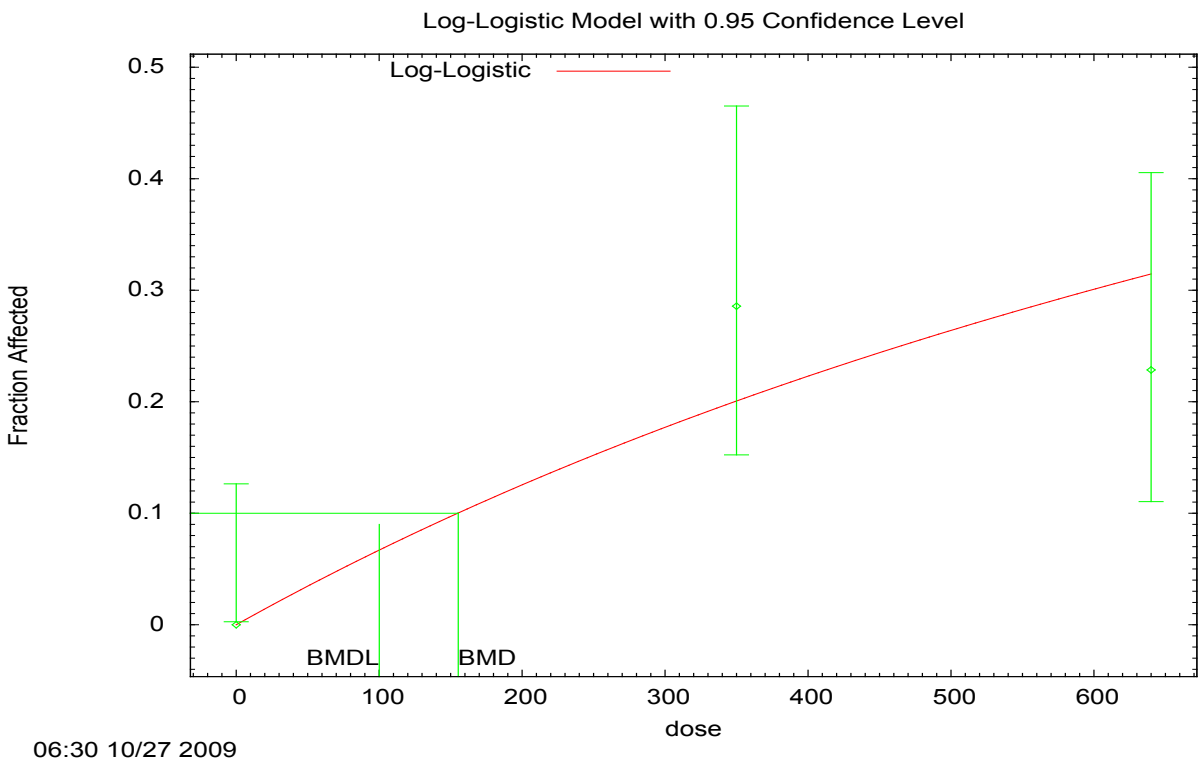
```
1   ==================================================================
2   Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
3   Input Data File:
4   L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_frat_hepato_ad_Msc-BMR10-1poly.(d)
5   Gnuplot Plotting File:
6   L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_frat_hepato_ad_Msc-BMR10-1poly.plt
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(-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 = 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
31
32  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

 95.0% Wald Confidence Interval
Variable Estimate Std. Err. 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
 Scaled
 Dose Est._Prob. Expected Observed Size 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-27 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	*p*-value	BMD_{10} mg/kg-day	$BMDL_{10}$ mg/kg-day	χ^{2a}	$BMD_{10\ HED}$ 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

[a]Maximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.
[b]Best-fitting model.
[c]Slope restricted ≥ 1.



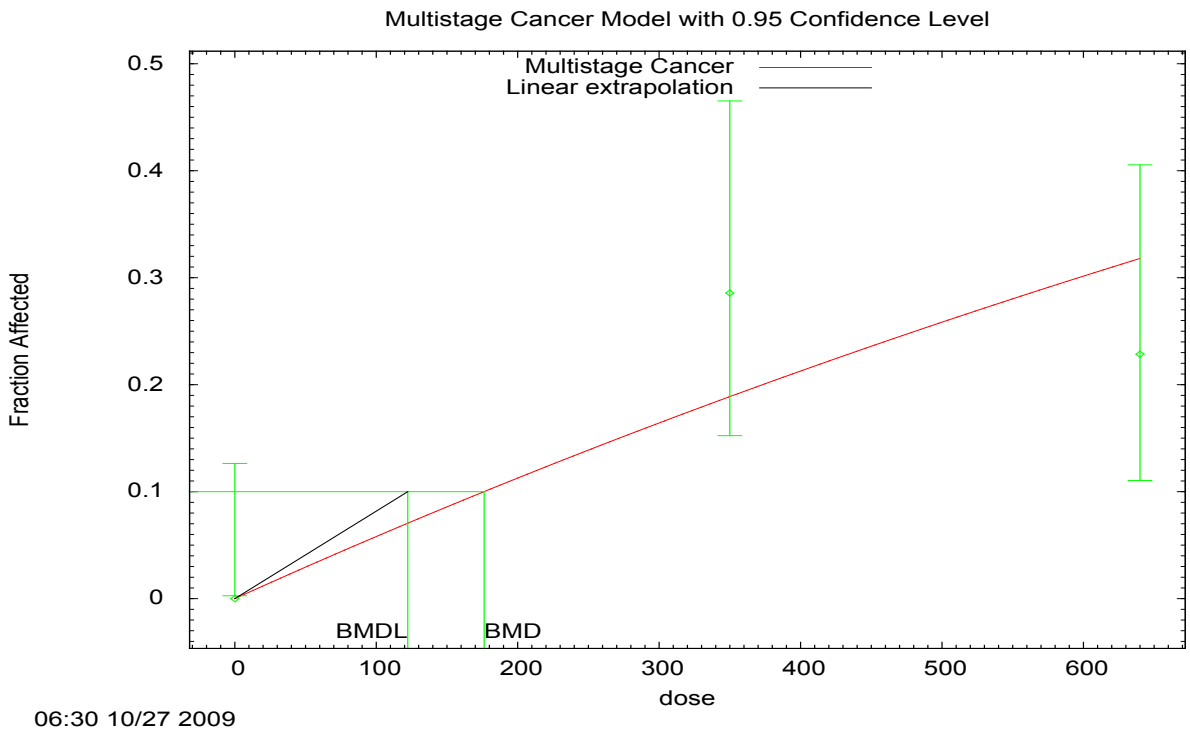
Source: NCI (1978).

Figure D-37 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.

```
==================================================================
Logistic Model. (Version: 2.12; Date: 05/16/2008)
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_nci_frat_nasal_car_Lnl-BMR10-Restrict.(d)
```

```
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_nci_frat_nasal_car_Lnl-BMR10-Restrict.plt
Tue Oct 27 07:30:09 2009
=================================================================
 BMDS Model Run
~~~
```

```
 Chi^2 = 2.78 d.f. = 2 P-value = 0.2486


 Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 155.324
 BMDL = 100.081
```



Source: NCI (1978).

Figure D-38 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.

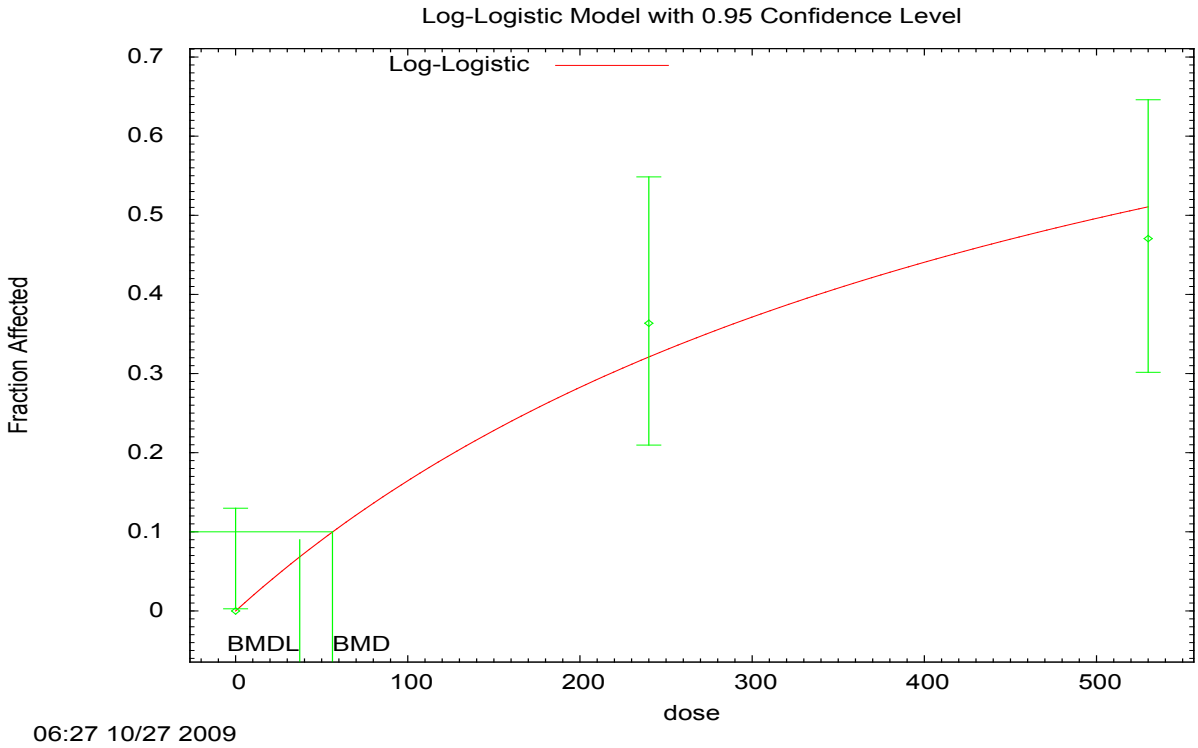
```
=================================================================
Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_frat_nasal_car_Msc-BMR10-1poly.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_frat_nasal_car_Msc-BMR10-1poly.plt
Tue Oct 27 07:30:12 2009
=================================================================
 BMDS Model Run
~~~
```

```
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 = 0.0569154
Beta(1) = 0.00042443

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

 95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
Background 0 * * *
Beta(1) 0.000597685 * * *

* - 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 -41.3998 1 3.29259 2 0.1928
 Reduced model -47.9161 1 16.3252 2 0.0002851

 AIC: 84.7996

 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size Residual
 ---------------------------------------------------------------------
 0.0000 0.0000 0.000 0.000 34 0.000
 350.0000 0.1888 6.607 10.000 35 1.466
 640.0000 0.3179 11.125 8.000 35 -1.134

 Chi^2 = 3.44 d.f. = 2 P-value = 0.1795

 Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 176.281
 BMDL = 122.274
 BMDU = 271.474

Taken together, (122.274, 271.474) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000817837
```

Table D-28 BMDS dose-response modeling results for the incidence of nasal cavity squamous cell carcinoma in male Osborne-Mendel rats (NCI, 1978) exposed to 1,4-dioxane in the drinking water for 2 years

Model	AIC	*p*-value	BMD_{10} mg/kg-day	$BMDL_{10}$ mg/kg-day	χ^{2a}	$BMD_{10\ HED}$ 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

[a]Maximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.
[b]Best-fitting model.
[c]Slope restricted ≥ 1.



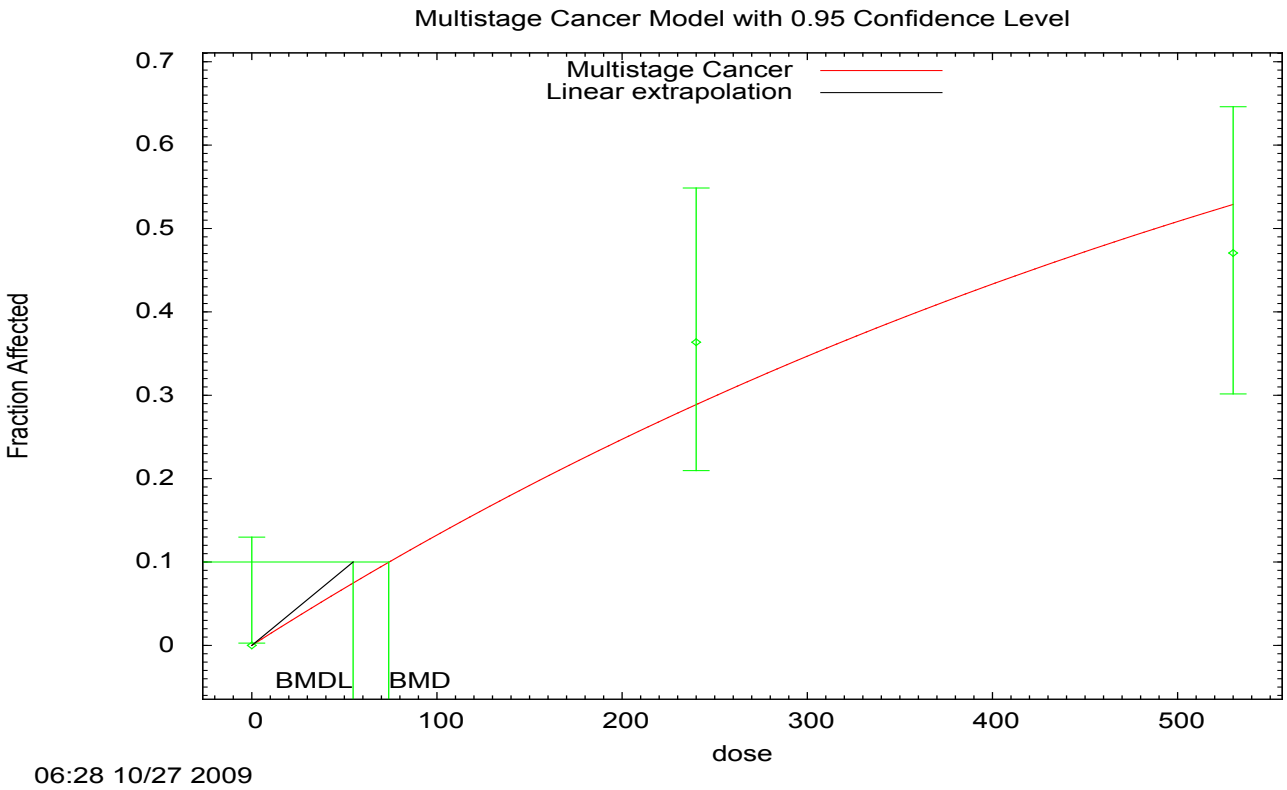
Source: NCI (1978).

Figure D-39 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:
4  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_nci_mrat_nasal_car_Lnl-BMR10-Restrict.(d)
5  Gnuplot Plotting File:
6  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_nci_mrat_nasal_car_Lnl-BMR10-Restrict.plt
```

```
Tue Oct 27 07:27:57 2009
=====================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the probability function is:
P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

Dependent variable = Effect
Independent variable = Dose
Slope parameter is restricted as slope >= 1

Total number of observations = 3
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

User has chosen the log transformed model

Default Initial Parameter Values
background = 0
intercept = -6.08408
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

 95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
background 0 * * *
intercept -6.2272 * * *
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 -45.139 3
 Fitted model -45.3835 1 0.488858 2 0.7832
 Reduced model -59.2953 1 28.3126 2 <.0001

 AIC: 92.7669

                                    Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size Residual
 ---------------------------------------------------------------------
 0.0000 0.0000 0.000 0.000 33 0.000
 240.0000 0.3216 10.612 12.000 33 0.517
 530.0000 0.5114 17.388 16.000 34 -0.476

 Chi^2 = 0.49 d.f. = 2 P-value = 0.7809
```

```
 Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 56.2596
 BMDL = 37.256
```



Source: NCI (1978).

Figure D-40 Multistage BMD model (1 degree) for the incidence of nasal cavity squamous cell carcinoma in male Osborne-Mendel ratsexposed to 1,4-dioxane in drinking water.

```
=================================================================
Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_mrat_nasal_car_Msc-BMR10-1poly.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_mrat_nasal_car_Msc-BMR10-1poly.plt
                                                Tue Oct 27 07:28:00 2009
=================================================================
 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 = 3
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 = 0.0578996
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)

 Beta(1)
 Beta(1) 1

                                  Parameter Estimates

 95.0% Wald Confidence Interval
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
 Scaled
 Dose Est._Prob. Expected Observed Size 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 B6C3F$_1$ Mice (NCI, 1978)

The incidence data for hepatocellular adenoma or carcinoma in male and female mice are presented in Table D-29. The 2-degree polynomial model (betas restricted ≥ 0) was the lowest degree polynomial that provided an adequate fit to the female mouse data (Figure D-41), while the gamma model

provided the best fit to the male mouse data (Figure D-42). The results of the BMDS modeling for the entire suite of models are presented in Table D-30 and Table D-31 for the female and male data, respectively.

Table D-29 Incidence of hepatocellular adenoma or carcinoma in male and female B6C3F$_1$ 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]

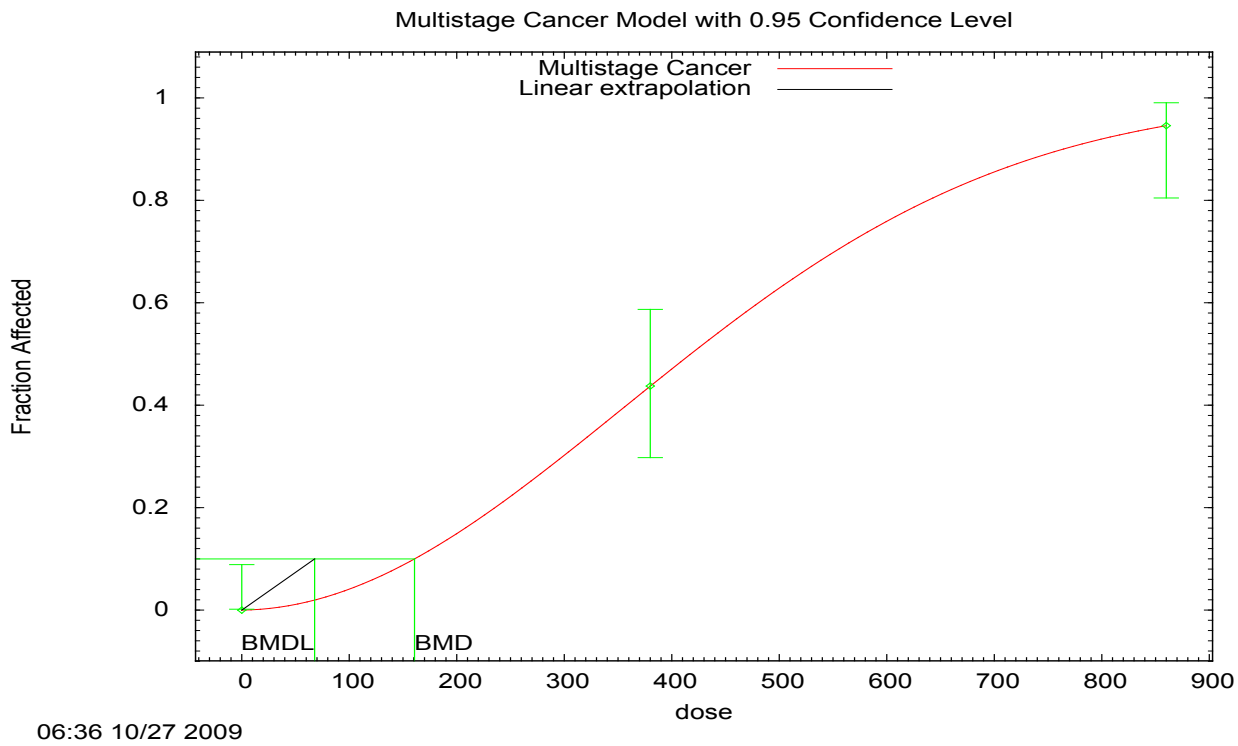
[a]Tumor incidence values were not adjusted for mortality.
[b]$p < 0.001$, positive dose-related trend (Cochran-Armitage test).
[c]$p < 0.001$ by Fisher's Exact test pair-wise comparison with controls.
[d]$p = 0.014$.

Source: NCI (1978).

Table D-30 BMDS dose-response modeling results for the combined incidence of hepatocellular adenoma or carcinoma in female B6C3F$_1$ mice (NCI, 1978) exposed to 1,4-dioxane in the drinking water for 2 years

Model	AIC	*p*-value	BMD_{10} mg/kg-day	$BMDL_{10}$ mg/kg-day	χ^{2a}	$BMD_{10\ HED}$ 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

[a]Maximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.
[b]Slope restricted ≥ 1.
[c]Best-fitting model.



Source: NCI (1978).

Figure D-41 Multistage BMD model (2 degree) for the incidence of hepatocellular adenoma or carcinoma in female B6C3F$_1$ mice exposed to 1,4-dioxane in drinking water.

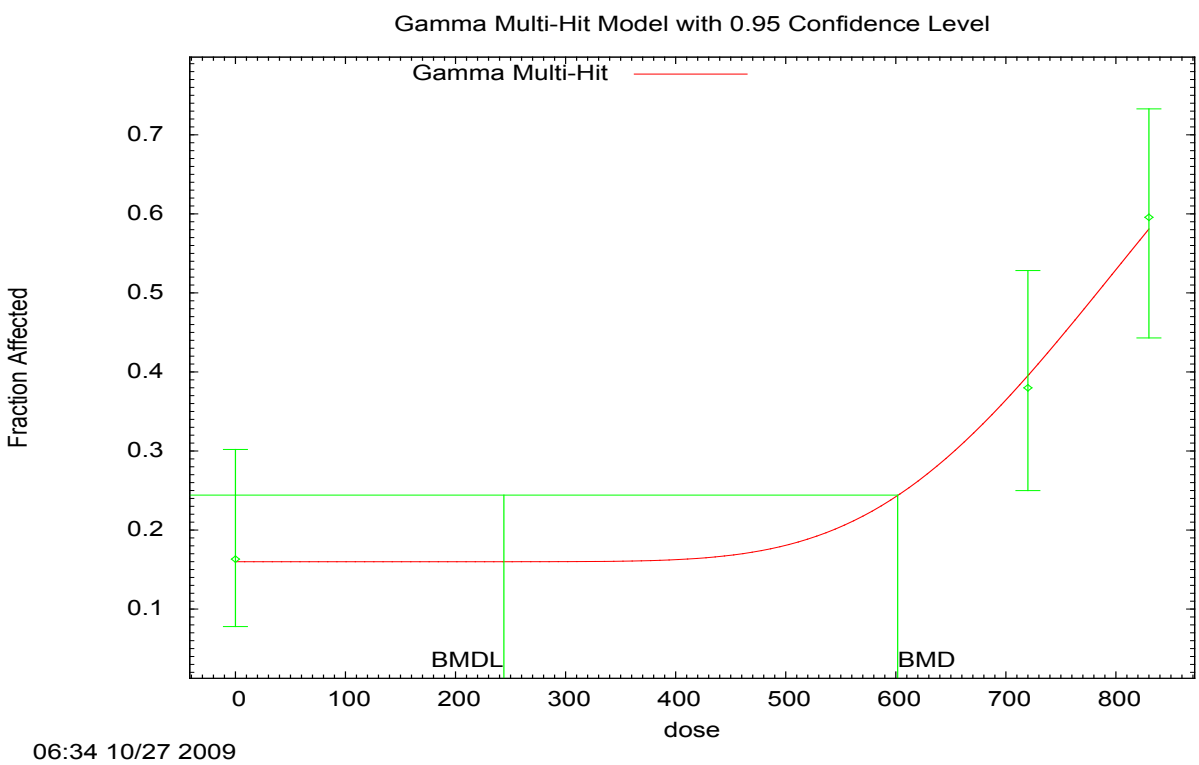
```
==================================================================
Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_fmouse_hepato_adcar_Msc-BMR10-2poly.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_fmouse_hepato_adcar_Msc-BMR10-2poly.plt
Tue Oct 27 07:36:26 2009
==================================================================
 BMDS Model Run
~~~
```

```
Default Initial Parameter Values
Background = 0
Beta(1) = 2.68591e-005
Beta(2) = 3.91383e-006


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(2)
 Beta(1) 1 -0.92
 Beta(2) -0.92 1


                                  Parameter Estimates

 95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
Background 0 * * *
Beta(1) 2.686e-005 * * *
Beta(2) 3.91382e-006 * * *

* - Indicates that this value is not calculated.


 Analysis of Deviance Table

 Model Log(likelihood) # Param's Deviance Test d.f. P-value
 Full model -40.6756 3
 Fitted model -40.6756 2 3.20014e-010 1 1
 Reduced model -91.606 1 101.861 2 <.0001

 AIC: 85.3511

 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size Residual
 ---------------------------------------------------------------------
 0.0000 0.0000 0.000 0.000 50 0.000
 380.0000 0.4375 21.000 21.000 48 0.000
 860.0000 0.9459 35.000 35.000 37 0.000

 Chi^2 = 0.00 d.f. = 1 P-value = 1.0000


 Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 160.678
 BMDL = 67.7635
 BMDU = 186.587

Taken together, (67.7635, 186.587) is a 90% two-sided confidence interval for the BMD

              Multistage Cancer Slope Factor = 0.00147572
```

Table D-31 BMDS dose-response modeling results for the combined incidence of hepatocellular adenoma or carcinoma in male B6C3F$_1$ mice (NCI, 1978) exposed to 1,4-dioxane in drinking water

Model	AIC	*p*-value	BMD_{10} mg/kg-day	$BMDL_{10}$ mg/kg-day	χ^{2a}	$BMD_{10\ HED}$ 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

[a]Maximum absolute χ^2 residual deviation between observed and predicted count. Values much larger than 1 are undesirable.
[b]Best-fitting model.
[c]Value unable to be calculated (NC: not calculated) by BMDS.
[d]Slope restricted ≥ 1.



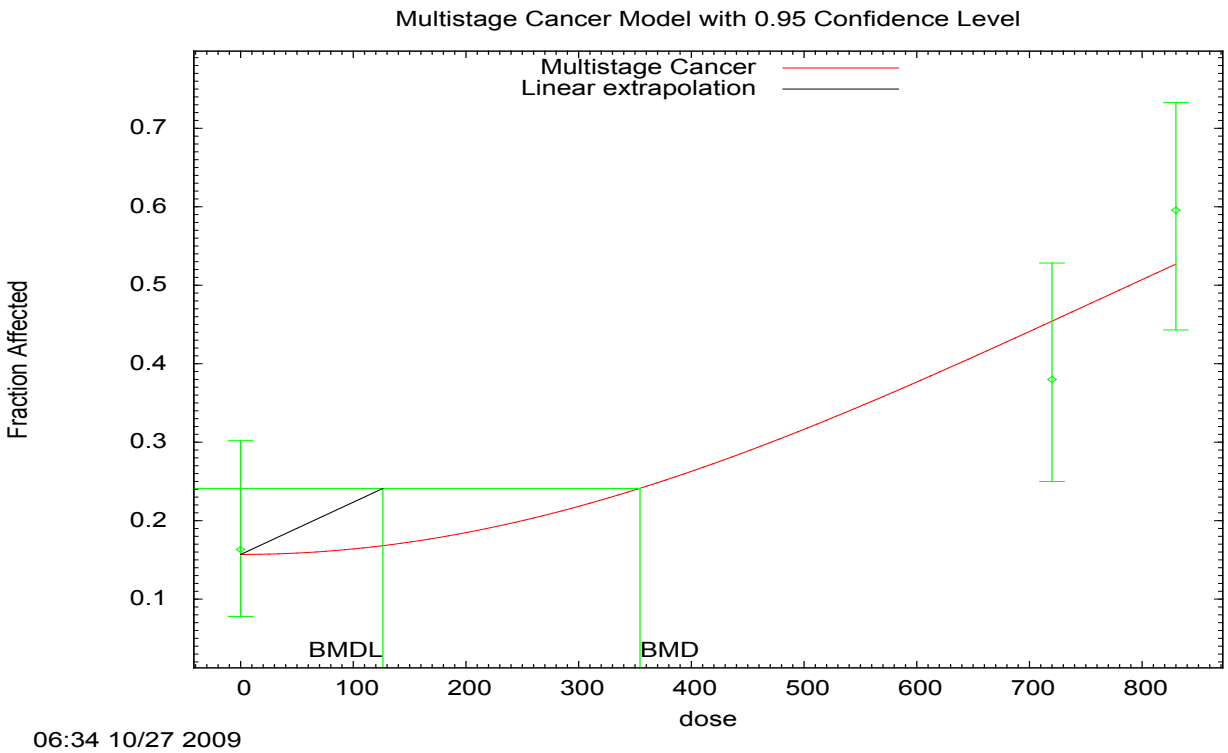
Source: NCI (1978).

Figure D-42 Gamma BMD model for the incidence of hepatocellular adenoma or carcinoma in male B6C3F$_1$ mice exposed to 1,4-dioxane in drinking water.

```
1  ==================================================================
2  Gamma Model. (Version: 2.13; Date: 05/16/2008)
3  Input Data File:
4  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\gam_nci_mmouse_hepato_adcar_Gam-BMR10-Restrict.(d)
```

```
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\gam_nci_mmouse_hepato_adcar_Gam-BMR10-Restrict.plt
Tue Oct 27 07:34:35 2009
=================================================================
BMDS Model Run
~~~
```

```
BMDL = 243.917
```



Source: NCI (1978).

Figure D-43 Multistage BMD model (2 degree) for the incidence of hepatocellular adenoma or carcinoma in male B6C3F$_1$ mice exposed to 1,4-dioxane in drinking water.

```
=====================================================================
Multistage Cancer Model. (Version: 1.7; Date: 05/16/2008)
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_mmouse_hepato_adcar_Msc-BMR10-2poly.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_mmouse_hepato_adcar_Msc-BMR10-2poly.plt
Tue Oct 27 07:34:42 2009
=====================================================================
BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

The form of the probability function is: P[response] = background +
(1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2)]

The parameter betas are restricted to be positive

Dependent variable = Effect
Independent variable = Dose

Total number of observations = 3
Total number of records with missing values = 0
Total number of parameters in model = 3
Total number of specified parameters = 0
Degree of polynomial = 2
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 = 0.131156
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

 95.0% Wald Confidence Interval
Variable Estimate Std. Err. 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
 Scaled
 Dose Est._Prob. Expected Observed Size 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

As described in detail in Section 4.2.1.2.6 of this *Toxicological Review of 1,4-Dioxane*, the JBRC conducted a 2-year drinking water study on the effects of 1,4-dioxane in both sexes of rats and mice. The results from this study have been reported three times, once as conference proceedings (Yamazaki et al., 1994a), once as a detailed laboratory report (JBRC, 1998), and once as a published manuscript (Kano et al., 2009). After the External Peer Review draft of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2009a) had been released, the Kano et al. (2009) manuscript was published; thus, minor changes to the *Toxicological Review of 1,4-Dioxane* occurred.

The purpose of this appendix is to provide a clear and transparent comparison of the reporting of this 2-year 1,4-dioxane drinking water study. The variations included: (1) the level of detail on dose information reported; (2) categories for incidence data reported (e.g., all animals or sacrificed animals); and (3) analysis of non- and neoplastic lesions. Even though the data contained in the reports varied, the differences were minor and did not did not significantly affect the qualitative or quantitative cancer assessment.

Tables contained within this appendix provide a comparison of the variations in the reported data (Kano et al., 2009; JBRC, 1998; Yamazaki et al., 1994a). Table E-32 and Table E-2 show the histological nonneoplastic findings provided for male and female F344 rats, respectively. Table E-34 and Table E-4 show the histological nonneoplastic findings provided for male and female F344 rats, respectively. Table E-34 and Table E-4 show the histological neoplastic findings provided for male and female F344 rats, respectively. Table E-5 and Table E-6 show the histological nonneoplastic findings provided for male and female F344 rats, respectively. Table E-7 and Table E-8 show the histological neoplastic findings provided for male and female Crj:BDF1 mice, respectively.

Table E-32 Nonneoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in male F344 rats

		Yamazaki et al. (1994a)[a]				JBRC (1998)[d]				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[g]	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	9/35f	12/22[e]	Not reported			
Spongiosis hepatis; 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[g]	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[g]	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[g]	All animals	Not reported				7/50	11/50	6/50	16/50	7/50	11/50	8/50	16/50'
	Sacrificed animals	Not reported				7/40	11/45	6/35	8/22[f]	Not reported			
Mixed-cell foci; liver[g]	All animals	Not reported				2/50	8/50	14/50	13/50	2/50	8/50	14/50e	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			

[a]Dose rates (mg/kg-day) were not provided in Yamazaki et al. (1994a). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.
[b]JBRC (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, 2009a) .
[c]Kano et al. (2009) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in the 2010 Toxicolgical Review of 1,4-Dioxane (U.S. EPA, 2010b).
[d]JBRC did not report statistical significance for the "All animals" comparison.
[e]$p \leq 0.01$ by $\chi 2$ test.
[f]$p \leq 0.05$ by $\chi 2$ test.
[g]The samples associated with liver hyperplasia for rats and mice in Yamazaki et al. (1994a) and JBRC (1998) were re-examined according to updated criteria for liver lesions and were afterwards classified as either hepatocellular adenoma or altered hepatocellular foci in Kano et al. (2009).

Table E-33 Nonneoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in female F344 rats

		Yamazaki et al. (1994a)[a]				JBRC (1998)[bd]				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; proliferation	All animals	0/50	0/50	0/50	11/50	0/50	0/50	0/50	11/50	Not reported			
	Sacrificed animals	Not reported				0/38	0/37	0/38	8/24[e]	Not reported			
Nasal olfactory epithelium; nuclear enlargement	All animals	Not reported				0/50	0/50	28/50	39/50	0/50	0/50	28/50[e]	39/50[e]
	Sacrificed animals	Not reported				0/38	0/37	24/38[e]	22/24[e]	Not reported			
Nasal olfactory epithelium; respiratory metaplasia	All animals	Not reported				2/50	0/50	2/50	42/50	Not reported			
	Sacrificed animals	Not reported				1/38	0/37	1/38	24/24[e]	Not reported			
Nasal olfactory epithelium; atrophy	All animals	Not reported				0/50	0/50	1/50	40/50	Not reported			
	Sacrificed animals	Not reported				0/38	0/37	1/38	22/24[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				0/38	0/37	0/38	23/24[e]	Not reported			
Lamina propria; slerosis	All animals	Not reported				0/50	0/50	0/50	48/50	Not reported			
	Sacrificed animals	Not reported				0/38	0/37	0/38	23/24[e]	Not reported			
Nasal cavity; adhesion	All animals	Not reported				0/50	0/50	0/50	46/50	Not reported			
	Sacrificed animals	Not reported				0/38	0/37	0/38	24/24[e]	Not reported			
Nasal cavity; inflammation	All animals	Not reported				0/50	0/50	1/50	15/50	Not reported			
	Sacrificed animals	Not reported				0/38	0/37	1/38	7/24[e]	Not reported			
Liver; hyperplasia[g]	All animals	3/50	2/50	11/50	47/50	3/50	2/50	11/50	47/50	Not reported			
	Sacrificed animals	Not reported				2/38	2/37	9/38	24/24[e]	Not reported			
Liver; spongiosis hepatis	All animals	0/50	0/50	1/50	20/50	0/50	0/50	1/50	20/50	Not reported			
	Sacrificed animals	Not reported				0/38	0/37	1/38	14/24[e]	Not reported			
Liver; cyst formation	All animals	Not reported				0/50	1/50	1/50	8/50	Not reported			
	Sacrificed animals	Not reported				0/38	1/37	0/38	5/24[f]	Not reported			
Liver; clear cell foci[g]	All animals	Not reported				Not reported				1/50	1/50	5/50	4/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Liver; acidophilic cell foci[g]	All animals	Not reported				Not reported				1/50	1/50	1/50	1/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Liver; basophilic cell foci[g]	All animals	Not reported				Not reported				23/50	27/50	31/50	8/50[e]
	Sacrificed animals	Not reported				Not reported				Not reported			
Liver; mixed-cell foci[g]	All animals	Not reported				1/50	1/50	3/50	11/50	1/50	1/50	3/50	11/50[e]
	Sacrificed animals	Not reported				1/38	1/37	3/38	7/24[f]	Not reported			
Kidney proximal tubule; nuclear enlargement	All animals	Not reported				0/50	0/50	6/50	39/50	Not reported			
	Sacrificed animals	Not reported				0/38	0/37	6/38	22/24[e]	Not reported			

[a]Dose rates (mg/kg-day) were not provided in Yamazaki et al. (1994a). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.
[b]JBRC (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, 2009a) .
[c]Kano et al. (2009) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in the 2010 Toxicolgical Review of 1,4-Dioxane (U.S. EPA, 2010b).
[d]JBRC did not report statistical significance for the "All animals" comparison.
[e]p ≤ 0.01 by χ2 test.
[f]p ≤ 0.05 by χ2 test.
[g]The samples associated with liver hyperplasia for rats and mice in Yamazaki et al. (1994a) and JBRC (1998) were re-examined according to updated criteria for liver lesions and were afterwards classified as either hepatocellular adenoma or altered hepatocellular foci in Kano et al. (2009).

Table E-34 Neoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in male F344 rats

		Yamazaki et al. (1994a)[a]				JBRC (1998)[b]				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[f]	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			
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			

[a]Dose rates (mg/kg-day) were not provided in Yamazaki et al. (1994a). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.
[b]JBRC (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, 2009a) .
[c]Kano et al. (2009) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in the 2010 Toxicolgical Review of 1,4-Dioxane (U.S. EPA, 2010b).
[d]p ≤ 0.01 by Fisher's Exact test.
[e]Significantly increased by Peto test for trend p < 0.01.
[f]The samples associated with liver hyperplasia for rats and mice in Yamazaki et al. (1994a) and JBRC (1998) were re-examined according to updated criteria for liver lesions and were afterwards classified as either hepatocellular adenoma or altered hepatocellular foci in Kano et al. (2009).

Table E-35 Neoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in female F344 rats

		Yamazaki et al. (1994a)[a]				JBRC (1998)[b]				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			
Sarcoma NOS	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[g]	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[g]	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			

[a]Dose rates (mg/kg-day) were not provided in Yamazaki et al. (1994a). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.
[b]JBRC (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, 2009a) .
[c]Kano et al. (2009) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in the 2010 Toxicolgical Review of 1,4-Dioxane (U.S. EPA, 2010b).
[d]$p \leq 0.05$ by Fisher's Exact test.
[e]$p \leq 0.01$ by Fisher's Exact test.
[f]Significantly increased by Peto test for trend $p < 0.01$.
[g]The samples associated with liver hyperplasia for rats and mice in Yamazaki et al. (1994a) and JBRC (1998) were re-examined according to updated criteria for liver lesions and were afterwards classified as either hepatocellular adenoma or altered hepatocellular foci in Kano et al. (2009).

Table E-36 Nonneoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in male Crj:BDF1 mice

		Yamazaki et al. (1994a)[a]				JBRC (1998)[b,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 0	37-94 (66)	144-358 (251)	451-1086 (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			
Bronhcial 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; accumlation 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			
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			

[a]Dose rates (mg/kg-day) were not provided in Yamazaki et al. (1994a). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.
[b]JBRC (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, 2009a) .
[c]Kano et al. (2009) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in the 2010 Toxicolgical Review of 1,4-Dioxane (U.S. EPA, 2010b).
[d]JBRC did not report statistical significance for the "All animals" comparison.
[e]$p \leq 0.01$ by $\chi 2$ test.
[f]$p \leq 0.05$ by $\chi 2$ test.

Table E-37 Nonneoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in female Crj:BDF1 mice

		Yamazaki et al. (1994a)[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])[D,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			
Bronhcial 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; Accumlation 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			

[a]Dose rates mg/kg-day]) were not provided in Yamazaki et al. (1994a). Drinking water concentrations (ppm) of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.
[b]Statistical analysis was not performed for data on 'All animals' in the JBRC (1998) report.
[c]JBRC (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, 2009a) .
[d]Kano et al. (2009) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in the 2010 Toxicolgical Review of 1,4-Dioxane (U.S. EPA, 2010b).
[e]$p \leq 0.01$ by chi-square test.

Table E-38 Neoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in male Crj:BDF1 mice

		Yamazaki et al. (1994a)[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	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			

[a]Dose rates (mg/kg-day) were not provided in Yamazaki et al. (1994a). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.
[b]JBRC (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, 2009a) .
[c]Kano et al. (2009) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in the 2010 Toxicolgical Review of 1,4-Dioxane (U.S. EPA, 2010b).
[d]$p \leq 0.05$ by Fisher's Exact test.
[e]Significantly increased by Peto test for trend $p < 0.01$.
[f]$p \leq 0.01$ by Fisher's Exact test.

Table E-39 Neoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in female Crj:BDF1 mice

		Yamazaki et al. (1994a)[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 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[u]	20/50[u]	2/50[e]	5/50	31/50[u]	20/50[u]	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[u]	45/50[u,g]	0/50	6/50[f]	30/50[u]	45/50[u,g]
	Sacrificed animals	Not reported				Not reported				Not reported			
Either adenoma or carcinoma	All animals	Not reported				4/50	34/50[u]	41/50[u]	46/50[u,g]	5/50	35/50[u]	41/50[u]	46/50[u,g]
	Sacrificed animals	Not reported				Not reported				Not reported			

[a]Dose rates (mg/kg-day) were not provided in Yamazaki et al. (1994a). Drinking water concentrations (ppm) of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.
[b]JBRC (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, 2009a) .
[c]Kano et al. (2009) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in the 2010 Toxicolgical Review of 1,4-Dioxane (U.S. EPA, 2010b).
[d]$p \leq 0.01$ by Fisher's Exact test.
[e]Significantly decreased by Cochran-Armitage test for trend $p < 0.05$
[f]$p \leq 0.05$ by Fisher's Exact test.
[g]Significantly 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

All available dichotomous models in the Benchmark Dose Software (version 2.1.2) were fit to the incidence data shown in Table F-40, for centrilobular necrosis of the liver in 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-40 Incidence of centrilobular necrosis of the liver in male F344/DuCrj rats exposed to 1,4-dioxane via inhalation for 2 years

1,4-dioxane vapor concentration (ppm)			
0	**50**	**250**	**1,250**
1/50 (2%)	3/50 (6%)	6/50 (12%)	12/50[a] (24%)

[a]p ≤ 0.01 by Fisher's exact test.

Source: Kasai et al. (2009).

As assessed by the χ^2 goodness-of-fit test, several models in the software provided adequate fits to the incidence data of centrilobular necrosis of the liver in male rats (χ^2 $p \geq 0.1$) (Table F-41). Comparing across adequately fitting models, the BMDL estimates were not within threefold difference of each other. Therefore, in accordance with EPA BMD technical guidance (U.S. EPA, 2012a), the adequately fitting model that resulted in the lowest BMDL was selected as appropriate for deriving a POD which was the Dichotomous-Hill model. BMDS modeling results for all dichotomous models are shown in Table F-41 and the model plot (Figure F-44) and output for the selected Dichotomous-Hill model are included immediately after the table.

Table F-41 Goodness-of-fit statistics and BMD_{10} and $BMDL_{10}$ 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	BMD_{10} (ppm)	$BMDL_{10}$ (ppm)
Male					
Gamma[b]	129.692	0.5099	0.786	502.444	308.113
Logistic	131.043	0.2794	-0.142	794.87	609.269
Log-logistic[c]	129.465	0.568	0.676	453.169	258.687
Log-probit[c]	132.067	0.1645	-0.175	801.17	539.489
Multistage (2 degree)[d]	129.692	0.5099	0.786	502.445	308.112
Probit	130.889	0.2992	-0.167	756.192	567.169
Weibull[b]	129.692	0.5099	0.786	502.461	308.113
Quantal-Linear	129.692	0.5099	0.786	502.461	308.113
Dichotomous-Hill[c, e]	**130.404**	**0.7459**	**-0.179**	**219.51**	**59.5598**

[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).

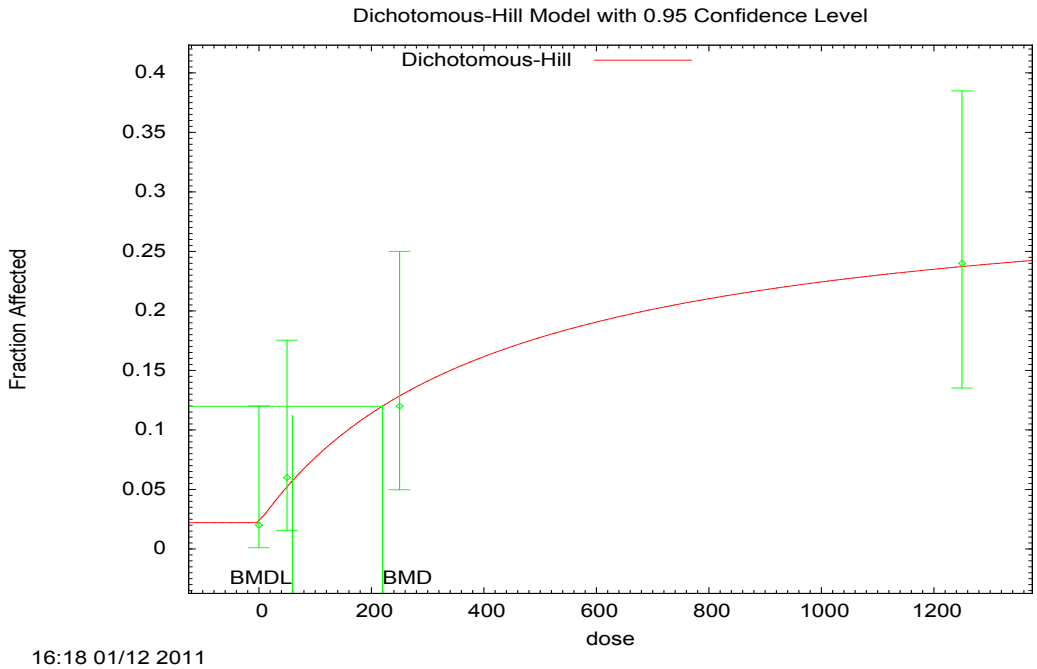


Figure F-44 BMD Dichotomous Hill model of centrilobular necrosis incidence data for male rats exposed to 1,4-dioxane vapors for 2 years to support the results in Table F-2.

```
=================================================================
Dichotomous Hill Model. (Version: 1.2; Date: 12/11/2009)
```

```
Input Data File: C:/Documents and Settings/pgillesp/Desktop/BMDS
files/dhl_Centr_necrosis_liver_Dhl-BMR10-Restrict.(d)
        Gnuplot Plotting File: C:/Documents and Settings/pgillesp/Desktop/BMDS
files/dhl_Centr_necrosis_liver_Dhl-BMR10-Restrict.plt
                                                Wed Jan 12 16:34:41 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 = -8.08245
 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)

 v g intercept
 v 1 -0.25 -0.89
 g -0.25 1 0.016
 intercept -0.89 0.016 1


 Parameter Estimates

 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. 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

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 -62.1506 4
 Fitted model -62.2022 3 0.103279 1 0.7479
 Reduced model -69.3031 1 14.305 3 0.002518

 AIC: 130.404

 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size 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

 Chi^2 = 0.10 d.f. = 1 P-value = 0.7459


 Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 219.51
 BMDL = 59.5598
```

F.2 Squamous Cell Metaplasia

All available dichotomous models in the Benchmark Dose Software (version 2.1.2) were fit to the incidence data shown in Table F-42, for squamous cell metaplasia of the respiratory epithelium in male F344/DuCrj rats exposed to 1,4-dioxane vapors for 2 years (NCI, 1978). Doses associated with a BMR of a 10% extra risk were calculated.

Table F-42 Incidence of squamous cell metaplasia of the respiratory epithelium in male 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	0/50	7/50[b] (14%)	44/50[a] (88%)

[a]p ≤ 0.01 by Fisher's exact test.
[b]p ≤ 0.05 by Fisher's exact test.

Source: Kasai et al. (2009).

For incidence of squamous cell metaplasia in F344/DuCrj male rats, the logistic and probit models all exhibited a statistically significant lack of fit (i.e., χ^2 *p*-value < 0.1; see Table F-43), and thus should not be considered further for identification of a POD. All of the remaining models exhibited adequate fit. The BMDL estimates for all appropriately fitting models were within threefold difference of each other, indicating that BMDL selection should be made based on model fit (U.S. EPA, 2012a). As assessed by the AIC, the Log-probit model provided the best fit to the squamous cell metaplasia data for male rats (Table F-43, Figure F-45), and could be used to derive a POD for this endpoint.

Table F-43 Goodness-of-fit statistics and BMD_{10} and $BMDL_{10}$ 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)

Model	AIC	*p*-value[a]	Scaled Residual of Interest	BMD_{10} (ppm)	$BMDL_{10}$ (ppm)
Male					
Gamma[b]	81.687	0.8682	0.24	218.38	150.329
Logistic	89.4148	0.0464	1.806	370.443	288.535
Log-logistic[c]	81.5252	0.9142	0.131	218.218	158.293
Log-probit[c, e]	**81.23**	**0.9894**	**0.032**	**217.79**	**159.619**
Multistage (2 degree)[d]	82.6875	0.6188	0.605	231.294	141.025
Probit	87.9361	0.0779	1.681	337.732	268.424
Weibull[b]	82.1236	0.7679	0.33	218.435	145.383
Quantal-Linear	92.9215	0.0198	-1.76	87.682	68.8015
Dichotomous-Hill[c]	83.1888	0.9995	0	240.867	161.945

[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 AIC.

Source: Kasai et al. (2009).

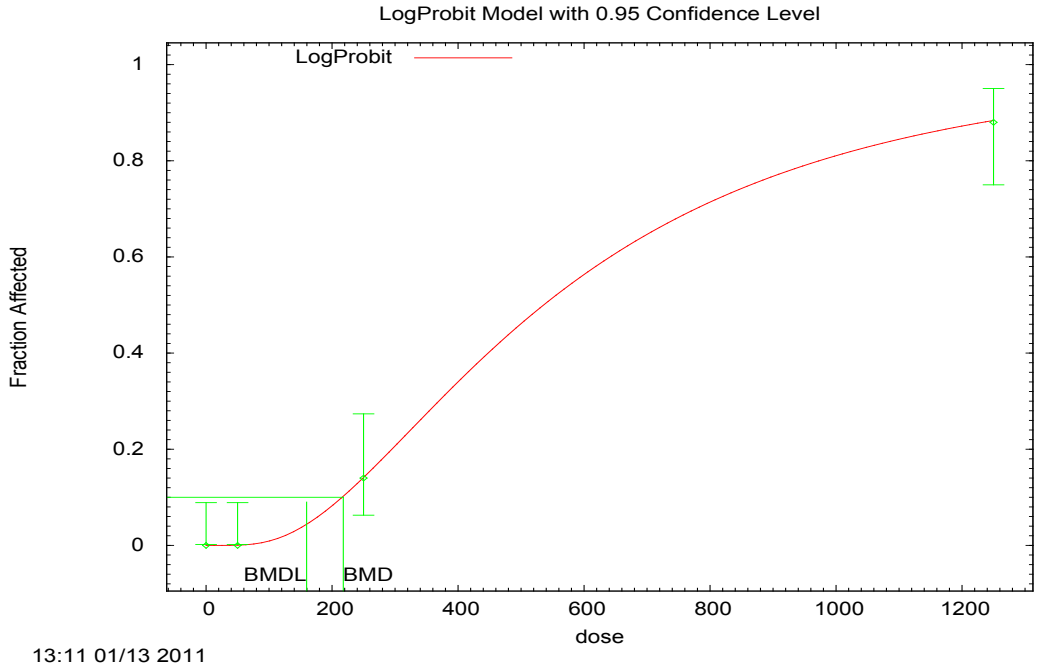


Figure F-45 BMD Log-probit model of squamous cell metaplasia of the respiratory epithelium incidence data for male rats exposed to 1,4-dioxane vapors for 2 years to support the results in Table F-6.

```
=================================================================
Probit Model. (Version: 3.2; Date: 10/28/2009)
Input Data File: C:/Documents and Settings/pgillesp/Desktop/BMDS
files/lnp_squ_cell_meta_re_Lnp-BMR10-Restrict.(d)
```

```
        Gnuplot Plotting File: C:/Documents and Settings/pgillesp/Desktop/BMDS
files/lnp_squ_cell_meta_re_Lnp-BMR10-Restrict.plt
                                                Thu Jan 13 13:11:09 2011
=================================================================
 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 The form of the probability function is:

 P[response] = Background + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),
        where CumNorm(.) is the cumulative normal distribution function

 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

 User has chosen the log transformed model

 Default Initial (and Specified) Parameter Values
 background = 0
 intercept = -6.76507
 slope = 1.09006

 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

 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
background 0 NA
 intercept -8.86173 1.2226 -11.258 -6.46548
 slope 1.40803 0.193057 1.02965 1.78642

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 -38.5944 4
 Fitted model -38.615 2 0.041197 2 0.9796
 Reduced model -113.552 1 149.916 3 <.0001

 AIC: 81.23

 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size Residual
 ---------------------------------------------------------------------
 0.0000 0.0000 0.000 0.000 50 0.000
 50.0000 0.0004 0.020 0.000 50 -0.141
 250.0000 0.1384 6.922 7.000 50 0.032
 1250.0000 0.8808 44.038 44.000 50 -0.017

 Chi^2 = 0.02 d.f. = 2 P-value = 0.9894
```

```
 Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 217.79
 BMDL = 159.619
```

F.3 Squamous Cell Hyperplasia

All available dichotomous models in the Benchmark Dose Software (version 2.1.2) were fit to the incidence data shown in Table F-44, for squamous cell hyperplasia of the respiratory epithelium in male F344/DuCrj rats exposed to 1,4-dioxane vapors for 2 years (NCI, 1978). Doses associated with a BMR of a 10% extra risk were calculated.

Table F-44 Incidence of squamous cell hyperplasia of the respiratory epithelium in male 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	0/50	1/50 (2%)	10/50[a] (20%)

[a]p ≤ 0.01 by Fisher's exact test.

Source: Kasai et al. (2009).

For incidence of squamous cell hyperplasia in F344/DuCrj male rats, the logistic, probit, and quantal-linear models all exhibited a statistically significant lack of fit (i.e., χ^2 p-value < 0.1; see Table F-45), and thus should not be considered further for identification of a POD. All of the remaining models exhibited adequate fit. The BMDL estimates for all appropriately fitting models were within threefold difference of each other, indicating that BMDL selection should be made based on model fit (U.S. EPA, 2012a). As assessed by the AIC, the Log-probit model provided the best fit to the squamous cell hyperplasia data for male rats (Table F-45, Figure F-46 and subsequent textual model output), and could be used to derive a POD for this endpoint.

Table F-45 Goodness-of-fit statistics and BMD_{10} and $BMDL_{10}$ 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)

Model	AIC	*p*-value[a]	Scaled Residual of Interest	BMD_{10} (ppm)	$BMDL_{10}$ (ppm)
Male					
Gamma[b]	81.687	0.8682	0.24	218.38	150.329
Logistic	89.4148	0.0464	1.806	370.443	288.535
Log-logistic[c]	81.5252	0.9142	0.131	218.218	158.293
Log-probit[c, e]	**81.23**	**0.9894**	**0.032**	**217.79**	**159.619**
Multistage (2 degree)[d]	82.6875	0.6188	0.605	231.294	141.025
Probit	87.9361	0.0779	1.681	337.732	268.424
Weibull[b]	82.1236	0.7679	0.33	218.435	145.383
Quantal-Linear	92.9215	0.0198	-1.76	87.682	68.8015
Dichotomous-Hill[c]	83.1888	0.9995	0	240.867	161.945

[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 AIC.

Source: Kasai et al. (2009).

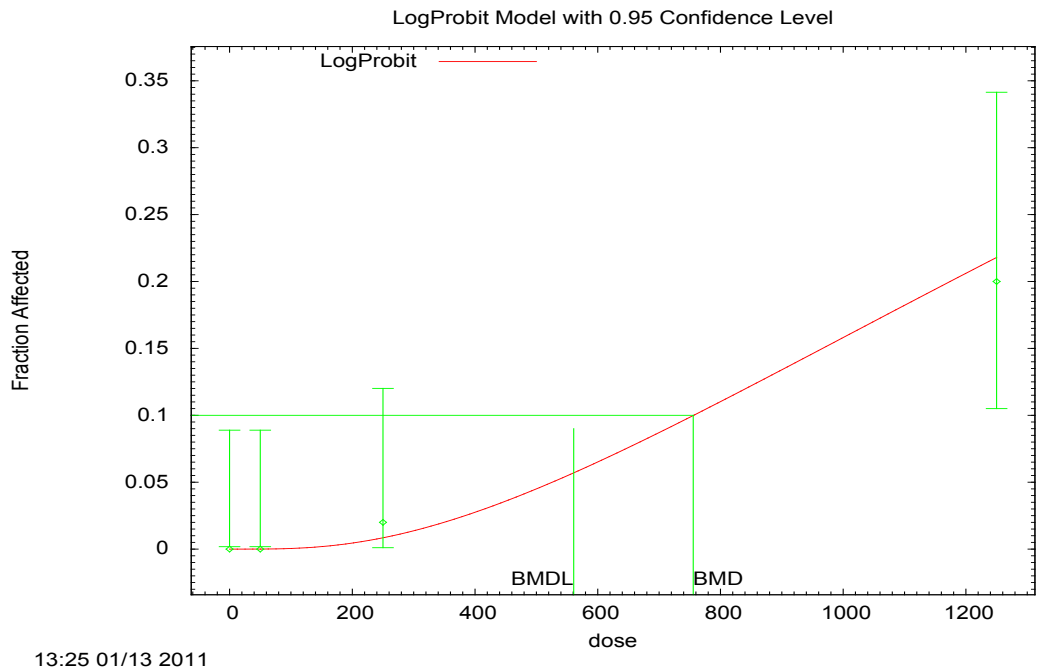


Figure F-46 BMD Log-probit model of squamous cell hyperplasia of the respiratory epithelium incidence data for male rats exposed to 1,4-dioxane vapors for 2 years to support the results in Table F-8.

```
================================================================
Probit Model. (Version: 3.2; Date: 10/28/2009)
Input Data File: C:/Documents and Settings/pgillesp/Desktop/BMDS
files/lnp_squ_cell_hyper_re_Lnp-BMR10-Restrict.(d)
        Gnuplot Plotting File: C:/Documents and Settings/pgillesp/Desktop/BMDS
files/lnp_squ_cell_hyper_re_Lnp-BMR10-Restrict.plt
```

```
                                                Thu Jan 13 13:25:05 2011
==================================================================
 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 The form of the probability function is:

 P[response] = Background + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),
        where CumNorm(.) is the cumulative normal distribution function

 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

 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

 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. 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
 Scaled
 Dose Est._Prob. Expected Observed Size 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.4 Respiratory Metaplasia

All available dichotomous models in the Benchmark Dose Software (version 2.1.2) were fit to the incidence data shown in Table F-46, for respiratory metaplasia of the olfactory epithelium in male F344/DuCrj rats exposed to 1,4-dioxane vapors for 2 years (NCI, 1978). Doses associated with a BMR of a 10% extra risk were calculated.

Table F-46 Incidence of respiratory metaplasia of the olfactory epithelium in male F344/DuCrj rats exposed to 1,4-dioxane via inhalation for 2 years

1,4-dioxane vapor concentration (ppm)			
0	**50**	**250**	**1,250**
11/50 (22%)	34/50 (68%)	49/50 [a] (98%)	48/50[a] (96%)

[a]p ≤ 0.01 by Fisher's exact test.

Source: Kasai et al. (2009).

As assessed by the χ^2 goodness-of-fit test, no models in the software provided adequate fits to the data for the incidence of respiratory metaplasia of the olfactory epithelium in male rats (χ^2 $p \geq 0.1$) (Table F-47). However, given that first non-control dose had a response level substantially above the desired BMR (i.e. 10%), the use of BMD methods included substantial model uncertainty. The model uncertainty associated with this dataset is related to low-dose extrapolation and consistent with BMD Technical Guidance Document (U.S. EPA, 2012a) all available dichotomous models in the Benchmark Dose Software (version 2.1.2) were fit to the incidence data shown in Table F-46 with the highest dose group omitted. As assessed by the χ^2 goodness-of-fit test , the logistic, log-logistic, log-probit, and probit models all exhibited a statistically significant lack of fit (i.e., χ^2 p-value < 0.1;See Table F-48), and thus should not be considered further for identification of a POD. The BMDL estimates for all appropriately fitting models were within threefold difference of each other, indicating that BMDL selection should be made based on model fit (U.S. EPA, 2012a). The AIC values for gamma, multistage, quantal-linear, and Weibull models in Table F-48 are equivalent and the lowest and, in this case, essentially represent the same model. Therefore, consistent with the Benchmark Dose Technical Guidance (U.S. EPA, 2012a), any of them with equal AIC values (gamma, multistage, quantal-linear, or Weibull) could be used to identify a POD for this endpoint. The model plot for the gamma model (Figure F-47) and output are included immediately after the table.

Table F-47 Goodness-of-fit statistics and BMD_{10} and $BMDL_{10}$ 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

Model	AIC	*p*-value[a]	Scaled Residual of Interest	BMD_{10} (ppm)	$BMDL_{10}$ (ppm)
Male					
Gamma[b]	179.68	0	-2.07	17.4082	12.3829
Logistic	191.339	0	1.788	34.2946	24.5917
Log-logistic[c]	152.72	0.0285	0.039	4.05465	1.90233
Log-probit[c]	161.267	0	-0.39	14.3669	10.3023
Multistage (2 degree)[d]	179.68	0	-2.07	17.4082	12.3829
Probit	198.785	0	1.479	61.4378	45.9091
Weibull[b]	179.68	0	-2.07	17.4082	12.3829
Quantal-Linear	179.68	0	-2.07	17.4082	12.3829
Dichotomous-Hill[c]	150.466	NA	0	38.8552	31.4727

[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-48 Goodness-of-fit statistics and BMD_{10} and $BMDL_{10}$ 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

Model	AIC	*p*-value[a]	Scaled Residual of Interest	BMD_{10} (ppm)	$BMDL_{10}$ (ppm)
Male					
Gamma[b, e]	**129.463**	**0.5815**	**-0.106**	**6.46848**	**4.73742**
Logistic	133.583	0.0119	-1.031	12.5197	9.34421
Log-logistic[c]	131.182	NA	0	14.2075	3.77044
Log-probit[c]	131.182	NA	0	12.2114	7.80131
Multistage (2 degree)[d, e]	**129.463**	**0.5815**	**-0.106**	**6.46847**	**4.73742**
Probit	136.121	0.0066	-1.511	15.2883	11.6855
Weibull[b]	129.463	0.5815	-0.106	6.46847	4.73742
Quantal-Linear[e]	**129.463**	**0.5815**	**-0.106**	**6.46847**	**4.73742**

[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 models based on lowest AIC.

Source: Kasai et al. (2009).

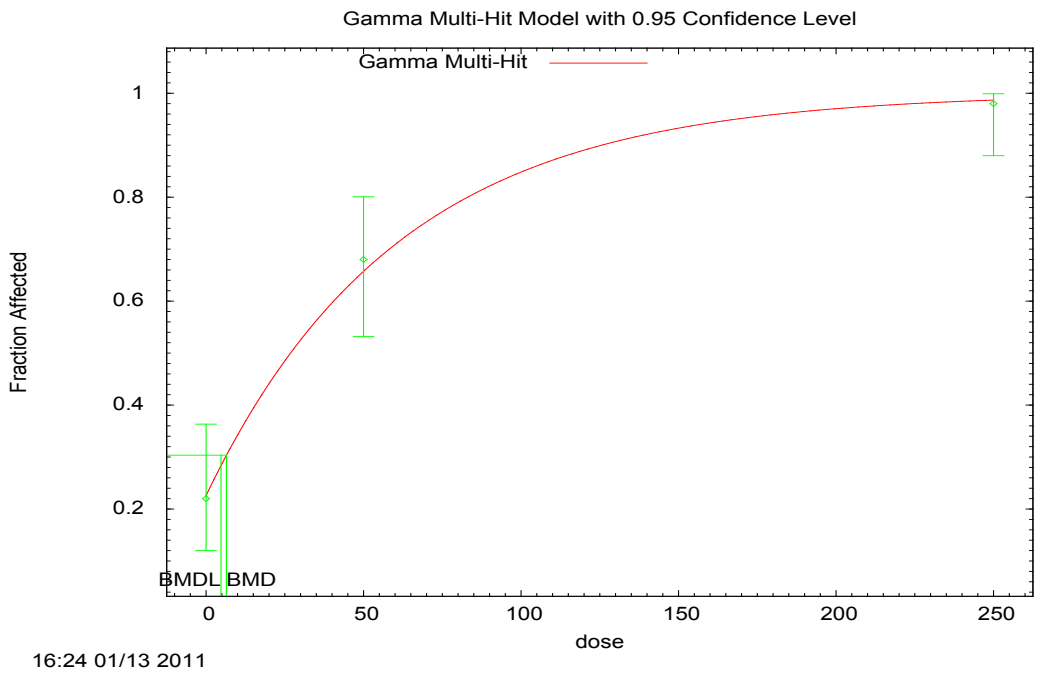


Figure F-47 BMD Gamma model of respiratory metaplasia of olfactory epithelium incidence data for male rats exposed to 1,4-dioxane vapors for 2 years

```
    ==================================================================
    Gamma Model. (Version: 2.15; Date: 10/28/2009)
    Input Data File: C:/Documents and Settings/pgillesp/Desktop/BMDS
    files/gam_resp_meta_no high dose_Gam-BMR10-Restrict.(d)
            Gnuplot Plotting File: C:/Documents and Settings/pgillesp/Desktop/BMDS
    files/gam_resp_meta_no high dose_Gam-BMR10-Restrict.plt
                                                  Thu Jan 13 16:24:15 2011
    ==================================================================
     BMDS_Model_Run
    ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
     The form of the probability function is:

     P[response]= background+(1-background)*CumGamma[slope*dose,power],
            where CumGamma(.) is the cummulative Gamma distribution function

     Dependent variable = Effect
     Independent variable = Dose
     Power parameter is restricted as power >=1

     Total number of observations = 3
     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 (and Specified) Parameter Values
     Background = 0.230769
     Slope = 0.022439
     Power = 1.3

     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 -0.33
     Slope -0.33 1
```

```
 Parameter Estimates

 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. 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

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 -62.5908 3
 Fitted model -62.7313 2 0.280907 1 0.5961
 Reduced model -99.1059 1 73.0301 2 <.0001

 AIC: 129.463

 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size 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

 Chi^2 = 0.30 d.f. = 1 P-value = 0.5815

 Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 6.46848
 BMDL = 4.73742
```

F.5 Atrophy

All available dichotomous models in the Benchmark Dose Software (version 2.1.2) were fit to the incidence data shown in Table F-49, for atrophy of the olfactory epithelium in 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-49 Incidence of atrophy of the olfactory epithelium in male 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%)

[a]p ≤ 0.01 by Fisher's exact test.

Source: Kasai et al. (2009).

As assessed by the χ^2 goodness-of-fit test, the gamma, logistic, log-probit, multistage, probit, Weibull, and quantal-linear models all exhibited a statistically significant lack of fit (i.e., χ^2 *p*-value < 0.1; see Table F-50), and thus should not be considered further for identification of a POD. The BMDL estimates for all appropriately fitting models were within threefold difference of each other, indicating that BMDL selection should be made based on model fit (U.S. EPA, 2012a). As assessed by the AIC, the Log-logistic model provided the best fit to the atrophy data for male rats (Table F-50, Figure F-48), and could be used to derive a POD for this endpoint. However, given that first non-control dose had a response level substantially above the desired BMR (i.e. 10%), the use of BMD methods included substantial model uncertainty.

Table F-50 Goodness-of-fit statistics and BMD_{10} and $BMDL_{10}$ 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	BMD_{10} (ppm)	$BMDL_{10}$ (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,e]	**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.
[b]Power restricted to ≥ 1.
[c]Slope restricted to ≥ 1.
[d]Betas restricted to ≥0.
[e]Bold indicates best-fit model based on lowest AIC.

Source: Kasai et al. (2009).

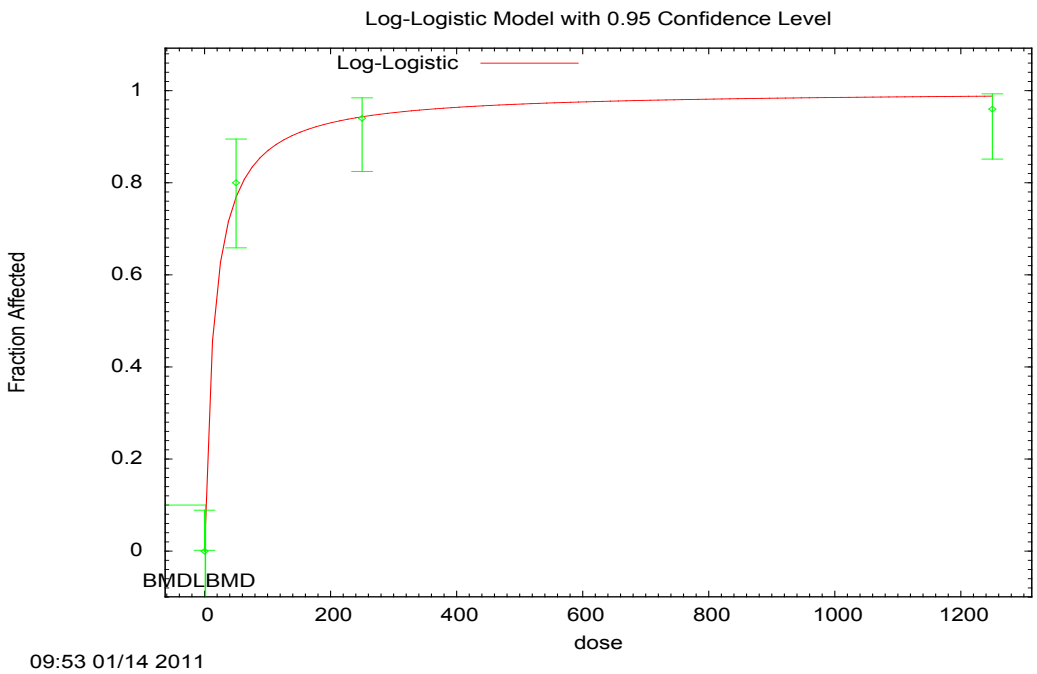


Figure F-48 BMD Log-Logistic model of atrophy of olfactory epithelium incidence data for male rats exposed to 1,4-dioxane vapors for 2 years to support the results in Table F-13.

```
    ==================================================================
 1  Logistic Model. (Version: 2.13; Date: 10/28/2009)
 2  Input Data File: C:/Documents and Settings/pgillesp/Desktop/BMDS
 3  files/lnl_atrophy_Lnl-BMR10-Restrict.(d)
 4          Gnuplot Plotting File: C:/Documents and Settings/pgillesp/Desktop/BMDS
 5  files/lnl_atrophy_Lnl-BMR10-Restrict.plt
 6                                                  Fri Jan 14 09:53:22 2011
 7  ==================================================================
 8   BMDS_Model_Run
 9  ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
10   The form of the probability function is:
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
27   intercept = -3.48908
28   slope = 1
29
30   Asymptotic Correlation Matrix of Parameter Estimates
31  (*** The model parameter(s) -background -slope have been estimated at a boundary
32  point, or have been specified by the user, and do not appear in the correlation
33  matrix)
34
```

```
 intercept
 intercept 1

 Parameter Estimates
 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. 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
 Scaled
 Dose Est._Prob. Expected Observed Size 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.6 Hydropic Change

All available dichotomous models in the Benchmark Dose Software (version 2.1.2) were fit to the incidence data shown in Table F-51, 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-51 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**
0/50	2/50 (4%)	36/50 [a] (72%)	49/50[a] (98%)

[a]p ≤ 0.01 by Fisher's exact test.

Source: Kasai et al., (2009).

For incidence of hydropic change of the lamina propria in F344/DuCrj male rats, the gamma, logistic, multistage, probit, Weibull, and quantal-linear models all exhibited a statistically significant lack of fit (i.e., χ^2 *p*-value < 0.1; see Table F-53), and thus should not be considered further for identification of a POD. The BMDL estimates for all appropriately fitting models were within threefold difference of each other, indicating that BMDL selection should be made based on model fit (U.S. EPA, 2012a). As assessed by the AIC, the Log-logistic model provided the best fit to the hydropic change of the lamina propria data for male rats (Table F-52, Figure F-49 and subsequent text output), and could be used to derive a POD of for this endpoint.

Table F-52 Goodness-of-fit statistics and BMD_{10} and $BMDL_{10}$ 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	BMD_{10} (ppm)	$BMDL_{10}$ (ppm)
Male					
Gamma[b]	98.3441	0.0002	-1.321	51.979	28.7632
Logistic	117.957	0	-1.143	89.2909	70.6131
Log-logistic[c,e]	**90.5388**	**0.6819**	**-0.333**	**68.5266**	**46.7808**
Log-probit[c]	91.5881	0.3458	-0.538	63.0852	44.5657
Multistage (2 degree)[d]	99.3482	0.0256	-2.411	28.7899	22.6831
Probit	136.585	0	-2.099	92.6118	74.3784
Weibull[b]	100.225	0.0033	-1.899	39.1371	23.9762
Quantal-Linear	99.3482	0.0256	-2.411	28.7899	22.6831
Dichotomous-Hill[c]	91.8937	1	0	73.1032	49.2687

[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 AIC.

Source: Kasai et al. (2009).

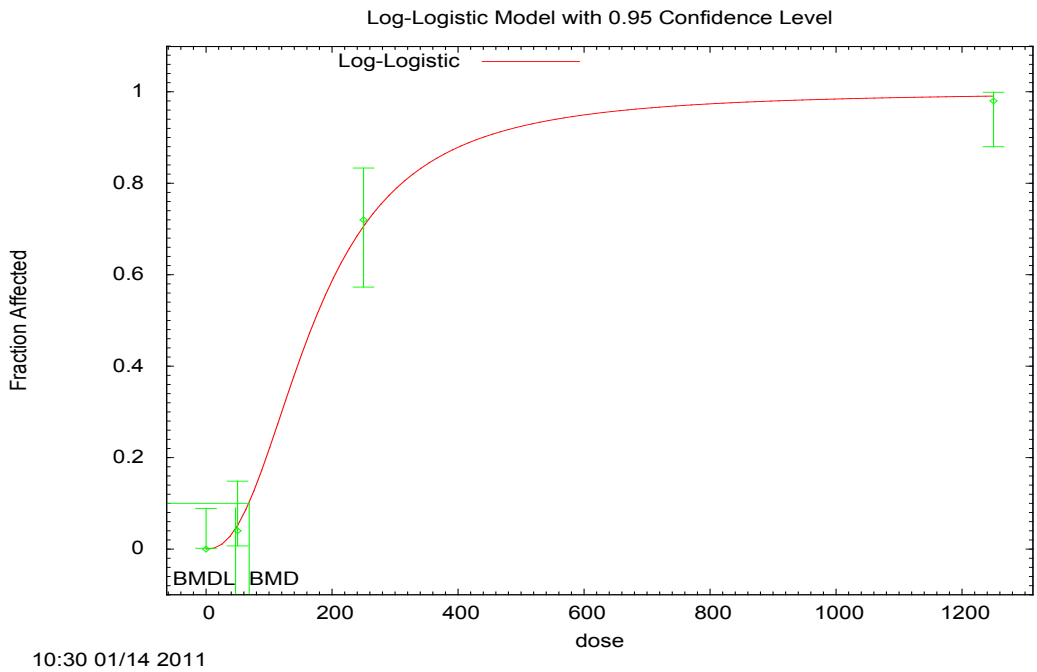


Figure F-49 BMD Log-logistic model of hydropic change of lamina propria (nasal cavity) incidence data for male rats exposed to 1,4-dioxane vapors for 2 years to support the results in Table F-16.

```
================================================================
Logistic Model. (Version: 2.13; Date: 10/28/2009)
Input Data File: C:/Documents and Settings/pgillesp/Desktop/BMDS
files/lnl_hydrpic_Lnl-BMR10-Restrict.(d)
        Gnuplot Plotting File: C:/Documents and Settings/pgillesp/Desktop/BMDS
files/lnl_hydrpic_Lnl-BMR10-Restrict.plt
```

```
Fri Jan 14 10:30:47 2011
====================================================================
 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 The form of the probability function is:
 P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]

 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

 User has chosen the log transformed model

 Default Initial Parameter Values
 background = 0
 intercept = -11.5745
 slope = 2.19638

 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
 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. 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
 Scaled
 Dose Est._Prob. Expected Observed Size 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.7 Sclerosis

All available dichotomous models in the Benchmark Dose Software (version 2.1.2) were fit to the incidence data shown in Table F-53, 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-53 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**
0/50	0/50	22/50 [a] (44%)	40/50[a] (80%)

[a]p ≤ 0.01 by Fisher's exact test.

Source: Kasai et al. (2009).

As assessed by the χ^2 goodness-of-fit test , all models with the exception of the dichotomous-hill model, exhibited a statistically significant lack of fit (i.e., χ^2 *p*-value < 0.1;See Table F-54), and thus should not be considered further for identification of a POD. Since the dichotomous-hill model provided the only fit to the sclerosis of the lamina propria data for male rats as assessed by the χ^2 goodness-of-fit test (Table F-54, Figure F-50 and subsequent text output), it could be considered to derive a POD for this endpoint; however, the model output warned that the BMDL estimate was "imprecise at best".

Table F-54 Goodness-of-fit statistics and BMD_{10} and $BMDL_{10}$ 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	BMD_{10} (ppm)	$BMDL_{10}$ (ppm)
Male					
Gamma[b]	134.416	0.0123	-1.89	75.4489	57.6938
Logistic	161.562	0	4.542	244.217	196.446
Log-logistic[c]	130.24	0.0683	-1.579	86.3863	52.4762
Log-probit[c]	127.784	0.0829	-0.995	109.558	88.1232
Multistage (2 degree)[d]	132.436	0.0356	-1.949	71.9719	57.6471
Probit	159.896	0	4.619	231.856	191.419
Weibull[b]	132.436	0.0356	-1.949	71.9719	57.6471
Quantal-Linear	132.436	0.0356	-1.949	71.9719	57.6471
Dichotomous-Hill[c,e]	124.633	0.9994	0	206.74	167.46

[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).

```
=================================================================
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

 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size Residual
 ---------------------------------------------------------------------
 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
```

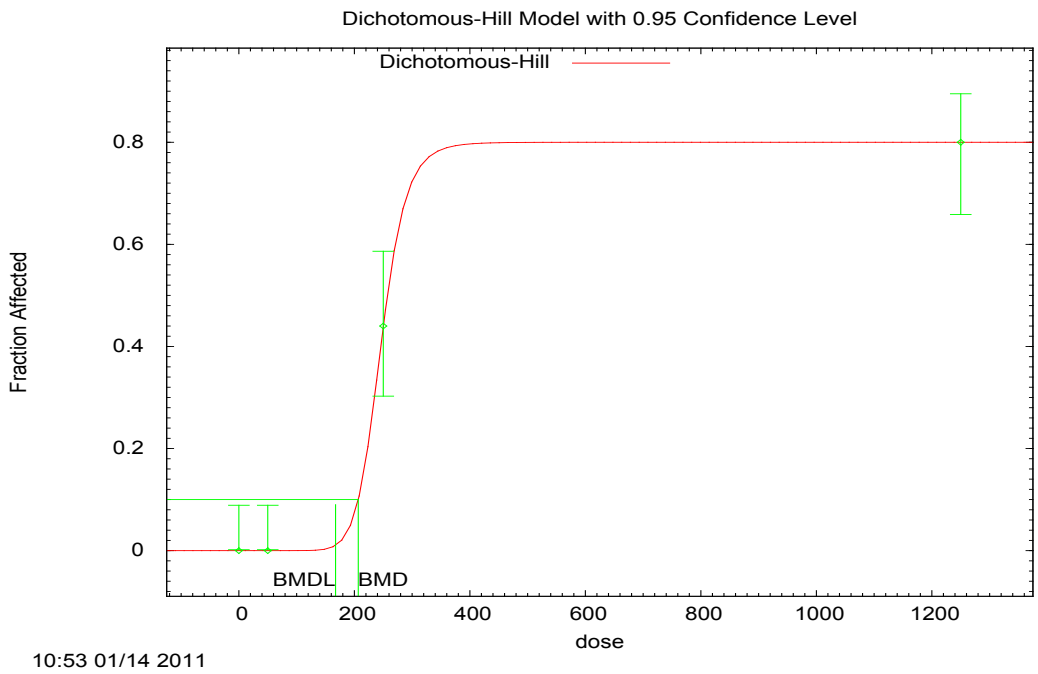


Figure F-50 BMD Log-logistic model of sclerosis of lamina propria (nasal cavity) incidence data for male rats exposed to 1,4-dioxane vapors for 2 years to support the results in Table F-18.

APPENDIX G. DETAILS OF BMD ANALYSIS FOR INHALATION UNIT RISK FOR 1,4-DIOXANE

Multistage cancer models available in the Benchmark Dose Software (BMDS) (version 2.2beta) were fit to the incidence data for hepatocellular carcinoma and/or adenoma, nasal cavity squamous cell carcinoma, renal cell carcinoma, peritoneal mesothelioma, and mammary gland fibroadenoma, Zymbal gland adenoma, and subcutis fibroma in rats exposed to 1,4-dioxane vapors for 2 years (Kasai et al., 2009). Concentrations associated with a benchmark response (BMR) of a 10% extra risk were calculated. BMC_{10} and $BMCL_{10}$ values from the best fitting model, determined by adequate global- fit ($\chi^2 p \geq 0.1$) and AIC values, are reported for each endpoint (U.S. EPA, 2012a). Given the multiplicity of tumor sites, basing the IUR on one tumor site will underestimate the carcinogenic potential of 1,4-dioxane. Multitumor BMD analysis was conducted using BMDS (version 2.2beta) MS_Combo program; model output is shown in Section G.3. Additionally, a Bayesian analysis was performed using WinBUGS (Spiegelhalter et al., 2003), freeware developed by the MRC Biostatistical Unit, Cambridge, United Kingdom (available at http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/contents.shtml) and reported in detail in Section G.3. The results of both analyses were comparable and resulted in equivalent IURs.

A summary of the BMDS model predictions for the Kasai et al. (2009) study are shown in Table G-1.

G.1 General Issues and Approaches to BMDS and Multitumor Modeling

G.1.1 Combining Data tumor types

The incidence of adenomas and the incidence of carcinomas within a dose group at a site or tissue in rodents are sometimes combined. This practice is based upon the hypothesis that adenomas may develop into carcinomas if exposure at the same dose was continued (U.S. EPA, 2005a; McConnell et al., 1986). In the same manner and was done for the oral cancer assessment (Appendix D), the incidence of hepatic adenomas and carcinomas was summed without double-counting them so as to calculate the combined incidence of either a hepatic carcinoma or a hepatic adenoma in rodents.

The remaining of the tumor types were assumed to occur independently.

G.1.2 Summary

The BMDS models recommended to calculate rodent BMC_{10} and $BMCL_{10}$ values for individual tumor types and combined tumor analysis are summarized in Table G-1. The first order multistage models for most tumor types were selected because they resulted in the lowest AIC values; however, for renal cell

carcinoma and Zymbal gland adenoma, the lowest AIC model was not the first order model. In BMDS, the third order model resulted in the lowest AIC (first (1°)-, second (2°)-, and third (3°)-degree models were evaluated); however, using the MCMC approach in WinBUGS, the third order (3°) multistage model did not converge while the second order(2°) model did converge. Thus, for renal cell carcinoma and Zymbal gland adenoma, the second order (2°) multistage model was used in both the MCMC (WinBugs) approach and the BMDS (Version 2.2 beta) MS_Combo approach for direct comparison of results. These results are shown below in Table G-1.

Table G-55 Summary of BMC_{10} and $BMCL_{10}$ model results for individual tumor types and combined tumor analysis for male rats exposed to 1,4-dioxane vapors (Kasai et al., 2009)

Endpoint	Multistage Model Degree	AIC	p-value	χ2 Residual of Interest	BMC10 (ppm)	BMCL10 (ppm)
Nasal squamous cell carcinoma	First (1°)	49.03	0.9607	0.176	1107.04	629.95
Hepatocellular adenoma/carcinoma	First (1°)	127.9	0.6928	-0.763	252.80	182.26
Renal cell carcinoma	Third (3°)	29.99	0.9984	0.017	1355.16	16.15
Peritoneal mesothelioma	First (1°)	155.4	0.8509	-0.204	82.21	64.38
Mammary gland fibroadenoma	First (1°)	86.29	0.7904	-0.149	1635.46	703.03
Zymbal gland adenoma	Third (3°)	29.99	0.9984	0.017	1355.16	16.15
Subcutis fibroma[a]	First (1°)	89.2	0.5245	0.537	141.762	81.9117
BMDS Version 2.2beta MS_Combo					40.4	30.3
WinBUGS multitumor analysis[b]					39.2	31.4

[a]High-dose dropped. See Section G.2.6 for details.
[b]In MCMC approach, the simulations for the four-parameter third order(3°) multistage model did not converge for renal cell carcinomas and Zymbal gland adenomas. Second order (2°) multistage model was used instead.

G.2 BMDS Model Output for Multistage Cancer Models for Individual Tumor Types

For tumor incidence data reported in the Kasai et al. (2009) 2-year inhalation bioassay, multistage cancer models of first (1°)-, second (2°)-, and third (3°)degrees were implemented BMDS (Version 2.2Beta). Incidence data used for BMD analysis are shown in Table G-2. Tumor incidence for mammary gland adenoma was excluded from this analysis since only 1 tumor of this type was found across all doses.

Table G-56 Incidence of tumors in male F344/DuCrj rats exposed to 1,4-dioxane vapor by whole-body inhalation for 2 years

Effect	1,4-dioxane vapor concentration (ppm)			
	0 (clean air)	50	250	1,250
Nasal squamous cell carcinoma	0/50	0/50	1/50	6/50[b,c]
Hepatocellular adenoma	1/50	2/50	3/50	21/50[a,c]
Hepatocellular carcinoma	0/50	0/50	1/50	2/50
Hepatocellular adenoma or carcinoma	1/50	2/50	4/50	22/50[a,c]
Renal cell carcinoma	0/50	0/50	0/50	4/50[c]
Peritoneal mesothelioma	2/50	4/50	14/50[a]	41/50[a,c]
Mammary gland fibroadenoma	1/50	2/50	3/50	5/50[d]
Zymbal gland adenoma	0/50	0/50	0/50	4/50[c]
Subcutis fibroma	1/50	4/50	9/50[a]	5/50

[a]$p \leq 0.01$ by Fisher's exact test.
[b]$p \leq 0.05$ by Fisher's exact test.
[c]$p \leq 0.01$ by Peto's test for dose-related trend.
[d]$p \leq 0.05$ by Peto's test for dose-related trend.
[e]Provided via personal communication from Dr. Tatsuya Kasai (2008) to Dr. Reeder Sams on 12/23/2008. 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

The incidence data for nasal squamous cell carcinoma were monotonic non-decreasing functions of dose; therefore, these data are appropriate for dose-response modeling using BMDS. The results of the BMDS modeling for the multistage cancer model for first (1°)-, second (2°)-, and third (3°)-degree polynomials are shown in Table G-3. The first (1°)-degree polynomial was the best fitting model based on AIC. The plot (Figure G-1) and model output for the first (1°)-degree model are shown below.

Table G-57 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)

Polynomial Degree	AIC	*p*-value	χ^2 Residual of Interest	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)
(1°) First[a]	49.0308	0.9607	0.176	1,107.04	629.95
(2°) Second	50.8278	0.9087	-0.021	1,086.94	642.43
(3°) Third	50.8278	0.9087	-0.021	1,086.94	642.43

[a]Best-fitting model based on AIC.

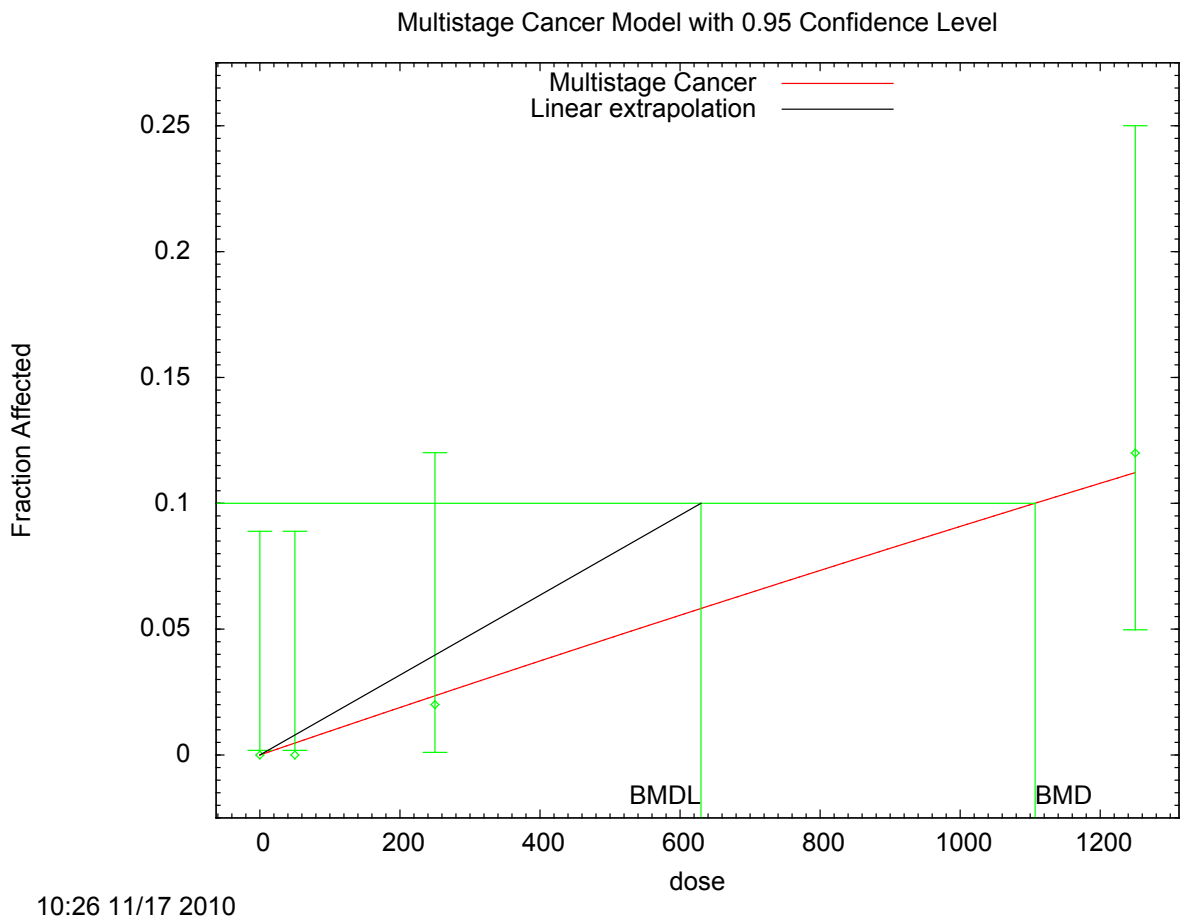


Figure G-51 Multistage model (First (1°)-degree) for male rat nasal squamous cell carcinomas.

```
    ======================================================================
    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
Beta(1) = 0.000104666

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
95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
Background 0 * * *
Beta(1) 9.51733e-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 -23.2482 4
Fitted model -23.5154 1 0.534383 3 0.9113
Reduced model -30.3429 1 14.1894 3 0.002658

AIC: 49.0308

Log-likelihood Constant 20.493267595834471


              Goodness of Fit
Scaled
Dose Est._Prob. Expected Observed Size Residual
-----------------------------------------------------------------------
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
1,250.0000 0.1122 5.608 6 50 0.176

Chi^2 = 0.30 d.f. = 3 P-value = 0.9607
```

```
 Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 1107.04
 BMDL = 629.948
 BMDU = 2215.11

Taken together, (629.948, 2215.11) is a 90% two-sided confidence interval for the BMD
```

G.2.2 Hepatocellular Adenoma and Carcinoma

The incidence data for the occurrence of either hepatocellular adenoma or carcinoma were combined for this analysis as explained in G.1.1. The incidence data were monotonic non-decreasing functions of dose; therefore, these data are appropriate for dose-response modeling using BMDS. The results of the BMDS modeling for the multistage cancer model for first-, second-, and third-degree polynomials are shown in Table G-4. The 1st-degree polynomial was the best fitting model based on AIC. The plot (Figure G-2) and model output for the 1st-degree model are shown below.

Table G-58 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)

Polynomial Degree	AIC	*p*-value	χ^2 Residual of Interest	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)
(1°) First[a]	127.86	0.6928	-0.763	252.80	182.26
(2°) Second	129.157	0.7636	-0.094	377.16	190.28
(3°) Third	129.131	0.8	-0.068	397.426	190.609

[a]Best-fitting model based on AIC.

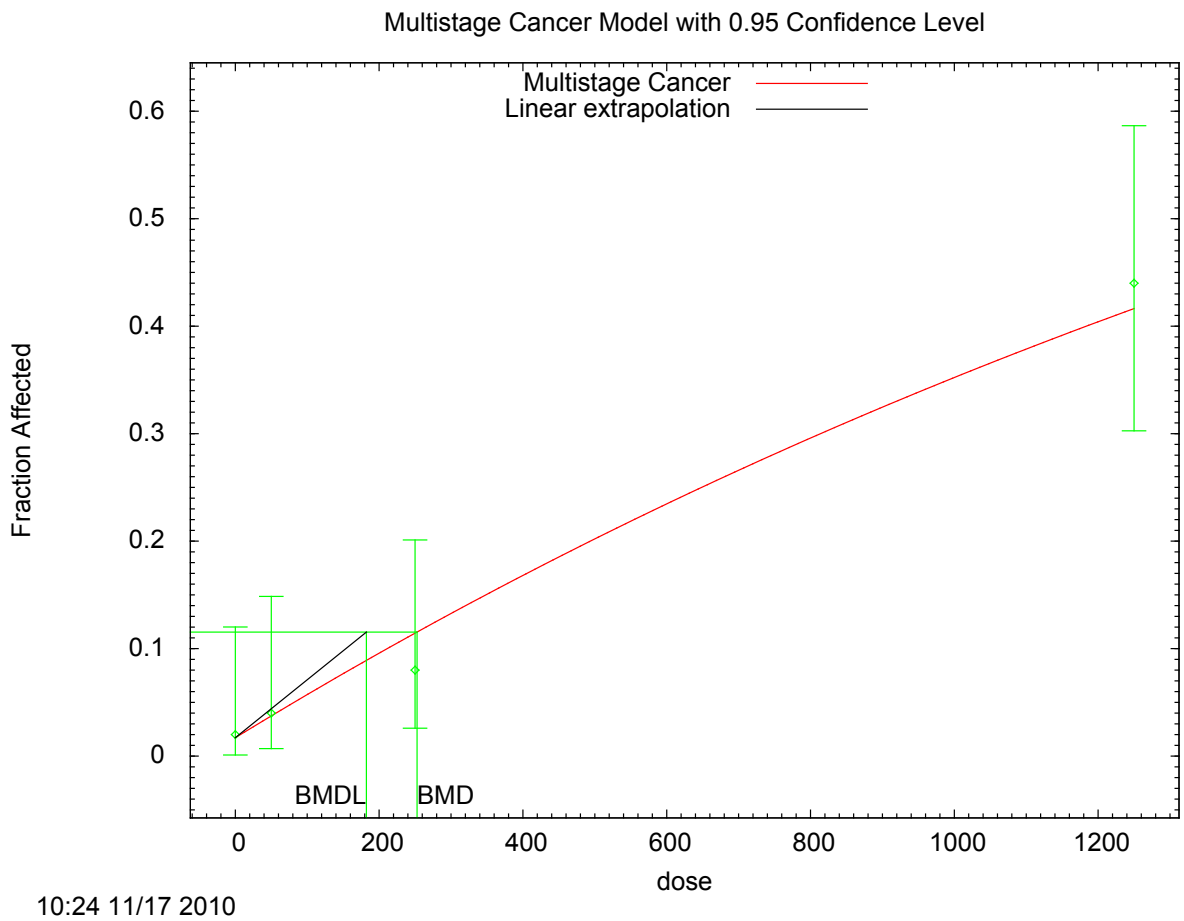


Figure G-52 Multistage model (First-degree (1°)) for male rat hepatocellular adenomas and carcinomas.

```
=====================================================================
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

 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
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
 Scaled
 Dose Est._Prob. Expected Observed Size 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
 1,250.0000 0.4162 20.810 22 50 0.342
 Chi^2 = 0.73 d.f. = 2 P-value = 0.6928


 Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 252.799
 BMDL = 182.256
 BMDU = 371.457

Taken together, (182.256, 371.457) is a 90% two-sided confidence interval for the BMD
```

G.2.3 Renal Cell Carcinoma and Zymbal Gland Adenoma

The incidence data for renal cell carcinomas and Zymbal gland adenomas were the same. These data were monotonic non-decreasing functions of dose; therefore, these data are appropriate for dose-response modeling using BMDS. The results of the BMDS modeling for the multistage cancer model for first (1°)-, second (2°)- and third-degree (3°) polynomials are shown in Table G-5. The third-degree (3°)polynomial was the best fitting model based on AIC; however, when conducting the multitumor analysis, WinBUGS was unable to converge using the third-degree (3°) model. Thus, the second degree (2°) model was used in the multitumor analyses. The plots (Figure G-3 and Figure G-4) and model outputs for both the second (2°)- and third-degree (3°) models are shown below.

Table G-59 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)

Polynomial Degree	AIC	*p*-value	χ^2 Residual of Interest	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)
(1°) First	31.6629	0.8004	0.446	1,974.78	957.63
(2°) Second	30.2165	0.9817	0.085	1,435.28	999.44
(3°) Third[a]	29.9439	0.9984	0.017	1,355.16	1,016.15

[a]Best-fitting model based on AIC.

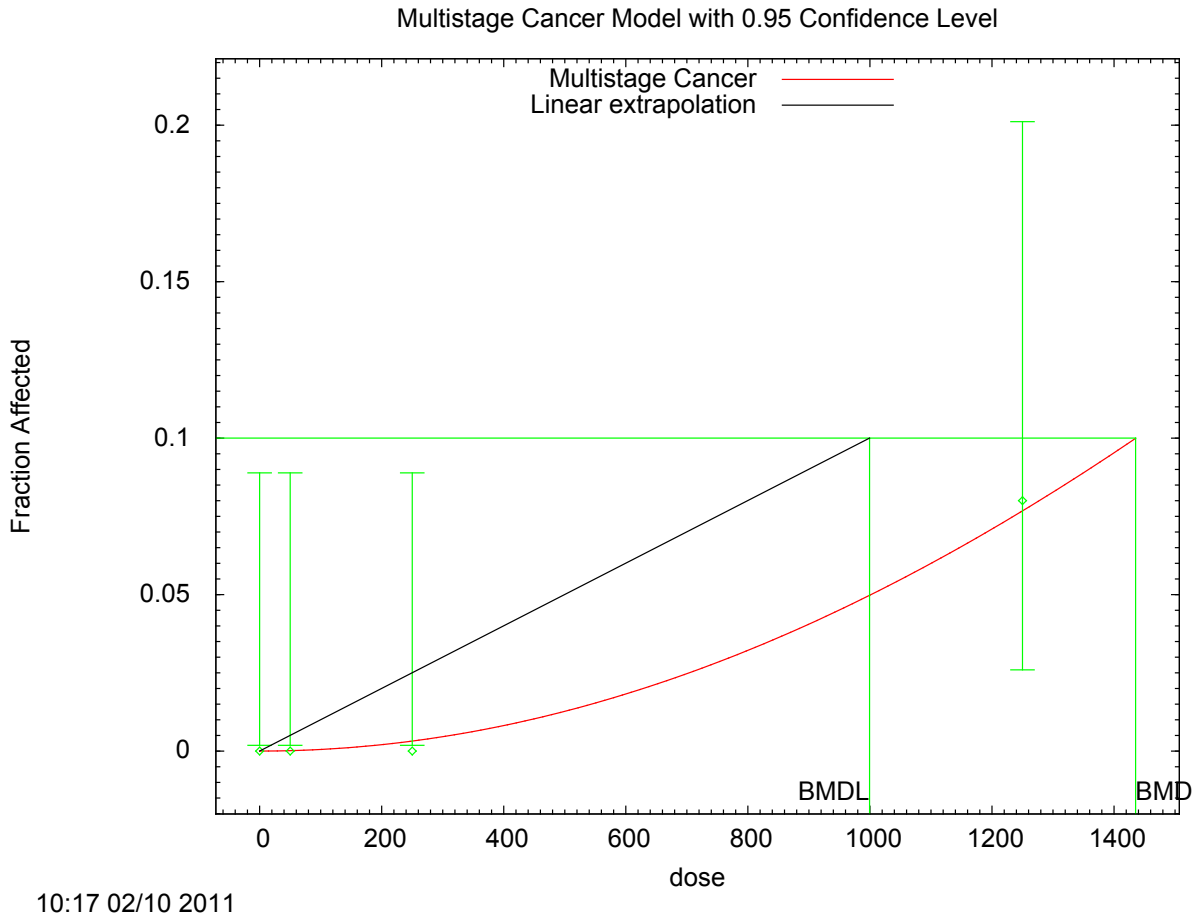


Figure G-53 Multistage model (Second-degree (2°)) for male rat renal cell carcinomas and Zymbal gland adenomas.

```
=====================================================================
Multistage Cancer Model. (Version: 1.9; Date: 05/26/2010)
Input Data File: C:/Documents and
Settings/emclanah/Desktop/BMD_14D_Cancer/Data/msc_Kasai2009_renal_Msc2-BMR10.(d)
        Gnuplot Plotting File: C:/Documents and
Settings/emclanah/Desktop/BMD_14D_Cancer/Data/msc_Kasai2009_renal_Msc2-BMR10.plt
                                                Thu Feb 10 10:17:39 2011
=====================================================================
 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
 The form of the probability function is:

```

```
P[response] = background + (1-background)*[1-EXP(-beta1*dose^1-beta2*dose^2)]

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 = 3
Total number of specified parameters = 0
Degree of polynomial = 2

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) = 5.40386e-008

Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -Background -Beta(1) have been estimated at a boundary
point, or have been specified by the user, and do not appear in the correlation
matrix)

Beta(2)
Beta(2) 1

Parameter Estimates
95.0% Wald Confidence Interval
Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
Background 0 * * *
Beta(1) 0 * * *
Beta(2) 5.11454e-008 * * *

* - Indicates that this value is not calculated.

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

AIC: 30.2165

Goodness of Fit
Scaled
Dose Est._Prob. Expected Observed Size 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

Chi^2 = 0.17 d.f. = 3 P-value = 0.9817
```

```
 Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 1,435.28
 BMDL = 999.44

 BMDU = 3,666.87

Taken together, (999.44 , 3,666.87) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000100056
```

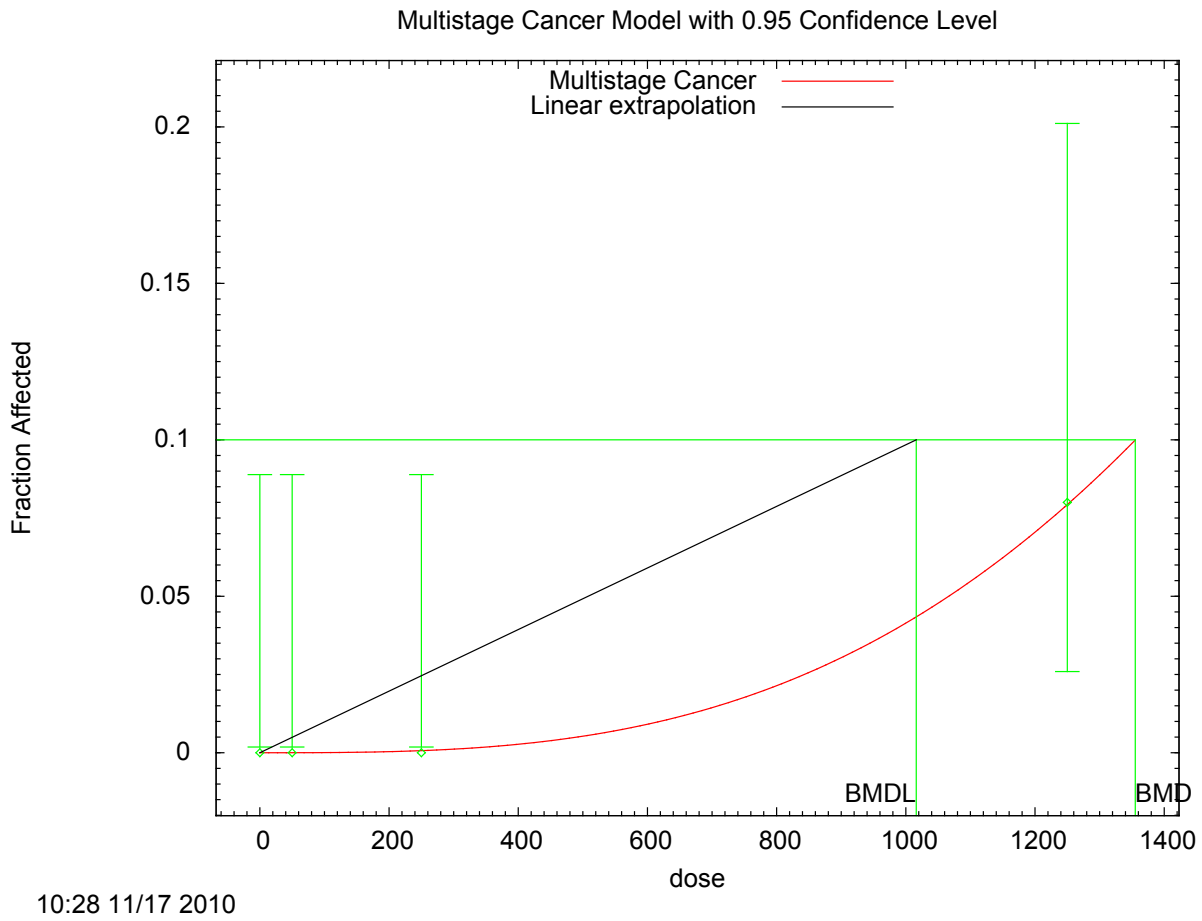


Figure G-54 Multistage model (Third-degree (3°)) for male rat renal cell carcinomas.

```
=================================================================
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-beta2*dose^2-
                          beta3*dose^3)]

 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 = 4
 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) = 4.2804e-011


 Asymptotic Correlation Matrix of Parameter Estimates
(*** The model parameter(s) -Background -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)

 Beta(3)
 Beta(3) 1

 Parameter Estimates

 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
Background 0 * * *
 Beta(1) 0 * * *
 Beta(2) 0 * * *
 Beta(3) 4.23353e-011 * * *

* - Indicates that this value is not calculated.

 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

 AIC: 29.9439

 Log-likelihood Constant 12.347138085809094


 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size 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

 Chi^2 = 0.03 d.f. = 3 P-value = 0.9984


 Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
```

```
Confidence level = 0.95
 BMD = 1,355.16
 BMDL = 1,016.15
 BMDU = 3,393.6

Taken together, (1016.15, 3393.6 ) is a 90% two-sided confidence interval for the BMD
```

G.2.4 Peritoneal Mesothelioma

The incidence data for peritoneal mesotheliomas were monotonic non-decreasing functions of dose; therefore, these data are appropriate for dose-response modeling using BMDS. The results of the BMDS modeling for the multistage cancer model for 1st, 2nd, and 3rd-degree polynomials are shown in Table G-6. The 1st-degree polynomial was the best fitting model based on AIC. The plot (Figure G-5) and model output for the 1st-degree model are shown below.

Table G-60 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)

Polynomial Degree	AIC	*p*-value	χ^2 Residual of Interest	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)
(1°) First[a]	155.433	0.8509	-0.204	82.21	64.38
(2°) Second	157.168	0.8053	-0.204	96.23	65.15
(3°) Third	157.168	0.8053	0	96.23	65.15

[a] Best-fitting model based on AIC.

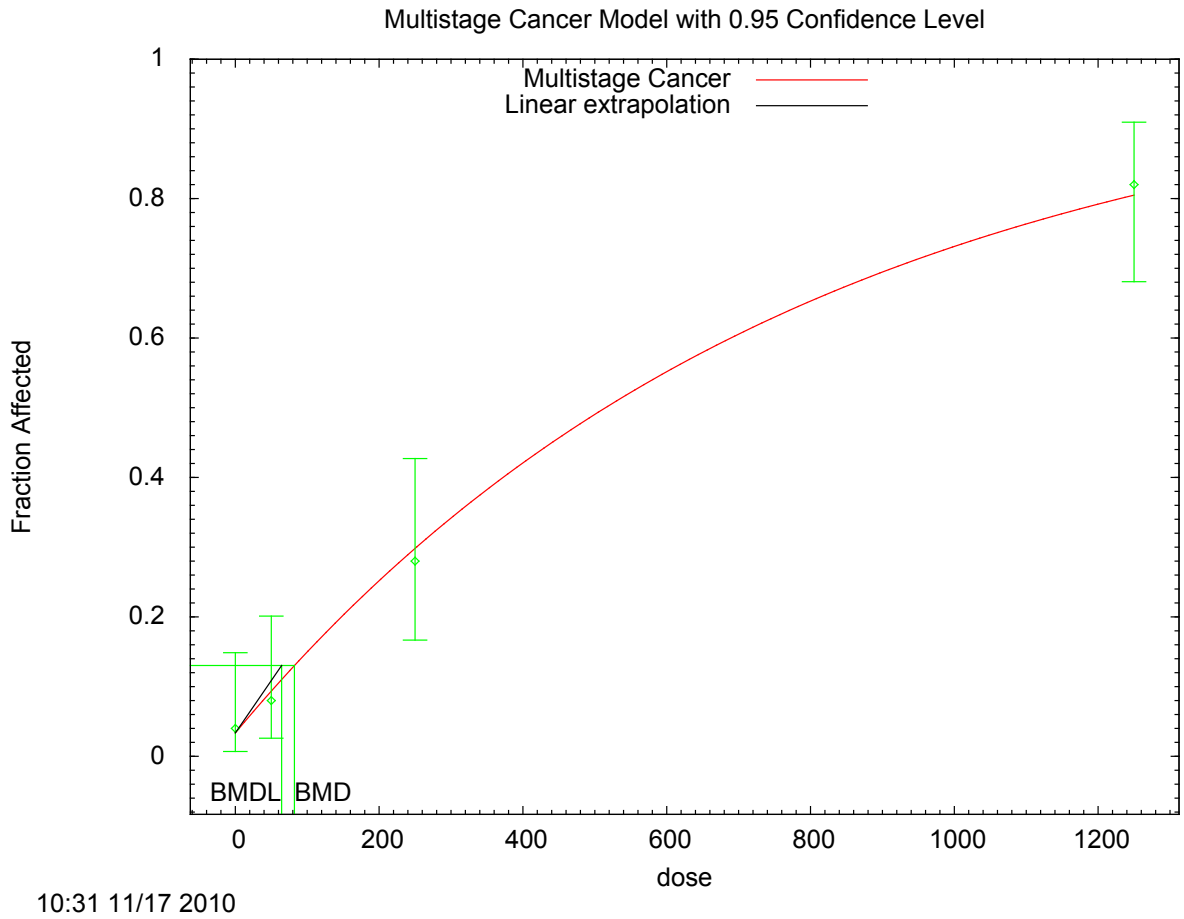


Figure G-55 Multistage model (First-degree (1°)) for male rat peritoneal mesotheliomas.

```
=================================================================
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 = 0.0172414
 Beta(1) = 0.00135351

 Asymptotic Correlation Matrix of Parameter Estimates

 Background Beta(1)
Background 1 -0.45
 Beta(1) -0.45 1

 Parameter Estimates
 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
Background 0.033631 * * *
 Beta(1) 0.00128167 * * *

* - Indicates that this value is not calculated.

 Analysis of Deviance Table

 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

 AIC: 155.433

 Log-likelihood Constant 68.666413125908832

 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size 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
 1,250.0000 0.8053 40.265 41 50 0.263

 Chi^2 = 0.32 d.f. = 2 P-value = 0.8509

 Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 82.2057
 BMDL = 64.3808
 BMDU = 107.497

Taken together, (64.3808, 107.497) is a 90% two-sided confidence interval for the BMD
```

G.2.5 Mammary Gland Fibroadenoma

The incidence data for mammary gland fibroadenomas were monotonic non-decreasing functions of dose; therefore, these data are appropriate for dose-response modeling using BMDS. The results of the BMDS modeling for the multistage cancer model for first (1°)-, second (2°), and third (3°)-degree polynomials are shown in Table G-7. Since quadratic and cubic terms of the multistage models evaluated resulted in the estimates on the boundary, i.e. equal to 0, the first (1°)-degree polynomial was selected based on model parsimony. The plot (Figure G-6) and model output for the first (1°)-degree model are shown below.

Table G-61 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)

Polynomial Degree	AIC	*p*-value	χ^2 Residual of Interest	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)
(1°) First[a]	86.29	0.7904	-0.149	1,635.46	703.03
(2°) Second	86.29	0.7904	-0.149	1,635.46	703.03
(3°) Third	86.29	0.7904	-0.149	1,635.46	703.03

[a]All model fits were equivalent based on AIC. Selected 1st-degree model based on parsimony.

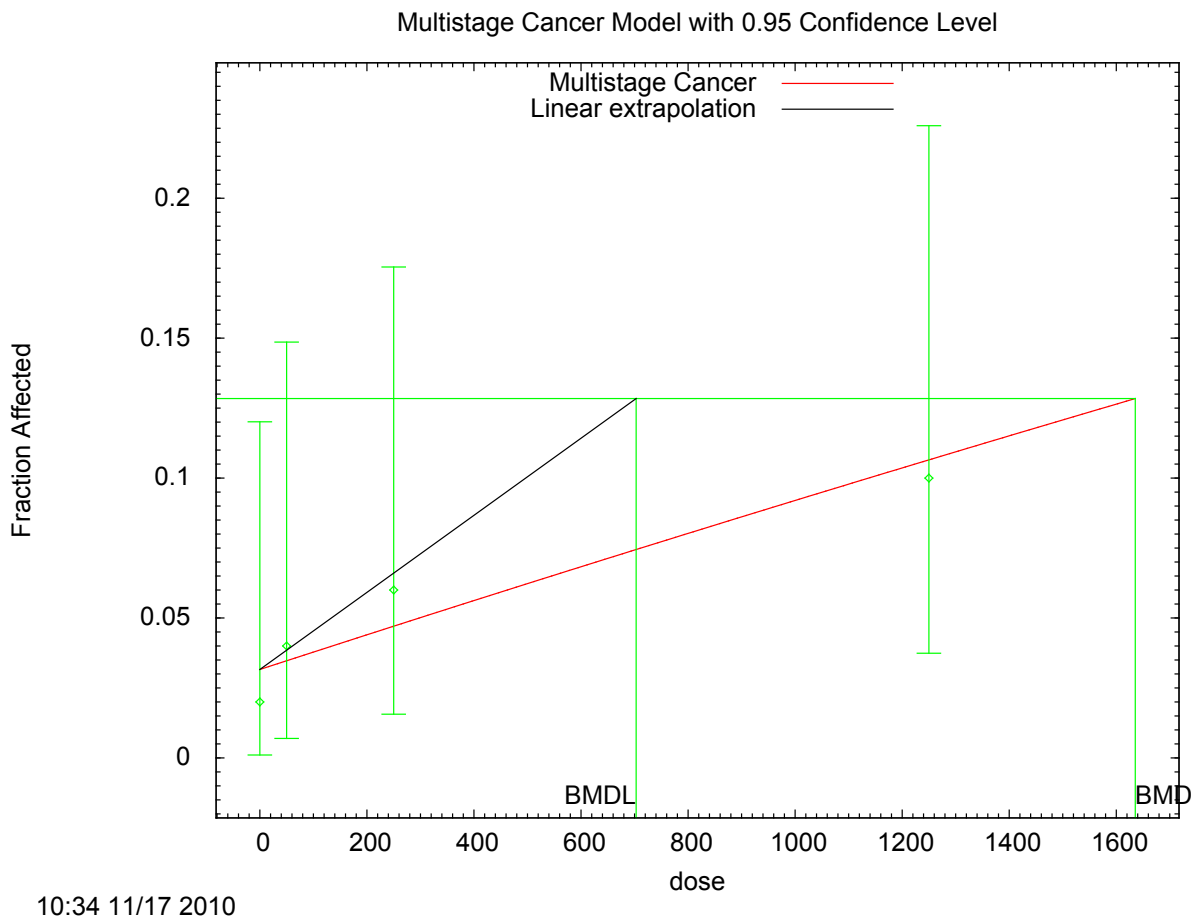


Figure G-56 Multistage model (First-degree (1°)) for male rat mammary gland fibroadenoma.

```
=================================================================
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 = 0.0335609
 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

 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. 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
 Scaled
 Dose Est._Prob. Expected Observed Size 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
 1,250.0000 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
Confidence level = 0.95
 BMD = 1,635.46
 BMDL = 703.034
 BMDU = 1.9523e+009

Taken together, (703.034, 1.9523e+009) is a 90% two-sided confidence interval for the
BMD
```

G.2.6 Subcutis Fibroma

The incidence data for subcutis fibroma were monotonic non-decreasing functions of dose for the control (0 ppm), low (50 ppm), and mid-dose (250 ppm); however, the incidence rate at the high dose (1,250 ppm) was lower than observed at the mid-dose. No BMDS model had reasonable fit to the data without dropping the high dose. The results of the BMDS modeling for the multistage cancer model for first (1°)-, second (2°), and third (3°)-degree polynomials with the high dose dropped are shown in Table G-8. Since quadratic and cubic terms of multistage models evaluated resulted in the estimates on the boundary, i.e. equal to 0, , the first (1°)-degree polynomial was selected based on model parsimony. The plot (Figure G-7) and model output for the first (1°)-degree model are shown below.

Table G-62 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)

Polynomial Degree	AIC	*p*-value	χ^2 Residual of Interest	BMC_{10} (ppm)	$BMCL_{10}$ (ppm)
(1°) First[a]	89.2094	0.5245	0.537	141.76	81.92
(2°) Second	89.2094	0.5245	0.537	141.76	81.92
(3°) Third	89.2094	0.5245	0.537	141.76	81.92

[a]All model fits were equivalent based on AIC. Selected 1st-degree model based on parsimony.

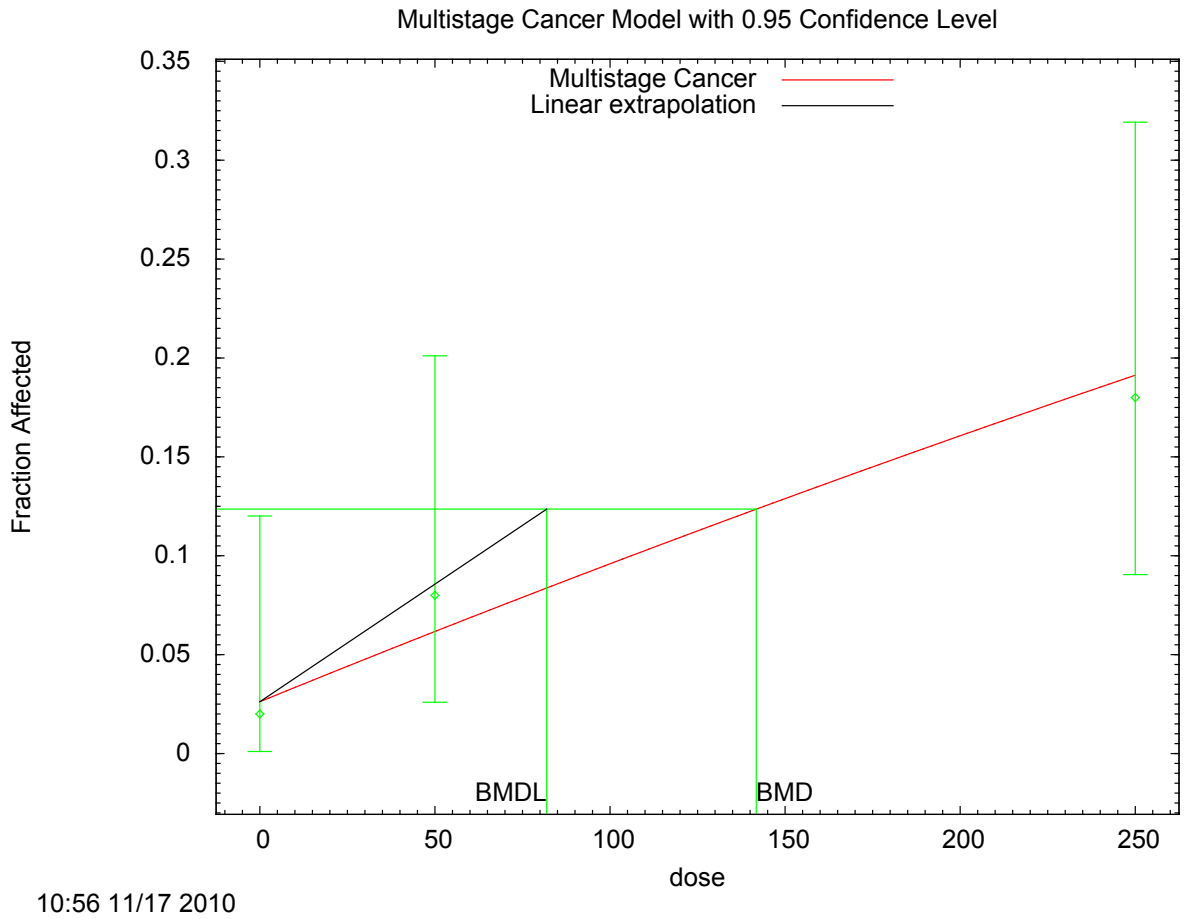


Figure G-57 Multistage model (First-degree (1°)) for male rat subcutis fibroma (high dose dropped).

```
    ==================================================================
    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 = 3
     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 = 0.0327631
 Beta(1) = 0.000673665


 Asymptotic Correlation Matrix of Parameter Estimates

 Background Beta(1)
Background 1 -0.68
 Beta(1) -0.68 1

 Parameter Estimates

 95.0% Wald Confidence Interval
 Variable Estimate Std. Err. Lower Conf. Limit Upper Conf. Limit
Background 0.0262054 * * *
 Beta(1) 0.00074322 * * *

* - Indicates that this value is not calculated.

 Analysis of Deviance Table

 Model Log(likelihood) # Param's Deviance Test d.f. P-value
 Full model -42.4101 3
 Fitted model -42.6047 2 0.389155 1 0.5327
 Reduced model -46.5274 1 8.23466 2 0.01629

 AIC: 89.2094

 Log-likelihood Constant 37.900888781466982

 Goodness of Fit
 Scaled
 Dose Est._Prob. Expected Observed Size 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
 Chi^2 = 0.41 d.f. = 1 P-value = 0.5245


 Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 141.762
 BMDL = 81.9117
 BMDU = 364.364

Taken together, (81.9117, 364.364) is a 90% two-sided confidence interval for the BMD
```

G.3 Multitumor Analysis Using BMDS MS_Combo

The combined tumor analysis was also performed with beta version of the MS_Combo model in BMDS (Version 2.2beta). The model resulted in similar results to the Bayesian method and model output is shown below for the combined calculation.

```
**** Start of combined BMD and BMDL Calculations.****
 Combined Log-Likelihood -277.79874987953076
 Combined Log-likelihood Constant 246.62591390071873
```

```
 Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
 BMD = 40.4937
 BMDL = 32.331
```

G.4 Multitumor analysis using Bayesian Methods

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

Kopylev et al. (2009) describe a Markov Chain Monte Caro (MCMC) computational approach to calculating the dose associated with a specified composite risk under assumption of independence of tumors. The current *Guidelines for Carcinogen Risk Assessment* recommend calculation of an upper bound to account for uncertainty in the estimate (U.S. EPA, 2005a). For uncertainty characterization, MCMC methods have the advantage of providing information about the full distribution of risk and/or benchmark dose, which can be used in generating a confidence bound. This MCMC approach building on the re-sampling approach recommended by Bogen (1990), and also provides a distribution of the combined potency across sites.

For individual tumor data modeled using the multistage model:

$$P(d \mid \mathbf{q}) = 1 - exp[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)],\ q_i \geq 0$$

the model for the combined tumor risk is still multistage, with a functional form that has the sum of stage-specific multistage coefficients as the corresponding multistage coefficient;

$$P_c(d \mid \mathbf{q}) = 1 - exp[-(q_{\Sigma 0i} + q_{\Sigma 1i} d + q_{\Sigma 2i} d^2 + \ldots + q_{\Sigma ki} d^k)],$$

The resulting equation for fixed extra risk (BMR) is polynomial in dose (when logarithms of both sides are taken) and can be straightforwardly solved for a combined BMC. Computation of the confidence bound on combined risk BMC can be accomplished via likelihood methods (BMDS-MS_Combo), re-sampling (bootstrap) or Bayesian methods.

The MCMC computations were conducted using WinBUGS (Spiegelhalter et al., 2003)(freeware developed by the MRC Biostatistical Unit, Cambridge, United Kingdom, available at http://www.mrc-bsu.cam.ac.uk/bugs/winbugs/contents.shtml). The model code was checked and compiled within, and the data read into, WinBUGS. Three chains were used for the analysis. Initial values

for each variable were generated using a Uniform(0,1) distribution and read into WinBUGS. The WinBUGS code calculates the BMC directly (U.S. EPA, 2013).

In a Bayesian analysis, the choice of an appropriate prior probability is important. In the examples developed by Kopylev et al. (2009), a diffuse (i.e., high variance or low tolerance) Gaussian prior restricted to be nonnegative was used; such diffuse priors performed reasonably well.

The mean and the 5th percentile of the posterior distribution of combined BMC provide estimates of the mean BMC and the lower bound on the BMC (BMCL), respectively, for the combined tumor risk. The values calculated using this method were: mean BMC_{10} 39.2 ppm, and $BMCL_{10}$ 31.4 ppm.