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TOXICOLOGICAL REVIEW

OF

1,4-Dioxane

(CAS No. 123-91-1)

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

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U.S. Environmental Protection Agency
Washington, DC

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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
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
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
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 coeffecient
LAP	leucine aminopeptidase
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LOAEL	lowest-observed-adverse-effect-level
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. EPA	U.S. Environmental Protection Agency
V	volts
VAS	visual analogue scale

$\mathbf{V_d}$	volume of distribution
$\mathbf{V_{max}}$	maximal rate of metabolism
$\mathbf{V_{maxC}}$	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 the data and related uncertainties. The discussion is intended to convey the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

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

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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 subchronic 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 ($\leq$ 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, 194806). 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, 1986,

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

001468), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986, 001466), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988, 064560), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991, 008567), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994, 076133), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994, 006488), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995, 005992), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996, 030019), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998, 030021), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000, 052149), *Benchmark Dose Technical Guidance Document* (External Review Draft) (U.S. EPA, 2000, 052150), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixture s* (U.S. EPA, 2000, 004421; U.S. EPA, 2000, 196144), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002, 088824), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005, 086237), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005, 088823), *Science Policy Council Handbook*: *Peer Review* (U.S. EPA, 2006, 194566), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006, 194567).

The literature search strategy employed for this compound was based on the Chemical Abstracts Service Registry Number (CASRN) and at least one common name. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through September 2009. Note that during the development of this assessment, new data regarding the toxicity of 1,4-dioxane through the inhalation route of exposure became available. These data have not been included in the current assessment and will be evaluated in a separate IRIS assessment.

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, 196089; Lewis, 2000, 625540). Synonyms include diethylene ether, 1,4-diethylene dioxide, diethylene oxide, dioxyethylene ether, and dioxane (Hawley and Lewis, 2001, 196089). The chemical structure of 1,4-dioxane is shown in Figure 2-1. Selected chemical and physical properties of this substance are listed in Table 2-1 below:

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

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

CASRN:	123-91-1 (CRC, 2000, 196090)
Molecular weight:	88.10 (Merck, 2001, 595055)
Chemical formula:	$C_4H_8O_2$ (Merck, 2001, 595055)
Boiling point:	101.1°C (Merck, 2001, 595055)
Melting point:	11.8°C (CRC, 2000, 196090)
Vapor pressure:	40 mmHg at 25°C (Lewis, 2000, 625540)
Density:	1.0337 g/mL at 20°C (CRC, 2000, 196090)
Vapor density:	3.03 (air = 1) (Lewis, 2000, 625540)
Water solubility:	Miscible with water (Hawley and Lewis, 2001, 196089)
Other solubilities:	Miscible with ethanol, ether, and acetone (CRC, 2000, 196090)
Log K_{ow}:	–0.27 (Hansch et al., 1995, 051424)
Henry's Law constant:	4.80×10^{-6} atm-m^3/molecule at 25°C (Park et al., 1987, 194328)
OH reaction rate constant:	1.09×10^{-11} cm^3/molecule sec at 25°C (Atkinson, 1989, 042876)
K_{oc}:	17 (estimated using log K_{ow}) (ACS, 1990, 004237)
Bioconcentration factor:	0.4 (estimated using log K_{ow}) (Meylan et al., 1999, 194377)
Conversion factors (in air):	1 ppm = 3.6 mg/m^3; 1 mg/m^3 = 0.278 ppm (25^oC and 1 atm) (HSDB, 2007, 196232)

1,4-Dioxane is produced commercially through the dehydration and ring closure of diethylene glycol (Surprenant, 2002, 196117). Concentrated sulfuric acid is used as a catalyst (Surprenant, 2002, 196117). This is a continuous distillation process with operating temperatures and pressures of 130–200°C and 188–825 mmHg, respectively (Surprenant, 2002, 196117). 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, 2002, 594597). The production volume reported during the years 1994, 1998, and 2002 was within the range of 1–10 million pounds (U.S. EPA, 2002, 594597).

Historically, 1,4-dioxane has been used as a stabilizer for the solvent 1,1,1-trichloroethane (Surprenant, 2002, 196117). 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, 196127; U.S. EPA, 1990, 196139; UNEP, 2000, 196125). 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, 196089; IARC, 1999, 196238; Merck, 2001, 595055). 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, 196117). Reaction products of 1,4-dioxane are used in the manufacture of insecticides, herbicides, plasticizers, and monomers (Surprenant, 2002, 196117).

When 1,4-dioxane enters the air, it will exist as a vapor, as indicated by its vapor pressure (HSDB, 2007, 196232). It is expected to be degraded in the atmosphere through photooxidation with hydroxyl radicals (HSDB, 2007, 196232; Surprenant, 2002, 196117). The estimated half-life for this reaction is 6.7 hours (HSDB, 2007, 196232). 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, 196213). 1,4-Dioxane is not expected to undergo direct photolysis (Wolfe and Jeffers, 2000, 196109). 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, 196086). 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 (ACS, 1990, 004237; ATSDR, 2007, 196127). This substance may volatilize from dry soil surfaces based on its vapor pressure (HSDB, 2007, 196232). The estimated bioconcentration factor value indicates that 1,4-dioxane will not bioconcentrate in aquatic or marine organisms (Franke et al., 1994, 194356; Meylan et al., 1999, 194377). 1,4-Dioxane is not expected to undergo hydrolysis or to biodegrade readily in the environment (ATSDR, 2007, 196127; HSDB, 2007, 196232). 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 (ACS, 1990, 004237; HSDB, 2007, 196232; Park et al., 1987, 194328). 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 (Lesage et al., 1990, 195913; Yasuhara et al., 1997, 195909; Yasuhara et al., 2003, 195095). 1,4-Dioxane has been detected in contaminated surface and groundwater samples collected near hazardous waste sites and industrial facilities (Derosa et al., 1996, 194371).

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., 1976, 062953). 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. (1977, 062956) 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., 1978, 062955; Young et al., 1978, 625640). 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

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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., 1978, 062955; Young et al., 1978, 625640). 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 (1982, 196146) 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 (1982, 196146) 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, 194326). 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, 195061) 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. (1977, 194355) 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]).

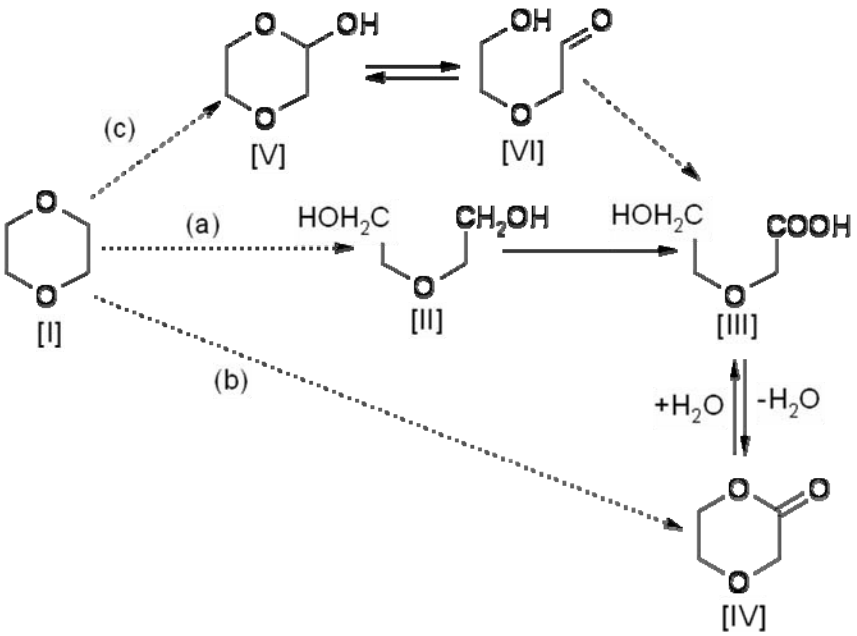
Liver, kidney, spleen, lung, colon, and skeletal muscle tissues were collected from 1, 2, 6, and 12 hours after dosing. Distribution was generally uniform across tissues, with blood concentrations higher than tissues at all times except for 1 hour post dosing, when kidney levels were approximately 20% 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 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., 1977, 194355). 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, 194370). Braun and Young (1977, 194370) 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., 1977, 062956; 1978, 062955; Young et al., 1978, 625640) 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., 1977, 194355) 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., 1977,

062951; Woo et al., 1978, 194345). 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, 194344). 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, 194370). Additionally, i.p. injection of 100–400 mg/kg diethylene glycol in Sprague Dawley rats resulted in urinary elimination of HEAA (Woo et al., 1977, 062950).



Source: Adapted with permission from Elsevier Ltd., Woo et al. (1977, 194355; 1977, 062951).

Figure 3-1. Suggested metabolic pathways of 1,4-dioxane in the rat. I = 1,4-dioxane; II = diethylene glycol; III = β-hydroxyethoxy acetic acid (HEAA); IV = 1,4-dioxane-2-one; V = 1,4-dioxane-2-ol; VI = β-hydroxyethoxy acetaldehyde. Note: Metabolite [V] is a likely intermediate in pathway b as well as pathway c. The proposed pathways are based on the metabolites identified; the enzymes responsible for each reaction have not been determined. The proposed pathways do not account for metabolite degradation to the labeled carbon dioxide (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 1,4-dioxane plant workers exposed to a TWA of 1.6 ppm of 1,4-dioxane for 7.5 hours, Young et al. (1976, 062953) 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., 1977, 062956), 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., 1978, 062955; Young et al., 1978, 625640). 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., 1978, 062955; Young et al., 1978, 625640). 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.

Source: Used with permission from Taylor and Francis, Young et al. (1978, 062955).

Figure 3-2. Plasma 1,4-dioxane levels in rats following i.v. doses of 3-5,600 mg/kg.

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., 2005, 195067). 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. The increase in 2C11 activity 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, 196102). 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, 196132; Waxman et al., 1991, 196102). 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, 196132). 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., 1976, 062953). 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., 1977, 062956). There are no data for 1,4-dioxane elimination in humans from oral exposures.

Elimination of 1,4-dioxane in rats (Young et al., 1978, 062955; Young et al., 1978, 625640) was primarily via urine. Like 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 and humans (Leung and Paustenbach, 1990, 062932; Reitz et al., 1990, 094806) and lactating women (Fisher et al., 1997, 194390). 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, 063020). 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., 1977, 062956; 1978, 062955) (Young et al., 1978, 625640), 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).

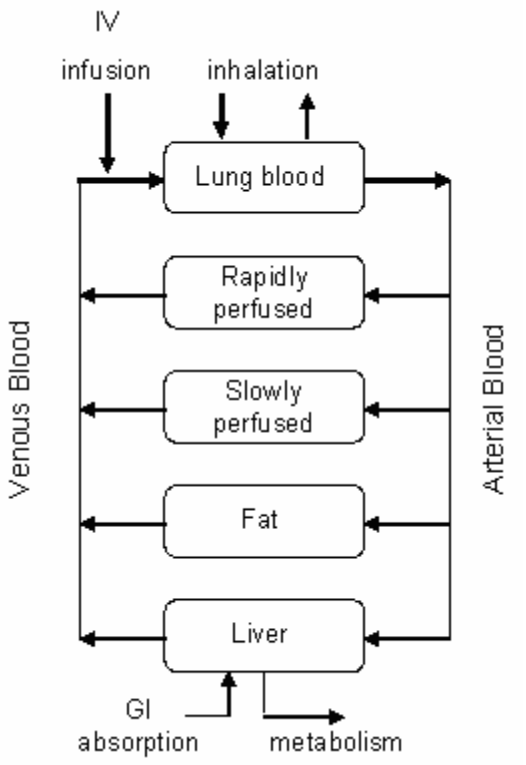


Figure 3-3. General PBPK model structure consisting of blood-flow limited tissue compartments connected via arterial and venous blood flows. Note: Orally administered chemicals are absorbed directly into the liver while inhaled and intravenously infused chemicals enter directly into the arterial and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

3.5.1. Available Pharmacokinetic Data

Animal and human data sets available for model calibration derive from Young et al. (1977, 062956; 1978, 062955; 1978, 625640), Mikheev et al. (1990, 195061), and Woo et al. (1977, 062950; 1977, 194355). Young et al. (1978, 062955; 1978, 625640) 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., 1978, 062955) (Young et al., 1978, 625640). 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., 1978, 062955; Young et al., 1978, 625640). 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. (1978, 062955; 1978, 625640) corroborate previous studies describing the saturable nature of 1,4-dioxane metabolism in rats (Woo et al., 1977, 062950; 1977, 194355) 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., 1978, 062955; Young et al., 1978, 625640). These data identify the region (10–100 mg/kg) in which oral exposures will result in nonlinear metabolism of 1,4-dioxane and can 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., 1978, 062955; Young et al., 1978, 625640). The observed linear elimination of 1,4-dioxane after inhalation exposure suggests that, via this route, metabolism is in the linear region at this exposure level.

The only human data adequate for use in PBPK model development (Young et al., 1977, 062956) 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. (1976, 062953) 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, 195061) 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. (1977, 062950; 1977, 194355) 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 (1990, 062932) 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, 063020), 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, 1990, 062932) 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., 1977, 062956; 1978, 062955; 1978, 625640); 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., 1977, 062956) 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. (1990, 094806) 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, 063020), 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, 001938), 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., 1977, 062956). 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, 1990, 062932) 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 (1990, 062932) 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., 1990, 094806). 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., 1990, 094806). The Reitz et al. (1990, 094806) 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. (1990, 094806) optimized values for human V_{max} and K_m against the experimental human 1,4-dioxane inhalation data (Young et al., 1977, 062956). 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., 1978, 062955; Young et al., 1978, 625640). As with the Leung and Paustenbach (1990, 062932) model, the Reitz et al. (1990, 094806) 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, 194390) 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 (1990, 062932) and Reitz et al. (1990, 094806) 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. (1990, 094806). 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.

3.5.3. 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., 1978, 062955; Young et al., 1978, 625640) and humans (Young et al., 1977, 062956) 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 (Fisher et al., 1997, 194390; Leung and Paustenbach, 1990, 062932; Reitz et al., 1990, 094806; Sweeney et al., 2008, 195085).

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. (1990, 094806) and Leung and Paustenbach (1990, 062932) PBPK models were both developed from a PBPK model of styrene (Ramsey and Andersen, 1984, 063020), with the exception of minor differences in the use of partition coefficients and biological parameters. The model code for Leung and Paustenbach (1990, 062932) was unavailable in contrast to Reitz et al. (1990, 094806). The model of Reitz et al. (1990, 094806) was identified for further consideration to assist in the derivation of toxicity values, and the Sweeney et al. (2008, 195085) PBPK model was also evaluated.

The biological plausibility of parameter values in the Reitz et al. (1990, 094806) human model were examined. The model published by Reitz et al. (1990, 094806) was able to predict the only available human inhalation data (50 ppm 1,4-dioxane for 6 hours; Young et al., (1977, 062956)) 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, 020304), 14 L/hour/kg$^{0.74}$ (Brown et al., 1997, 020304), and 1,825 (Leung and Paustenbach, 1990, 062932), respectively. Furthermore, Reitz et al. (1990, 094806) 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. (1977, 062956) 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. (1990, 094806) 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. (1977, 062956; 1978, 062955; 1978, 625640) empirical rat and human models using the acslXtreme simulation software, re-calibration of the Reitz et al. (1990, 094806) human PBPK model, and evaluation of the model parameters published by Sweeney et al. (2008, 195085). Using the model descriptions and equations given in Young et al. (1977, 062956; 1978, 062955; 1978, 625640), 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. (1977, 062956; 1978, 062955; 1978, 625640).

The PBPK model of Reitz et al. (1990, 094806) 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. (2008, 195085) 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. (1977, 062956; 1978, 062955; 1978, 625640) were successfully implemented in acslXtreme and perform identically to the models reported in the published papers (Figures B-3 through B-7), 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. (1978, 062955; 1978, 625640) 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 lower than observations, this is due to parameter fitting of Young et al. (1977, 062956). No model output was published in Young et al. (1977, 062956) for comparison. The empirical models were modified to allow for user-defined inhalation exposure levels. However, no modifications were made to model oral exposures as adequate data to parameterize such modifications do not exist for rats or humans.

Several procedures were applied to the Reitz et al. (1990, 094806) 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 do not come within 10-fold of the experimental values using measured tissue:air partition coefficients from Leung and Paustenbach (1990, 062932) or Sweeney et al. (2008, 195085) (Figures B-8 and B-9). 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-10). Recalibration 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 (Figures B-12 and B-13). Exploration of the model space using an assumption of zero-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-16). 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_{maxC} 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 (Table B-1). Therefore, some other, as yet unknown, modification to model structure may be necessary.

Similarly, Sweeney et al. (2008, 195085) also evaluated the available PBPK models (Leung and Paustenbach, 1990, 062932; Reitz et al., 1990, 094806) 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. (2008, 195085) resulted in partition coefficients that were consistent with previously measured values and those used in the Leung and Paustenbach (1990, 062932) model. Of noteworthy significance, the laboratory results of Sweeney et al. (2008, 195085) did not confirm the human blood:air partition coefficient Reitz et al. (1990, 094806) reported. Furthermore, Sweeney et al. (2008, 195085) estimated metabolic rate constants (V_{maxC} and K_m) within the range used in the previous models (Leung and Paustenbach, 1990, 062932; Reitz et al., 1990, 094806). Overall, the Sweeney et al. (2008, 195085) 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. (1977, 062956).

Updated PBPK models were developed based on these new data and data from previous kinetic studies in rats, workers, and human volunteers reported by Young et al. (1976, 062953; 1977, 062956; 1978, 062955; 1978, 625640). The optimized rate of metabolism for the mouse was significantly higher than the value previously estimated. The optimized rat kinetic parameters were similar to those in the 1990 models. Of the two available human studies (Young et al., 1976, 062953; 1977, 062956), model predictions were consistent with one study, but did not fit the second as well.

3.6. RAT NASAL EXPOSURE VIA DRINKING WATER

Sweeney et al. (2008, 195085) 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 (Barber, 1934, 062913; Johnstone, 1959, 062927). Barber (1934, 062913) 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, 062927) 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, 062952). 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., 1934, 062919), 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, 196105), of the Institute of Pharmacology, University of Wurzburg, reported slight mucous membrane irritation

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

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, 063013) 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, 063013) 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 (Fairley et al., 1934, 062919; Silverman et al., 1946, 063013; Wirth and Klimmer, 1936, 196105; Yant et al., 1930, 062952) 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. (1977, 062956) 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., 1976, 062953).

Ernstgård et al. (2006, 195034) 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., 1978, 062914; Thiess et al., 1976, 062943). 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. (1976, 062943) 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 g 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. (1978, 062914) 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 oral drinking water studies. Longer-term inhalation studies consisted of only one subchronic study (Fairley et al., 1934, 062919) and one chronic study (Torkelson et al., 1974, 094807). These studies were not sufficient to characterize the inhalation risks of 1,4-dioxane (see Section 4.2.2.).

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., 1934, 062919). Using reference BWs and drinking water ingestion rates for rats and mice (U.S. EPA, 1988, 064560), 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 sacrificed 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, 064678)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, 063021) 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, 063021) 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, 196245) 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 1554 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. 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, 196245)

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. 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, 196245).

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

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

[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, 196245).

4.2.1.1.4. *Yamamoto et al.* Studies (Yamamoto et al., 1998, 196114; Yamamoto et al., 1998, 594544) 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., 1965, 017009) 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, 064560), 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., 1973, 062912; Hoch-Ligeti et al., 1970, 062926). 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 autopsies 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, 064560) 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 killed 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. (1973, 062912) 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 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 numbers of incipient liver tumors and hepatomas in rats from this study (treated for 13 months and observed at 13–16 months) are presented in Table 4-3.

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

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

[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. (1973, 062912).

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 (1970, 029386) 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, 064560) 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., 1974, 062929). 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. (1974, 062929) 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. (1974, 062929) 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. (1974, 062929) 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: Used with permission from Elsevier, Ltd., Kociba et al. (1974, 062929).

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 hyperplasia 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, 062935). 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, 062935).

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, 062925) 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, 062925)).

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

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

[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, 062935).

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 commonly found in dosed mice, but the incidences were not significantly different from controls and showed no dose-response trend. 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]$p < 0.001$ by Fisher's Exact test pair-wise comparison with controls.
[d]$p = 0.014$.

Source: NCI (1978, 062935).

In addition to liver tumors, a variety of other benign and malignant neoplasms occurred. However, the report (NCI, 1978, 062935) 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, 062935) 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., 1994, 196120), once as a laboratory report (JBRC, 1998, 196240), and most recently as a peer-reviewed manuscript (Kano et al., 2009, 594539). Dr. Yamazaki also provided some detailed information (Yamazaki, 2006, 626614). 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. (1994, 196120) only included drinking water concentrations for each dose group. In contrast, JBRC (1998, 196240) included drinking water concentrations (ppm), in addition using body weights and water consumption measurements to calculate daily chemical intake (mg/kg-day). JBRC (1998, 196240) 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, 2009, 628630), the midpoint of the range was used. Kano et al. (2009, 594539) 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, 594539) calculated mean chemical intake (mg/kg-day) is used for quantitiative analysis of this data.

The categories for which incidence rates were described also varied among the reports. Yamazaki et al. (1994, 196120) and Kano et al. (2009, 594539) reported histopathological results for all animals, including dead and moribund animals; however, the detailed JBRC laboratory findings (1998, 196240) 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, 594539). The manuscript by Kano et al. (2009, 594539) stated that the lesions diagnosed in the earlier reports (JBRC, 1998, 196240; Yamazaki et al., 1994, 196120) were re-examined and recategorized as appropriate according to current pathological diagnostic criteria (see references in Kano et al. (2009, 594539)).

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, 196245) 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, 594539) 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, 594539) 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, 626614). 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, 594539) 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, 594539). 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, 626614); 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, 626614).

Several hematological changes were noted in the JBRC report (1998, 196240): Decreases in RBC (male rats only), hemoglobin, hematocrit, and MCV; and increases in platelets in high-dose groups were observed (JBRC, 1998, 196240). 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, 196240). 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, 196240). Also, blood in the urine was seen in female rats at 83 and 429 mg/kg-day (JBRC, 1998, 196240). In male rats, relative liver weights were increased at 55 and 274 mg/kg-day (Kano et al., 2009, 594539). In female rats, relative liver weight was increased at 429 mg/kg-day (Kano et al., 2009, 594539).

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 s 4-8 and 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, hyperplasia, and clear and mixed cell foci) and at 429 mg/kg-day in female rats (hyperplasia, 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, 196240).

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

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

[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, 594539).
[c]Data from Kano et al. (2009, 594539).
[d]Data from JBRC (1998, 196240). JBRC did not report statistical significance for the "All animals" comparison.
[e]$p < 0.01$ by χ^2 test.
[f]$p < 0.05$ by χ^2 test.
Sources: Kano et al. (2009, 594539) and JBRC (1998, 196240).

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

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

[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, 594539).
[c]Data from Kano et al. (2009, 594539).
[d]Data from JBRC (1998, 196240). JBRC did not report statistical significance for the "All animals" comparison.
[e]$p < 0.01$ by χ^2 test.
[f]$p < 0.05$ by χ^2 test.

Sources: Kano et al. (2009, 594539) and JBRC (1998, 196240).

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, 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, 196112) 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, 196140; Karbe and Kerlin, 2002, 196246; Stroebel et al., 1995, 196101). 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, 196140; Stroebel et al., 1995, 196101). 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, 2002, 196246). In the case of the JBRC (1998, 196240) 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, 196240) in the high dose groups, 274 and 429 mg/kg-day, respectively. These serum chemistry changes seen in terminal blood samples from high-dose male and female rats are likely related to tumor formation in these dose groups.

Significantly increased incidences of liver tumors (adenomas and carcinomas) and tumors of the nasal cavity occurred in high-dose male and female rats (Tables 4-10 and 4-11) treated with 1,4-dioxane for 2 years (Kano et al., 2009, 594539). 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, 626614). 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, 626614). 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 Tables 4-10 and 4-11 were not adjusted for survival.

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

	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: Used with permission from Elsevier, Ltd., Kano et al. (2009, 594539).

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: Used with permission from Elsevier, Ltd., Kano et al. (2009, 594539).

For mice, growth and mortality rates were reported in Kano et al. (2009, 594539) 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, 594539) 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, 626614). Dr. Yamazaki 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, 626614). 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, 626614). For mice, growth and mortality rates were reported in Kano et al. (2009, 594539) 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, 196240). 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

(Tables 4-12 and 4-13). Commonly seen alterations included nuclear enlargement, atrophy, and inflammation of the epithelium. Other notable 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, 594539).
[c]Data from Kano et al. (2009, 594539).
[d]Data from JBRC (1998, 196240). JBRC did not report statistical significance for the "All animals" comparison.
[e]$p < 0.01$ by χ^2 test.

Sources: Kano et al. (2009, 594539) and JBRC (1998, 196240).

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, 594539).
[c]Data from Kano et al. (2009, 594539).
[d]Data from JBRC (1998, 196240). JBRC did not report statistical significance for the "All animals" comparison.
[e]$p < 0.01$ by χ^2 test.

Sources: Kano et al. (2009, 594539) and JBRC (1998, 196240).

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. The serum chemistry changes seen in terminal blood samples from male and female mice (mid- and high-dose groups) are likely related to tumor formation in these animals. 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, 626614). 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, 626614). 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, 594539) that included lesions in animals that became moribund or died prior to the completion of the 2-year study.

Katagiri et al. (1998, 193804) 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, 594539) 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, 193804). 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, 020667). 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: Used with permission from Elsevier, Ltd., Kano et al. (2009, 594539).

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

4.2.2. Inhalation Toxicity

4.2.2.1. *Subchronic Inhalation Toxicity*

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., 1934, 062919). 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 killed 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.

4.2.2.2. *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., 1974, 094807). 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 of 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. The true NOAEL was likely to be higher.

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 or nasal cavity tumors were seen in any rat.

4.2.3. Initiation/Promotion Studies

4.2.3.1. *Bull et al.*

Bull et al. (1986, 194336) 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., 1973, 029390). 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, 062933) 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 concentrations 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., 1985, 062924). 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 4-15. 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 (Laug et al., 1939, 195055; see Table 4-15; Pozzani et al., 1959, 063019; Smyth et al., 1941, 060695). Clinical signs of CNS depression were observed, including staggered gait, narcosis, paralysis, coma, and death (de Navasquez, 1935, 196174; Laug et al., 1939, 195055; Nelson, 1951, 196087; Schrenk and Yant, 1936, 195076). Severe liver and kidney degeneration and necrosis were often seen in acute studies (David, 1964, 195954; de Navasquez, 1935, 196174; JBRC, 1998, 196242; Kesten et al., 1939, 194972; Laug et al., 1939, 195055; Schrenk and Yant, 1936, 195076). JBRC (1998, 196242) 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 4-15. Mortality occurred in many high-concentration studies (Nelson, 1951, 196087; Pozzani et al., 1959, 063019; Wirth and Klimmer, 1936, 196105). Inhalation of 1,4-dioxane caused eye and nasal irritation, altered respiration, and pulmonary edema and congestion (Yant et al., 1930,

062952). Clinical signs of CNS depression were observed, including staggered gait, narcosis, paralysis, coma, and death (Nelson, 1951, 196087; Wirth and Klimmer, 1936, 196105). Liver and kidney degeneration and necrosis were also seen in acute and short-term inhalation studies (Drew et al., 1978, 067913; Fairley et al., 1934, 062919).

Table 4-15. 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, 195954)
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, 194972)
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, 196242)
Female Sprague Dawley rat	Gavage	0, 168, 840, 2550, or 4,200 mg/kg by gavage, 21 and 4 hours prior to sacrifice	Increased ODC activity, hepatic CYP450 content, and DNA single-strand breaks	840 mg/kg (ODC activity only)	Kitchin and Brown (1990, 062928)
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, 063019)
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, 060695)

Animal	Exposure route	Test conditions	Results	Dose[a]	Reference
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, 195055)
Rabbit	Gavage	Single gavage dose of 0, 207, 1,034, or 2,068 mg/kg-day	Clinical signs of CNS depression, mortality at 2068 mg/kg, renal toxicity (polyuria followed by anuria), histopathological changes in liver and kidneys	1,034 mg/kg-day	de Navasquez (1935, 196174)
Rat, rabbit	Gavage	Single dose; mortality after 2 weeks	Mortality and narcosis	3,160 mg/kg	Nelson (1951, 196087)
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, 196242)
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, 195076)
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, 067913)
Rat	Vapor inhalation	5 hours of exposure	Mortality and narcosis	6,000 ppm	Nelson (1951, 196087)
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, 063019)
Mouse, cat	Vapor inhalation	8 hours/day for 17 days	Paralysis and death	8,400 ppm	Wirth and Klimmer (1936, 196105)
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, 062952)
Rabbit, guinea pig, rat, mouse	Vapor inhalation	3 hours exposure, for 5 days; 1.5 hour exposure for 1 day	Degeneration and necrosis in the kidney and liver, vascular congestion in the lungs	10,000 ppm	Fairley et al.(1934, 062919)

Animal	Exposure route	Test conditions	Results	Dose[a]	Reference
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, 194970)
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, 196174)
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, 062934)
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, 018767)

[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, 062927). 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 (de Navasquez, 1935, 196174; Laug et al., 1939, 195055; Nelson, 1951, 196087; Schrenk and Yant, 1936, 195076; Yant et al., 1930, 062952). The neurotoxicity of 1,4-dioxane was further investigated in several studies described below (Frantik et al., 1994, 067510; Goldberg et al., 1964, 058035; Kanada et al., 1994, 078052; Knoefel, 1935, 195914).

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, 067510). 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, 058035) 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, 078052). 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, 195914). 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 Tables 4-16 and 4-17 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 the majority of 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.

Negative findings were reported for mutagenicity in in vitro assays with the prokaryotic organisms *Salmonella typhimurium*, *Escherichia coli*, and *Photobacterium phosphoreum* (Mutatox assay) (Haworth et al., 1983, 028947; Hellmér and Bolcsfoldi, 1992, 194717;

Khudoley et al., 1987, 194949; Kwan et al., 1990, 196078; Morita and Hayashi, 1998, 195065; Nestmann et al., 1984, 194339; Stott et al., 1981, 063021). 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, 194373; Zimmermann et al., 1985, 194343). In the presence of toxicity, positive results were reported for meiotic nondisjunction in Drosophila (Munoz and Barnett, 2002, 195066).

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 (McGregor et al., 1991, 194381; Morita and Hayashi, 1998, 195065). 1,4-Dioxane did not produce chromosomal aberrations or micronucleus formation in Chinese hamster ovary (CHO) cells (Galloway et al., 1987, 007768; Morita and Hayashi, 1998, 195065). Results were negative in one assay for sister chromatid exchange (SCE) in CHO (Morita and Hayashi, 1998, 195065) and were weakly positive in the absence of metabolic activation in another (Galloway et al., 1987, 007768). 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, 007323) 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, 195078).

1,4-Dioxane was not genotoxic in the majority of available in vivo mammalian assays. 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, 195060; Mirkova, 1994, 195062; Tinwell and Ashby, 1994, 195086) and micronucleus formation in peripheral blood of CD1 mice (Morita, 1994, 196085; Morita and Hayashi, 1998, 195065). Mirkova (1994, 195062) 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, 195086) 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, 1994, 195062) using the same mouse strain, 1,4-dioxane dose (3,600 mg/kg) and sampling time (24 hours). Morita and Hayashi (1998, 195065) 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, 1990, 062928).

Roy et al. (2005, 196094) 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, 062925; Stott et al., 1981, 063021), but increased hepatocyte DNA synthesis indicative of cell proliferation in several in vivo studies (Goldsworthy et al., 1991, 062925; Miyagawa et al., 1999, 195063; Stott et al., 1981, 063021; Uno et al., 1994, 194385). 1,4-Dioxane caused a transient inhibition of RNA polymerase A and B in the rat liver (Kurl et al., 1981, 195054), 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., 1977, 062950). 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, 063021).

Rosenkranz and Klopman (1992, 004321) 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, 004321) 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, 195087). Van Delft et al. (2004, 195087) 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, 195087), 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 4-16. Genotoxicity studies of 1,4-dioxane; in vitro

Test system	Endpoint	Test conditions	Results[a]		Dose[b]	Source
			Without activation	With activation		
Prokaryotic organisms in vitro						
S. typhimurium strains TA98, TA100, TA1535, TA1537	Reverse mutation	Plate incorporation assay	–	–	10,000 µg/plate	Haworth et al. (1983, 028947)
S. typhimurium strains TA98, TA100, TA1530, TA1535, TA1537	Reverse mutation	Plate incorporation assay	–	–	ND	Khudoley et al. (1987, 194949)
S. typhimurium strains TA98, TA100, TA1535, TA1537	Reverse mutation	Plate incorporation and preincubation assays	–	–	5,000 µg/plate	Morita and Hayashi (1998, 195065)

Test system	Endpoint	Test conditions	Results[a]		Dose[b]	Source
			Without activation	With activation		
S. typhimurium strains TA100, TA1535	Reverse mutation	Preincubation assay	–	–	103 mg	Nestmann et al. (1984, 194339)
S. typhimurium strains TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	Plate incorporation assay	–	–	103 mg	Stott et al. (1981, 063021)
E. coli K-12 uvrB/recA	DNA repair	Host mediated assay	–	–	1,150 mmol/L	Hellmer and Bolcsfoldi (1992, 194717)
E. coli WP2/WP2uvrA	Reverse mutation	Plate incorporation and preincubation assays	–	–	5,000 µg/plate	Morita and Hayashi (1998, 195065)
P. phosphoreum M169	Mutagenicity, DNA damage	Mutatox assay	–	ND	ND	Kwan et al. (1990, 196078)
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. (1985, 194343)
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, 195066)
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, 194373)
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, 007323)
Primary hepatocyte culture from male F344 rats	DNA repair	Autoradiography	–	ND[d]	1 mM	Goldsworthy et al. (1991, 062925)
L5178Y mouse lymphoma cells	Forward mutation assay	Thymidine kinase mutagenicity assay (trifluorothymidine resistance)	–	–	5,000 µg/mL	McGregor et al. (1991, 194381)

Test system	Endpoint	Test conditions	Results[a]		Dose[b]	Source
			Without activation	With activation		
L5178Y mouse lymphoma cells	Forward mutation assay	Thymidine kinase mutagenicity assay (trifluorothymidine resistance)	–	–T	5,000 µg/mL	Morita and Hayashi (1998, 195065)
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, 195078)
CHO cells	SCE	BrdU was added 2 hours after 1,4-dioxane addition; chemical treatment was 2 hours with S9 and 25 hours without S9	±[g]	–	10,520 µg/mL	Galloway et al. (1987, 007768)
CHO cells	Chromosomal aberration	Cells were harvested 8–12 hours or 18–26 hours after treatment (time of first mitosis)	–	–	10,520 µg/mL	Galloway et al. (1987, 007768)
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, 195065)
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, 195065)
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, 195065)

Test system	Endpoint	Test conditions	Results[a]		Dose[b]	Source
			Without activation	With activation		
Calf thymus DNA	Covalent binding to DNA	Incubation with microsomes from 3-methylcholanthrene treated rats	–	–	0.04 pmol/mg DNA (bound)	Woo et al. (1977, 062950)

[a]+ = positive, ± = equivocal or weak positive, – = negative, T = toxicity. 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.

Table 4-17. Genotoxicity studies of 1,4-dioxane; mammalian in vivo

Test system	Endpoint	Test Conditions	Results[a]	Dose[b]	Source
Female Sprague Dawley Rat	DNA damage; single-strand breaks measured by alkaline elution	Two gavage doses given 21 and 4 hours prior to sacrifice	+[c]	2,550 mg/kg	Kitchin and Brown (1990, 062928)
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, 063021)
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, 195060)
Male and female C57BL6 Mouse; male BALB/c Mouse	Micronucleus formation in bone marrow	Gavage; analysis of polychromatic erythrocytes 24 or 48 hours after dosing	+ (C57BL6)[d] – (BALB/c)	900 mg/kg (C57BL6); 5,000 mg/kg (BALB/c)	Mirkova (1994, 195062)
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, 196085)
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, 195065)
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, 195065)
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, 195086)
Male CD1 Mouse	Micronuclei formation in bone marrow	Gavage; analysis for micronucleated erythrocytes 24 hours after dosing	+[f]	1,500 mg/kg-day for 5 days	Roy et al. (2005, 196094)
Male CD1 Mouse	Micronuclei formation in hepatocytes	Gavage; analysis for micronuclei 24 hours after dosing	+[g]	2,500 mg/kg-day for 5 days	Roy et al.(2005, 196094)
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, 063021)

Test system	Endpoint	Test Conditions	Results[a]	Dose[b]	Source
Male F344 Rat	DNA repair in hepatocytes (autoradiography)	Gavage and drinking water exposure; thymidine incorporation	–	1,000 mg/kg for 2 or 12 hours; 1,500 mg/kg-day for 2 weeks or 3,000 mg/kg-day for 1 week	Goldsworthy et al. (1991, 062925)
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, 062925)
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, 062925)
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, 062925)
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, 195054)
Male F344 Rat	DNA synthesis in hepatocytes	Gavage; thymidine and BrdU incorporation	+[j]	1,000 mg/kg	Miyagawa (1999, 195063)
Male F344 Rat	DNA synthesis in hepatocytes	Thymidine incorporation	±[k]	2,000 mg/kg	Uno et al. (1994, 194385)
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, 063021)

[a]+ = positive, ± = equivocal or weak positive, – = negative, T = toxicity. 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*

Burmistrov et al. (2001, 195972) investigated the effect of 1,4-dioxane inhalation on free radical processes 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., 1978, 062955; Young et al., 1978, 625640). Nannelli et al. (2005, 195067) (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. (1975, 062930) who evaluated toxicity from a chronic drinking water study alongside data providing a pharmacokinetic profile for 1,4-dioxane. Kociba et al. (1975, 062930) 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. (2005, 195067) 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, 062925; Kitchin and Brown, 1990, 062928; Stott et al., 1981, 063021). Stott et al. (1981, 063021) 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, 063021). 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, 062925) 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, 062935) 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, 062925). 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. (2005, 195067) 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, 062925). 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, 062935) 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, 062925). 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, 1990, 062928). 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 and kidney toxicity were 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 (Barber, 1934, 062913; Johnstone, 1959, 062927). Neurological changes were also reported in one case; including, headache, elevation in blood pressure, agitation and restlessness, and coma (Johnstone, 1959, 062927). Perivascular widening was observed in the brain of this worker, with small foci of demyelination in several regions (e.g., cortex, basal nuclei). Liver and kidney degeneration and necrosis were observed in acute oral and inhalation studies (David, 1964, 195954; de Navasquez, 1935, 196174; Drew et al., 1978, 067913; Fairley et al., 1934, 062919; JBRC, 1998, 196242; Kesten et al., 1939, 194972; Laug et al., 1939, 195055; Schrenk and Yant, 1936, 195076). The results of subchronic and chronic studies are discussed below.

4.6.1. Oral

Table 4-18 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 (Argus et al., 1965, 017009; Fairley et al., 1934, 062919; Kociba et al., 1974, 062929; NCI, 1978, 062935). Renal cell degeneration generally began with cloudy swelling of cells in the cortex (Fairley et al., 1934, 062919). Nuclear enlargement of proximal tubule cells was observed at doses below those producing renal necrosis (JBRC, 1998, 196240; Kano et al., 2008, 196245), 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. (1974, 062929) study. Cortical tubule degeneration was seen at higher doses in the NCI (1978, 062935) bioassay

(240 mg/kg-day, male rats), and glomerulonephritis was reported for rats given doses of ≥ 430 mg/kg-day (Argus et al., 1965, 017009; 1973, 062912).

Table 4-18. Oral toxicity studies (noncancer effects) for 1,4-dioxane

Species	Dose/duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effect	Reference
Subchronic studies					
Rat and mouse (6/species); unknown strain	Rats 0 or 1,900 mg/kg-day; mice 0 or 3,300 mg/kg-day for 67 days	NA	1,900 rats 3,300 mice	Renal cortical degeneration and necrosis, hemorrhage; hepatocellular degeneration	Fairley et al. (1934, 062919)
Male Sprague Dawley Rat (4–6/group)	0, 10, or 1,000 mg/kg-day for 11 weeks	10	1,000	Minimal centrilobular hepatocyte swelling; increased DNA synthesis	Stott et al. (1981, 063021)
F344/DuCrj rat (10/sex/group)	Males 0, 52, 126, 274, 657, or 1,554 mg/kg-day; females 0, 83, 185, 427, 756, or 1,614 mg/kg-day for 13 weeks	52	126	Nuclear enlargement of nasal respiratory epithelium; hepatocyte swelling	Kano et al. (2008, 196245)
Crj:BDF1 Mouse (10/sex/group)	Males 0, 86, 231, 585, 882, or 1,570 mg/kg-day; females 0, 170, 387, 898, 1,620, or 2,669 mg/kg-day for 13 weeks	170	387	Nuclear enlargement of bronchial epithelium	Kano et al. (2008, 196245)
Chronic studies					
Male Wistar Rat (26 treated, 9 controls)	0 or 640 mg/kg-day for 63 weeks	NA	640	Hepatocytes with enlarged hyperchromic nuclei; glomerulonephritis	Argus et al. (1965, 017009)
Male Sprague Dawley rats (30/group)	0, 430, 574, 803, or 1,032 mg/kg-day for 13 months	NA	430	Hepatocytomegaly; glomerulonephritis	Argus et al. (1973, 062912)
Sherman rat (60/sex/dose group)	Males 0, 9.6, 94, or 1,015 mg/kg-day; females 0, 19, 148, or 1,599 mg/kg-day for 2 years	9.6	94	Degeneration and necrosis of renal tubular cells and hepatocytes	Kociba et al. (1974, 062929)
Osborne-Mendel rat (35/sex/dose level)	Males 0, 240, or 530 mg/kg-day; females 0, 350, or 640 mg/kg-day for 110 weeks	NA	240	Pneumonia, gastric ulcers, and cortical tubular degeneration in the kidney	NCI (1978, 062935)
B6C3F$_1$ mouse (50/sex/dose level)	Males 0, 720, or 830 mg/kg-day; females 0, 380, or 860 mg/kg-day for 90 weeks	NA	380	Pneumonia and rhinitis	NCI (1978, 062935)

Species	Dose/duration	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Effect	Reference
F344/DuCrj rat (50/sex/dose level)	Males 0, 11, 55, or 274 mg/kg-day; females 0, 18, 83, or 429 mg/kg-day for 2 years	55	274	Atrophy of nasal olfactory epithelium; nasal adhesion and inflammation	JBRC (1998, 196240); Kano et al. (2009, 594539)
F344/DuCrj rat (50/sex/dose level)	Males 0, 11, 55, or 274 mg/kg-day; females 0, 18, 83, or 429 mg/kg-day for 2 years	11	55	Liver hyperplasia	JBRC (1998, 196240); Kano et al. (2009, 594539)
F344/DuCrj rat (50/sex/dose level)	Males 0, 11, 55, or 274 mg/kg-day; females 0, 18, 83, or 429 mg/kg-day for 2 years	55	274	Increases in serum liver enzymes (GOT, GPT, LDH, and ALP)	JBRC (1998, 196240); Kano et al. (2009, 594539)
Crj:BDF1 mouse (50/sex/dose level)	Males 0, 49, 191 or 677 mg/kg-day; females 0, 66, 278, or 964 mg/kg-day for 2 years	66	278	Nasal inflammation	JBRC (1998, 196240); Kano et al. (2009, 594539)
Crj:BDF1 mouse (50/sex/dose level)	Males 0, 49, 191 or 677 mg/kg-day; females 0, 66, 278, or 964 mg/kg-day for 2 years	49	191	Increases in serum liver enzymes (GOT, GPT, LDH, and ALP)	JBRC (1998, 196240); Kano et al. (2009, 594539)
Developmental studies					
Sprague Dawley rat (18–20/group)	Pregnant dams 0, 250, 500, or 1,000 mg/kg-day on gestation days 6–15	500	1,000	Delayed ossification of the sternebrae and reduced fetal BWs	Giavani et al. (1985, 062924)

Liver effects included degeneration and necrosis, hepatocyte swelling, cells with hyperchromic nuclei, spongiosis hepatis, hyperplasia, and clear and mixed cell foci of the liver (Argus et al., 1965, 017009; Argus et al., 1973, 062912; Fairley et al., 1934, 062919; Kano et al., 2008, 196245; Kociba et al., 1974, 062929; NCI, 1978, 062935). Hepatocellular degeneration and necrosis were seen at high doses in a subchronic study (1,900 mg/kg-day in rats) (Fairley et al., 1934, 062919) and at lower doses in a chronic study (94 mg/kg-day, male rats) (Kociba et al., 1974, 062929). Argus et al. (1973, 062912) 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, hyperplasia, and clear and mixed-cell foci were also observed in the liver of rats (doses >55 mg/kg-day in male rats) (JBRC, 1998, 196240; Kano et al., 2009, 594539). 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, 196140; Karbe and Kerlin, 2002, 196246; Stroebel et al., 1995, 196101). 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, 2002, 196246), but may also occur in combination with preneoplastic foci, or hepatocellular adenoma or carcinoma (Bannasch, 2003, 196140; Stroebel et al., 1995, 196101). In the case of the JBRC (1998, 196240) study, spongiosis hepatis was associated with other preneoplastic changes in the liver (hyperplasia, 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, 196240) study only. Mean doses are reported based on information provided in Kano et al. (2009, 594539). 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, 196112) 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, 062935) (mice only, dose ≥ 380 mg/kg-day) and JBRC (1998, 196240) studies (≥ 274 mg/kg-day in rats, >278 mg/kg-day in mice). The JBRC (1998, 196240) 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 (Goldsworthy et al., 1991, 062925; Sweeney et al., 2008, 195085) 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, 062935) study. The significance of this effect is unclear, as it was not observed in other studies that evaluated lung histopathology (JBRC, 1998, 196240; Kano et al., 2008, 196245; Kociba et al., 1974, 062929). 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, 196240); 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, 594539); 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, 196240).

4.6.2. Inhalation

Only one subchronic study (Fairley et al., 1934, 062919) and one chronic inhalation study (Torkelson et al., 1974, 094807) were identified. In the subchronic study, 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 liver and kidney damage and acute vascular congestion of the lungs were noted for all exposure concentrations tested. 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. Torkelson et al. (1974, 094807) 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. These studies were not sufficient to characterize the inhalation risks of 1,4-dioxane, due to the nature of the available data (i.e., free-standing LOAEL and NOAEL values).

4.6.3. 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 (1976, 062953; Young et al., 1977, 062956). Like humans, rats extensively metabolized inhaled 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., 1978, 062955; Young et al., 1978, 625640).

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., 1977, 062951; Woo et al., 1978, 194345). 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,

194344). The differences between single and multiple doses in urinary and expired radiolabel support the notion that 1,4-dioxane may induce its own metabolism. 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., 2005, 195067). 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. (2005, 195067) investigated the role of CYP450 isozymes in the liver toxicity of 1,4-dioxane. Hepatic CYPB1/2 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 toxic 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., 1977, 062950). 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 accumulation of the parent compound may be related to 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., 1975, 062930). 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. 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. 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, 2005, 086237), 1,4-dioxane is "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 (Argus et al., 1965, 017009; Argus et al., 1973, 062912; Hoch-Ligeti and Argus, 1970, 029386; Hoch-Ligeti et al., 1970, 062926; JBRC, 1998, 196240; Kano et al., 2009, 594539;

Kociba et al., 1974, 062929; NCI, 1978, 062935; Yamazaki et al., 1994, 196120). Additionally, mesotheliomas of the peritoneum (JBRC, 1998, 196240; Kano et al., 2009, 594539; Yamazaki et al., 1994, 196120), mammary (JBRC, 1998, 196240; Kano et al., 2009, 594539; Yamazaki et al., 1994, 196120), and nasal tumors (Argus et al., 1973, 062912; Hoch-Ligeti et al., 1970, 062926; JBRC, 1998, 196240; Kano et al., 2009, 594539; Kociba et al., 1974, 062929; NCI, 1978, 062935; Yamazaki et al., 1994, 196120) have been observed in rats due to exposure to 1,4-dioxane. 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., 1978, 062914; Thiess et al., 1976, 062943).

The available evidence is inadequate to establish a mode of action (MOA) by which 1,4-dioxane induces liver tumors in rats and mice. 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 (King et al., 1973, 029390; Lundberg et al., 1987, 062933). Dose-response and temporal data support the occurrence of cell proliferation and hyperplasia prior to the development of liver tumors (JBRC, 1998, 196240; Kociba et al., 1974, 062929) 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, 196240; Kociba et al., 1974, 062929) 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 4-18) from cytotoxicity and cell proliferation to eventual liver tumor formation are not available.

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

U.S. EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005, 086237) 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. Information on the carcinogenic effects of 1,4-dioxane via the inhalation and dermal routes in humans and animals is absent. (Note: Note that during the development of this assessment, new data regarding the toxicity of 1,4-dioxane through the inhalation route of exposure became available. These data have not been included in the current assessment and will be evaluated in a separate IRIS assessment.) Based on the

observance of systemic tumors following oral exposure, and in the absence of information to indicate otherwise, it is assumed that an internal dose will be achieved regardless of the route of exposure. Therefore, 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 and number of reported cases were of limited size (Buffler et al., 1978, 062914; Thiess et al., 1976, 062943).

Several carcinogenicity bioassays have been conducted for 1,4-dioxane in mice, rats, and guinea pigs (Argus et al., 1965, 017009; Argus et al., 1973, 062912; Hoch-Ligeti and Argus, 1970, 029386; Hoch-Ligeti et al., 1970, 062926; JBRC, 1998, 196240; Kano et al., 2009, 594539; Kociba et al., 1974, 062929; NCI, 1978, 062935; Torkelson et al., 1974, 094807; Yamazaki et al., 1994, 196120). Liver tumors have been observed following drinking water exposure in male Wistar rats (Argus et al., 1965, 017009), male guinea pigs (Hoch-Ligeti and Argus, 1970, 029386), male Sprague Dawley rats (Argus et al., 1973, 062912; Hoch-Ligeti et al., 1970, 062926), male and female Sherman rats (Kociba et al., 1974, 062929), female Osborne-Mendel rats (NCI, 1978, 062935), male and female F344/DuCrj rats (JBRC, 1998, 196240; Kano et al., 2009, 594539; Yamazaki et al., 1994, 196120), male and female B6C3F$_1$ mice (NCI, 1978, 062935), and male and female Crj:BDF1 mice (JBRC, 1998, 196240; Kano et al., 2009, 594539; Yamazaki et al., 1994, 196120). In the earliest cancer bioassays, the liver tumors were described as hepatomas (Argus et al., 1965, 017009; Argus et al., 1973, 062912; Hoch-Ligeti and Argus, 1970, 029386; Hoch-Ligeti et al., 1970, 062926); however, later studies made a distinction between hepatocellular carcinoma and hepatocellular adenoma (JBRC, 1998, 196240; Kano et al., 2009, 594539; Kociba et al., 1974, 062929; NCI, 1978, 062935; Yamazaki et al., 1994, 196120). Both tumor types have been seen in rats and mice exposed to 1,4-dioxane. Kociba et al. (1974, 062929) 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. Findings from JBRC (1998, 196240) also provided evidence of liver hyperplasia in male F344/DuCrj rats at a dose level below the dose that induced a statistically significant increase in tumor formation.

Nasal cavity tumors were also observed in Sprague Dawley rats (Argus et al., 1973, 062912; Hoch-Ligeti et al., 1970, 062926), Osborne-Mendel rats (NCI, 1978, 062935), Sherman rats (Kociba et al., 1974, 062929), and F344/DuCrj rats (JBRC, 1998, 196240; Kano et al., 2009, 594539; Yamazaki et al., 1994, 196120). Most tumors were characterized as squamous cell

carcinomas. Nasal tumors were not elevated in B6C3F$_1$ or Crj:BDF1 mice. JBRC (1998, 196240) was the only study that evaluated nonneoplastic changes in nasal cavity tissue following prolonged exposure to 1,4-dioxane in the drinking water. Histopathological lesions in female F344/DuCrj rats were suggestive of toxicity and regeneration in this 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, 062935) 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. 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., 1974, 094807).

Tumor initiation and promotion studies in mouse skin and rat liver suggested that 1,4-dioxane does not initiate the carcinogenic process, but instead acts as a tumor promoter (Bull et al., 1986, 194336; King et al., 1973, 029390; Lundberg et al., 1987, 062933) (see Section 4.2.3).

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, 594539) study (also JBRC, 1998, 196240; Yamazaki et al., 1994, 196120). Female rats dosed with 429 mg/kg-day in drinking water for 2 years also showed a statistically significant increase in mammary gland adenomas (JBRC, 1998, 196240; Kano et al., 2009, 594539; Yamazaki et al., 1994, 196120). A significant increase in the incidence of these tumors was not observed in other chronic oral bioassays of 1,4-dioxane (Kociba et al., 1974, 062929; NCI, 1978, 062935).

4.7.3. Mode of Action Information

The MOA by which 1,4-dioxane produces liver, nasal, peritoneal (mesotheliomas), and mammary gland 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 clearly identify whether 1,4-dioxane or one of its metabolites is responsible for the observed effects. 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, 2005, 086237). MOA analyses were not conducted for peritoneal or mammary gland 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., 1974, 062929), 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 (JBRC, 1998, 196240; Kano et al., 2008, 196245; NCI, 1978, 062935). 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., 1975, 062930); however, no in vivo or in vitro assays have examined the toxicity of metabolites resulting from 1,4-dioxane to support this hypothesis. Nanelli et al. (2005, 195067) 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 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. 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 (King et al., 1973, 029390; Lundberg et al., 1987, 062933). This is consistent with the increase in hepatocyte cell proliferation observed in several studies (Goldsworthy et al., 1991, 062925; Miyagawa et al., 1999, 195063; Stott et al., 1981, 063021; Uno et al., 1994, 194385). These mechanistic studies provide evidence of cell proliferation, but do not indicate whether mitogenesis or cytotoxicity is responsible for increased cell turnover.

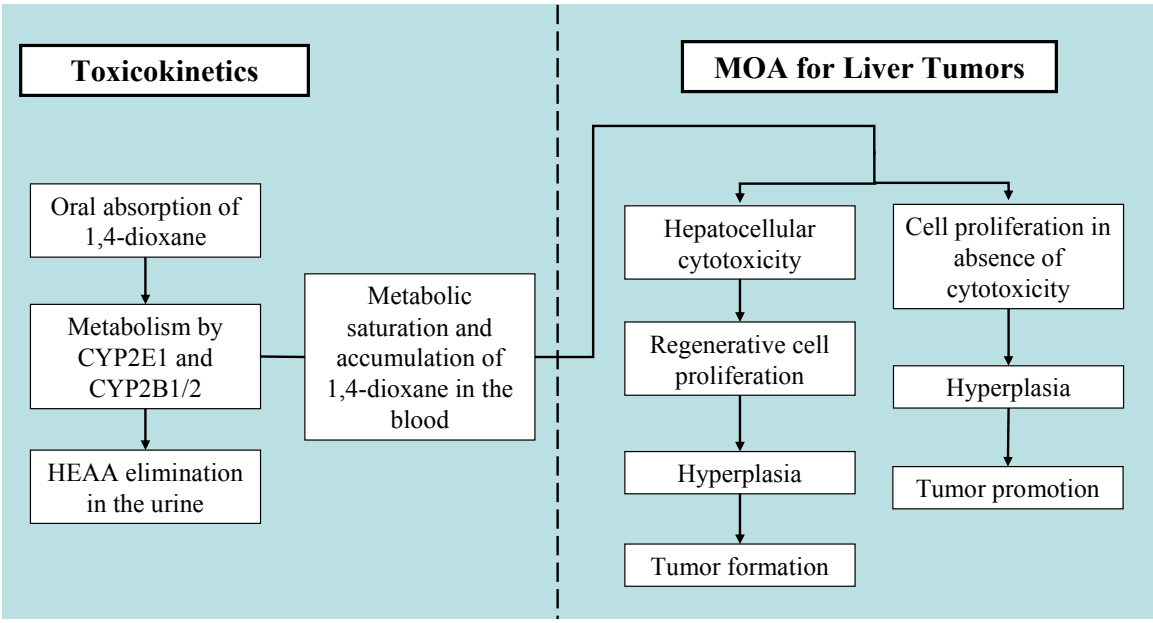

Figure 4-1. A schematic representation of the possible key events in the delivery of 1,4-dioxane to the liver and the hypothesized MOA(s) for liver carcinogenicity.

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. Precursor events in which 1,4-dioxane may promote proliferation of transformed nasal cells are highly uncertain. Figure 4-2 presents a schematic representation of possible key events leading to the formation of nasal cavity tumors. Histopathological lesions in female rats were suggestive of toxicity and regeneration in this tissue (i.e., atrophy, adhesion, inflammation, nuclear enlargement, and hyperplasia and metaplasia of respiratory and olfactory epithelium) (JBRC, 1998, 196240; Kano et al., 2009, 594539).

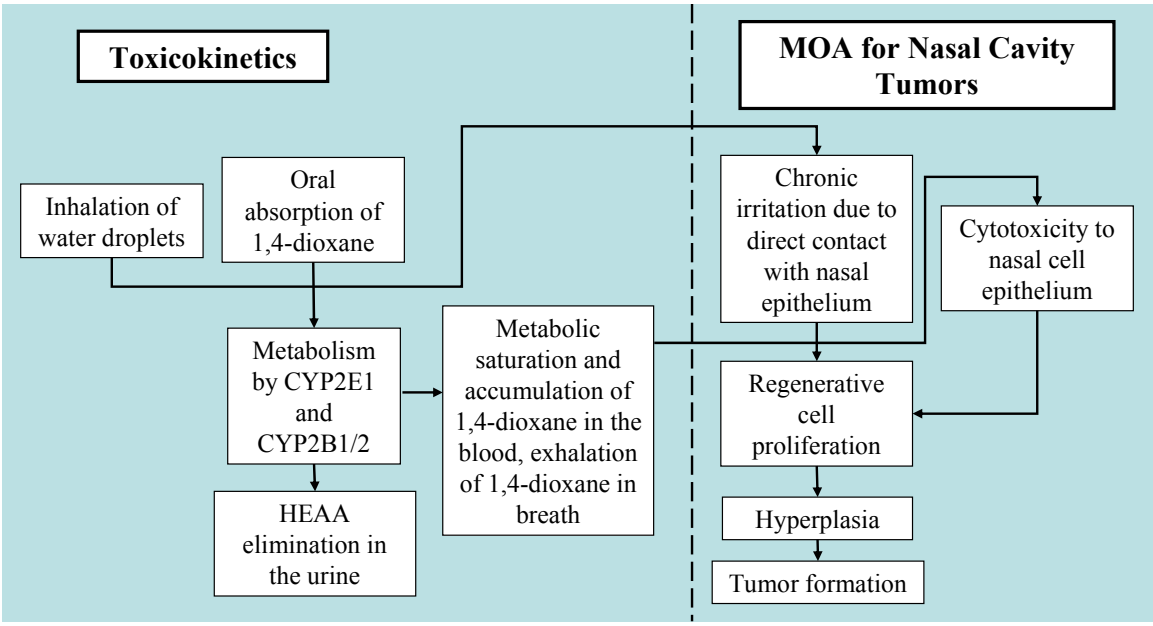

Figure 4-2. A schematic representation of the possible key events in the delivery of 1,4-dioxane to the nasal cavity and the hypothesized MOA(s) for nasal cavity carcinogenicity.

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., 1974, 062929) and the demonstration of nonneoplastic liver lesions in subchronic (Kano et al., 2008, 196245) and acute and short-term oral studies (see Table 4-15). Because the incidence of nonneoplastic lesions was not reported by Kociba et al. (1974, 062929), 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. (1974, 062929), liver tumors were observed in female rats and female mice in the absence of lesions indicative of cytotoxicity (JBRC, 1998, 196240; Kano et al., 2008, 196245; NCI, 1978, 062935). 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 (Goldsworthy et al., 1991, 062925; Miyagawa et al., 1999, 195063; Uno et al., 1994, 194385). 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 (JBRC, 1998, 196240; Kano et al., 2009, 594539; Kociba et al., 1974, 062929; NCI, 1978, 062935; Yamazaki et al., 1994, 196120), but were not elevated in two strains of mice (JBRC, 1998, 196240; Kano et al., 2009, 594539; NCI, 1978, 062935; Yamazaki et al., 1994, 196120). Chronic irritation was indicated by the observation of rhinitis and inflammation of the nasal cavity in rats from the JBRC (1998, 196240) study. This study also showed atrophy of the nasal epithelium and adhesion in rats. Regeneration of the nasal epithelium is demonstrated by metaplasia and hyperplasia observed in rats exposed to 1,4-dioxane (JBRC, 1998, 196240; Kano et al., 2009, 594539; Yamazaki et al., 1994, 196120).

4.7.3.3. ***Dose-Response Relationship***

4.7.3.3.1. ***Liver.*** Table 4-19 presents the temporal sequence 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. (1974, 062929) 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., 1974, 062929). Hepatic tumors were only observed at the highest dose (Kociba et al., 1974, 062929). Hepatic regeneration was indicated in the mid- and high-dose group by the formation of hepatocellular hyperplastic nodules. Liver hyperplasia was also seen in rats from the JBRC (1998, 196240) study, at or below the dose level that resulted in tumor formation (Kano et al., 2009, 594539); however, hepatocellular degeneration and necrosis were not observed. These results suggest that hepatic cell proliferation and hyperplasia 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 4-19. Temporal sequence and dose-response relationship for possible key events and liver tumors in rats and mice

Dose (mg/kg-day)	Key event (time →)				
	Metabolism 1,4-dioxane	Liver damage	Cell proliferation	Hyperplasia	Adenomas and/or carcinomas
Kociba et al., (1974, 062929)—Sherman rats (male and female combined)					
0	—[a]				—[a]
14	+[b]	—[a]	—[a]	—[a]	—[a]
121	+[b]	+[c]	—[a]	+[c]	—[a]
1,307	+[b]	+[c]	—[a]	+[c]	+[c]
NCI, (1978, 062935)—female Osborne-Mendel rats					
0	—[a]	—[a]	—[a]	—[a]	—[a]
350	+[b]	—[a]	—[a]	—[a]	+[c]
640	+[b]	—[a]	—[a]	—[a]	+[c]
NCI, (1978, 062935)—male B6C3F$_1$ mice					
0	—[a]	—[a]	—[a]	—[a]	—[a]
720	+[b]	—[a]	—[a]	—[a]	+[c]
830	+[b]	—[a]	—[a]	—[a]	+[c]
NCI, (1978, 062935)—female B6C3F$_1$ mice					
0	—[a]	—[a]	—[a]	—[a]	—[a]
380	+[b]	—[a]	—[a]	—[a]	+[c]
860	+[b]	—[a]	—[a]	—[a]	+[c]
Kano et al., (2009, 594539); JBRC, (1998, 196240)—male F344/DuCrj rats					
0	—[a]	—[a]	—[a]	—[a]	—[a]
11	+[b]	—[a]	—[a]	—[a]	—[a]
55	+[b]	—[a]	—[a]	+[c,e]	—[a]
274	+[b]	+[c,d]	—[a]	+[c,e]	+[c,e]
Kano et al., (2009, 594539); JBRC, (1998, 196240)—female F344/DuCrj rats					
0	—[a]	—[a]	—[a]	—[a]	—[a]
18	+[b]	—[a]	—[a]	—[a]	—[a]
83	+[b]	—[a]	—[a]	—[a]	—[a]
429	+[b]	—[a]	—[a]	+[c,e]	+[c,e]
Kano et al., (2009, 594539); JBRC, (1998, 196240)—male Crj:BDF1 mice					
0	—[a]	—[a]	—[a]	—[a]	—[a]
49	+[b]	—[a]	—[a]	—[a]	+[c,e]
191	+[b]	—[a]	—[a]	—[a]	+[c,e]
677	+[b]	+[c,d]	—[a]	—[a]	+[c,e]

Dose (mg/kg-day)	Key event (time →)				
	Metabolism 1,4-dioxane	Liver damage	Cell proliferation	Hyperplasia	Adenomas and/or carcinomas
Kano et al., (2009, 594539); JBRC, (1998, 196240)—female Crj:BDF1 mice					
0	—[a]	—[a]	—[a]	—[a]	—[a]
66	+[b]	—[a]	—[a]	—[a]	+[c,e]
278	+[b]	—[a]	—[a]	—[a]	+[c,e]
964	+[b]	+[c,d]	—[a]	—[a]	+[c,e]

[a]— No evidence demonstrating key event.
[b]+ 1,4-dioxane metabolism was not evaluated as part of the chronic bioassays. Data from pharmacokinetic studies suggest that metabolism of 1,4-dioxane by CYP2E1 and CYP2B2 occurs immediately and continues throughout the duration of exposure at all exposure levels.
[c]+ Evidence demonstrating key event.
[d]+ Single cell necrosis was observed in a 13 week bioassay for male rats (274 mg/kg-day), male mice (585 mg/kg-day), and female mice (898 mg/kg-day) exposed to 1,4-dioxane in drinking water (Kano et al., 2008, 196245).
[e]+ Kano et al. (2009, 594539) reported incidence rates for hepatocellular adenomas and carcinomas; however, information from JBRC (1998, 196240) on incidence of liver hyperplasia was used to create this table.

4.7.3.3.2. ***Nasal cavity.*** 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 (see also JBRC, 1998, 196240; Kano et al., 2009, 594539).

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 (JBRC, 1998, 196240; Kano et al., 2008, 196245; Kano et al., 2009, 594539). 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 could presumably lead to regenerative hyperplasia and tumor formation following chronic exposure. As discussed above, histopathological evidence of regenerative hyperplasia has been seen following long-term exposure to 1,4-dioxane (JBRC, 1998, 196240; Kociba et al., 1974, 062929). Tumors occurred earlier at high doses in both mice and rats from this study (Yamazaki, 2006, 626614); 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. (1973, 062912) 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.

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 (Bull et al., 1986, 194336; King et al., 1973, 029390; Lundberg et al., 1987, 062933). Further support for this hypothesis is provided by studies demonstrating that 1,4-dioxane increased hepatocyte DNA synthesis, indicative of cell proliferation (Goldsworthy et al., 1991, 062925; Miyagawa et al., 1999, 195063; Stott et al., 1981, 063021; Uno et al., 1994, 194385). 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, 195021); however, other MOAs have been proposed for nasal carcinogens that induce effects through other mechanisms (Green et al., 2000, 196210; Kasper et al., 2007, 195045).

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, 1997, 195983). 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., 1974, 094807).

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, 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 4-16. Negative findings were reported for mutagenicity in *Salmonella typhimurium*, *Escherichia coli*, and *Photobacterium phosphoreum* (Mutatox assay) (Haworth et al., 1983, 028947; Hellmér and Bolcsfoldi, 1992, 194717; Khudoley et al., 1987, 194949; Kwan et al., 1990, 196078; Morita and Hayashi, 1998, 195065; Nestmann et al., 1984, 194339; Stott et al., 1981, 063021). 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., 1985, 194343). In contrast, positive results were reported in assays for sister chromatid exchange (Galloway et al., 1987, 007768), DNA damage (Kitchin and Brown, 1990, 062928), and in in vivo micronucleus formation in bone marrow (Mirkova, 1994, 195062; Roy et al., 2005, 196094), and liver (Morita and Hayashi, 1998, 195065; Roy et al., 2005, 196094). Lastly, in the presence of toxicity, positive results were reported for meiotic nondisjunction in drosophila (Munoz and Barnett, 2002, 195066), DNA damage (Sina et al., 1983, 007323), and cell transformation (Sheu et al., 1988, 195078).

Additionally, 1,4-dioxane metabolism did not produce reactive intermediates that covalently bound to DNA (Stott et al., 1981, 063021; Woo et al., 1977, 062950) and DNA repair assays were generally negative (Goldsworthy et al., 1991, 062925; Stott et al., 1981, 063021). 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., 1974, 062929) and a

regenerative response to tissue injury was demonstrated by histopathology. Liver hyperplasia and tumor formation have also been observed in the absence of cytotoxicity (see also JBRC, 1998, 196240; Kano et al., 2009, 594539). Cell proliferation and tumor promotion have been shown to occur after prolonged exposure to 1,4-dioxane (Bull et al., 1986, 194336; Goldsworthy et al., 1991, 062925; King et al., 1973, 029390; Lundberg et al., 1987, 062933; Miyagawa et al., 1999, 195063; Stott et al., 1981, 063021; Uno et al., 1994, 194385).

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.

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. As was stated, the MOA by which 1,4-dioxane produces liver, nasal, peritoneal, and mammary gland tumors is unknown. 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 observed peritoneal or mammary gland tumors (see also JBRC, 1998, 196240; Kano et al., 2009, 594539; Yamazaki et al., 1994, 196120).

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

There is no direct evidence to establish that certain populations and lifestages may be potentially 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, 011956) 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, 080660). 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 (Hayashi et al., 1991, 196219; Watanabe et al., 1994, 196099) and were considered to be possible factors in the abnormal liver function seen in workers exposed to vinyl chloride (Huang et al., 1997, 005276). 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.

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 (Barber, 1934, 062913; Johnstone, 1959, 062927). In animals, liver and kidney degeneration and necrosis were observed frequently in acute oral and inhalation studies (David, 1964, 195954; de Navasquez, 1935, 196174; Drew et al., 1978, 067913; Fairley et al., 1934, 062919; JBRC, 1998, 196242; Kesten et al., 1939, 194972; Laug et al., 1939, 195055; Schrenk and Yant, 1936, 195076). Liver and kidney effects were also observed following chronic oral exposure to 1,4-dioxane in animals (Argus et al., 1965, 017009; Argus et al., 1973, 062912; JBRC, 1998, 196240; Kano et al., 2009, 594539; Kociba et al., 1974, 062929; NCI, 1978, 062935; Yamazaki et al., 1994, 196120) (see Table 4-18).

Liver toxicity in the available chronic studies was characterized by necrosis, spongiosis hepatic, hyperplasia, cyst formation, clear foci, and mixed cell foci. Kociba et al. (1974, 062929) demonstrated hepatocellular degeneration and necrosis at doses of 94 mg/kg-day (LOAEL in male rats) or greater. 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. (1973, 062912) described early preneoplastic changes in the liver and JBRC (1998, 196240) 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, 196240) 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, 196240); 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, 196240) study. The highest non-tumorigenic dose levels for

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

this study approximated the LOAEL derived from the Kociba et al. (1974, 062929) 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 (Argus et al., 1965, 017009; Argus et al., 1973, 062912; Fairley et al., 1934, 062919; Kociba et al., 1974, 062929; NCI, 1978, 062935). Kociba et al. (1974, 062929) 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., 1974, 062929). Doses of ≥ 430 mg/kg-day 1,4-dioxane induced marked kidney alterations (Argus et al., 1973, 062912). 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, 062935) 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, 196240) 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. (1974, 062929) 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. (1974, 062929) reported degenerative effects in the liver, while liver lesions reported in other studies (Argus et al., 1973, 062912; JBRC, 1998, 196240) appeared to be related to the carcinogenic process. Kociba et al. (1974, 062929) also reported degenerative changes in the kidney. NCI (1978, 062935) and Argus et al. (1973, 062912) provided supporting data for this endpoint; however, kidney toxicity was observed in these studies at higher doses. JBRC (1998, 196240) 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. (1974, 062929) 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 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.)

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 (1990, 062932) or Sweeney et al. (2008, 195085) (Figures B-8 and B-9). 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-10). 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 (Figures B-12 and B-13). Exploration of the model space using an assumption of zero-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-16). 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_{maxC} and K_m. This suggests that the model structure is insufficient to capture the apparent 10-fold species difference in the blood 1,4-dioxane between rats and humans. 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 (Table B-1). Therefore, some other, as yet unknown, modification to model structure may be necessary.

Kociba et al. (1974, 062929) did not provide quantitative incidence or severity data for liver and kidney degeneration and necrosis. Benchmark dose (BMD) modeling could not be performed for this study and 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.

Alternative PODs were calculated using incidence data reported for cortical tubule degeneration in male and female rats (NCI, 1978, 062935) and liver hyperplasia (JBRC, 1998, 196240). 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 for cortical tubule degeneration in the NCI (1978, 062935) study ($BMDL_{10}$ of 22.3 mg/kg-day) (Table 5-2). Incidence data (JBRC, 1998, 196240; Kano et al., 2009, 594539) for liver hyperplasia in male and female rats exposed to 1,4-dioxane in the drinking water for 2 years are presented in Table 5-3. Details of the BMD analysis of these data are presented in Appendix C. Male rats were more sensitive to developing liver hyperplasia due to exposure to 1,4-dioxane than females and the male rat data provided the lowest POD for hyperplasia in the JBRC (1998,

196240) study ($BMDL_{10}$ of 23.8 mg/kg-day) (Table 5-4). The $BMDL_{10}$ values of 22.3 mg/kg-day and 23.8 mg/kg-day from the NCI (1978, 062935) and JBRC (1998, 196240) studies, respectively, are about double the NOAEL (9.6 mg/kg-day) observed by Kociba et al. (1974, 062929).

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, 062935).

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

	BMD_{10} (mg/kg-day)	**$BMDL_{10}$ (mg/kg-day)**
Male rats	28.8	22.3
Female rats	596.4	452.4

Source: NCI (1978, 062935).

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

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

[a]Dose information from Kano et al. (2009, 594539) and incidence data for sacrificed animals from JBRC (1998, 196240).
[b]Statistically significant compared to controls by the Dunnett's test ($p < 0.05$).
[c]Incidence significantly elevated compared to control by χ^2 test ($p < 0.01$).

Sources: Kano et al. (2009, 594539); JBRC (1998, 196240).

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

	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Male rats	35.9	23.8
Female rats	137.3	88.5

Source: Kano et al. (2009, 594539); JBRC (1998, 196240).

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., 1974, 062929). The Kociba et al. (1974, 062929) 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., 1974, 062929) by a composite UF of 300, as follows:

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

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 was used to account for pharmacokinetic and pharmacodynamic differences across species. 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 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., 1985, 062924). This developmental study indicates that the developing fetus may be a target of toxicity.

An UF to extrapolate from a subchronic to a chronic 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 was not necessary because the RfD was based on a NOAEL. Kociba et al. (1974, 062929) 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 was not reported. Several additional oral studies (acute/short-term, subchronic, and chronic durations) were available that support liver and kidney toxicity as the critical effect (Argus et al., 1973, 062912; JBRC, 1998, 196240; Kano et al., 2008, 196245; NCI, 1978, 062935) (Tables 4-15 and 4-17). Although degenerative liver and kidney toxicity was not observed in rats from the JBRC (1998, 196240) study at doses at or below the LOAEL in the Kociba et al. (1974, 062929) 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 sample oral RfDs based on selected studies included in Table 4-18 are arrayed in Figures 5-1 to 5-3, and provide perspective on the RfD supported by Kociba et al. (1974, 062929). 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 sample 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 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. (1974, 062929) provided the data set most appropriate for deriving the RfD. For degenerative liver effects resulting from 1,4-dioxane exposure, the Kociba et al. (1974, 062929) 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., 1965, 017009; Argus et al., 1973, 062912) and degeneration of the cortical tubule (Kociba et al., 1974, 062929; NCI, 1978, 062935) 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., 1974, 062929). A comparison of the available datasets from which an RfD could potentially be derived is presented in Figure 5-2.

Rhinitis and inflammation of the nasal cavity were reported in both the NCI (1978, 062935) (mice only, dose ≥ 380 mg/kg-day) and JBRC (1998, 196240) studies (≥ 274 mg/kg-day in rats, >278 mg/kg-day in mice). JBRC (1998, 196240) 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 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) during gestation can produce delayed ossification of the sternebrae and reduced fetal BWs (Giavini et al., 1985, 062924). This graphical display (Figure 5-4) compares organ specific toxicity for 1,4-dioxane, including a single developmental study. The most sensitive measures of degenerative liver are 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., 1974, 062929) and are presented for completeness.

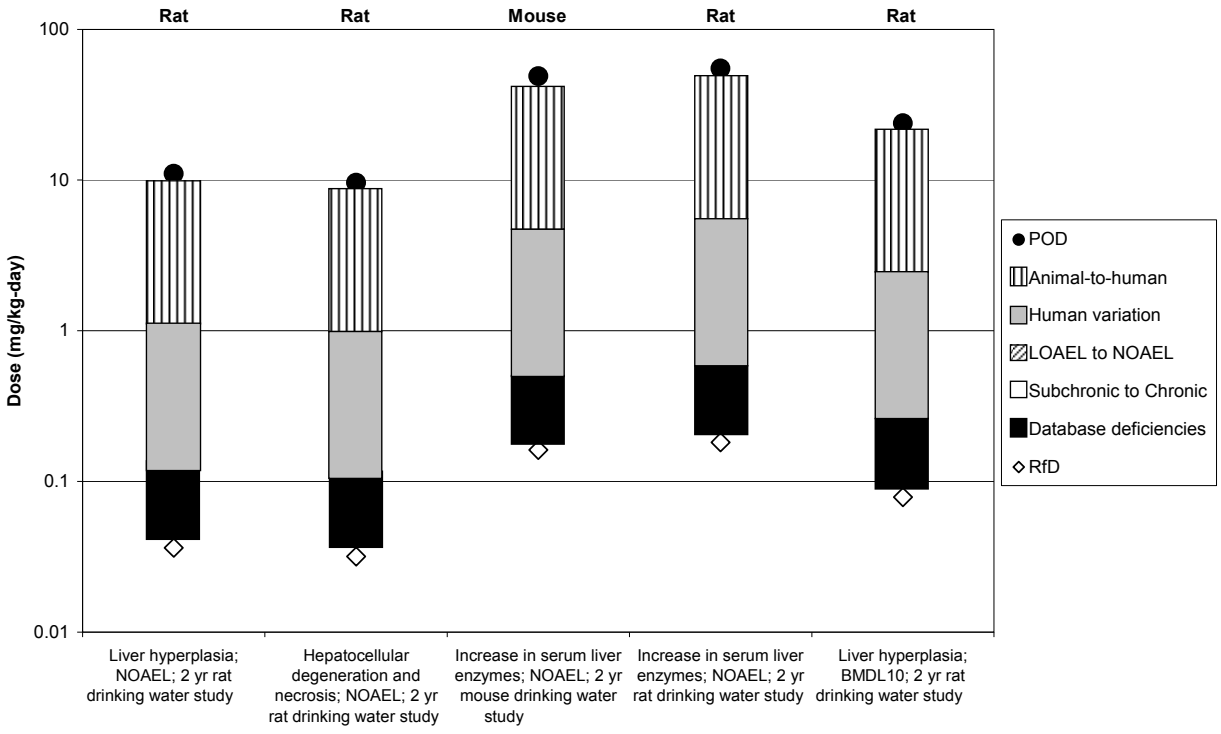

Figure 5-1. Potential points of departure (POD) for liver toxicity endpoints with corresponding applied uncertainty factors and derived RfDs following oral exposure to 1,4-dioxane.

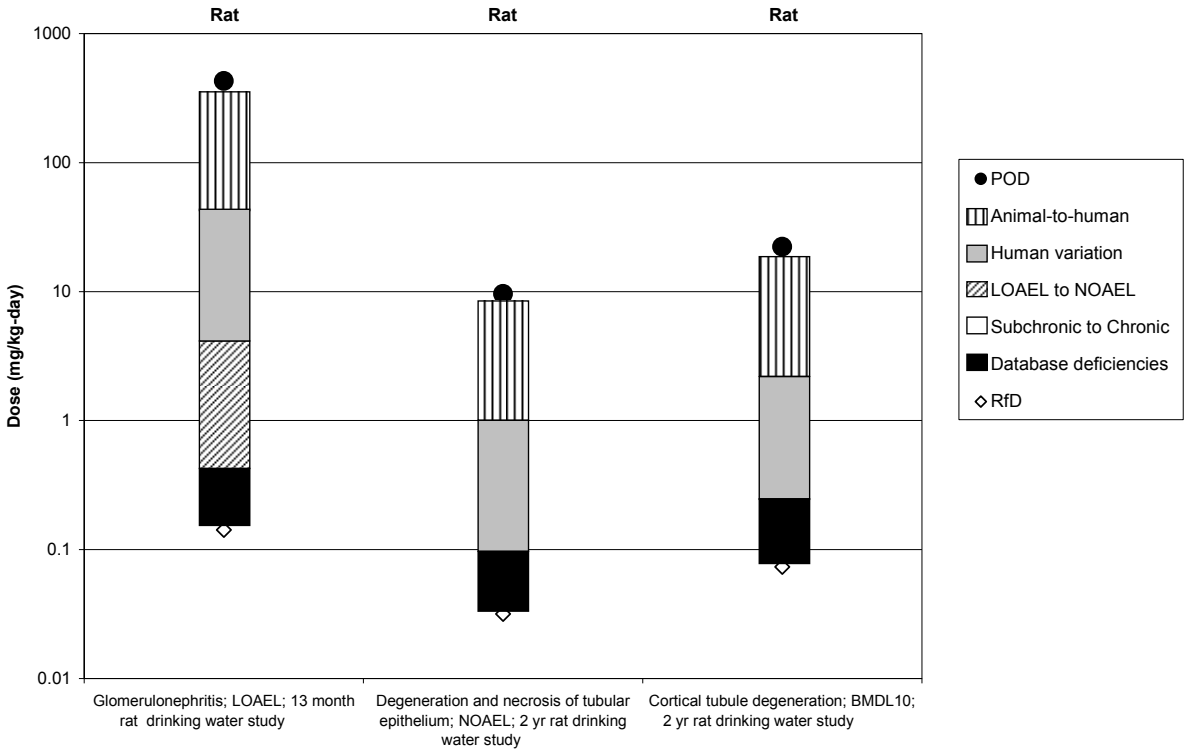

Figure 5-2. Potential points of departure (POD) for kidney toxicity endpoints with corresponding applied uncertainty factors and derived RfDs following oral exposure to 1,4-dioxane.

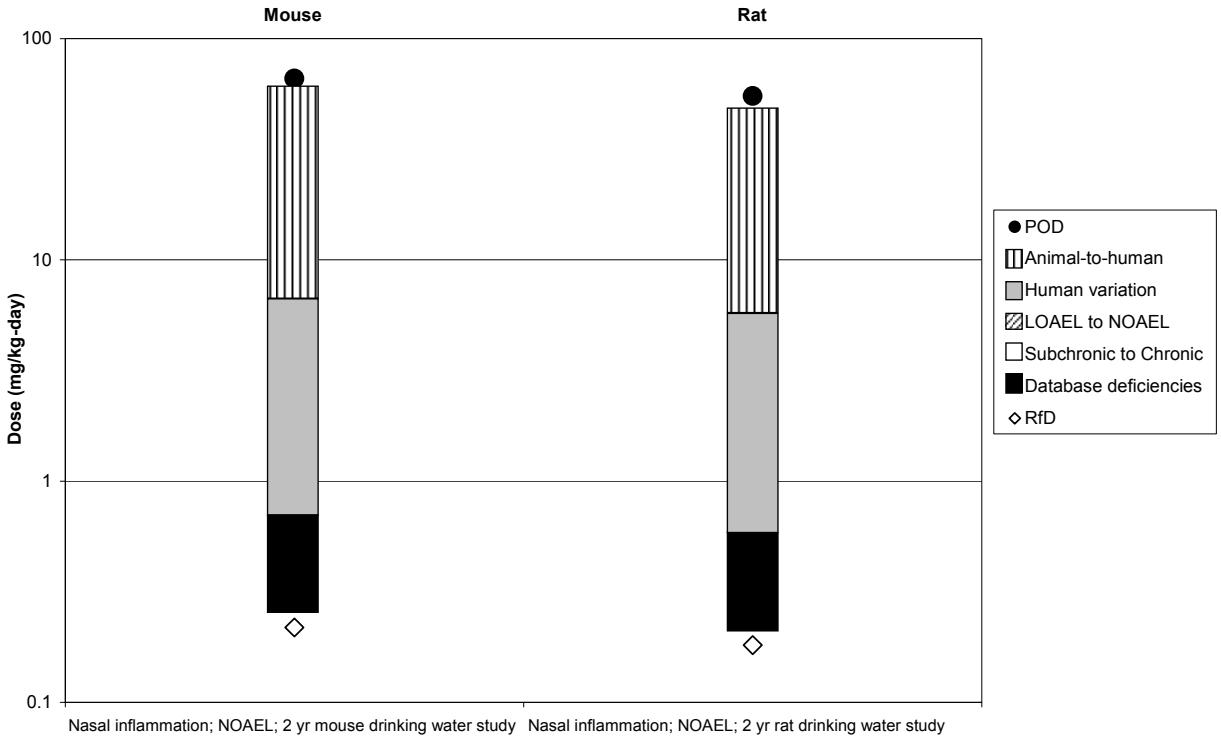

Figure 5-3. Potential points of departure (POD) for nasal inflammation with corresponding applied uncertainty factors and derived sample RfDs following oral exposure to 1,4-dioxane.

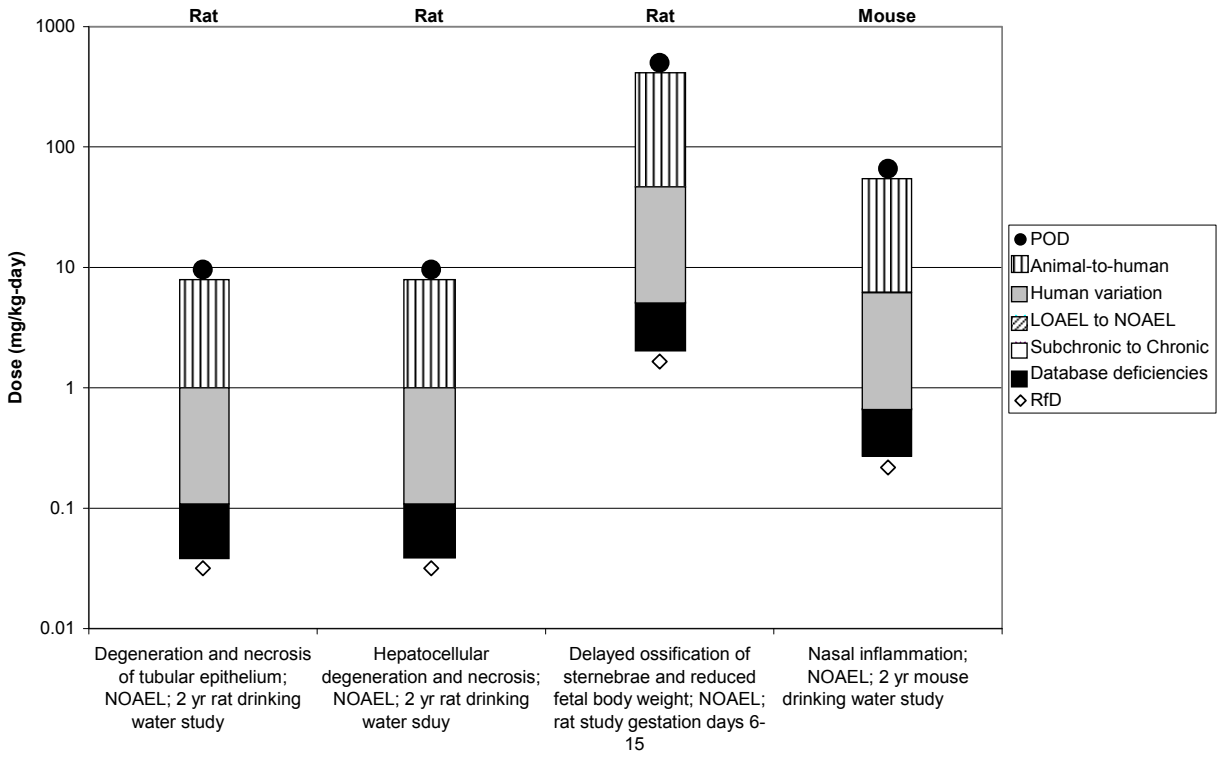

Figure 5-4. Potential points of departure (POD) for organ specific toxicity endpoints with corresponding applied uncertainty factors and derived sample RfDs following oral exposure to 1,4-dioxane.

5.1.5. Previous RfD Assessment

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)

NOTE: During the development of this assessment, new data regarding the toxicity of 1,4-dioxane through the inhalation route of exposure became available. The IRIS Program will evaluate the more recently published 1,4-dioxane inhalation data for the potential to derive an RfC in a separate assessment. A description of the studies that were available at the time that this assessment was under development are summarized below.

Inhalation studies for 1,4-dioxane evaluated in this assessment were not adequate for the determination of an RfC value. Only one subchronic study (Fairley et al., 1934, 062919) and one chronic inhalation study (Torkelson et al., 1974, 094807) were identified. In the subchronic study, 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 liver and kidney 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.

Torkelson et al. (1974, 094807) 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. Because Fairley et al. (1934, 062919) identified a free-standing LOAEL only, and Torkelson et al. (1974, 094807) identified a free-standing NOAEL only, neither study was sufficient to characterize the inhalation risks of 1,4-dioxane. A route extrapolation from 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) (Fairley et al., 1934, 062919; Wirth and Klimmer, 1936, 196105; Yant et al., 1930, 062952), which would not be accounted for in a cross-route extrapolation. In addition, available kinetic models are not suitable for this purpose (Appendix B).

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

5.3. UNCERTAINTIES IN THE ORAL REFERENCE DOSE (RfD)

Risk assessments need to portray associated uncertainty. The following discussion identifies uncertainties associated with the RfD for 1,4-dioxane. As presented earlier in this section (5.1.2 and 5.1.3), the uncertainty factor approach (U.S. EPA, 1994, 006488; U.S. EPA, 2002, 088824), was applied to a POD. Factors accounting for uncertainties associated with a number of steps in the analyses were adopted to account for extrapolating from an animal bioassay to human exposure, a diverse population of varying susceptibilities, and to account for database deficiencies. These extrapolations are carried out with current approaches given the paucity of experimental 1,4-dioxane data to inform individual steps.

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 one subchronic bioassay in rabbits, guinea pigs, and rats, and a chronic inhalation bioassay in rats. Although the subchronic bioassay observed degenerative effects in the liver, kidney, and lungs of all species tested, the

information reported from the study was insufficient to determine an exposure level below which these effects did not occur. The only available chronic inhalation bioassay did not indicate any treatment related effects due to exposure to 1,4-dioxane. Thus, the inhalation database lacked sufficient information to derive toxicity values relevant to this route of exposure for 1,4-dioxane. 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., 1974, 062929) 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, 062935) was also suitable for deriving a RfD, but it is associated with higher a POD and potential RfD compared to Kociba et al. (1974, 062929).

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., 1974, 062929), precluding modeling of the dose-response. However confidence in the LOAEL can be derived from additional studies (Argus et al., 1965, 017009; Argus et al., 1973, 062912; JBRC, 1998, 196240; NCI, 1978, 062935) 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, 062935) 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 was 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.

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. 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 ingested 1,4-dioxane are associated with deficiencies in reproductive toxicity information. The oral database lacks a multigeneration reproductive toxicity study. A single oral prenatal developmental toxicity study

in rats was available for 1,4-dioxane (Giavini et al., 1985, 062924). This developmental study indicates that the developing fetus may be a target of toxicity. The database of inhalation studies is of particular concern due to the lack of a basic toxicological studies, a multigenerational reproductive study, and developmental toxicity studies.

5.4. CANCER ASSESSMENT

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

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 (JBRC, 1998, 196240; Kano et al., 2009, 594539; Kociba et al., 1974, 062929; NCI, 1978, 062935; Yamazaki et al., 1994, 196120). The dose-response data from each of these studies are summarized in Table 5-5. With the exception of the NCI (1978, 062935) study, the incidence of nasal cavity tumors was generally lower than the incidence of liver tumors in exposed rats. The Kano et al. (2009, 594539) 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, 062935) bioassay (Table 5-5). The Kociba et al. (1974, 062929) study also used three dose groups and low exposures; however, the study authors only reported the incidence of hepatocellular carcinoma, which may underestimate the combined incidence of rats with adenoma or carcinoma. In addition to increased incidence of liver tumors, chosen as the most sensitive target organ for tumor formation, the Kano et al. (2009, 594539) study also noted increased incidence of peritoneal and mammary gland tumors. Nasal cavity tumors were also seen in high-dose male and female rats; however, the incidence of nasal tumors was much lower than the incidence of liver tumors in both rats and mice.

In a personal communication, Dr. Yamazaki (2006, 626614) provided 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, 626614). 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, 626614). Thus, the high mortality rates in the female mice were still considered to be relevant for this analysis.

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. (1974, 062929)	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, 062935)	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, 594539)	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[i,k]
	Male Crj:BDF1 mice[d]	0	23/50	0/50	NA	NA
		49	31/50	0/50	NA	NA
		191	37/50[i]	0/50	NA	NA
		677	40/50[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 (no significant change from controls)

5.4.2. Dose-Response Data

Table 5-6 summarizes the incidence of hepatocellular adenoma or carcinoma in rats and mice from the Kano et al. (2009, 594539) 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 significant increase in the incidence of peritoneal mesothelioma was observed in high-dose male rats only (28/50 rats, Table 5-5). The incidence of peritoneal mesothelioma was lower than the observed incidence of hepatocellular adenoma or carcinoma in male rats (Table 5-6); therefore, hepatocellular adenoma or carcinoma data were used to derive an oral CSF for 1,4-dioxane.

Table 5-6. Incidence of hepatocellular adenoma or carcinoma in rats and mice exposed to 1,4-dioxane in drinking water for 2 years

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

[a]Incidence of either hepatocellular adenoma or carcinoma.
[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: Used with permission from Elsevier, Ltd., Kano et al. (2009, 594539).

5.4.3. Dose Adjustments and Extrapolation Method(s)

5.4.3.1. *Dose Adjustments*

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

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

For all caculations, a human BW of 70 kg was used. HEDs for the principal study (Kano et al., 2009, 594539) are given in Table 5-7. HEDs were also calculated for supporting studies (Kociba et al., 1974, 062929; NCI, 1978, 062935) and are also shown in Table 5-7.

Table 5-7. 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, 594539)	Male F344/DuCrj rats	432[a]	11	3.1
		432[a]	81	23
		432[a]	398	112
	Female F344/DuCrj rats	267[a]	18	4.5
		267[a]	83	21
		267[a]	429	107
	Male Crj:BDF1 mice	47.9[a]	49	7.9
		47.9[a]	191	31
		47.9[a]	677	110
	Female Crj:BDF1 mice	35.9[a]	66	10
		35.9[a]	278	42
		35.9[a]	964	145
Kociba et al. (1974, 062929)	Male and female (combined) Sherman rats	325[b]	14	3.7
		325[b]	121	32
		285[c]	1,307	330
NCI (1978, 062935)	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, 594539); Kociba et al. (1974, 062929); and NCI (1978, 062935).

5.4.3.2. *Extrapolation Method(s)*

The U.S. EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005, 086237) 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, 2005, 086237). In the case of 1,4-dioxane, the mode of carcinogenic action for peritoneal, mammary, nasal, and liver tumors is unknown. Therefore, a linear low-dose extrapolation approach was used to estimate human carcinogenic risk associated with 1,4-dioxane exposure.

However, several of the external peer review panel members (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 4.7.3., 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., 1973, 029390). 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 (Bull et al., 1986, 194336; Goldsworthy et al., 1991, 062925; King et al., 1973, 029390; Lundberg et al., 1987, 062933; Miyagawa et al., 1999, 195063; Stott et al., 1981, 063021; Uno et al., 1994, 194385). 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, 2005, 086237), 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 curve fitting 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 (female mice; liver tumors; Kano et al., 2009, 594539).

The oral CSF was calculated using the following equation:

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

5.4.4. Oral Slope Factor and Inhalation Unit Risk

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 the drinking water (Kano et al., 2009, 594539; Kociba et al., 1974, 062929; NCI, 1978, 062935) (Table 5-5). Animal doses are used for BMD modeling and HED BMD and BMDL values are calculated using the animal TWAs (Table 5-7) and a human BW of 70kg. Doses associated with a BMR of 10% extra risk were calculated. BMDs and BMDLs from all models are reported, and the output

and plots corresponding to the best-fitting model 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 BMDS model predictions for the Kano et al. (2009, 594539), NCI (1978, 062935), and Kociba et al. (1974, 062929) studies is shown in Table 5-8.

Table 5-8. BMD_{HED} and $BMDL_{HED}$ values from models fit to tumor incidence data for rats and mice exposed to 1,4-dioxane in drinking water for 2 years and corresponding oral CSFs

Study	Gender/strain/species	Tumor type	BMD_{HED}[a] (mg/kg-day)	$BMDL_{HED}$[a] (mg/kg-day)	Oral CSF $(mg/kg\text{-}day)^{-1}$
Kano et al. (2009, 594539)	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	0.18
	Female Crj:BDF1 mice[d, e]		3.22[e]	2.12[e]	0.14
	Female Crj:BDF1 mice[d, f]		7.51[f]	4.95[f]	0.10
	Female F344/DuCrj rats[g]	Nasal squamous cell carcinoma	94.84	70.23	1.4×10^{-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. (1974, 062929)	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, 062935)	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 AIC, *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 5-8, 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 5-8.

A comparison of the model estimates derived for rats and mice from the Kano et al. (2009, 594539), NCI (1978, 062935), and Kociba et al. (1974, 062929) studies (Table 5-8) indicates that female mice are more sensitive to liver carcinogenicity induced by 1,4-dioxane compared to other species or tumor types. 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:

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

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

Inhalation studies for 1,4-dioxane evaluated in this assessment were not adequate for the determination of an inhalation unit risk. No treatment-related tumors were noted in a chronic inhalation study in rats; however, only a single exposure concentration was used (111 ppm 1,4-dioxane vapor for 7 hours/day, 5 days/week for 2 years) (Torkelson et al., 1974, 094807). A route extrapolation from oral bioassay data was not performed (Section 5.2). In addition, available kinetic models are not suitable for this purpose (Appendix B).

During the development of this assessment, new data regarding the toxicity of 1,4-dioxane through the inhalation route of exposure became available. The IRIS Program will evaluate the more recently published 1,4-dioxane inhalation data for the potential to derive an inhalation unit risk in a separate assessment.

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, 1986, 199530)) 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, 062935). The linearized multistage extra risk procedure was used for linear low dose extrapolation.

5.5. UNCERTAINTIES IN CANCER RISK VALUES

As in most risk assessments, extrapolation of study data to estimate potential risks to human populations from exposure to 1,4-dioxane has engendered some uncertainty in the results. Several types of uncertainty may be considered quantitatively, but other important uncertainties cannot be considered quantitatively. Thus an overall integrated quantitative uncertainty analysis is not presented. Principal uncertainties are summarized below and in Table 5-9.

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 for 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 (JBRC, 1998, 196240; Kano et al., 2009, 594539; Kociba et al., 1974, 062929; NCI, 1978, 062935). 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 a metabolite thereof, interacts with cells starting 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 they have a generic biological motivation based on long-established 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.

In the studies evaluated (Kano et al., 2009, 594539; Kociba et al., 1974, 062929; NCI, 1978, 062935), 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 (Appendix D for details). However, the multistage model did not provide an adequate fit for the female mouse liver tumor dataset based upon the following (U.S. EPA, 2000, 052150):

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

BMDS software typically implements the guidance in the external peer review draft BMD technical guidance document (U.S. EPA, 2000, 052150) by imposing 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.

The log-logistic model was selected because it provides an adequate fit for the female mouse data (Kano et al., 2009, 594539). A BMR of 50% was used because it is proximate to the response at the lowest dose tested and the $BMDL_{50\ HED}$ was derived by applying appropriate parameter constraints, consistent with recommended use of BMDS in the BMD technical guidance document (U.S. EPA, 2000, 052150).

The human equivalent oral CSFs estimated from tumor datasets with statistically significant increases ranged from 4.2×10^{-4} to 0.18 per mg/kg-day (Table 5-8), a range of about three orders of magnitude, with the extremes coming from the combined male and female rat data for hepatocellular carcinomas (Kociba et al., 1974, 062929) and the female mouse combined liver adenoma and carcinomas (Kano et al., 2009, 594539).

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 toxicity. 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.

5.5.1.3. *Cross-Species Scaling*

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 2005 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005, 086237). It is assumed that equal risks result from equivalent constant lifetime exposures.

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, there is a reasonably small degree of uncertainty at the 10% excess incidence level (the POD for linear low-dose extrapolation).

5.5.1.5. *Bioassay Selection*

The study by Kano et al. (2009, 594539) 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 (Kociba et al., 1974, 062929; NCI, 1978, 062935) were available and were fully considered for the derivation of the oral CSF.

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, 594539) 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, 626614) provided that the survival of mice 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, 626614). 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, 626614). 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 incidence of hepatocellular adenomas and carcinomas in historical controls was evaluated with the data from Kano et al. (2009, 594539). Katagiri et al. (1998, 193804) 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, 594539) 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

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.

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 5-9. Summary of uncertainty in the 1,4-dioxane cancer risk estimation

Consideration/ approach	Impact on oral slope factor	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, 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 ↓ CSF by an unknown extent	JBRC (1998, 196240)	Alternative bioassays were available and considered for derivation of oral CSF.
Species /gender combination	Human risk could ↓ or ↑, depending on relative sensitivity	Female mouse	There are no MOA data to guide extrapolation approach for any choice. It was assumed that humans are as sensitive as the most sensitive rodent gender/species tested; true correspondence is unknown. Calculation of the CSF for 1,4-dioxane was based on dose-response data from the most sensitive species and gender. The carcinogenic response occurs across species.
Human relevance of mouse tumor data	If rodent tumors proved not to be relevant to humans, unit risk would not apply i.e., could ↓ CSF	Liver adenomas and carcinomas are relevant to humans	1,4-dioxane is a multi-site carcinogen in rodents and the MOA(s) is unknown; carcinogenicity observed in the rodent studies is considered relevant to human exposure.
Human population variability in metabolism and response/ sensitive subpopulations	Low-dose risk ↑ or ↓ to an unknown extent	Considered qualitatively	No data to support range of human variability/sensitivity, including whether children are more sensitive.

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

6.1. HUMAN HAZARD POTENTIAL

1,4-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 and kidney 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 (Barber, 1934, 062913; Johnstone, 1959, 062927). Neurological changes were also reported in one case, including headache, elevation in blood pressure, agitation and restlessness, and coma (Johnstone, 1959, 062927). 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) (David, 1964, 195954; de Navasquez, 1935, 196174; Drew et al., 1978, 067913; Fairley et al., 1934, 062919; JBRC, 1998, 196242; Kesten et al., 1939, 194972; Laug et al., 1939, 195055; Schrenk and Yant, 1936, 195076).

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., 1974, 062929) and preneoplastic changes were noted in the liver following chronic administration of 1,4-dioxane in drinking water (Argus et al., 1973, 062912; JBRC, 1998, 196240; Kano et al., 2009, 594539). 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., 1975). Kidney damage was characterized by degeneration of the cortical tubule cells, necrosis with hemorrhage, and glomerulonephritis (Argus et al., 1965, 017009; Argus et al., 1973, 062912; Fairley et al., 1934, 062919; Kociba et al., 1974, 062929; NCI, 1978, 062935).

Several carcinogenicity bioassays have been conducted for 1,4-dioxane in mice, rats, and guinea pigs (Argus et al., 1965, 017009; Argus et al., 1973, 062912; Hoch-Ligeti and Argus, 1970, 029386; Hoch-Ligeti et al., 1970, 062926; JBRC, 1998, 196240; Kano et al., 2009, 594539; Kociba et al., 1974, 062929; NCI, 1978, 062935; Torkelson et al., 1974, 094807). Liver tumors (hepatocellular adenomas and carcinomas) have been observed following drinking water exposure in several species and strains of rats, mice, and guinea pigs. Nasal (squamous cell

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

carcinomas), peritoneal, and mammary tumors were also observed in rats, but were not seen in mice. With the exception of the NCI (1978, 062935) study, the incidence of nasal cavity tumors was generally lower than that of liver tumors in the same study population.

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005, 086237), 1,4-dioxane is "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 (Argus et al., 1965, 017009; Argus et al., 1973, 062912; Hoch-Ligeti and Argus, 1970, 029386; Hoch-Ligeti et al., 1970, 062926; JBRC, 1998, 196240; Kano et al., 2009, 594539; Kociba et al., 1974, 062929; NCI, 1978, 062935). 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., 1978, 062914; Thiess et al., 1976, 062943).

The available evidence is inadequate to establish a MOA by which 1,4-dioxane induces liver 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 (Galloway et al., 1987, 007768; Kitchin and Brown, 1990, 062928; Mirkova, 1994, 195062; Morita and Hayashi, 1998, 195065; Roy et al., 2005, 196094). A MOA hypothesis 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 (King et al., 1973, 029390; Lundberg et al., 1987, 062933). Some dose-response and temporal evidence support the occurrence of cell proliferation and hyperplasia prior to the development of liver tumors (JBRC, 1998, 196240; Kociba et al., 1974, 062929). 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. Conflicting data from rat and mouse bioassays (JBRC, 1998, 196240; Kociba et al., 1974, 062929) 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 (JBRC, 1998, 196240; Kano et al., 2009, 594539; NCI, 1978, 062935). Data regarding a plausible dose response and temporal progression from cytotoxicity to cell proliferation and eventual liver tumor formation are not available.

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., 1974, 062929). 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., 1974, 062929) 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

No inhalation RfC was derived for 1,4-dioxane. Inhalation data were inadequate and a route extrapolation from oral toxicity data was not performed, due to direct effects of 1,4-dioxane on the respiratory tract (i.e., respiratory irritation in humans, pulmonary congestion in animals) (Fairley et al., 1934, 062919; Wirth and Klimmer, 1936, 196105; Yant et al., 1930, 062952) and lack of a suitable kinetic model (Appendix B).

Note that during the development of this assessment, new data regarding the toxicity of 1,4-dioxane through the inhalation route of exposure became available and have not been included in the current assessment. The IRIS Program will evaluate the more recently published 1,4-dioxane inhalation in a separate assessment.

6.2.3. Cancer/Oral

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005, 086237), 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. An oral CSF for 1,4-dioxane of 0.10 $(mg/kg\text{-}day)^{-1}$ was based on liver tumors in female mice from a chronic study (Kano et al., 2009, 594539). 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, 2005, 086237), 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.

6.2.3.1. *Choice of Low-Dose Extrapolation Approach*

The range of possibilities for the low-dose extrapolation of tumor risk for 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, 594539). 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.

In the studies evaluated (Kano et al., 2009, 594539; Kociba et al., 1974, 062929; NCI, 1978, 062935) 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 (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, 2000, 052150):

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

BMDS software typically implements the guidance in the BMD technical guidance document (U.S. EPA, 2000, 052150) by imposing 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.

The log-logistic model was selected because it provides an adequate fit for the female mouse data (Kano et al., 2009, 594539). 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, 2000, 052150).

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 extremes coming from the combined male and female data for hepatocellular carcinomas (Kociba et al., 1974, 062929) and the female mouse liver adenoma and carcinoma dataset (Kano et al., 2009, 594539).

6.2.3.2. *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.

6.2.3.3. *Cross-Species Scaling*

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, 2005, 086237). It is assumed that equal risks result from equivalent constant lifetime exposures.

6.2.3.4. *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, there is a reasonably small degree of uncertainty at the 50% excess incidence level (the POD for linear low-dose extrapolation).

6.2.3.5. *Bioassay Selection*

The study by Kano et al. (2009, 594539) 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 (Kociba et al., 1974, 062929; NCI, 1978, 062935) were available and were fully considered for the derivation of the oral CSF.

6.2.3.6. *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, 594539) 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.

6.2.3.7. *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.

6.2.3.8. *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 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.

6.2.4. Cancer/Inhalation

Inhalation studies for 1,4-dioxane were not adequate for the determination of an inhalation unit risk value. No treatment-related tumors were noted in a chronic inhalation study in rats; however only a single exposure concentration was used (111 ppm 1,4-dioxane vapor for 7 hours/day, 5 days/week for 2 years) (Torkelson et al., 1974, 094807). Route extrapolation from oral bioassay data was not performed because available kinetic models were not considered suitable for this purpose.

Note that during the development of this assessment, new data regarding the toxicity of 1,4-dioxane through the inhalation route of exposure became available and have not been included in the current assessment. The IRIS Program will evaluate the more recently published 1,4-dioxane inhalation data in a separate assessment.

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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 formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 2000, 052149; U.S. EPA, 2006, 194566). 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. 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.

A.1. EXTERNAL PEER REVIEW PANEL COMMENTS

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.

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

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, 195061) 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. (1977, 062951; 1978, 194345) was added to the paragraph. In Section 4.5.2.2, study details for Nannelli et al. (2005, 195067) 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. (2008, 195044; 2009, 193803) 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, 195044) 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, 193803) 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, 628200) 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, 196127) 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, 195080) 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, 625635) 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, 195080) 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, 594539) was added to the assessment, which was an update and peer-reviewed published manuscript of the JBRC (1998, 196240) 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. (1974, 062929) 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, 594539) was published and this assessment was updated accordingly (previously JBRC (1998, 196240). It is assumed the uncertainty referred to by the reviewer was addressed by the published Kano et al. (2009, 594539) paper.

Clarification regarding the uncertainty surrounding the identification of the toxic moiety was added to Section 4.6.3 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., 1974, 062929) 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. (1974, 062929) study was the best choice for the principal study.

One reviewer stated that Kociba et al. (1974, 062929) 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, 062935) or JBRC (1998, 196240) study.

Response: The reviewer is correct that Kociba et al. (1974, 062929) did not provide incidence data; however, Kociba et al. (1974, 062929) identified a NOAEL (9.6 mg/kg-day) and LOAEL (94 mg/kg-day) within the text of the manuscript. Kociba et al. (1974, 062929) 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. (1974, 062929) as the principal study comes from comparison of the liver and kidney toxicity data reported by JBRC (1998, 196240) and NCI (1978, 062935), which was presented in

Section 5.1. The effects reported by JBRC (1998, 196240) and NCI (1978, 062935) were consistent with what was observed by Kociba et al. (1974, 062929) 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, 062935) or JBRC (1998, 196240) 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. (1974, 062929) 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. (1974, 062929) 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. (1974, 062929) 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. (1974, 062929) 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. (1974, 062929) 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., 1990, 094806; Young et al., 1977, 062956; Young et al., 1978, 625640; Young et al., 1978, 062955). 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. (2008, 195085).

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. (1990, 094806) 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 USEPA 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, 1990, 062932) or (Sweeney et al., 2008, 195085) (Figures B-8 and B-9). 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-10). 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 (Figures B-12 and B-13). 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-16). 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 (Table B-1). Therefore, some other, as yet unknown, modification to model structure may be necessary.

The results of USEPA's model evaluation were confirmed by other investigators (Sweeney et al., 2008, 195085). Sweeney et al. (2008, 195085) 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. (2008, 195085) 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. (1977, 062956). 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 USEPA during the evaluation of the model and should have been described as a clearance constant (zero-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. (1990, 094806) 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. (1990, 094806) 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 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

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, 2005, 086237). 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, 2005, 086237). 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, 2005, 086237), 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, 196240) 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, 196240) study as the principal study for the development of an OSF. However, two reviewers that agreed with the choice of JBRC (1998, 196240) 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, 196240) 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, 062935) study was not appropriate for use, but that

the Kociba et al. (1974, 062929) 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, 2009, 628630), the cancer portion of the study conducted by the JBRC laboratory was published in the peer-reviewed literature as Kano et al. (2009, 594539). This manuscript was reviewed by EPA. EPA determined that the data published by Kano et al. (2009, 594539) should be included in the assessment of 1,4-dioxane for several reasons: (1) while the JBRC (1998, 196240) 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, 594539) 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, 594539), 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, 594539) manuscript. Additional discussoin 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, 594539) 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, 196240) 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, 2005, 086237) 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 US EPA's 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 USEPA's Guidelines for Carcinogen Risk Assessment (2005, 086237) 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 USEPA's Guidelines for Carcinogen Risk Assessment (2005, 086237) 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

Comments on the *Toxicological Review of 1,4-Dioxane* submitted by the public 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, 594539)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, 626613). 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, 193804) 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, 594539) 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, 2005, 086237).

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, 2000, 052150), 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, 196240) 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, 196240) was published as conference proceedings as Yamazaki et al. (1994, 196120) and recently in the peer-reviewed literature as Kano et al. (2009, 594539). Additional study information was also gathered from the authors (Yamazaki, 2006, 626614) and is available upon request from the IRIS Hotline. The peer-reviewed and published data from Kano et al. (2009, 594539) 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. (2008, 195085) model. The PBPK model should be used for both noncancer and cancer dose extrapolation.

Response: The Agency evaluated the Sweeney et al. (2008, 195085) 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. (2008, 195085). 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. (1990, 094806) model and the updated input parameters provided in Sweeney et al. (2008, 195085) and add a compartment for the kidney

Response: See response to previous comment regarding evaluation of Sweeney et al. (2008, 195085). 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. (2008, 195044; 2009, 193803) studies for inhalation and MOA relevance.

Response: The 13 week and 2-year inhalation studies by Kasai et al. (2008, 195044; 2009, 193803) 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.

APPENDIX B. EVALUATION OF EXISTING PBPK 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., 1978, 062955; Young et al., 1978, 625640) and humans (Young et al., 1977, 062956) 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 (Fisher et al., 1997, 194390; Leung and Paustenbach, 1990, 062932; Reitz et al., 1990, 094806).

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. The model of Reitz et al. (1990, 094806) 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. (1990, 094806) human model were identified. The model was able to predict the only available human inhalation data set (50 ppm 1,4 dioxane for 6 hours; Young et al., 1977, 062956) 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. (1977, 062956) 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.

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

This appendix describes activities conducted in the evaluation of the empirical models (Young et al., 1977, 062956; Young et al., 1978, 062955; Young et al., 1978, 625640), and re-calibration and exercising of the Reitz et al. (1990, 094806) PBPK model, and evaluation of the Sweeney et al. (2008, 195085) model to determine the potential utility of the PBPK models for 1,4-dioxane for interspecies and route-to-route extrapolation.

B.2. SCOPE

The scope of this effort consisted of implementation of the Young et al. (1977, 062956; 1978, 062955; 1978, 625640) empirical rat and human models using the acslXtreme simulation software, re-calibration of the Reitz et al. (1990, 094806) human PBPK model, and evaluation of model parameters published by Sweeney et al. (2008, 195085). Using the model descriptions and equations given in Young et al. (1977, 062956; 1978, 062955; 1978, 625640), 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. (1977, 062956; 1978, 062955; 1978, 625640).

The PBPK model of Reitz et al. (1990, 094806) 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. (2008, 195085) were also tested to evaluate the ability of the PBPK model to predict human data following exposure to 1,4-dioxane.

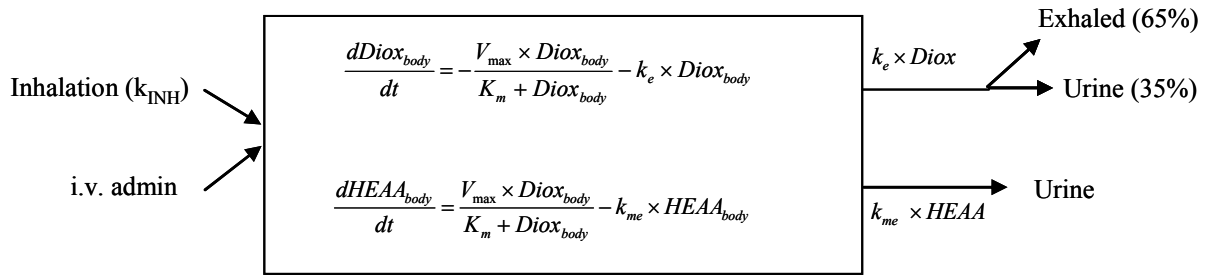
B.3. IMPLEMENTATION OF THE EMPIRICAL MODELS IN acslXtreme

The empirical models of Young et al. (1977, 062956; 1978, 062955; 1978, 625640) for 1,4-dioxane in rats and humans were reproduced using acslXtreme, version 2.3 (Aegis Technologies, Huntsville, AL). Model code files were developed using the equations described in the published papers. Additional files containing experiment-specific information (i.e., BWs, exposure levels, and duration) were also generated.

B.3.1. Model Descriptions

The empirical model of Young et al. (1978, 062955; 1978, 625640) 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. No information is reported

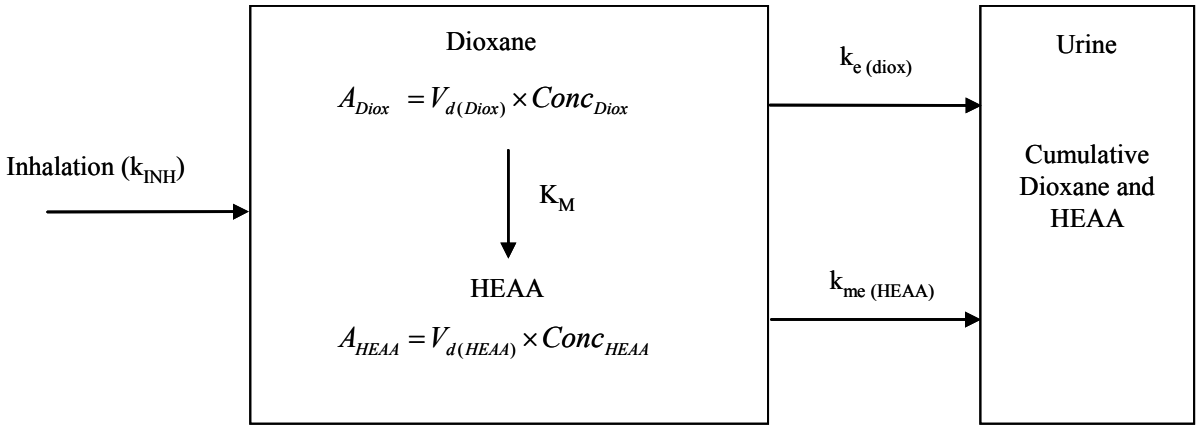
describing pulmonary absorption or intravenous (i.v.) injection/infusion of 1,4-dioxane. The metabolism of 1,4-dioxane and subsequent appearance of HEAA is described by Michaelis-Menten kinetics governed by a maximum rate (V_{max}, µg/mL-hour) and affinity constant (K_m, µg/mL) . Both 1,4-dioxane and HEAA are eliminated via the 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 is determined by dividing the instantaneous amount of 1,4-dioxane in blood by a V_d of 301 mL/kg BW.



Source: Used with permission from Taylor & Francis, Young et al. (1978, 062955; 1978, 625640).

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

Figure B-2 illustrates the empirical model for 1,4-dioxane in humans as described in Young et al. (1977, 062956). 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 by a fixed rate of 76.1 mg/hour (k_{INH}). Blood concentrations of 1,4-dioxane and HEAA are calculated as instantaneous amount (mg) divided by $V_{d(diox)}$ or $V_{d(HEAA)}$, respectively (104 and 480 mL/kg BW, respectively).



Source: Used with permission from Taylor & Francis, Young et al. (1977, 062956).

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

B.3.2. 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. (1977, 062956; 1978, 062955; 1978, 625640) 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. (1978, 062955; 1978, 625640) 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., 1978, 062955; 1978, 625640). The value for k_{INH} was estimated by optimization against the blood time course data of Young et al. (1978, 062955; 1978, 625640). 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. (1978, 062955; 1978, 625640) 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. (1978, 062955; 1978, 625640), a standard rat urine production rate of 0.00145 L/hour was used.

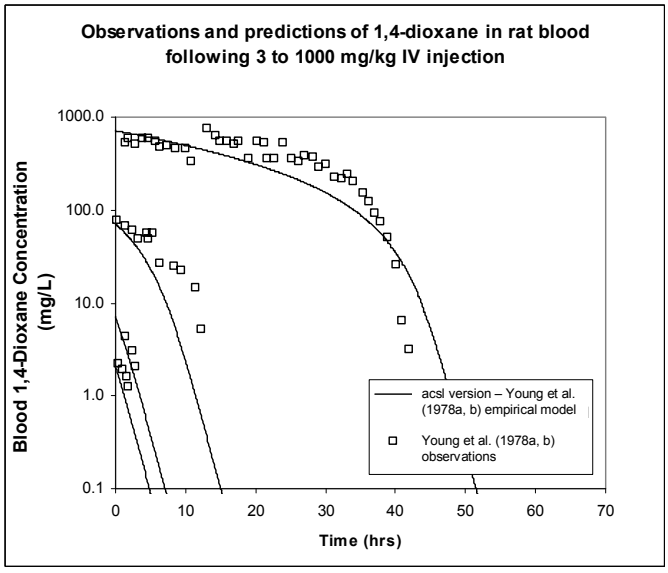
For humans, Young et al. (1977, 062956) used a fixed 1,4-dioxane inhalation uptake rate of 76.1 mg/hour, which corresponded to observations during a 50 ppm exposure. In order to facilitate user-specified inhalation concentrations, pulmonary absorption was modeled. The modeling was performed identically to the rat model, but using a human minute volume of 7 L/minute. Urinary HEAA data were reported by Young et al. (1977, 062956) 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. (1977, 062956; 1978, 062955; 1978, 625640) 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.

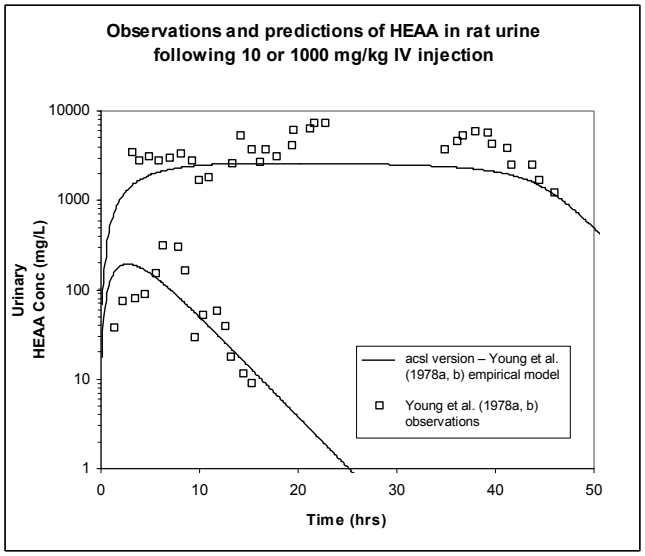
B.3.3. Results

The acslXtreme implementation of the Young et al. (1978, 062955; 1978, 625640) rat empirical model simulates the 1,4-dioxane blood levels from the i.v. experiments identically to the model output reported in the published paper (Figure B-3). However, the acslXtreme version predicts urinary HEAA concentrations in rats that are approximately threefold lower and reach a maximum sooner than the predicted levels reported in the paper (Figure B-4). These discrepancies may be due, at least in part, to the reliance in the acslXtreme 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: Used with permission from Taylor & Francis, Young et al. (1978, 062955; 1978, 625640).

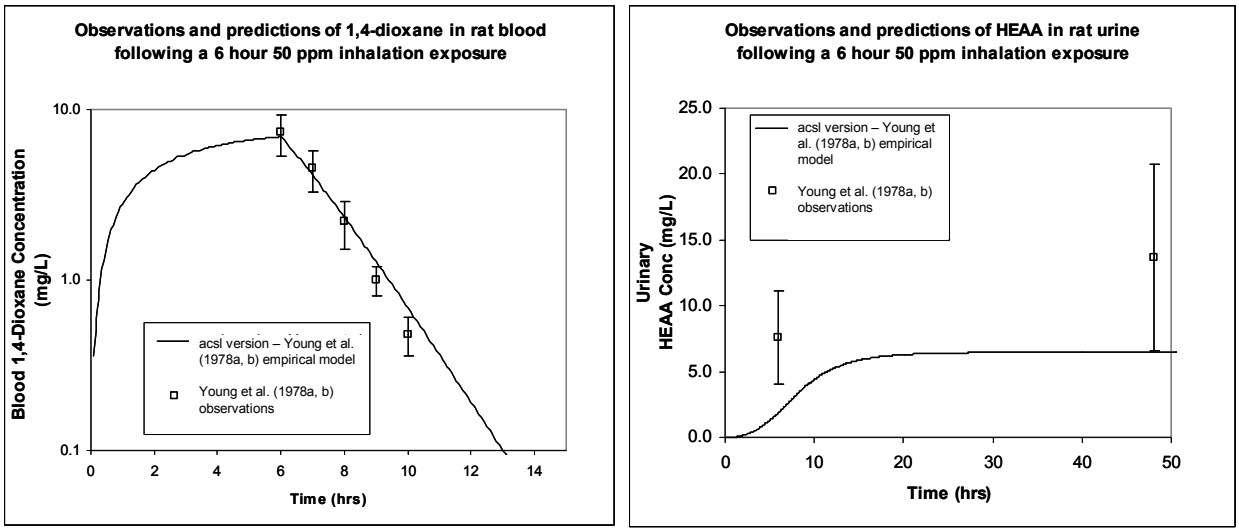
Figure B-3. Output of 1,4-dioxane blood level data from the acslXtreme implementation (left) and published (right) empirical rat model simulations of i.v. administration experiments.



Source: Used with permission from Taylor & Francis, Young et al. (1978, 062955; 1978, 625640).

Figure B-4. Output of HEAA urine level data from acslXtreme implementation (left) and published (right) empirical rat model simulations of i.v. administration experiments.

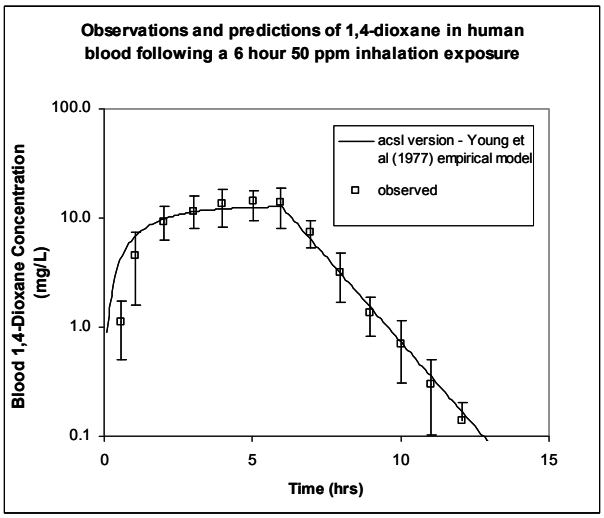
The Young et al. (1978, 062955; 1978, 625640) report did not provide model predictions for the 50-ppm inhalation experiment. However, the acslXtreme implementation produces blood 1,4-dioxane predictions that are quite similar to the reported observations (Figure B-5). As with the urine data from the i.v. experiment, the acslXtreme-predicted urinary HEAA concentrations are approximately threefold lower than the observations, presumably for the same reasons discussed above for the i.v. predictions.



Source: Used with permission from Taylor & Francis, Young et al. (1978, 062955; 1978, 625640).

Figure B-5. acslXtreme predictions of blood 1,4-dioxane and urine HEAA levels from the empirical rat model simulations of a 6-hour, 50-ppm inhalation exposure.

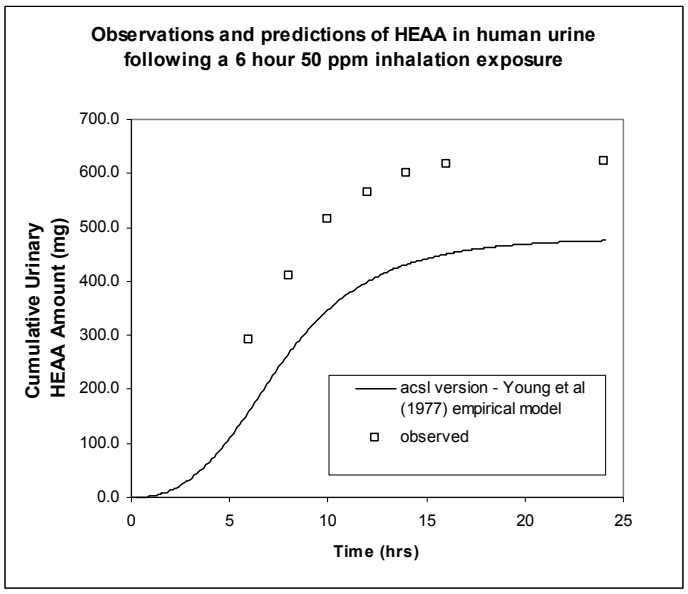
Inhalation data for a single exposure level (50 ppm) are available for humans. The acslXtreme predictions of the blood 1,4-dioxane observations are identical to the predictions reported in Young et al. (1977, 062956) (Figure B-6). Limited blood HEAA data were reported, and the specimen analysis was highly problematic (e.g., an analytical interference was sometimes present from which HEAA could not be separated). For this reason, Young et al. (1977, 062956) did not compare predictions of the blood HEAA data to observations in their manuscript.



Source: Used with permission from Taylor & Francis, Young et al. (1978, 062955; 1978, 625640).

Figure B-6. Output of 1,4-dioxane blood level data from the acslXtreme implementation (left) and published (right) empirical human model simulations of a 6-hour, 50-ppm inhalation exposure.

Data for cumulative urinary HEAA amounts are provided in Young et al. (1977, 062956), and no analytical problems for these data were reported. Nevertheless, model predictions for urinary HEAA were not presented in the manuscript. The acslXtreme prediction of the HEAA kinetics profile is similar to the observations, although predicted values are approximately 1.5- to 2-fold lower than the observed values (Figure B-7). 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 for humans cannot be attributed to uncertainties in urine volumes in the subjects.



Source: Used with permission from Taylor & Francis, Young et al. (1977, 062956).

Figure B-7. Observations and acslXtreme predictions of cumulative HEAA in human urine following a 6-hour, 50-ppm inhalation exposure.

B.3.4. Conclusions for Empirical Model Implementation

The empirical models described by Young et al. (1977, 062956; 1978, 062955; 1978, 625640) for rats and humans were implemented using acslXtreme. The models were modified to allow for user-defined inhalation levels by addition of a first-order rate constant for pulmonary uptake of 1,4-dioxane, fitted to the inhalation data. No modifications were made for oral absorption as adequate data are not available for parameter estimation. The acslXtreme predictions of 1,4-dioxane in the blood are identical to the published predictions for simulations of 6-hour, 50-ppm inhalation exposures in rats and humans and 3 to 1,000 mg/kg i.v. doses in rats (Figures B-3, B-5, and B-6). However, the acslXtreme version predicts lower urinary HEAA concentrations in rats appearing earlier than either the Young et al. (1978, 062955; 1978, 625640) model predictions or the experimental observations. The lower predicted urinary HEAA levels in the acslXtreme implementation for rats is likely due to use of default values for urine volume in the absence of measured volumes. The reason for differences in time-to-peak levels is unknown, but may be the result of an unreported adjustment by Young et al. (1978, 062955; 1978, 625640) in model parameter values. For humans, Young et al. (1977, 062956) did not report model predictions of urinary HEAA levels. The urinary HEAA levels predicted by acslXtreme were low relative to the observations. However, unlike the situation in rats, these data are not dependent on unreported urine volumes (observations were reported as cumulative HEAA amount rather than HEAA concentration), but reflect the model parameter values

reported by Young et al. (1977, 062956). Presently, there is no explanation for the lack of fit of the reported urinary HEAA elimination rate constant to the observations.

B.4. INITIAL RE-CALIBRATION OF THE PBPK MODEL

Concern regarding adjustments made to some of the parameter values in Reitz et al. (1990, 094806) prompted a re-calibration of the Reitz et al. (1990, 094806) human PBPK model using more biologically plausible values for all measured parameter values. Reitz et al. (1990, 094806) 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 cross-species dose 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.4.1. Sources of Values for Flow Rates

The cardiac output of 30 L/hour/kg$^{0.74}$ (Table B-1) reported by Reitz et al. (Reitz et al., 1990, 094806) 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, 020304). 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, 020304). Brown et al. (1997, 020304) also cite the ICRP (1975, 196239) as having a mean respiratory minute volume of 7.5 L/minute, which results in an alveolar ventilation rate of 5 L/minute (assuming 33% lung dead space), or 13 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. (1990, 094806). Young et al. (1977, 062956) 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.

Table B-1. Human PBPK model parameter values for 1,4-dioxane

Parameter	Reitz et al. (1990, 094806)	Leung and Paustenbach (1990, 062932)	Sweeney et al. (2008, 195085)	EPA[c]
Physiological Flows				
Cardiac output (QCC)[a]	30	--	--	17.0
Alveolar ventilation (QPC)[a]	30	--	--	17.7
Partition Coefficients (PCs)				
Blood:air (PB)	3,650	1,825 ± 94	1,666 ± 287	1,850
Fat:air (PFA)	851	851 ± 118	--	851
Liver:air (PLA)	1,557	1,557 ± 114	1,862 ± 739[b]	1,557
Rapidly perfused tissue:air (PRA)	1,557	--	--	1,557
Slowly perfused tissue:air (PSA)	1,557	997 ± 254	1,348 ± 290[b]	166
Metabolic Constants				
Maximum rate for 1,4-dioxane metabolism (V_{maxC})[d]	6.35	--	--	5.49
Metabolic affinity constant (K_m)[e]	3.00	--	--	9.8
HEAA urinary elimination rate constant (k_{me})[f]	0.56	--	--	0.44

[a]L/hour/kg $BW^{0.74}$
[b]Measurement for rat tissue
[c]Biologically plausible values utilized by EPA in this assessment
[d]mg/hour/kg $BW^{0.75}$
[e]mg/L
[f]$hour^{-1}$

Examination of the experimental data of Young et al. (1977, 062956) yields an estimated alveolar ventilation to be 7 L/minute (or 16 L/hour/$kg^{0.74}$) for volunteers having a mean BW of 84 kg. This rate is based on the Young et al. (1977, 062956) estimate of 76.1 mg/hour for 1,4-dioxane uptake. 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. (1977, 062956). Therefore, these flow values were chosen for the model re-calibration.

B.4.2. Sources of Values for Partition Coefficients

Two data sources are available for the tissue:air equilibrium partition coefficients for 1,4-dioxane: Leung and Paustenbach (1990, 062932) and Sweeney et al. (2008, 195085). Both investigators report mean values and standard deviations for human blood:air, rat liver:air, and rat muscle:air (e.g., slowly perfused tissue:air), while Leung and Paustenbach et al. (1990, 062932) also reported values for rat fat:air (Table B-1).

B.4.3. Calibration Method

The PBPK model was twice re-calibrated using the physiological flow values suggested values (current EPA assessment, see Table B-1) and the partition coefficients of Leung and Paustenbach (1990, 062932) and Sweeney et al. (2008, 195085) separately. For each calibration, the metabolic parameters V_{maxC} and K_m, were simultaneously fit (using the parameter estimation tool provided in the acslXtreme software) to the output of 1,4-dioxane blood concentrations generated by the acslXtreme implementation of the Young et al. (1977, 062956) 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, rather than experimental observations, were used to provide a more robust data set for model fitting, as 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. (1977, 062956).

B.4.4. Results

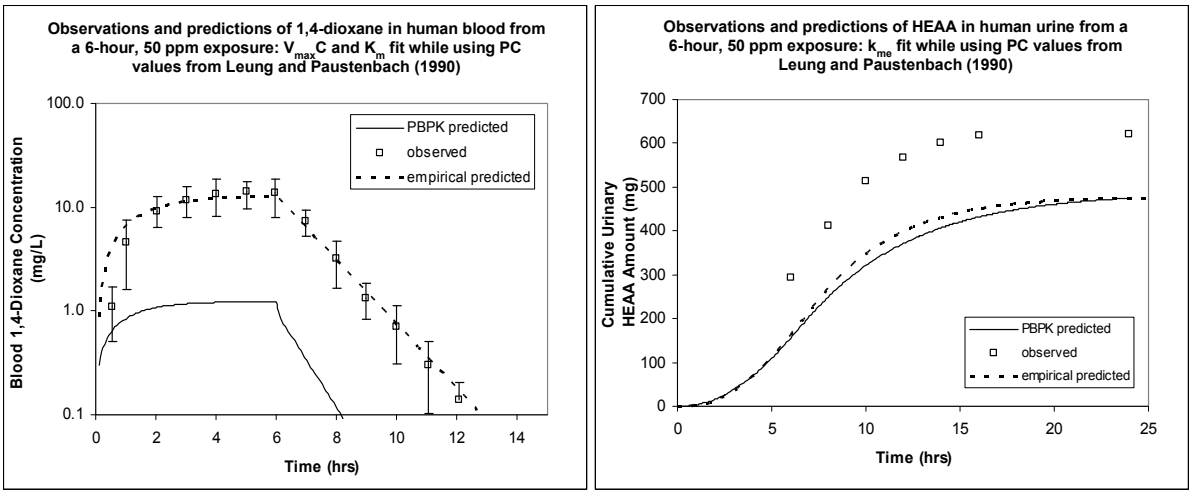
Results of the model re-calibration are provided in Table B-2. The re-calibrated values for V_{maxC} and k_{me} associated with the Leung and Paustenbach (1990, 062932) or Sweeney et al. (2008, 195085) tissue:air partition coefficients are very similar. However, the fitted value for K_m using the Sweeney et al. (2008, 195085) partition coefficients is far lower (0.0001 mg/L) than that resulting from use of the Leung and Paustenbach (1990, 062932) partition coefficients (2.5 mg/L). This appears to be due to the higher slowly perfused tissue:air partition coefficient determined by Sweeney et al. (2008, 195085) (1,348 vs. 997), resulting in a higher apparent V_d than if the Leung and Paustenbach (1990, 062932) value is used. Thus, the optimization algorithm selects a low K_m, artificially saturating metabolism in an effort to drive predicted blood 1,4-dioxane levels closer to the empirical model output. Saturation of metabolism during a 50 ppm inhalation exposure is inconsistent with the observed kinetics.

Table B-2. PBPK metabolic and elimination parameter values resulting from re-calibration of the human model using alternative values for physiological flow rates[a] and tissue:air partition coefficients

Source of Partition Coefficients	Leung and Paustenbach (1990, 062932)	Sweeney et al. (2008, 195085)
Maximum rate for 1,4-dioxane metabolism (V_{maxC})[b]	16.9	20.36
Metabolic affinity constant (K_m)[c]	2.5	0.0001
HEAA urinary elimination rate constant (k_{me})[d]	0.18	0.17

[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 $BW^{0.75}$
[c]mg/L
[d]$hour^{-1}$

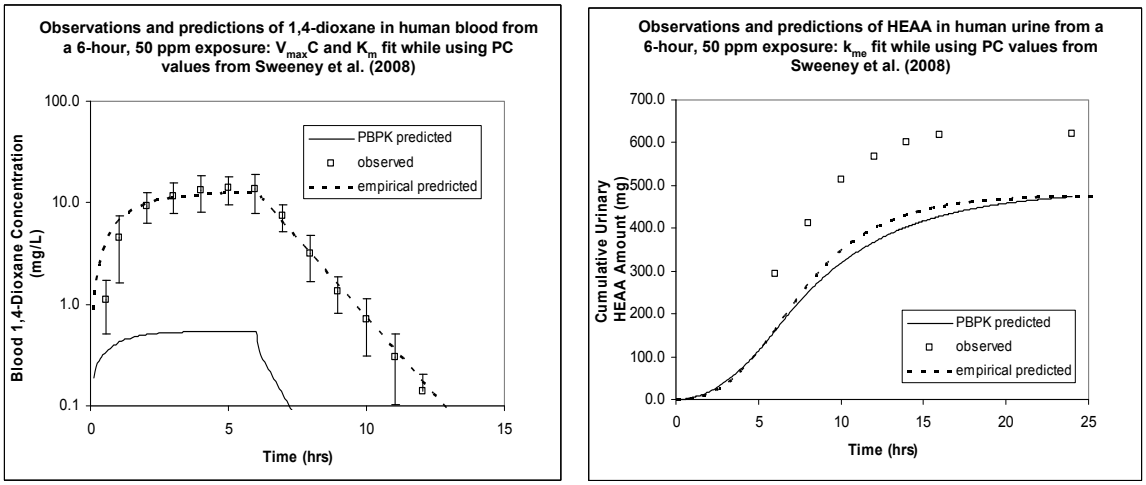
Plots of predicted and experimentally observed blood 1,4-dioxane and urinary HEAA levels are shown in Figures 4-1 and 4-2. Neither re-calibration resulted in an adequate fit to the blood 1,4-dioxane data from the empirical model output or the experimental observations. Re-calibration using either the Leung and Paustenbach (1990, 062932) or Sweeney et al. (2008, 195085) partition coefficients resulted in blood 1,4-dioxane predictions that were at least 10-fold lower than empirical model predictions or observations.



Source: Used with permission from Elsevier, Ltd., Leung and Paustenbach (1990, 062932).

Figure B-8. Predicted and observed blood 1,4-dioxane concentrations (left) and urinary HEAA levels (right) following re-calibration of the human PBPK model with tissue:air partition coefficient values.

The refitted values for k_{me} resulted in HEAA levels in urine that were very similar to the empirical model output (compare Figures B-7, B-8, and B-9), which was not surprising, given the fitting of a single parameter to the data.



Source: Used with permission of Oxford Journals, Sweeney et al. (2008, 195085).

Figure B-9. Predicted and observed blood 1,4-dioxane concentrations (left) and urinary HEAA levels (right) following re-calibration of the human PBPK model with tissue:air partition coefficient values.

Outputs of the blood 1,4-dioxane and urinary HEAA levels using the suggested (Table B-1) parameters are shown in Figure B-10. These outputs rely on a very low value for the slowly perfused tissue:air partition coefficient (166) that is six- to eightfold lower than the measured values reported in Leung and Paustenbach (1990, 062932) and Sweeney et al. (2008, 195085), and 10-fold lower than the value used by Reitz et al. (1990, 094806). While the predicted maximum blood 1,4-dioxane levels are much closer to the observations, the elimination kinetics are markedly different, producing higher predicted elimination rates compared to observations during the post-exposure phase of the experiment.

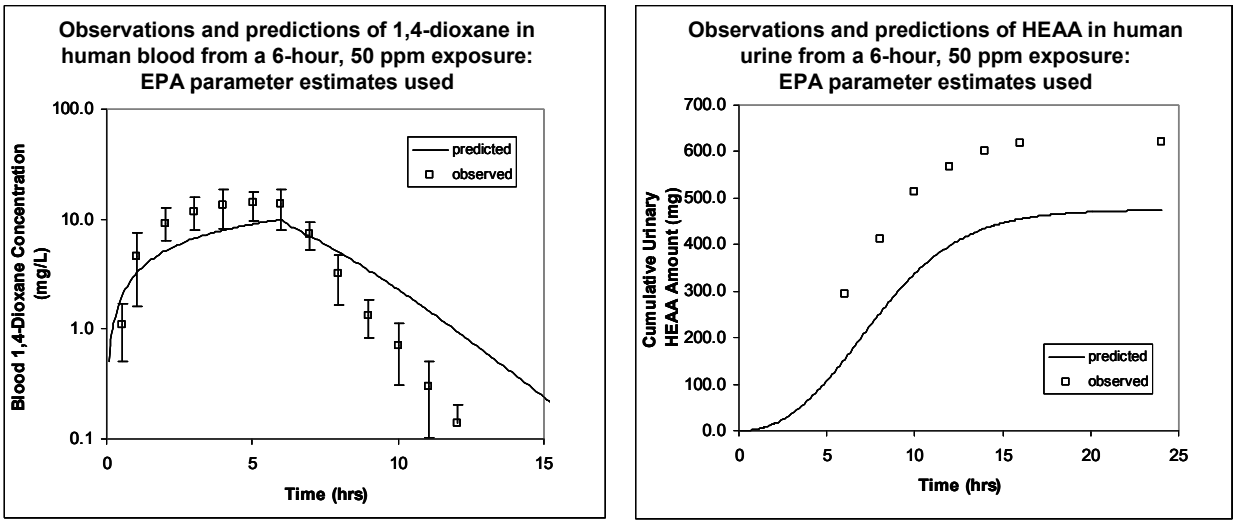


Figure B-10. Predicted and observed blood 1,4-dioxane concentrations (left) and urinary HEAA levels (right) using EPA estimated biologically plausible parameters (Table B-1).

B.4.5. Conclusions for PBPK Model Implementation

Re-calibration of the human PBPK model was performed using experiment-specific values for cardiac output and alveolar ventilation (values derived from Young et al., 1977, 062956) and measured mean tissue:air 1,4-dioxane partition coefficients reported by Leung and Paustenbach (1990, 062932) or Sweeney et al. (2008, 195085). The resulting predictions of 1,4-dioxane in blood following a 6-hour, 50-ppm inhalation exposure were 10-fold (or more) 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, but lower than observed values (Figures B-8 and B-9). Output from the model using biologically plausible parameter values (Table B-1), Figure B-10 shows that application of a value for the slowly perfused tissue:air partition coefficient, which is 10-fold lower than the measured value reported by Leung and Paustenbach (1990, 062932), results in closer agreement of the predictions to observations during the exposure phase, but not during the elimination phase. 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.

B.4.6. SENSITIVITY ANALYSIS

A sensitivity analysis of the Reitz et al. (1990, 094806) model was performed 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.4.7. 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 x is the model parameter, f(x) is the output variable, Δx is the perturbation of the parameter from the nominal value, and f'(x) is the sensitivity coefficient. The sensitivity coefficients were scaled to the nominal value of x and f(x) 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.4.8. Results

The sensitivity coefficients for the seven most influential model parameters at 1 and 4 hours of exposure are shown in Figure B-11. The three parameters with the highest sensitivity coefficients in descending order are alveolar ventilation (QPC) (1.0), the blood:air partition coefficient (PB) (0.65), and the slowly perfused tissue:air partition coefficient (PSA) (0.51). Not surprisingly, these were the parameters that were doubled or given surrogate values in the Reitz et al. (1990, 094806) 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.

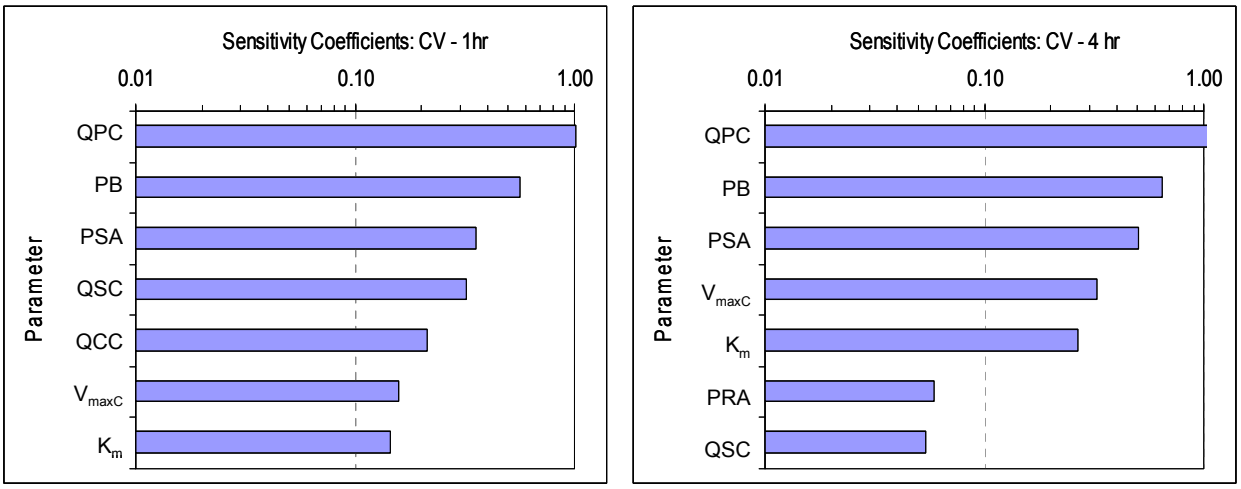


Figure B-11. The highest seven sensitivity coefficients (and associated parameters) for blood 1,4-dioxane concentrations (CV) at 1 (left) and 4 (right) hours of a 50-ppm inhalation exposure.

B.5. PBPK MODEL EXERCISES USING BIOLOGICALLY PLAUSIBLE PARAMETER BOUNDARIES

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.5.1. Observations Regarding the Volume of Distribution

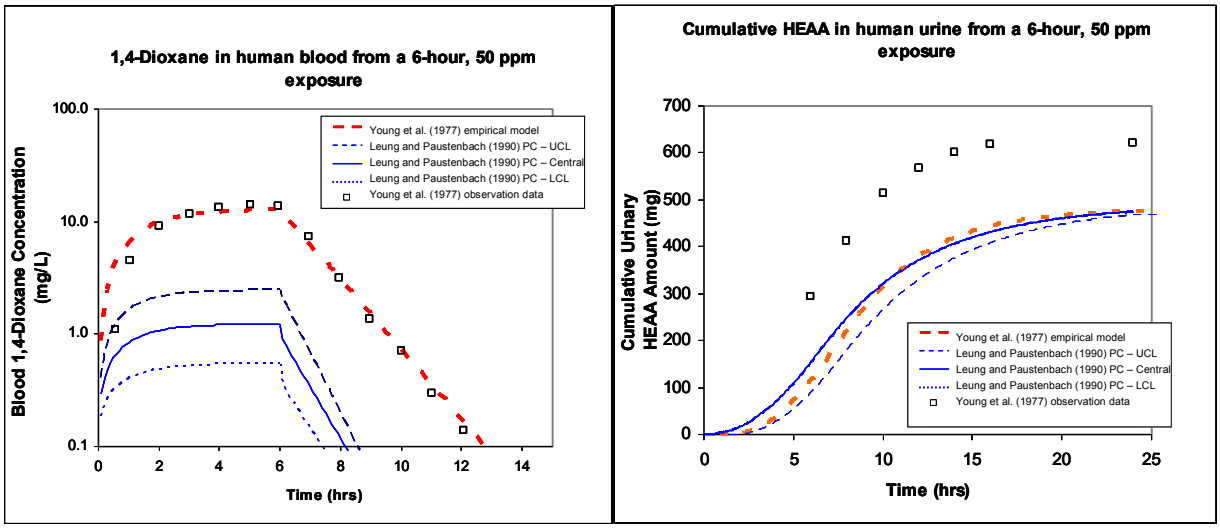
Young et al. (1978, 062955; 1978, 625640) used experimental observations to estimate a V_d for 1,4-dioxane in rats of 301 mL, or 1,204 mL/kg BW. For humans, the V_d was estimated to be 104 mL/kg BW (Young et al., 1977, 062956). It is possible that a very large volume of the slowly perfused tissues in 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.5.2. 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 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.4.3. This procedure was performed for both the Leung and Paustenbach (1990, 062932) and Sweeney et al. (2008, 195085) 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.

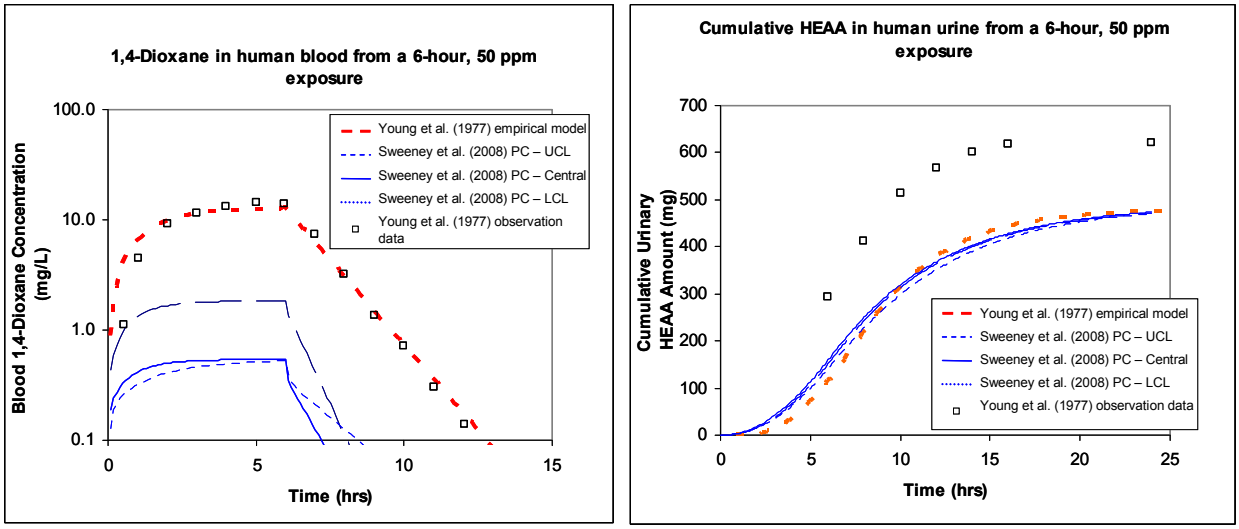
B.5.3. Results

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-12 for the Leung and Paustenbach (1990, 062932) values and Figure B-13 for the Sweeney et al. (2008, 195085) 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 (1990, 062932) or Sweeney et al. (2008, 195085), the model predictions are still at least six- to sevenfold lower than either the empirical model output or the experimental observations. The range of possible urinary HEAA predictions brackets 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. (1977, 062956) human empirical model or the experimental observations when constrained by biologically plausible values for physiological flow rates and tissue:air partition coefficients.



Source: Used with permission of Elsevier, Ltd., Leung and Paustenbach (1990, 062932)

Figure B-12. Comparisons of the range of PBPK model predictions from upper and lower boundaries on partition coefficients with empirical model predictions and experimental observations for blood 1,4-dioxane concentrations (left) and urinary HEAA levels (right) from a 6-hour, 50-ppm inhalation exposure.



Source: Used with permission of Oxford Journals, Sweeney et al. (2008, 195085); Used with permission of Taylor & Francis, Young et al. (1977, 062956).

Figure B-13. Comparisons of the range of PBPK model predictions from upper and lower boundaries on partition coefficients with empirical model predictions and experimental observations for blood 1,4-dioxane concentrations (left) and urinary HEAA levels (right) from a 6-hour, 50-ppm inhalation exposure.

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 Pausenbach (1990, 062932)		Sweeney et al. (2008, 195085)	
	For maximal V_d	For minimal V_d	For maximal V_d	For minimal V_d
Maximum rate for 1,4-dioxane metabolism (V_{maxC})[b]	14.95	18.24	17.37	21.75
Metabolic dissociation constant (K_m)[c]	5.97	0.0001	4.88	0.0001
HEAA urinary elimination rate constant (k_{me})[d]	0.18	0.17	0.26	0.19

[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 $BW^{0.75}$
[c]mg/L
[d]$hour^{-1}$

B.5.4. Alternative Model Parameterization

Since the PBPK model does not predict the experimental observations of Young et al. (1977, 062956) 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., 1977, 062956), the parameters V_{maxC} and K_m were replaced by a zero-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. (2008, 195085) to minimize the V_d (i.e., maximize the blood 1,4-dioxane levels). The resulting model predictions are shown in Figure B-14. As before, the maximum blood 1,4-dioxane levels were approximately sevenfold lower than the observed values.

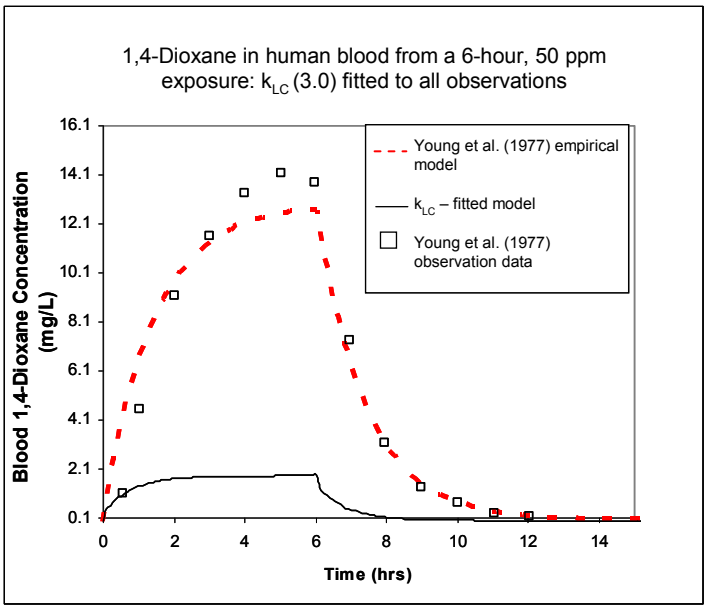


Figure B-14. Predictions of blood 1,4-dioxane concentration following calibration of a zero-order metabolism rate constant, k_{LC}, to the experimental data.

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-15. 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 (compare Figures B-10 and B-15).

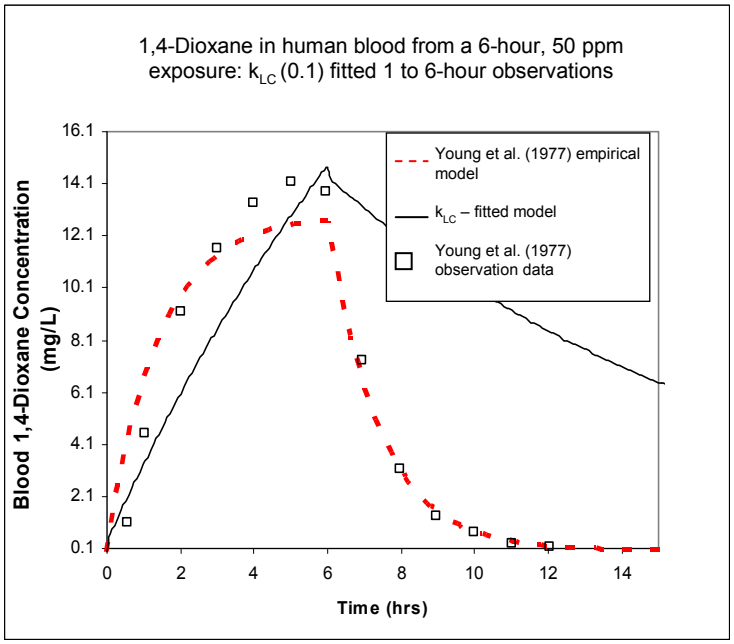


Figure B-15. Predictions of blood 1,4-dioxane concentration following calibration of a zero-order metabolism rate constant, k_{LC}, to only the exposure phase of the experimental data.

Finally, the model was re-calibrated by simultaneously fitting k_{LC} and the slowly perfused tissue:air partition 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 an extremely low (and biologically unlikely) value of 0.0001. The resulting model predictions, however, were closer to the observations than even the empirical model predictions (Figure B-16). 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. 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.

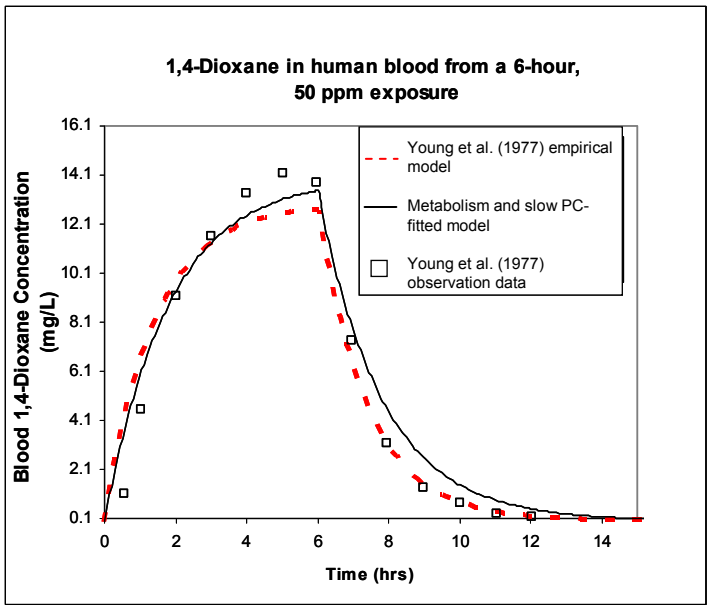


Figure B-16. Predictions of blood 1,4-dioxane concentration following simultaneous calibration of a zero-order metabolism rate constant, k_{LC}, and slowly perfused tissue:air partition coefficient to the experimental data.

B.6. CONCLUSIONS

The rat and human empirical models of Young et al. (1977, 062956; 1978, 062955; 1978, 625640) were successfully implemented in acslXtreme and perform identically to the models reported in the published papers (Figures 3-3 through 3-6), 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. (1978, 062955; 1978, 625640) 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 lower than observations, this is due to parameter fitting of Young et al. (1977, 062956). No model output was published in Young et al. (1977, 062956) for comparison. The empirical models were modified to allow for user-defined inhalation exposure levels. However, no modifications were made to model oral exposures because adequate data to parameterize such modifications do not exist for rats or humans.

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 do not come within 10-fold of the experimental values using measured tissue:air partition coefficients from Leung and Paustenbach (1990, 062932) or Sweeney et al. (2008, 195085) (Figures B-8 and B-9). Use 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-10). 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 (Figures B-12 and B-13). 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-16). Artificially low values for the other tissue:air partition coefficients are not expected to improve the model fit, because the sensitivity analysis to exert less influence on blood 1,4-dioxane than V_{maxC} and K_m. This suggests that the model structure is insufficient to capture the apparent 10-fold species difference in the blood 1,4-dioxane V_d between rats and humans. 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 (Table B-1). Therefore, some other, as yet unknown, modification to model structure may be necessary.

B.7. RECOMMENDATIONS FOR UTILIZING EXISTING PBPK MODELS

The use of empirical or PBPK models to reduce uncertainty in extrapolation of dose-responses (in terms of internal dosimetry) requires accurate representation of exposure and biological reality. In the case of the empirical models of Young et al. (1977, 062956; 1978, 062955; 1978, 625640), the acslXtreme implementations are adequate for predicting blood 1,4-dioxane levels for a variety of inhalation exposure levels in rats and up to 50 ppm in humans. However, the absence of data with which to evaluate simulated oral absorption in either species precludes the inclusion of this route of exposure in the models. Therefore, the empirical models may be useful for assessment of toxicity by inhalation exposure, but not by oral exposure, and not for route-to-route extrapolation. For the PBPK model, an apparent gap in the model structure exists such that experimental observations of blood 1,4-dioxane levels in humans during and following inhalation exposures to 1,4-dioxane cannot be reproduced under the constraints of biologically plausible parameter values for all parameters. Therefore, the use of the PBPK model (in its present form) is not recommended for application to the derivation of toxicity values for 1,4-dioxane.

B.8. acslXtreme CODE FOR THE YOUNG ET AL. EMPIRCAL MODEL FOR 1,4-DIOXANE IN RATS

```
PROGRAM: Young (1978, 062955) rat.csl
!-------------------------------------------------------------
! Created by Michael Lumpkin, Syracuse Research Corporation, 08/06
! This program implements the 1-compartment empirical model for 1,4-dioxane
! in rats, developed by Young et al. 1978a, b. Program was modified to run
! in ACSL Xtreme and to include user-defined i.v. and inhalation concentrations
!(MLumpkin, 08/06)
!-------------------------------------------------------------

INITIAL

!*****Timing and Integration Commands*****
ALGORITHM IALG=2        !Gear integration algorithm for stiff systems
!MERROR %%%%=0.01       !Relative error for lead in plasma
NSTEPS NSTP=1000 !Number of integration steps per communication interval
CINTERVAL CINT=0.1      !Communication interval
CONSTANT TSTART=0.      !Start of simulation (hr)
CONSTANT TSTOP=70.      !End of simulation (hr)

!*****MODEL PARAMETERS*****
CONSTANT BW=0.215       !Body weight (kg)
CONSTANT MINVOL=0.238 !respiratory minute volume (L/min) estimated from Young et al.
(1978)
CONSTANT IVDOSE = 0.   !IV dose (mg/kg)!
CONSTANT CONC = 0.   !inhalation concentration (ppm)

CONSTANT MOLWT=88.105 !mol weight of 1,4-dioxane
CONSTANT TCHNG=6.0      !Exposure pulse 1 width (hr)
CONSTANT TDUR=24.0    !Exposure duration (hr)
CONSTANT TCHNG2=120.0 !Exposure pulse 2 width (hr)
CONSTANT TDUR2=168.0  !Exposure duration 2 (hr)

CONSTANT Vmax=4.008   !(mcg/mL/hr)
CONSTANT Km=6.308     !(mcg/mL)
CONSTANT Kinh=0.43         !pulmonary absorption constant (/hr)
CONSTANT Ke=0.0149    !(/hr)
CONSTANT Kme=0.2593   !(/hr)
CONSTANT Vd=0.3014     !(L)

IV = IVDOSE*BW
AmDIOXi=IV

END                     !Of Initial Section

DYNAMIC
```

```
DERIVATIVE

!*** Dioxane inhalation concentration ***
CIZONE=PULSE(0.0, TDUR, TCHNG) * PULSE(0.0, TDUR2, TCHNG2)
        !First pulse is hours/day, second pulse is hours/week
CI=CONC*CIZONE*MOLWT/24450.        !Convert to mg/L

!*** Dioxane metabolism/1st order elimination ***
dAmDIOX=(Kinh*CI*(MINVOL*60))-((Vmax*(AmDIOX))/(Km+(AmDIOX)))-
(Ke*(AmDIOX))
AmDIOX=INTEG(dAmDIOX,AmDIOXi)
ConcDIOX=AmDIOX/Vd     !plasma dioxane concentration (mcg/mL)
AUCDIOX=INTEG(ConcDIOX,0) !plasma dioxane AUC

!*** HEAA production and 1st order metabolism ***
dAmHEAA=((Vmax*(AmDIOX))/(Km+(AmDIOX)))-(Kme*(AmHEAA))
AmHEAA=INTEG(dAmHEAA,0.)
ConcHEAA=AmHEAA/Vd !plasma HEAA concentration

!*** 1st order dioxane elimination to urine ***
dAmDIOXu=(Ke*(AmDIOX))*0.35
AmDIOXu=INTEG(dAmDIOXu,0.)
ConcDIOXu=Ke*AmDIOX*0.35/1.45e-3 !urine production approx 1.45e-3 L/hr in SD rats

!*** 1st order dioxane exhaled ***
dAmDIOXex=(Ke*(AmDIOX))*0.65
AmDIOXex=INTEG(dAmDIOXex,0.)

!*** 1st order HEAA elimination to urine ***
dAmHEAAu=(Kme*(AmHEAA))
AmHEAAu=INTEG(dAmHEAAu,0.)
ConcHEAAu=Kme*AmHEAA/1.45e-3 !urine production approx 1.45e-3 L/hr in SD rats

END !of Derivative Section

DISCRETE

END    !of Discrete Section

TERMT (T .GT. TSTOP)

END !of Dynamic Section

TERMINAL

END    !of Terminal Section

END    !of Program
```

B.9. acslXtreme CODE FOR THE YOUNG ET AL. EMPIRICAL MODEL FOR 1,4-DIOXANE IN HUMANS

```
PROGRAM: Young  (1977, 062956) human.csl
!------------------------------------------------------------
! Created by Michael Lumpkin, Syracuse Research Corporation, 01/06
! This program implements the 1-compartment model for 1,4-dioxane in humans,
! developed by Young et al., 1977. Program was modified to run
! in acslXtreme (MLumpkin, 08/06)
!------------------------------------------------------------

INITIAL

!*****Timing and Integration Commands*****
ALGORITHM IALG=2        !Gear integration algorithm for stiff systems
!MERROR %%%%=0.01       !Relative error for lead in plasma
NSTEPS NSTP=1000 !Number of integration steps per communication interval
CINTERVAL CINT=0.1      !Communication interval
CONSTANT TSTART=0.      !Start of simulation (hr)
CONSTANT TSTOP=120.     !End of simulation (hr)

!*****MODEL PARAMETERS*****
!CONSTANT DATA=1        !Optimization dataset
CONSTANT MOLWT=88.105 !mol weight for 1,4-dioxane
CONSTANT DOSE=0.        !Dose (mg/kg
CONSTANT CONC=0.        !Inhalation concentration (ppm)
CONSTANT BW=84.1        !Body weight (kg)
CONSTANT MINVOL=7.0  !pulmonary minute volume (L/min)
CONSTANT F=1.0          !Fraction of dose absorbed
CONSTANT kinh=1.06      !Rate constant for inhalation (mg/hr); optimized by MHL
CONSTANT ke=0.0033      !Rate constant for dioxane elim to urine (hr-1)
CONSTANT km=0.7096      !Rate constant for metab of dioxane to HEAA (hr-1)
CONSTANT kme=0.2593     !Rate constant for transfer from rapid to blood (hr-1)
CONSTANT VdDkg=0.104   !Volume of distribution for dioxane (L/kg BW)

CONSTANT VdMkg=0.480  !Volume of distribution for HEAA (L/kg BW)
CONSTANT OStart=0.      !Time of first oral dose (hr)
CONSTANT OPeriod=120.   !Oral Dose pulse period (hr)
CONSTANT OWidth=1.      !Width (gavage/drink time) of oral dose (hr)

CONSTANT IStart=0.      !Time of inhalation onset (hr)
CONSTANT IPeriod=120.   !Inhalation pulse period (hr)
CONSTANT IWidth=6.      !Width (duration) of inhalation exposure (hr)

END                     !Of Initial Section

DYNAMIC

DERIVATIVE
```

```
!****VARIABLES and DEFINED VALUES*****
VdD=BW*VdDkg     !Volume of distribution for dioxane
VdM=BW*VdMkg     !Volume of distribution for HEAA

InhalePulse=PULSE(IStart,IPeriod,IWidth)
Inhale=CONC*InhalePulse*MOLWT/24450.          !Convert to mg/L

!*****DIFFERENTIAL EQUATIONS FOR COMPARTMENTS****

!*** Dioxane in the body (plasma) ***
dAMTbD=(Kinh*Inhale*(MINVOL*60))-(AMTbD*km)-(AMTbD*ke)
AMTbD=INTEG(dAMTbD,0.)
CbD=AMTbD/VdD
AUCbD=INTEG(CbD,0)

!*** HEAA in the body  (plasma)***
dAMTbM=AMTbD*km-AMTbM*kme
AMTbM=INTEG(dAMTbM,0.)
CbM=AMTbM/VdM

!*** Cumulative Dioxane in the urine ***
dAMTuD=(AMTbD*ke)
AMTuD=INTEG(dAMTuD,0.)

!*** Cumulative HEAA in the urine ***
dAMTuM=(AMTbM*kme)
AMTuM=INTEG(dAMTuM,0.)

END                  !Of Derivative Section

DISCRETE

END                  !of Discrete Section

TERMT (T .GT. TSTOP)

END                  !Of Dynamic Section

TERMINAL

END                  !of Terminal Section

END                  !of Program
```

B.10. acslXtreme CODE FOR THE REITZ ET AL. PBPK MODEL FOR 1,4- DIOXANE

(Reitz et al., 1990, 094806)

```
PROGRAM: DIOXANE.CSL (Used in Risk Estimation Procedures)
  !Added a venous blood compartment and 1st order elim of metab.'
  !Mass Balance Checked OK for Inhal, IV, Oral, and Water RHR'
  !Defined Dose Surrogates for Risk Assessment 01/04/89'
  !Modified the Inhal Route to use PULSE for exposure conditions'
  !Modifications by GLDiamond, Aug2004, marked as !**
  !
  !Metabolism of dioxane modified by MLumpkin, Oct2006, to include 1st order
  !or saturable kinetics. For 1st order, set VmaxC=0; for M-Menten, set KlC=0.
  !
INITIAL

  INTEGER I
   I=1
!  ARRAY TDATA(20)  !  CONSTANT TDATA=999, 19*1.0E-6 !**
  CONSTANT    BW = 0.40   !'Body weight (kg)'
  CONSTANT   QPC = 15.    !'Alveolar ventilation rate (l/hr)'
  CONSTANT   QCC = 15.    !'Cardiac output (l/hr)'

!Flows to Tissue Compartments'
  CONSTANT   QLC = 0.25   !'Fractional blood flow to liver'
  CONSTANT   QFC = 0.05   !'Fractional blood flow to fat'
  CONSTANT   QSC = 0.18   !'Fractional blood flow to slow'
         QRC = 1.0 - (QFC + QSC + QLC)
  CONSTANT   SPDC = 1.0  ! diffusion constant for slowly perfused tissues

!Volumes of Tissue/Blood Compartments'
  CONSTANT   VLC = 0.04   !'Fraction liver tissue'
  CONSTANT   VFC = 0.07   !'Fraction fat tissue'
  CONSTANT   VRC = 0.05   !'Fraction Rapidly Perf tissue'
  CONSTANT   VBC = 0.05   !'Fraction as Blood'
         VSC = 0.91 - (VLC + VFC + VRC + VBC)

!Partition Coefficients'
  CONSTANT   PLA = 1557.  !'Liver/air partition coefficient'
  CONSTANT   PFA =  851.  !'Fat/air partition coefficient'
  CONSTANT   PSA = 2065.  !'Muscle/air (Slow Perf) partition'
  CONSTANT   PRA = 1557.  !'Richly perfused tissue/air partition'
  CONSTANT    PB = 1850.  !'Blood/air partition coefficient'

!Other Compound Specific Parameters'
  CONSTANT    MW = 88.1   !'Molecular weight (g/mol)'
  CONSTANT   KLC = 12.0   ! temp zero-order metab constant
  CONSTANT VMAXC = 13.8   !'Maximum Velocity of Metabol.'
  CONSTANT    KM = 29.4   !'Michaelis Menten Constant'
  CONSTANT  ORAL = 0.0    !'Oral Bolus Dose (mg/kg)'
```

```
  CONSTANT    KA = 5.0    !'Oral uptake rate (/hr)'
  CONSTANT WATER = 0.0    !'Conc in Water (mg/liter, ppm)'
  CONSTANT WDOSE=0.0    !Water dose (mg/kg/day) **
  CONSTANT    IV = 0.0    !'IV dose (mg/kg)'
  CONSTANT  CONC = 0.0    !'Inhaled concentration (ppm)'
  CONSTANT   KME = 0.276  !'Urinary Elim constant for met (hr-1)'

!Timing commands'
  CONSTANT   TSTOP = 50   !'Length of experiment (hrs)'
  CONSTANT   TCHNG =  6   !'Length of inhalation exposure (hrs)'
  CINTERVAL CINT=0.1
  CONSTANT WIDD=24.    !**
  CONSTANT PERD=24.    !**
  CONSTANT PERW=168.  !**
  CONSTANT WIDW=168.  !**
  CONSTANT DAT=0.017   !**

!Scaled parameters calculated in this section of Program'
   QC=QCC*BW**0.74
        QP=QPC*BW**0.74
   QL=QLC*QC
        QF=QFC*QC
        QS=QSC*QC
        QR=QRC*QC
   VL=VLC*BW
        VF=VFC*BW
        VS=VSC*BW
        VR=VRC*BW
        VB=VBC*BW
   PL=PLA/PB
        PR=PRA/PB
        PS=PSA/PB
        PF=PFA/PB
        KL = KLC*bw**0.7 ! Zero-order metab constant
        VMAX = VMAXC*BW**0.7
   DOSE = ORAL*BW              !'Initial Amount in Stomach'
   AB0 = IV*BW                 !'Initial Amount in Blood'
   !DRINK = 0.102*BW**0.7*WATER/24 !'Input from water (mg/hr)' !**
   !DRINKA = 0.102*BW**0.7*WATER/DAT !'Input from water (mg/hr)'  !**
        DRINKA=WDOSE*BW/DAT
   CV = AB0/VB             !'Initialize CV'

END    !'End of INITIAL'

DYNAMIC

        ALGORITHM IALG = 2       !'Gear method for stiff systems'
        TERMT(T .GE. TSTOP )
        CR = AR/VR
```

```
          CS = AS/VS
          CF = AF/VF
          BODY = AL + AR + AS + AF + AB + TUMMY
          BURDEN = AM + BODY
          TMASS = BURDEN + AX + AMEX

!Calculate the Interval Excretion Data here:'
 !        DAX = AMEX-AMEX2
 !        IF(DOSE .LE. 0.0 .AND. IV .LE. 0.0 ) GO TO SKIP1
 !        PCTAX = 100*(AX - AX2)/(DOSE + IV*BW)
 !        PCTMX = 100*(AMEX - AMEX2)/(DOSE + IV*BW)
!         SKIP1.. CONTINUE
 !        IF(T .LT. TDATA(I) .OR. I .GE. 20 ) GO TO SKIP
  !       AX2=AX
!         AMEX2=AMEX
!         I=I+1
!         SKIP.. CONTINUE

!DISCRETE EXPOSE
!   CIZONE = 1.0  ! CALL LOGD(.TRUE.) Turns on inhalation exposure?
!END
!DISCRETE CLEAR
!   CIZONE = 0.0  ! CALL LOGD(.TRUE.)
!END

DERIVATIVE

!Use Zero-Crossing Form of DISCRETE Function Here'
!  SCHEDULE command must be in DERIVATIVE section'
 !  DAILY = PULSE (0.0, PER1, TCHNG )
 !  WEEKLY = PULSE (0.0, PER2, LEN2  )
  ! SWITCHY = DAILY * WEEKLY
!SCHEDULE EXPOSE .XP. SWITCHY - 0.995
!SCHEDULE CLEAR  .XN. SWITCHY - 0.005

DAILY=PULSE(0.0,PERD,WIDD)
WEEKLY=PULSE(0.0,PERW,WIDW)
SWITCHY = DAILY * WEEKLY

!**********************Modified Here for Wong*****************'
          CI = CONC * MW / 24451.0 * SWITCHY!**

      !CA = Concentration in arterial blood (mg/l)'
       CA = (QC*CV+QP*CI)/(QC+(QP/PB))
       CX = CA/PB

          DRINK=DRINKA*SWITCHY         !**

   !TUMMY = Amount in stomach'
```

```
  RTUMMY = -KA*TUMMY
   TUMMY = INTEG(RTUMMY,DOSE)
    !RAX = Rate of Elimination in Exhaled air'
     RAX = QP*CX
      AX = INTEG(RAX, 0.0)

     !AS = Amount in slowly perfused tissues (mg)'
     RAS = SPDC*(CA-CVS) !now governed by diffusion-limited constant, SPDC, instead of QS
      AS = INTEG(RAS,0.)
     CVS = AS/(VS*PS)

     !AR = Amount in rapidly perfused tissues (mg)'
     RAR = QR*(CA-CVR)
      AR = INTEG(RAR,0.)
     CVR = AR/(VR*PR)

     !AF = Amount in fat tissue (mg)'
     RAF = QF*(CA-CVF)
      AF = INTEG(RAF,0.)
     CVF = AF/(VF*PF)

     !AL = Amount in liver tissue (mg)'
     RAL = QL*(CA-CVL) - KL*CVL - VMAX*CVL/(KM+CVL) + KA*TUMMY + DRINK
           AL = INTEG(RAL,0.)
     CVL = AL/(VL*PL)

!Metabolism comments updated by EDM on 2/1/10
     !AM = Amount metabolized (mg)'
    RMEX = (KL*CVL)+(VMAX*CVL/(KM+CVL))   !Rate of 1,4-dioxane metabolism
     RAM = (KL*CVL)+(VMAX*CVL)/(KM+CVL) - KME*AM  !Rate of change of metabolite
in body

          AM = INTEG(RAM, 0.0)              !'Amt Metabolite in body
     CAM = AM/BW                      !'Conc Metabolite in body'
    AMEX = INTEG(KME*AM, 0.0)           !'Amt Metabolite Excreted via urine'

     !AB = Amount in Venous Blood'
     RAB = QF*CVF + QL*CVL + QS*CVS + QR*CVR  - QC*CV
      AB = INTEG(RAB, AB0)
      CV = AB/VB
     AUCV = INTEG(CV, 0.0)

!Possible Dose Surrogates for Risk Assessment Defined Here'

     CEX = 0.667*CX + 0.333*CI         !'Conc in Exhal Air'
  AVECON = PLA * (CEX+CI)/2            !'Ave Conc in Nose Tissue'
  AUCCON = INTEG(AVECON, 0.0)          !'Area under Curve (Nose)'

  AUCMET = INTEG(CAM, 0.0)             !'Area under Curve (Metab)'
```

```
      CL = AL/VL                    !'Conc Liver Tissue'
     AUCL = INTEG(CL, 0.0)          !'Area under Curve (Liver)'
          AAUCL=AUCL/TIME

!  Dose Surrogates are Average Area under Time/Conc Curve per 24 hrs'
IF (T .GT. 0) TIME=T
     dayS = TIME/24.0
     NOSE = AUCCON/DAYS             !'Nasal Turbinates'
    LIVER = AUCL/DAYS               !'Liver Tissues'
    METAB = AUCMET/DAYS             !'Stable Metabolite'

END       !'End of dynamic'

END  ! End of TERMINAL

END       !'End of PROGRAM
```

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-1, for cortical tubule degeneration in male and female Osborne-Mendel rats exposed to 1,4-dioxane in the drinking water (NCI, 1978, 062935). Doses associated with a BMR of a 10% extra risk were calculated.

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

Males (mg/kg-day)			Females (mg/kg-day)		
0	**240**	**530**	**0**	**350**	**640**
0/31[a]	20/31[b] (65%)	27/33[b] (82%)	0/31[a]	0/34	10/32[b] (31%)

[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, 062935).

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-2). Comparing across models, a better fit is indicated by a lower AIC value (U.S. EPA, 2000, 052150). 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-2, Figure C-1) and could be used to derive a POD of 38.5 mg/kd-ay for this endpoint. The Weibull model provided the best fit to the data for female rats (Table C-2, Figure 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-2.

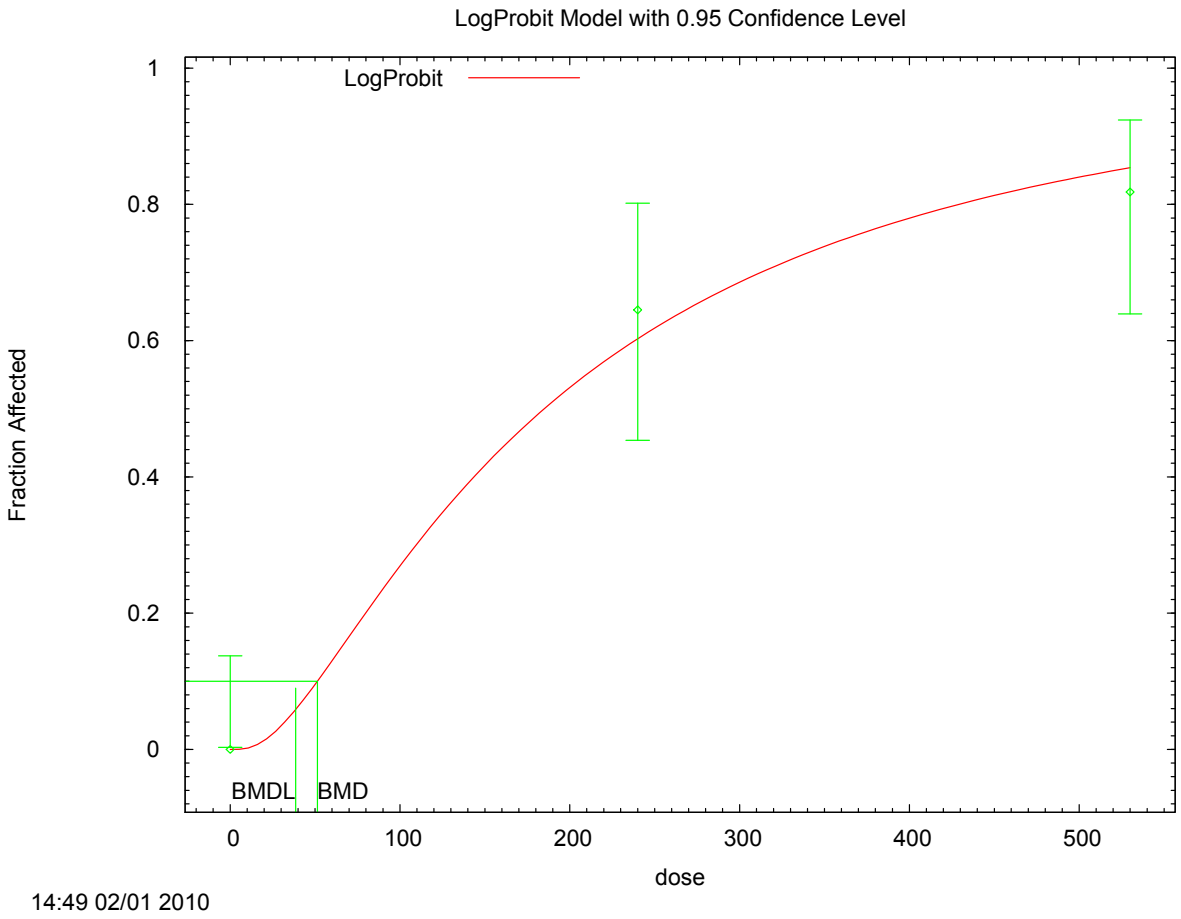
Note: Hyperlinks to the reference citations throughout this document will take you to the NCEA HERO database (Health and Environmental Research Online) at http://epa.gov/hero. HERO is a database of scientific literature used by U.S. EPA in the process of developing science assessments such as the Integrated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

Table C-2. Goodness-of-fit statistics and 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, 062935) 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, 062935).



Source: NCI (1978, 062935).

Figure C-1. BMD Log-probit model of cortical tubule degeneration incidence data for male rats exposed to 1,4-dioxane in drinking water for 2 years to support the results in Table C-2.

```
=================================================================
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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	NA		
intercept	-5.22131	0.172682	-5.55976	-4.88286
slope	1	NA		

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

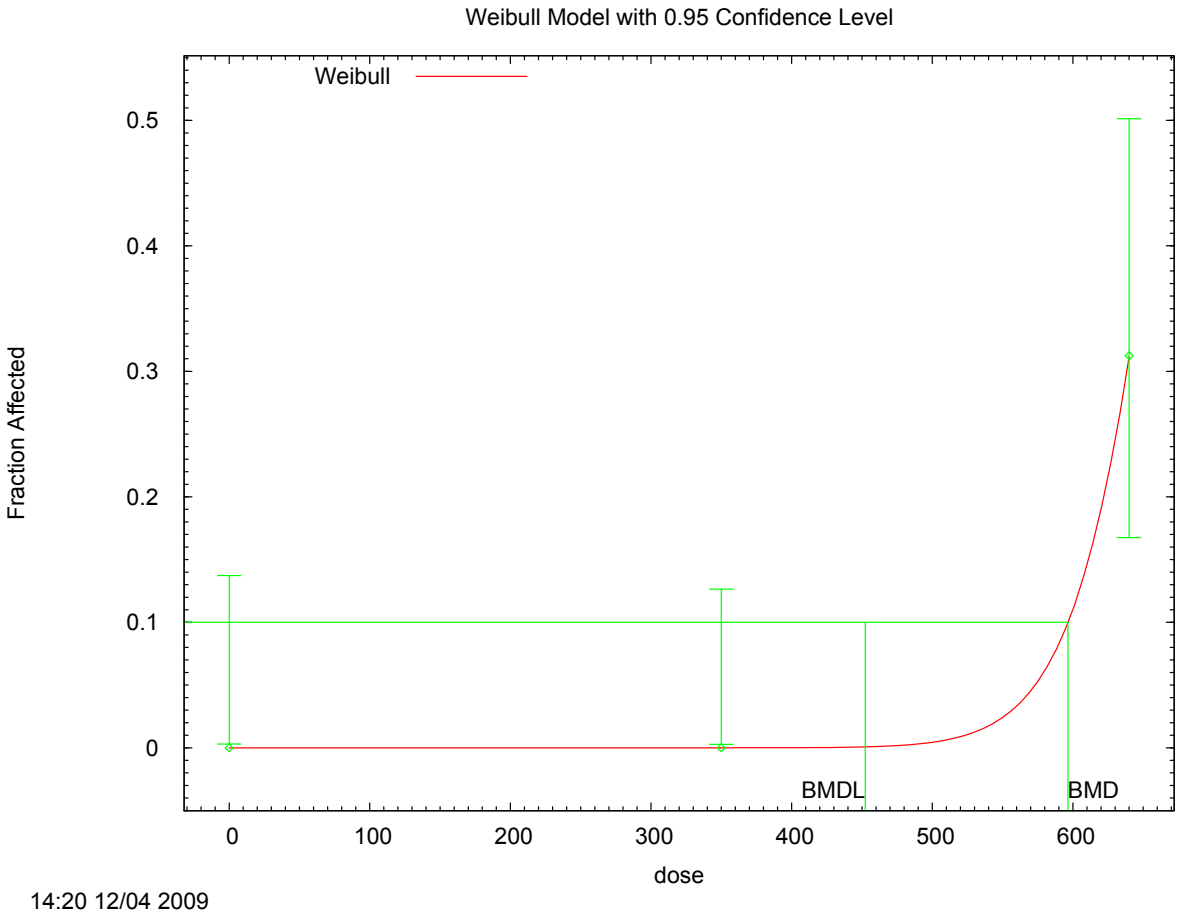
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	31	0.000
240.0000	0.6023	18.672	20.000	31	0.487
530.0000	0.8535	28.166	27.000	33	-0.574

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, 062935).

Figure C-2. BMD Weibull model of cortical tubule degeneration incidence data for female rats exposed to 1,4-dioxane in drinking water for 2 years to support the results in Table C-2.

```
=================================================================
Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
Input Data File: Z:\14Dioxane\BMDS\wei_nci_frat_cortdeg_Wei-BMR10-Restrict.(d)
Gnuplot Plotting File:  Z:\14Dioxane\BMDS\wei_nci_frat_cortdeg_Wei-BMR10-Restrict.plt
                                    Fri Dec 04 14:20:41 2009
=================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
    The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]

   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 = 0.015625
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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	NA		
Slope	1.15454e-051	1.#QNAN	1.#QNAN	1.#QNAN
Power	18	NA		

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

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-19.8748	3			
Fitted model	-19.875	1	0.000487728	2	0.9998
Reduced model	-32.1871	1	24.6247	2	<.0001

AIC: 41.75

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	31	0.000
350.0000	0.0000	0.000	0.000	34	-0.016
640.0000	0.3125	9.999	10.000	32	0.000

Chi^2 = 0.00 d.f. = 2 P-value = 0.9999

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 596.445
BMDL = 452.359

C.2. LIVER HYPERPLASIA

All available dichotomous models in the Benchmark Dose Software (version 2.1.1) were fit to the incidence data shown in Table C-3, for liver hyperplasia in male and female F344/DuCrj rats exposed to 1,4-dioxane in the drinking water (JBRC, 1998, 196240; Kano et al., 2009, 594539). Benchmark doses associated with a BMR of a 10% extra risk were calculated.

Table C-3. Incidence of liver hyperplasia in F344/DuCrj rats exposed to 1,4-dioxane in drinking water[a]

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

[a]Dose information from Kano et al. (2009, 594539) and incidence data from sacrificed animals from JBRC (1998, 196240).
[b]Incidence significantly elevated compared to control by χ^2 test ($p < 0.05$).
[c]Incidence significantly elevated compared to control by χ^2 test ($p < 0.01$).

Sources: Kano et al. (2009, 594539); JBRC (1998, 196240).

For incidence of liver hyperplasia in F344 male rats, the logistic, probit, and dichotomous-Hill models all exhibited a statistically significant lack of fit (i.e., χ^2 p-value < 0.1; see Table C-4), and thus should not be considered further for identification of a POD. All of the remaining models exhibited adequate fit, but the AIC values for the gamma, multistage, quantal-linear, and Weibull models were lower than the AIC values for the log-logistic and log-probit models. Finally, the AIC values for gamma, multistage, quantal-linear, and Weibull models in Table C-4 are equivalent and, in this case, essentially represent the same model. Therefore, consistent with the external review draft Benchmark Dose Technical Guidance (U.S. EPA, 2000, 052150), any of them with equal AIC values (gamma, multistage, quantal-linear, or Weibull) could be used to identify a POD for this endpoint of 23.8 mg/kg-day.

For liver hyperplasias in F344 female rats exposed to 1,4-dioxane, the quantal-linear and dichotomous-Hill models did not result in a good fit (i.e., χ^2 p-value < 0.1; See Table C-4). The multistage (3-degree) model had the lowest AIC value and was selected as the best-fitting model. Therefore, consistent with the BMD technical guidance document (U.S. EPA, 2000, 052150), the BMDL from the multistage (3-degree) model was selected to yield a POD for this endpoint of 27.1 mg/kg-day.

Table C-4. Benchmark dose modeling results based on the incidence of liver hyperplasias in male and female F344 rats exposed to 1,4-dioxane in drinking water for 2 years

Model	AIC	*p*-value[a]	Scaled Residual of Interest	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Male					
Gamma[b]	114.172	0.3421	0.886	35.90	23.81
Logistic	117.047	0.0706	1.869	83.56	63.29
Log-logistic[c]	115.772	0.1848	0.681	33.39	16.96
Log-probit[c]	115.57	0.1431	1.472	54.91	37.05
Multistage[d] (2 degree)	114.172	0.3421	0.886	35.90	23.81
Probit	116.668	0.0859	1.804	76.69	58.57
Weibull[b]	114.172	0.3421	0.886	35.90	23.81
Quantal-Linear	114.172	0.3421	0.886	35.90	23.81
Dichotomous-Hill	117.185	NC[e]	-0.2398	32.01	14.84
Female					
Gamma[b]	78.8357	0.9783	0	70.78	40.51
Logistic	77.0274	0.9174	-0.016	54.66	41.11
Log-logistic[c]	78.8357	0.9781	0	77.72	51.21
Log-probit[c]	78.8357	0.9781	0	74.64	50.97
Multistage[d] (2 degree)	76.9718	0.9563	-0.107	56.06	31.17
Multistage[d] (3 degree)	76.8351	0.9999	0	65.28	27.08
Probit	77.0308	0.9095	0.017	52.53	38.44
Weibull[b]	78.8349	0.9995	0	66.47	36.14
Quantal-Linear	87.3833	0.0245	-1.116	21.52	15.61
Dichotomous-Hill	2972.99	NC[e]	0	NC[e]	NC[e]

[a]*p*-Value from the χ^2 goodness-of-fit test for the selected model. Values < 0.1 indicate that the model exhibited a statistically significant lack of fit, and thus a different model should be chosen.
[b]Power restricted to ≥ 1.
[c]Slope restricted to ≥ 1.
[d]Betas restricted to ≥0.
[e]NC=Not calculated.

Sources: Kano et al. (2009, 594539); JBRC (1998, 196240).

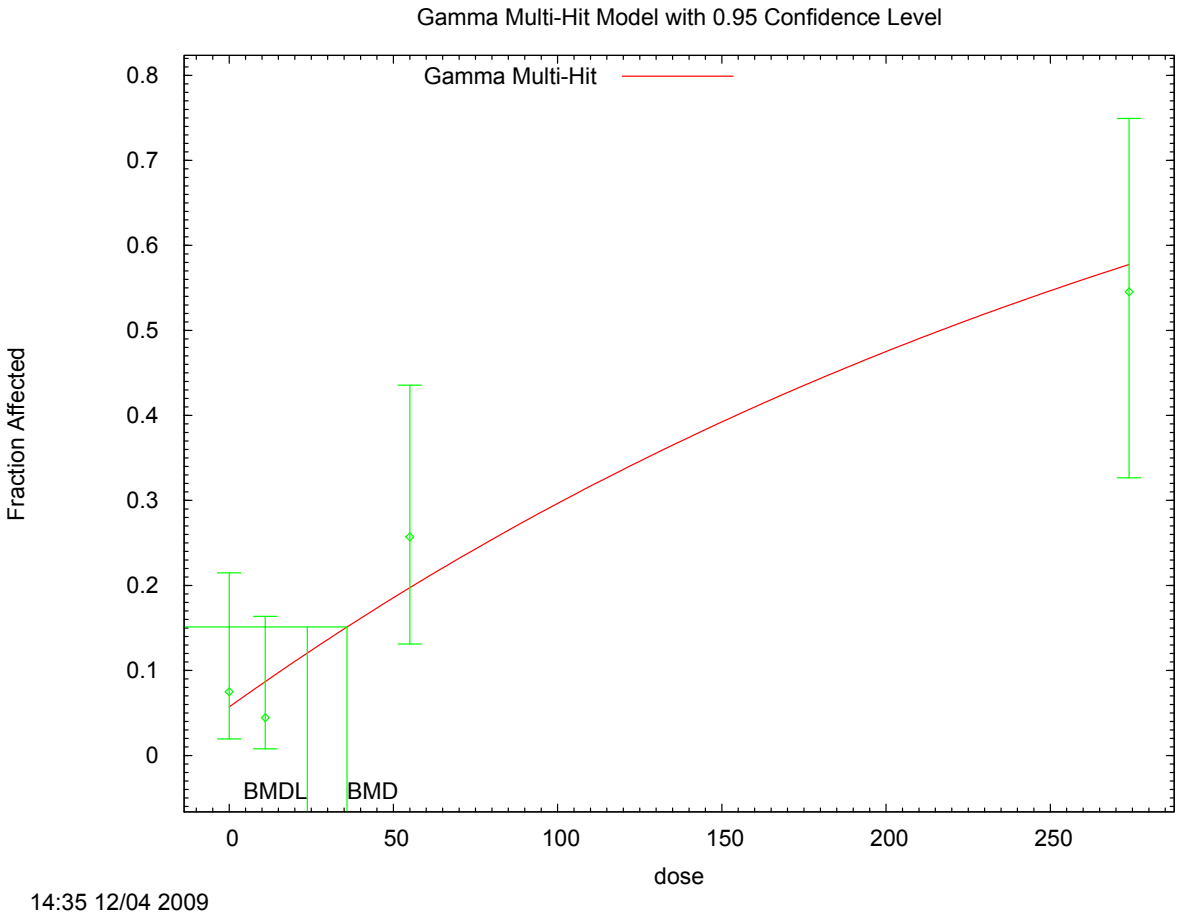


Figure C-3. BMD gamma model of liver hyperplasia incidence data for F344 male rats exposed to 1,4-dioxane in drinking water for 2 years to support results Table C-4.

```
=====================================================================
Gamma Model. (Version: 2.13;  Date: 05/16/2008)
Input Data File: Z:\14Dioxane\BMDS\gam_jbrc1998_mrat_liver_hyper_Gam-BMR10-
Restrict.(d)
Gnuplot Plotting File:  Z:\14Dioxane\BMDS\gam_jbrc1998_mrat_liver_hyper_Gam-BMR10-
Restrict.plt
                                    Fri Dec 04 14:35:02 2009
=====================================================================
 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 = 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 = 0.0853659
Slope = 0.00479329
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	-0.36
Slope	-0.36	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0569658	0.0278487	0.00238329	0.111548
Slope	0.00293446	0.000814441	0.00133818	0.00453073
Power	1	NA		

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

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-53.9471	4			
Fitted model	-55.0858	2	2.27725	2	0.3203
Reduced model	-67.6005	1	27.3066	3	<.0001

AIC: 114.172

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0570	2.279	3.000	40	0.492
11.0000	0.0869	3.911	2.000	45	-1.011
55.0000	0.1975	6.913	9.000	35	0.886
274.0000	0.5780	12.715	12.000	22	-0.309

Chi^2 = 2.15 d.f. = 2 P-value = 0.3421

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 35.9046
BMDL = 23.8065

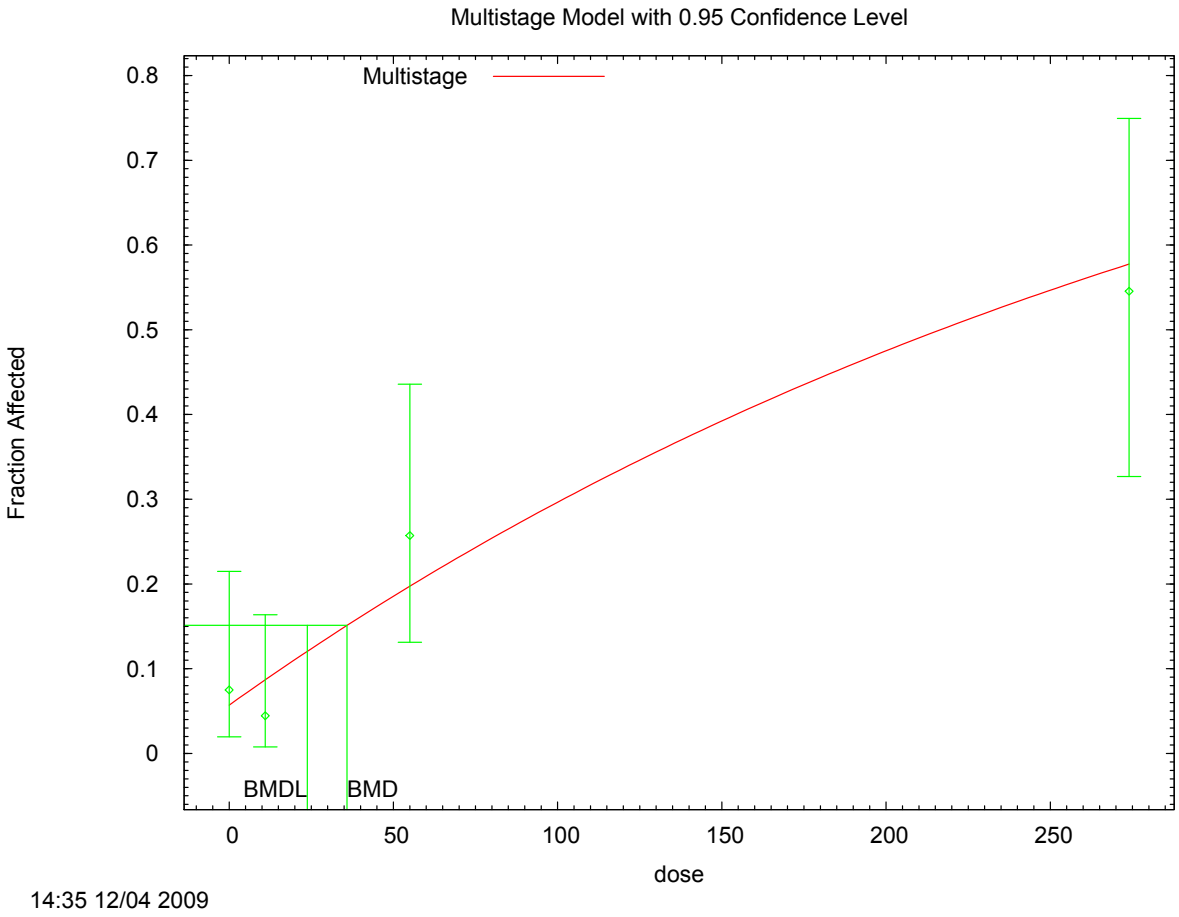


Figure C-4. BMD multistage (2 degree) model of liver hyperplasia incidence data for F344 male rats exposed to 1,4-dioxane in drinking water for 2 years to support results Table C-4.

```
=================================================================
Multistage Model. (Version: 3.0;  Date: 05/16/2008)
Input Data File: Z:\14Dioxane\BMDS\mst_jbrc1998_mrat_liver_hyper_Mst-BMR10-
restrict.(d)
Gnuplot Plotting File:  Z:\14Dioxane\BMDS\mst_jbrc1998_mrat_liver_hyper_Mst-BMR10-
Restrict.plt
                                        Fri Dec 04 14:35:06 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 = 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 =    0.0750872
                       Beta(1) =   0.00263797
                       Beta(2) =            0


          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)
Background            1        -0.49
   Beta(1)        -0.49             1


                                Parameter Estimates
                                                    95.0% Wald Confidence Interval
Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf. Limit
Background        0.0569658            *                 *                   *
   Beta(1)       0.00293446            *                 *                   *
   Beta(2)                0            *                 *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -53.9471          4
   Fitted model         -55.0858          2       2.27725      2          0.3203
  Reduced model         -67.6005          1       27.3066      3         <.0001

           AIC:          114.172


                               Goodness  of  Fit
                                                                    Scaled
    Dose      Est._Prob.     Expected     Observed      Size       Residual
  ---------------------------------------------------------------------------
    0.0000      0.0570          2.279      3.000          40        0.492
   11.0000      0.0869          3.911      2.000          45       -1.011
   55.0000      0.1975          6.913      9.000          35        0.886
  274.0000      0.5780         12.715     12.000          22       -0.309

 Chi^2 = 2.15      d.f. = 2        P-value = 0.3421


   Benchmark Dose Computation
Specified effect =            0.1
Risk Type        =      Extra risk
Confidence level =           0.95
             BMD =        35.9046
            BMDL =        23.8065
            BMDU =        82.1206

Taken together, (23.8065, 82.1206) is a 90% two-sided confidence interval for the BMD
```

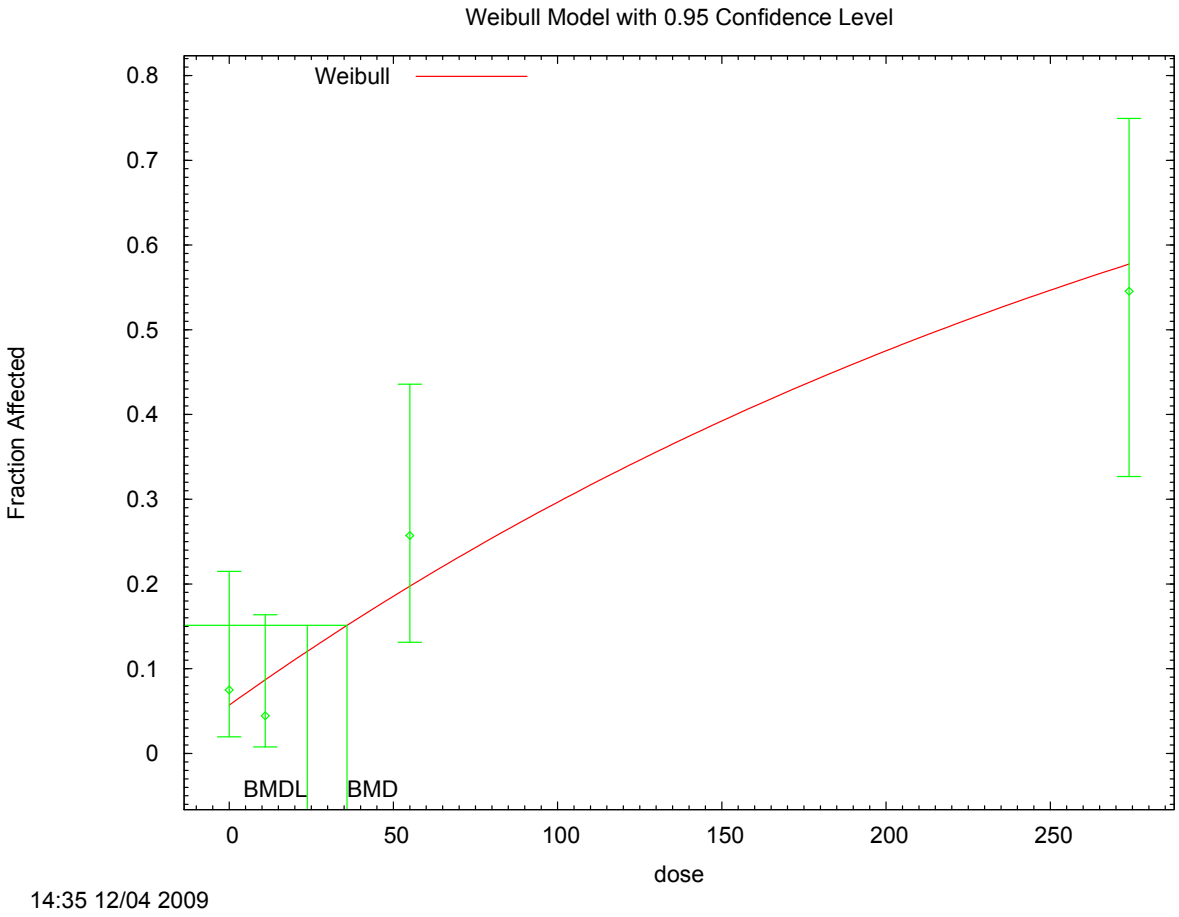


Figure C-5. BMD Weibull model of liver hyperplasia incidence data for F344 male rats exposed to 1,4-dioxane in drinking water for 2 years to support the results in Table C-4.

```
=====================================================================
Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
Input Data File: Z:\14Dioxane\BMDS\wei_jbrc1998_mrat_liver_hyper_Wei-BMR10-
Restrict.(d)
Gnuplot Plotting File:  Z:\14Dioxane\BMDS\wei_jbrc1998_mrat_liver_hyper_Wei-BMR10-
Restrict.plt
                                        Fri Dec 04 14:35:08 2009
=====================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]

   Dependent variable = Effect
   Independent variable = Dose
   Power parameter is restricted as power >=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 (and Specified) Parameter Values
Background = 0.0853659
Slope = 0.00253609
Power = 1

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

	Background	Slope
Background	1	-0.36
Slope	-0.36	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0569661	0.0278498	0.00238155	0.111551
Slope	0.00293445	0.000814445	0.00133816	0.00453073
Power	1	NA		

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

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-53.9471	4			
Fitted model	-55.0858	2	2.27725	2	0.3203
Reduced model	-67.6005	1	27.3066	3	<.0001

AIC: 114.172

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0570	2.279	3.000	40	0.492
11.0000	0.0869	3.911	2.000	45	-1.011
55.0000	0.1975	6.913	9.000	35	0.886
274.0000	0.5780	12.715	12.000	22	-0.309

Chi^2 = 2.15 d.f. = 2 P-value = 0.3421

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 35.9047
BMDL = 23.8065

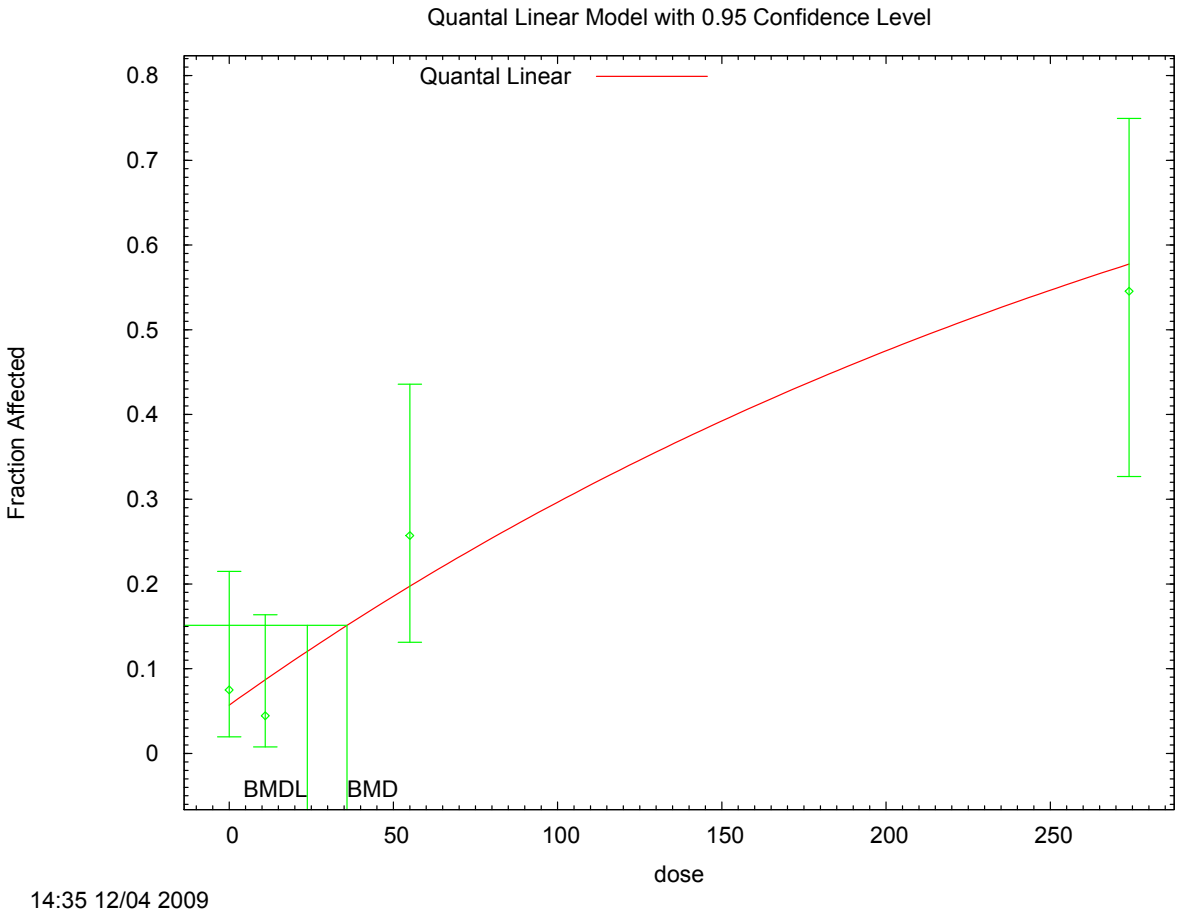


Figure C-6. BMD quantal-linear model of liver hyperplasia incidence data for F344 male rats exposed to 1,4-dioxane in drinking water for 2 years to support the results in Table C-4.

```
=====================================================================
Quantal Linear Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
Input Data File: Z:\14Dioxane\BMDS\qln_jbrc1998_mrat_liver_hyper_Qln-BMR10.(d)
Gnuplot Plotting File:  Z:\14Dioxane\BMDS\qln_jbrc1998_mrat_liver_hyper_Qln-BMR10.plt
                                    Fri Dec 04 14:35:09 2009
=====================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose)]


   Dependent variable = Effect
   Independent variable = Dose

   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 =    0.0853659
                          Slope =   0.00253609
                          Power =            1   Specified
```

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

	Background	Slope
Background	1	-0.36
Slope	-0.36	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
Background	0.0569665	0.02785	0.00238157	0.111551
Slope	0.00293447	0.000814452	0.00133818	0.00453077

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-53.9471	4			
Fitted model	-55.0858	2	2.27725	2	0.3203
Reduced model	-67.6005	1	27.3066	3	<.0001

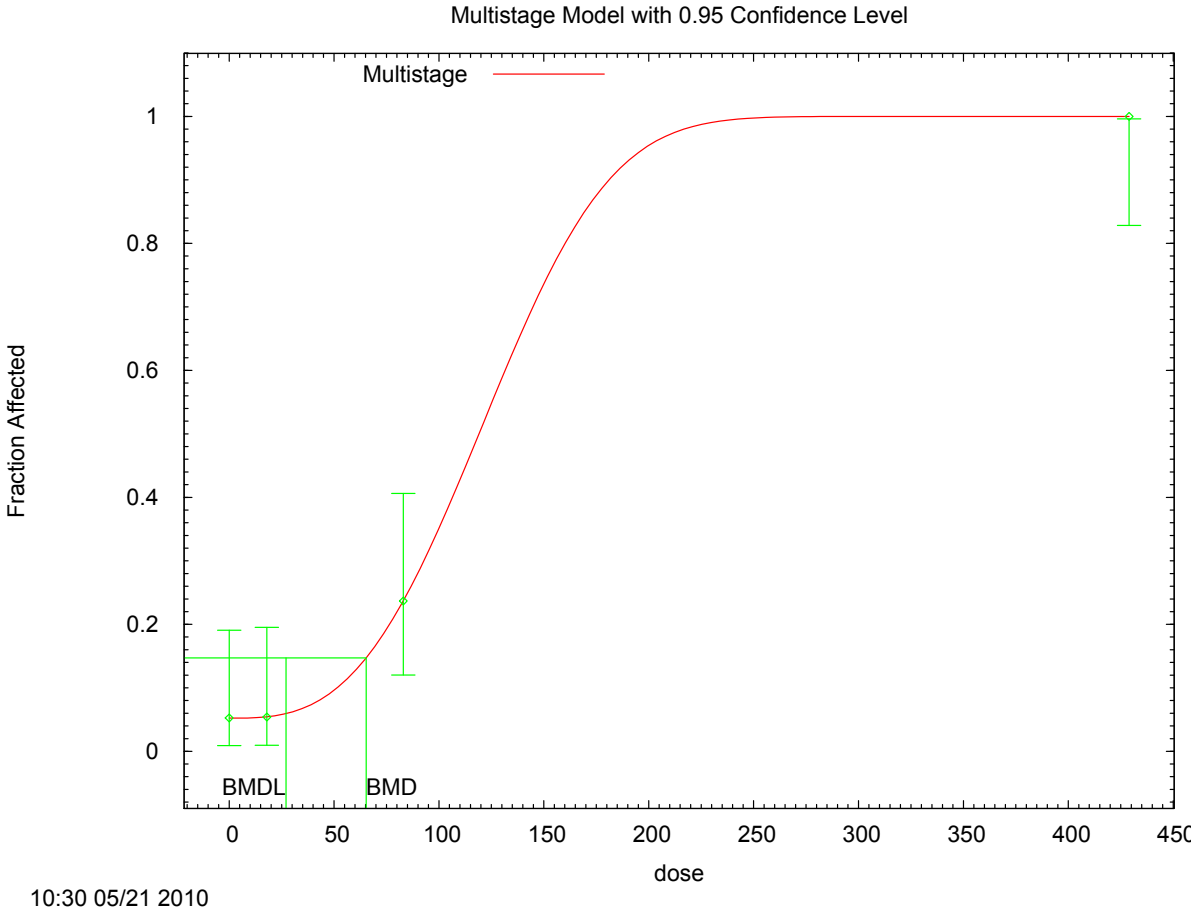
AIC: 114.172

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0570	2.279	3.000	40	0.492
11.0000	0.0869	3.911	2.000	45	-1.011
55.0000	0.1975	6.913	9.000	35	0.886
274.0000	0.5780	12.716	12.000	22	-0.309

Chi^2 = 2.15 d.f. = 2 P-value = 0.3421

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 35.9044
BMDL = 23.8065



Source: JBRC (1998, 196240).

Figure C-7. BMD log-probit model of liver hyperplasia incidence data for F344 female rats exposed to 1,4-dioxane in drinking water for 2 years to support the results in Table C-4.

```
=====================================================================
Multistage Model. (Version: 3.0;  Date: 05/16/2008)
Input Data File: H:\14Dioxane\BMDS\mst_jbrc1998_frat_liver_hyper_Mst-BMR10-Restrict-
3deg.(d)
Gnuplot Plotting File:  H:\14Dioxane\BMDS\mst_jbrc1998_frat_liver_hyper_Mst-BMR10-
Restrict-3deg.plt
                                                Fri May 21 10:30:14 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) =	1.2696e+012

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(1), -Beta(2) have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	Background	Beta(3)
Background	1	-0.55
Beta(3)	-0.55	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0523101	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	3.78712e-007	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-36.4175	4			
Fitted model	-36.4175	2	0.00016582	2	0.9999
Reduced model	-79.9164	1	86.9979	3	<.0001

AIC: 76.8351

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0523	1.988	2.000	38	0.009
18.0000	0.0544	2.013	2.000	37	-0.009
83.0000	0.2368	8.999	9.000	38	0.000
429.0000	1.0000	24.000	24.000	24	0.000

Chi^2 = 0.00 d.f. = 2 P-value = 0.9999

Benchmark Dose Computation

Specified effect =	0.1
Risk Type =	Extra risk
Confidence level =	0.95
BMD =	65.2814
BMDL =	27.0766
BMDU =	91.3457

Taken together, (27.0766, 91.3457) is a 90% two-sided confidence interval for the BMD

APPENDIX D. DETAILS OF BMD ANALYSIS FOR ORAL CSF FOR 1,4-DIOXANE

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, 2000, 052150). 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, 594539) study are shown in Table D-1. The data and BMD modeling results are presented separately for each dataset as follows:

- Hepatic adenomas and carcinomas in female F344 rats (Tables D-2 and D-3; Figure D-1)
- Hepatic adenomas and carcinomas in male F344 rats (Tables D-4 and D-5; Figures D-2 and D-3)
- 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-6)
  - Nasal cavity tumors in female F344 rats (Table D-7; Figure D-4)
  - Nasal cavity tumors in male F344 rats (Table D-8; Figure D-5)
  - Mammary gland adenomas in female F344 rats (Table D-9; Figures D-6 and D-7)
  - Peritoneal mesotheliomas in male F344 rats (Table D-10; Figures D-8 and D-9)
- Hepatic adenomas and carcinomas in female BDF1 mice (Tables D-11, D-12, and D-13; Figures D-10, D-11, D-12, and D-13)
- Hepatic adenomas and carcinomas in male BDF1 mice (Tables D-14 and D-15; Figures D-14 and D-15)

Data and BMD modeling results from the additional chronic bioassays (Kociba et al., 1974, 062929; NCI, 1978, 062935) were evaluated for comparison with the data from Kano et al. (2009, 594539). 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-16)

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

- Incidence of hepatocellular carcinoma and nasal squamous cell carcinoma in male and female Sherman rats (combined) (Kociba et al., 1974, 062929) treated with 1,4-dioxane in the drinking water for 2 years (Table D-17)
  - BMDS dose-response modeling results for incidence of hepatocellular carcinoma in male and female Sherman rats (combined) (Kociba et al., 1974, 062929) exposed to 1,4-dioxane in drinking water for 2 years (Table D-18; Figures D-16 and D-17)
  - BMDS dose-response modeling results for incidence of nasal squamous cell carcinoma in male and female Sherman rats (combined) (Kociba et al., 1974, 062929) exposed to 1,4-dioxane in the drinking water for 2 years (Table D-19; Figure D-18)
- Incidence of nasal cavity squamous cell carcinoma and hepatocellular adenoma in Osborne-Mendel rats (NCI, 1978, 062935) exposed to 1,4-dioxane in the drinking water (Table D-20)
  - BMDS dose-response modeling results for incidence of hepatocellular adenoma in female Osborne-Mendel rats (NCI, 1978, 062935) exposed to 1,4-dioxane in the drinking water for 2 years (Table D-21; Figures D-19 and D-20)
  - BMDS dose-response modeling results for incidence of nasal cavity squamous cell carcinoma in female Osborne-Mendel rats (NCI, 1978, 062935) exposed to 1,4-dioxane in the drinking water for 2 years (Table D-22; Figures D-21 and D-22)
  - BMDS dose-response modeling results for incidence of nasal cavity squamous cell carcinoma in male Osborne-Mendel rats (NCI, 1978, 062935) exposed to 1,4-dioxane in the drinking water for 2 years (Table D-23; Figures D-23 and D-24)
- Incidence of hepatocellular adenoma or carcinoma in male and female $B6C3F_1$ mice (NCI, 1978, 062935) exposed to 1,4-dioxane in drinking water (Table D-24)
  - BMDS dose-response modeling results for the combined incidence of hepatocellular adenoma or carcinoma in female $B6C3F_1$ mice (NCI, 1978, 062935) exposed to 1,4-dioxane in the drinking water for 2 years (Table D-25; Figure D-25)
  - BMDS dose-response modeling results for incidence of combined hepatocellular adenoma or carcinoma in male $B6C3F_1$ mice (NCI, 1978, 062935) exposed to 1,4-dioxane in the drinking water for 2 years (Table D-26; Figures D-26 and D-27).

D.1. GENERAL ISSUES AND APPROACHES TO BMDS MODELING

D.1.1. Combining Data on Adenomas and Carcinomas

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 are a severe endpoint by themselves and most would have developed into carcinomas if exposure at the same dose was continued (U.S. EPA, 2005, 086237). The incidence at high doses of both tumors in rat and mouse liver is high in the key study (Kano et al., 2009, 594539).

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, 2000, 052150) 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-1.

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-1.

Table D-1. Recommended models for rodents exposed to 1,4-dioxane in drinking water (Kano et al., 2009, 594539)

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 R344 Rat								
Hepatic Tumors	Lowest AIC	Probit	147.787	0.9867	62.20	51.12	17.43	14.33
Peritoneal Meso-thelioma	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, 594539) are shown in Table D-2.

Table D-2. Data for hepatic adenomas and carcinomas in female F344 rats (Kano et al., 2009, 594539)

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, 594539).

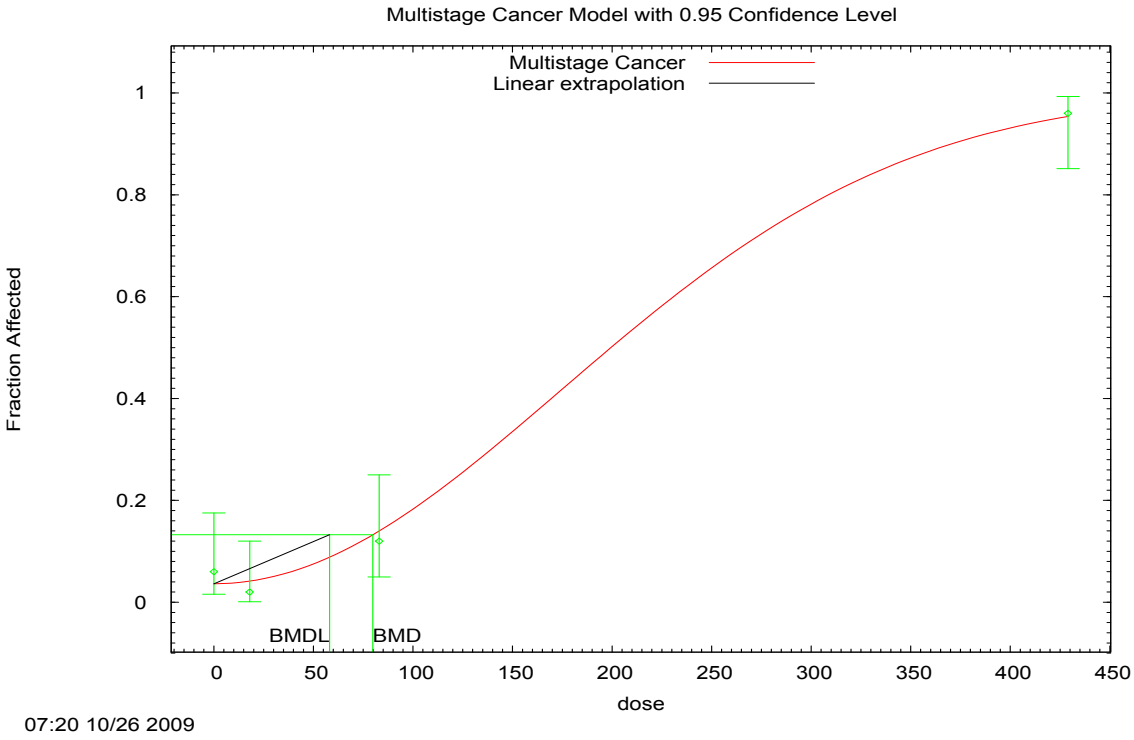
Note that the incidence of rats with adenomas, with carcinomas, and with either adenomas or carcinomas are monotone non-decreasing functions of dose except for 3 female rats in the control group. These data therefore appear to be appropriate for dose-response modeling using BMDS.

The results of the BMDS modeling for the entire suite of models are presented in Table D-3.

Table D-3. BMDS dose-response modeling results for the combined incidence of hepatic adenomas and carcinomas in female F344 rats (Kano et al., 2009, 594539)

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 permisison of Elservier, Ltd., Kano et al. (2009, 594539).

Figure D-1. Multistage BMD model (2 degree) for the combined incidence of hepatic adenomas and carcinomas 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_hepato_adcar_Msc-
BMR10-2poly.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_frat_hepato_adcar_Msc-BMR10-2poly.plt
Mon Oct 26 08:20:52 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 = 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 =    0.0281572
```

```
Beta(1) =            0
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, 594539) are shown in Table D-4.

Table D-4. Data for hepatic adenomas and carcinomas in male F344 rats (Kano et al., 2009, 594539)

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, 594539).

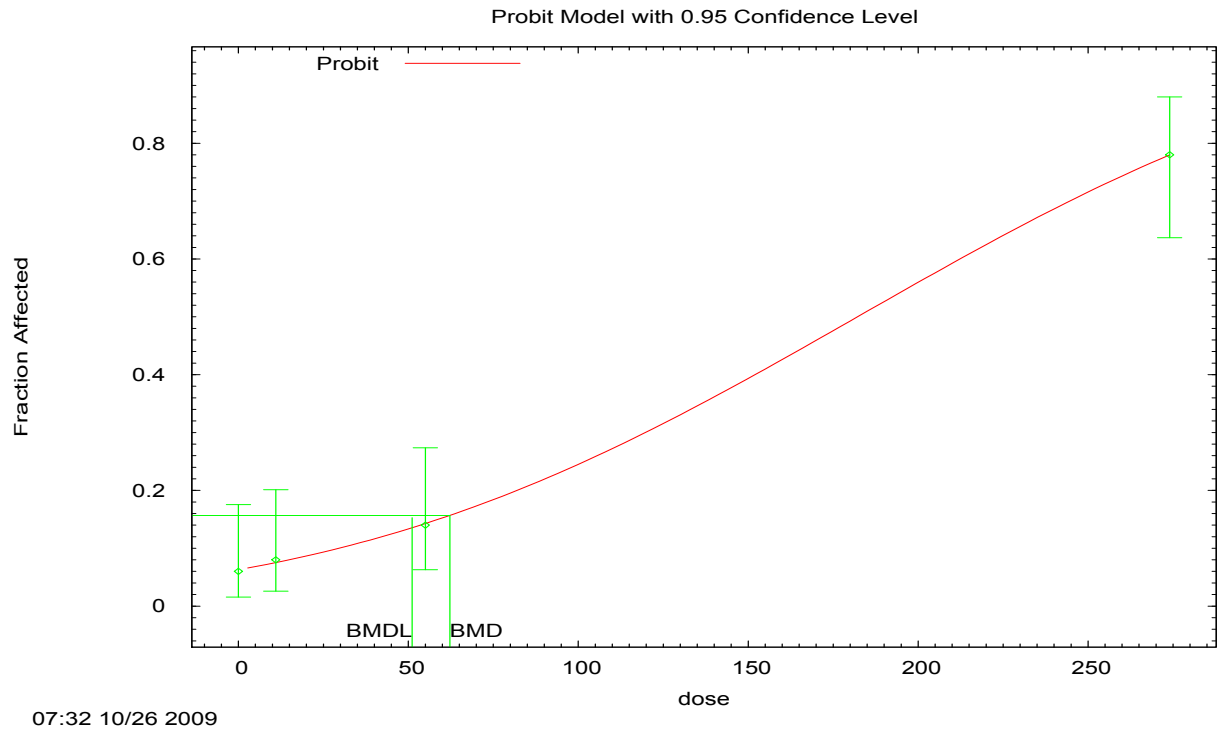
Note that the incidence of rats with hepatic adenomas, carcinomas, and with either adenomas or carcinomas are monotone non-decreasing functions of dose. These data therefore appear to be appropriate for dose-response modeling using BMDS.

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-5.

Table D-5. BMDS dose-response modeling results for the combined incidence of adenomas and carcinomas in livers of male F344 rats (Kano et al., 2009, 594539)

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, 594539).

Figure D-2. 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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

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 =     -1.51718
slope =          0.00831843
```

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	-0.69
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

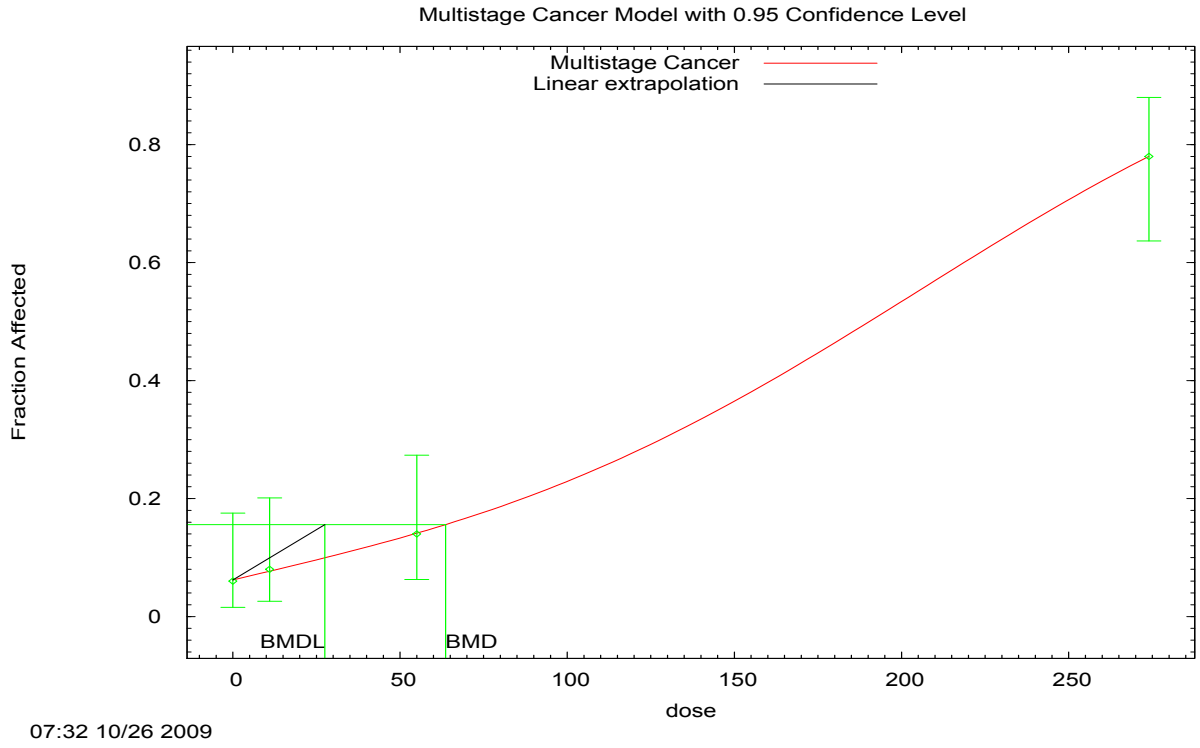
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0628	3.142	3.000	50	-0.083
11.0000	0.0751	3.754	4.000	50	0.132
55.0000	0.1425	7.125	7.000	50	-0.050
274.0000	0.7797	38.985	39.000	50	0.005

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, 594539).

Figure D-3. Multistage BMD model (3 degree) for the combined incidence of hepatic adenomas and carcinomas 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_hepato_adcar_Msc-
BMR10-3poly.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mrat_hepato_adcar_Msc-BMR10-3poly.plt
Mon Oct 26 08:32:08 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 =   0.0623822
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

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0620	3.100	3.000	50	-0.058
11.0000	0.0769	3.844	4.000	50	0.083
55.0000	0.1412	7.059	7.000	50	-0.024
274.0000	0.7799	38.997	39.000	50	0.001

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, 594539) are shown in Table D-6. 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-6. Data for significant tumors at other sites in male and female F344 rats (Kano et al., 2009, 594539)

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

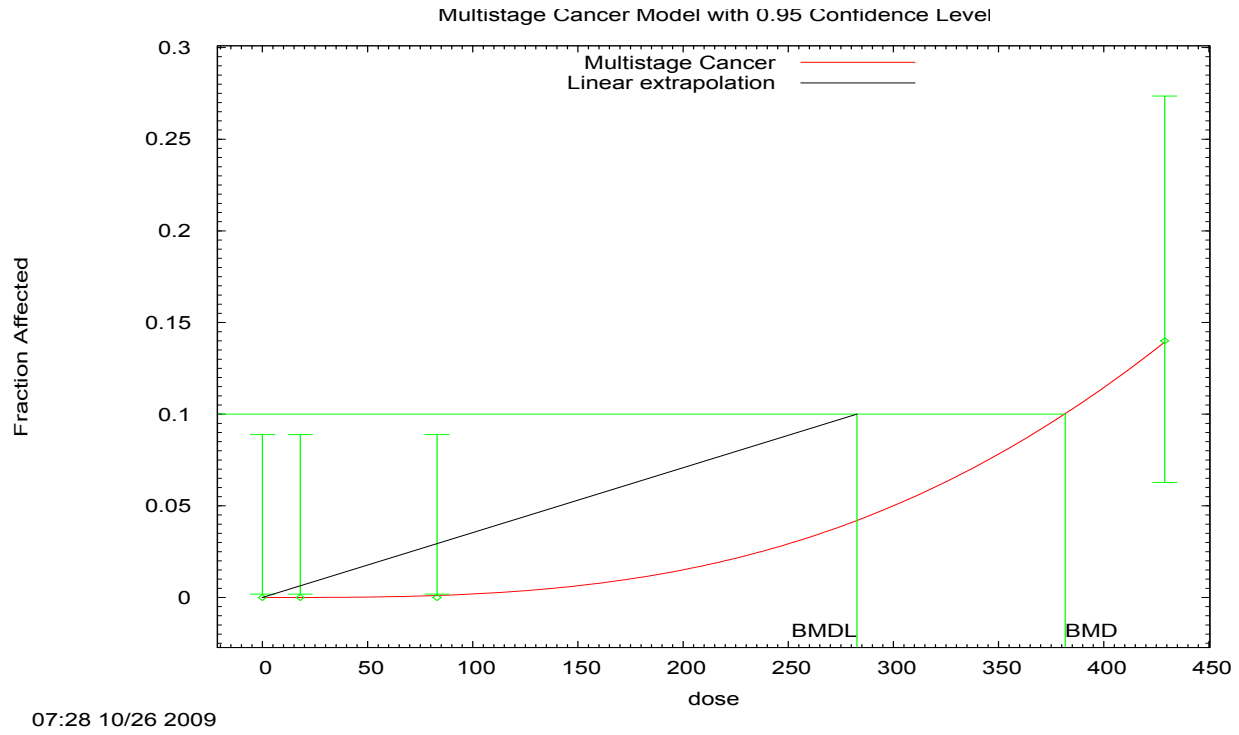
Source: Used with permission from Elsevier, Ltd., Kano et al., (2009, 594539).

The results of the BMDS modeling for the entire suite of models are presented in Tables D-7 through Table D-10 for tumors in the nasal cavity, mammary gland, and peritoneal cavity.

Table D-7. BMDS dose-response modeling results for the incidence of nasal cavity tumors in female F344 rats[a] (Kano et al., 2009, 594539)

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, 594539).

Figure D-4. Multistage BMD model (3 degree) for nasal cavity tumors 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_nasal_car_Msc-
BMR10-3poly.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_frat_nasal_car_Msc-BMR10-3poly.plt
Mon Oct 26 08:28:58 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) = 1.91485e-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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0	*	*	*
Beta(3)	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

Dose	Est._Prob.	Expected	Observed	Size	Scaled 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

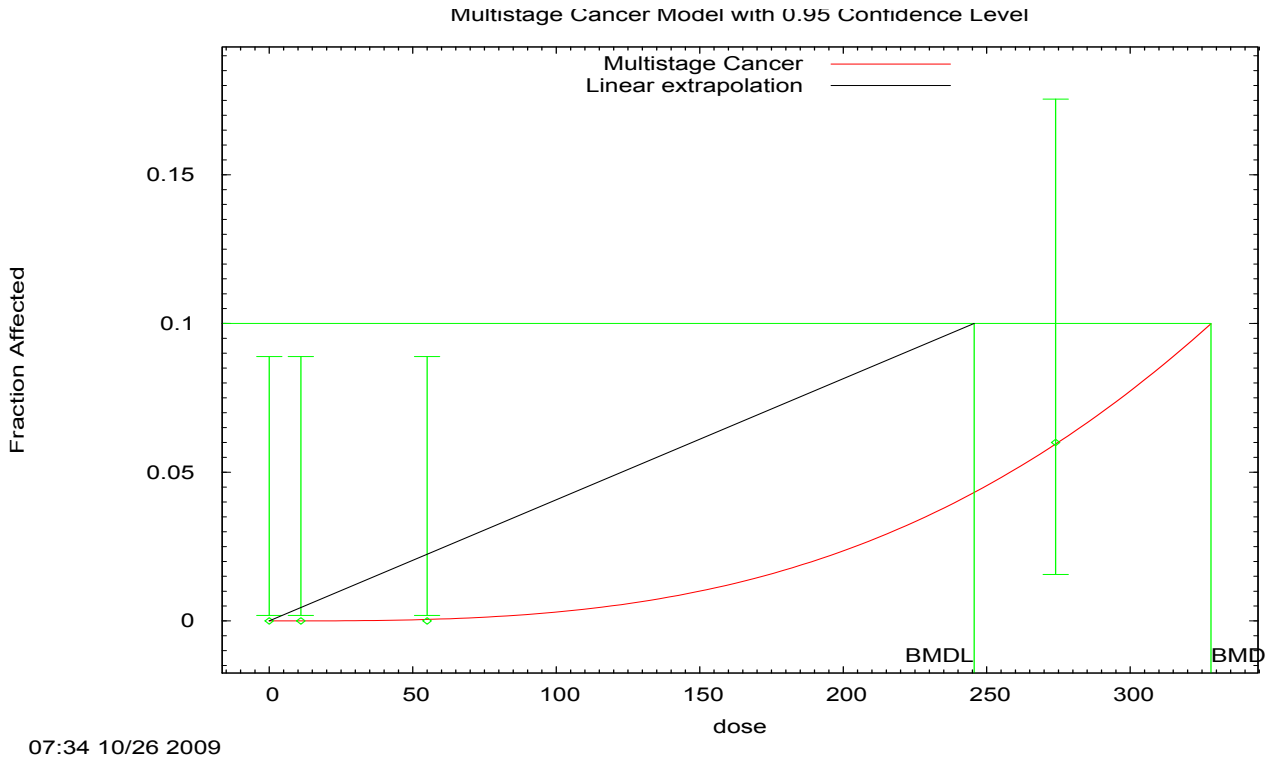
Taken together, (282.609, 500.178) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000353846

Table D-8. BMDS dose-response modeling results for the incidence of nasal cavity tumors in male F344 rats[a] (Kano et al., 2009, 594539)

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 permisison from Elsevier, Ltd., Kano et al. (2009, 594539).

Figure D-5. Multistage BMD model (3 degree) for nasal cavity tumors in male F344 rats.

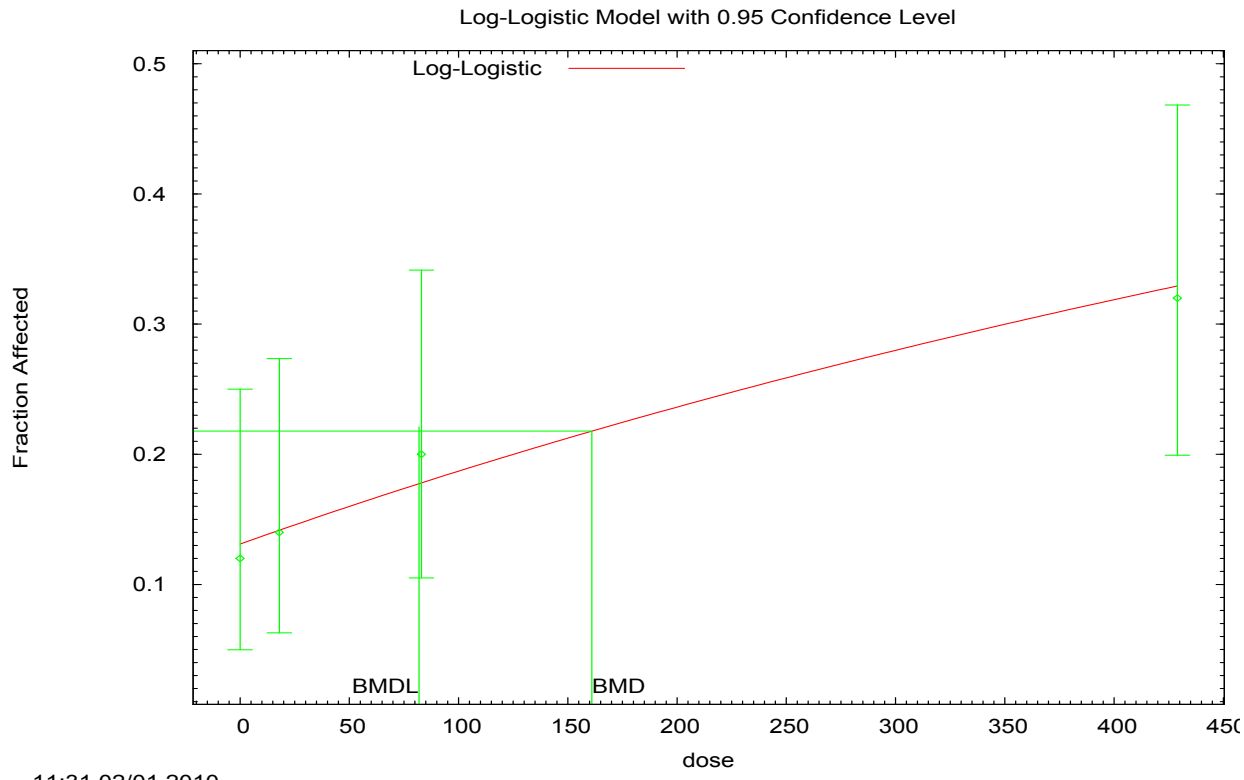
```
=====================================================================
Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mrat_nasal_car_Msc-
BMR10-3poly.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mrat_nasal_car_Msc-BMR10-3poly.plt
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-9. BMDS dose-response modeling results for the incidence of mammary gland adenomas in female F344 rats (Kano et al., 2009, 594539)

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, 594539).

Figure D-6. LogLogistic BMD model for mammary gland adenomas in female F344 rats.

```
================================================================
Logistic Model. (Version: 2.12; Date: 05/16/2008)
Input Data File: C:\14DBMDS\lnl_kano2009_frat_mamm_ad_Lnl-BMR10-Restrict.(d)
Gnuplot Plotting File:  C:\14DBMDS\lnl_kano2009_frat_mamm_ad_Lnl-BMR10-Restrict.plt
                                          Mon Feb 01 11:31:31 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 =         0.12
                     intercept =     -7.06982
                         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	-0.53
intercept	-0.53	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.130936	*	*	*
intercept	-7.2787	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-94.958	4			
Fitted model	-95.0757	2	0.235347	2	0.889
Reduced model	-98.6785	1	7.4409	3	0.0591

AIC: 194.151

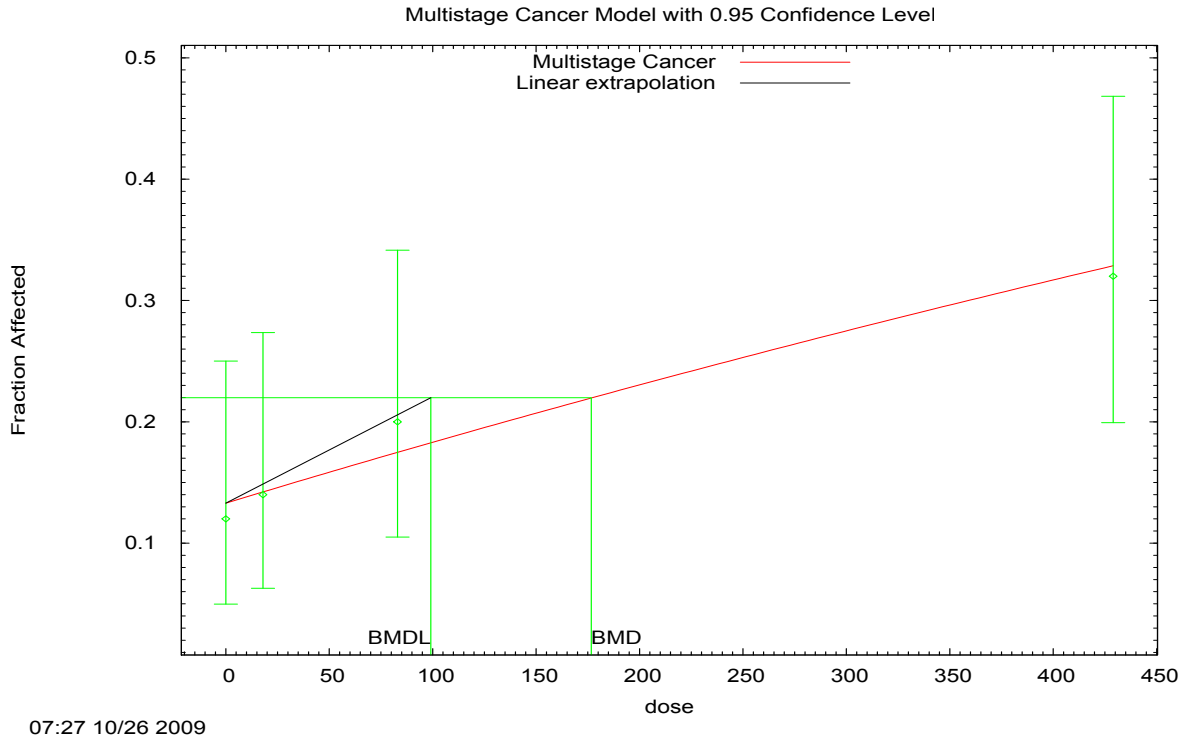
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1309	6.547	6.000	50	-0.229
18.0000	0.1416	7.080	7.000	50	-0.032
83.0000	0.1780	8.901	10.000	50	0.406
429.0000	0.3294	16.472	16.000	50	-0.142

Chi^2 = 0.24 d.f. = 2 P-value = 0.8874

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 161.012
BMDL = 81.9107



Source: Used with permission from Elsevier, Ltd., Kano et al. (2009, 594539).

Figure D-7. 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

Dose	Est._Prob.	Expected	Observed	Size	Scaled 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

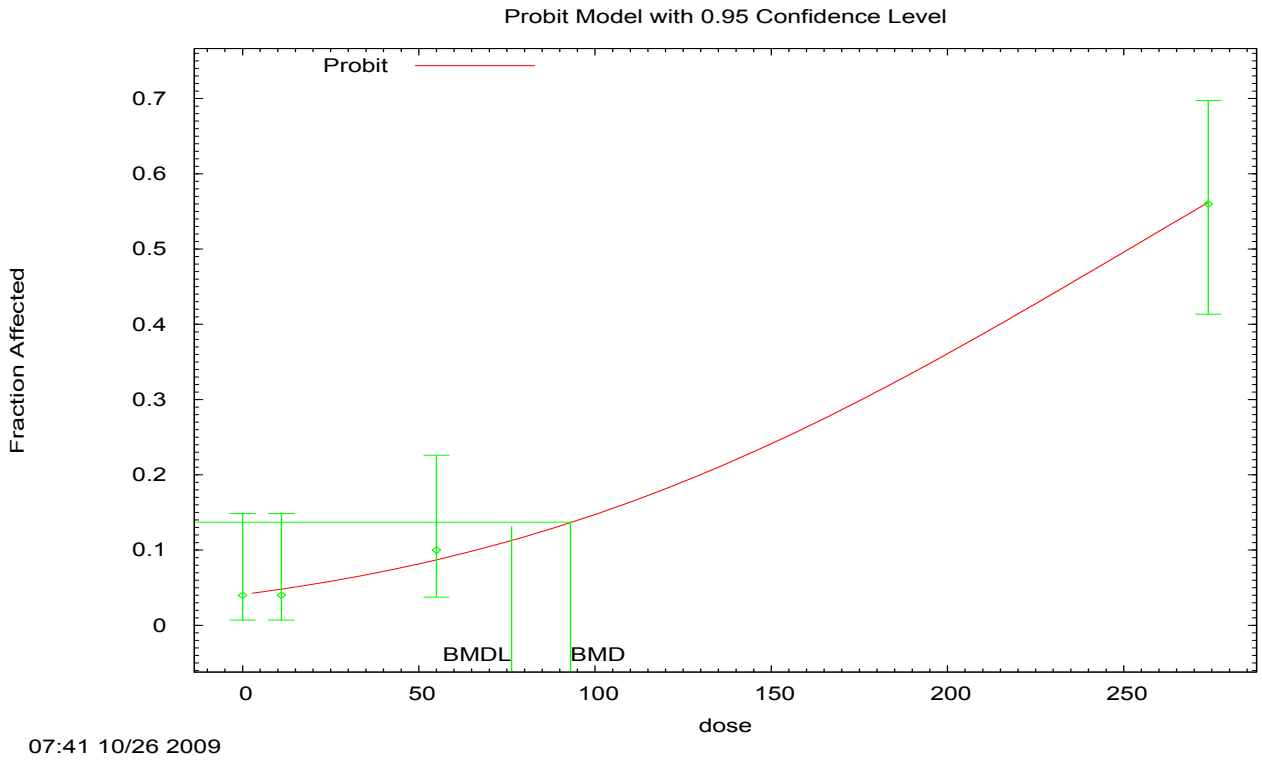
Taken together, (99.1337, 501.523) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00100874

Table D-10. BMDS dose-response modeling results for the incidence of peritoneal mesotheliomas in male F344 rats (Kano et al., 2009, 594539)

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, 594539).

Figure D-8. 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				

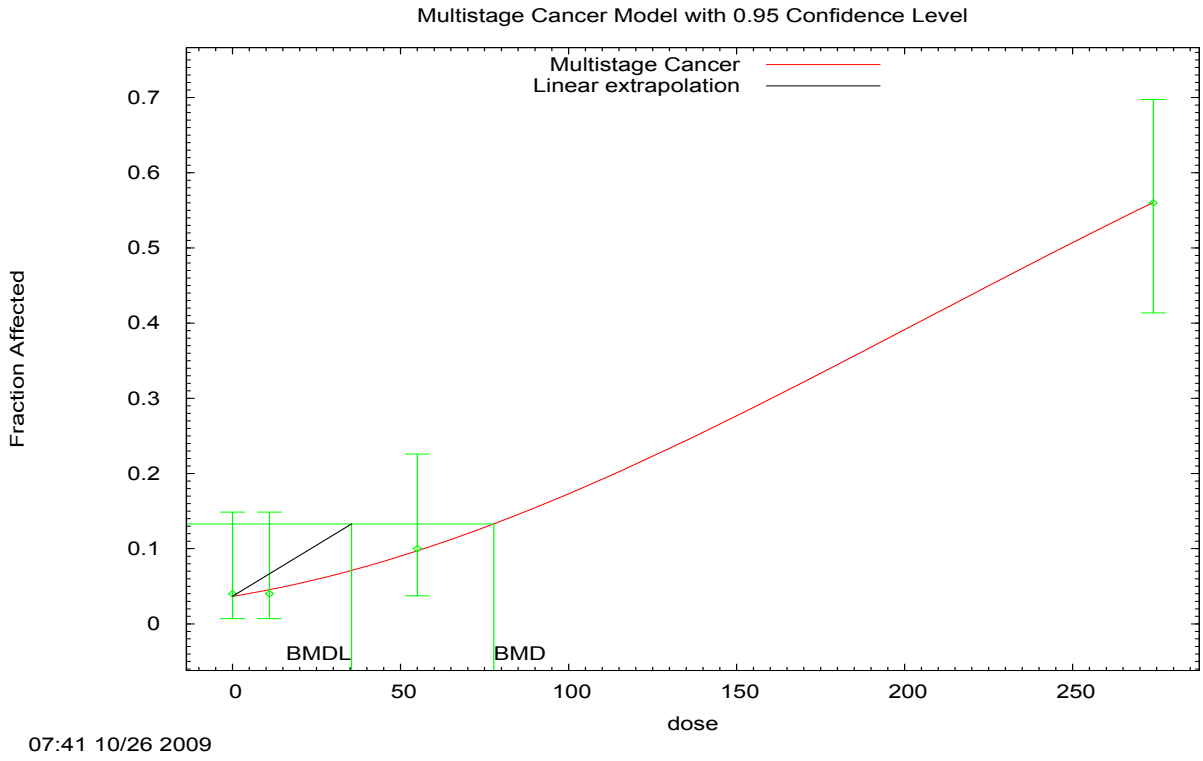
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled 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, 594539).

Figure D-9. Multistage BMD (2 degree) model for peritoneal mesotheliomas 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_peri_meso_Msc-
BMR10-2poly.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mrat_peri_meso_Msc-BMR10-2poly.plt
Mon Oct 26 08:41:28 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 = 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 =   0.0358706
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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0366063	*	*	*
Beta(1)	0.000757836	*	*	*
Beta(2)	7.6893e-006	*	*	*

* - 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

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0366	1.830	2.000	50	0.128
11.0000	0.0455	2.275	2.000	50	-0.186
55.0000	0.0972	4.859	5.000	50	0.067
274.0000	0.5605	28.027	28.000	50	-0.008

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-11. 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-11. Data for hepatic adenomas and carcinomas in female BDF1 mice (Kano et al., 2009, 594539)

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, 594539).

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-12. 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, 594539). 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-13. 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-12. BMDS dose-response modeling results for the combined incidence of hepatic adenomas and carcinomas in female BDF1 mice (Kano et al., 2009, 594539)

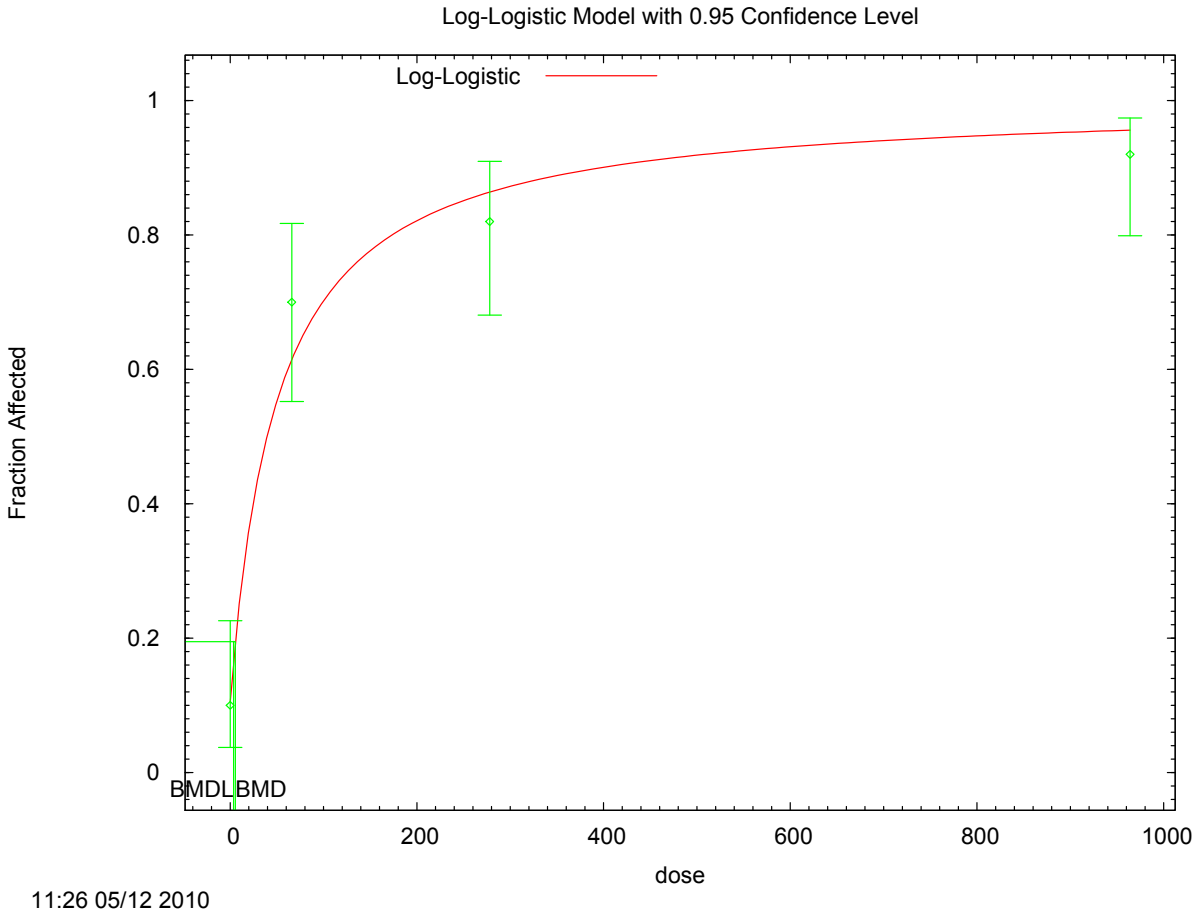
Model	AIC	*p*-value	BMD_{10} mg/kg-day	$BMDL_{10}$ mg/kg-day	χ^{2a}	$BMD_{10\ HED}$ mg/kg-day	$BMDL_{10\ HED}$ mg/kg-day
Gamma	203.331	0	26.43	19.50	-2.654	3.98	2.94
Logistic	214.951	0	58.05	44.44	3.201	8.74	6.69
LogLogistic[b]	176.214	0.1421	5.54	3.66	-0.121	0.83	0.55
LogProbit[c]	198.354	0	26.37	19.57	-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-13. BMDS LogLogistic dose-response modeling results using BMRs of 10, 30, and 50% for the combined incidence of hepatic adenomas and carcinomas in female BDF1 mice (Kano et al., 2009, 594539).

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.

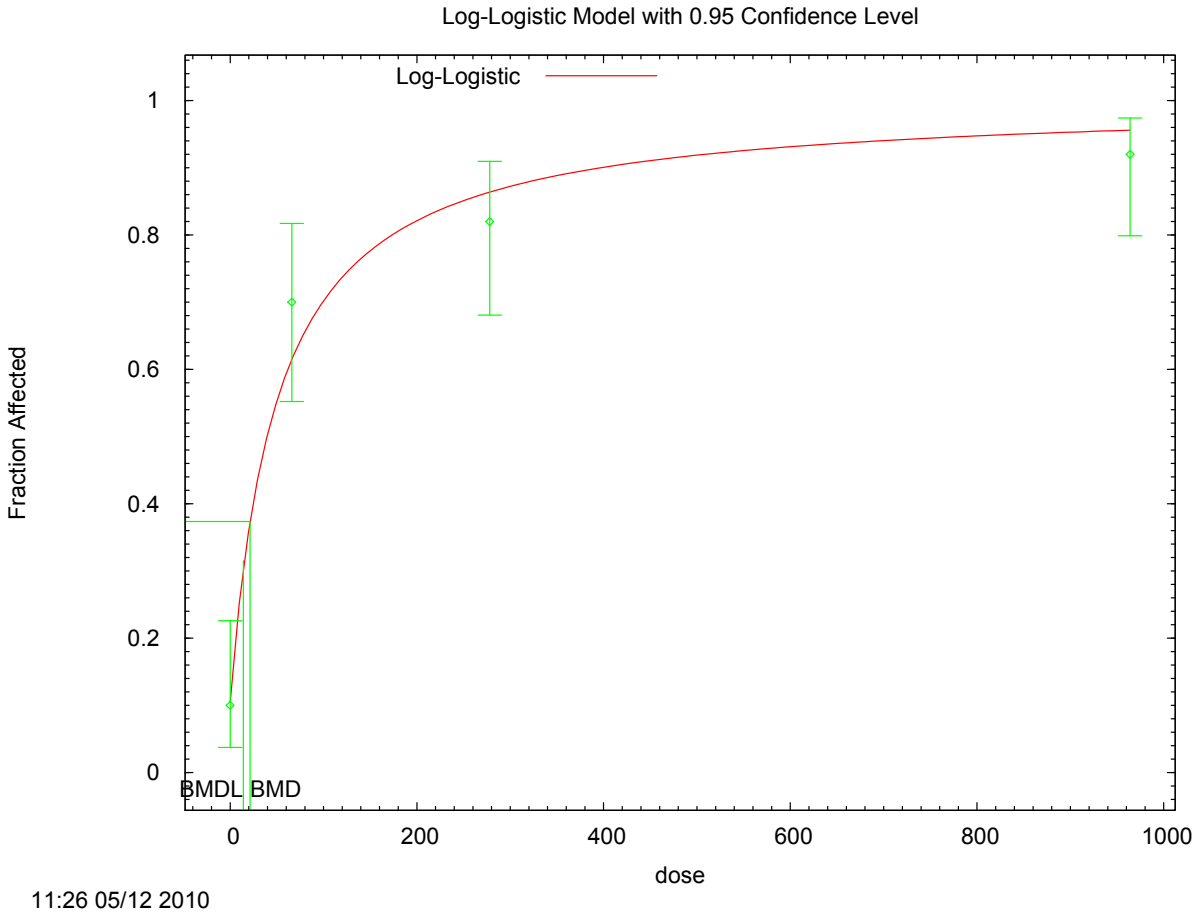


Source: Used with permission from Elsevier, Ltd., Kano et al. (2009, 594539).

Figure D-10. 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.plt
                                          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, 594539).

Figure D-11. 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.plt
                                        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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.105265	*	*	*
intercept	-3.90961	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-84.3055	4			
Fitted model	-86.107	2	3.6029	2	0.1651
Reduced model	-131.248	1	93.8853	3	<.0001

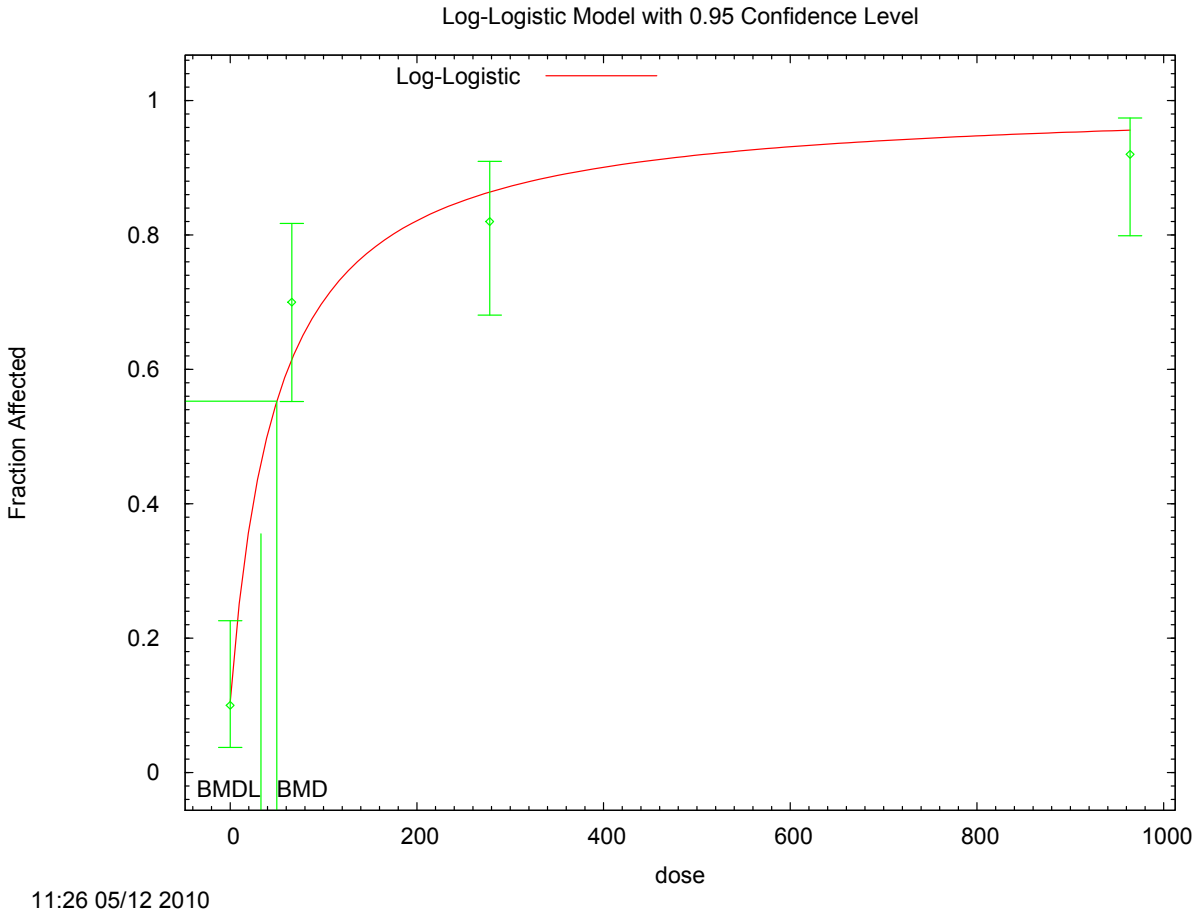
AIC: 176.214

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1053	5.263	5.000	50	-0.121
66.0000	0.6149	30.743	35.000	50	1.237
278.0000	0.8639	43.194	41.000	50	-0.905
964.0000	0.9560	47.799	46.000	50	-1.240

Chi^2 = 3.90 d.f. = 2 P-value = 0.1421

Benchmark Dose Computation
Specified effect = 0.3
Risk Type = Extra risk
Confidence level = 0.95
BMD = 21.377
BMDL = 14.1113

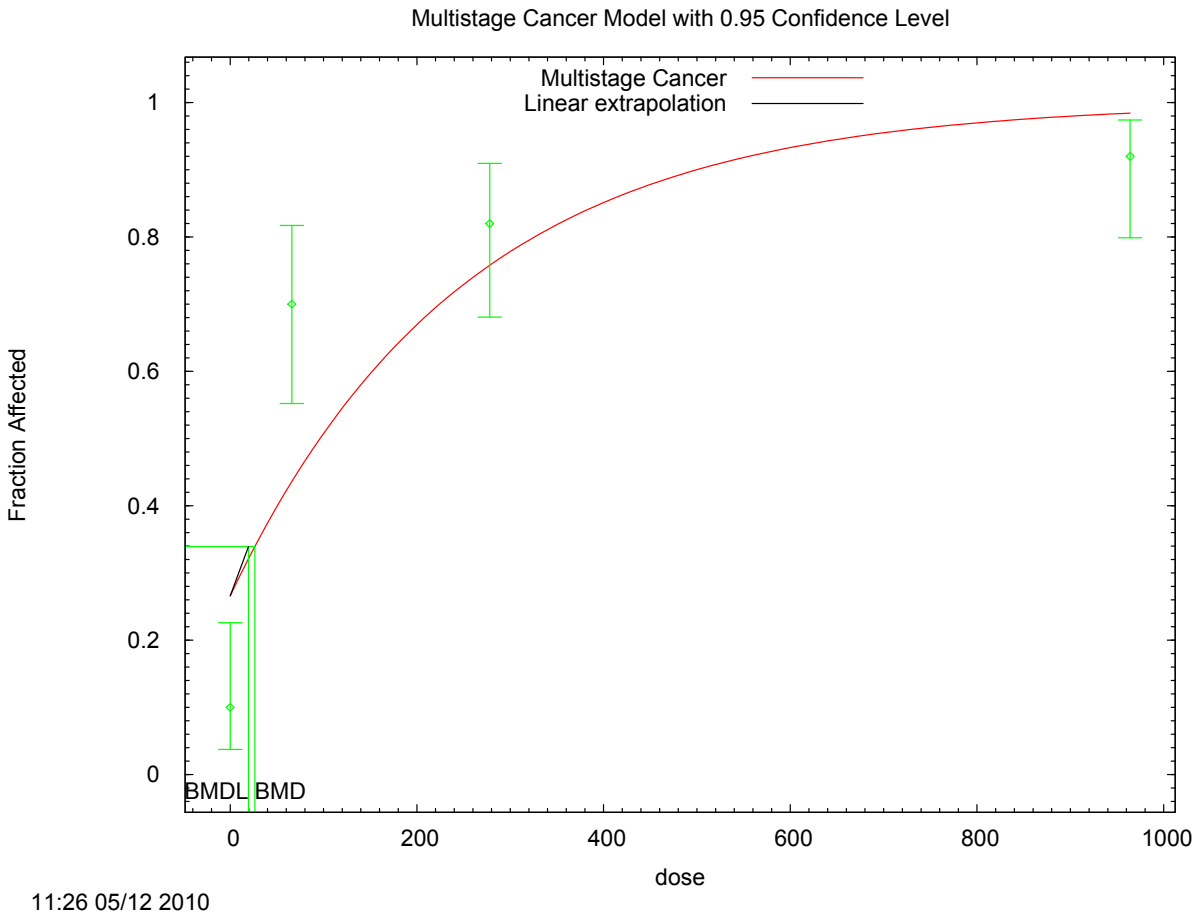


Source: Used with permission from Elsevier, Ltd., Kano et al. (2009, 594539).

Figure D-12. 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.plt
                                        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, 594539).

Figure D-13. Multistage BMD model (1 degree) for the combined incidence of hepatic adenomas and carcinomas in female BDF1 mice.

```
=================================================================
Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_fmouse_hepato_adcar_Msc-BMR10-1poly.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_fmouse_hepato_adcar_Msc-BMR10-1poly.plt
                                          Wed May 12 11:26:31 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 = 0.51713
Beta(1) = 0.00201669

Asymptotic Correlation Matrix of Parameter Estimates

	Background	Beta(1)
Background	1	-0.65
Beta(1)	-0.65	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.265826	*	*	*
Beta(1)	0.00398627	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-84.3055	4			
Fitted model	-99.6653	2	30.7195	2	2.1346928e-007
Reduced model	-131.248	1	93.8853	3	<.0001

AIC: 203.331

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.2658	13.291	5.000	50	-2.654
66.0000	0.4357	21.783	35.000	50	3.770
278.0000	0.7576	37.880	41.000	50	1.030
964.0000	0.9843	49.213	46.000	50	-3.651

Chi^2 = 35.65 d.f. = 2 P-value = 0.0000

Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Extra risk
Confidence level = 0.95
BMD = 26.4309
BMDL = 19.5045
BMDU = 37.5583

Taken together, (19.5045, 37.5583) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00512702

D.6. MALE BDF1 MICE: HEPATIC CARCINOMAS AND ADENOMAS

Data for hepatic carcinomas and adenomas in male BDF1 mice (Kano et al., 2009, 594539) are shown in Table D-14. 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-14. Data for hepatic adenomas and carcinomas in male BDF1 mice (Kano et al., 2009, 594539)

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

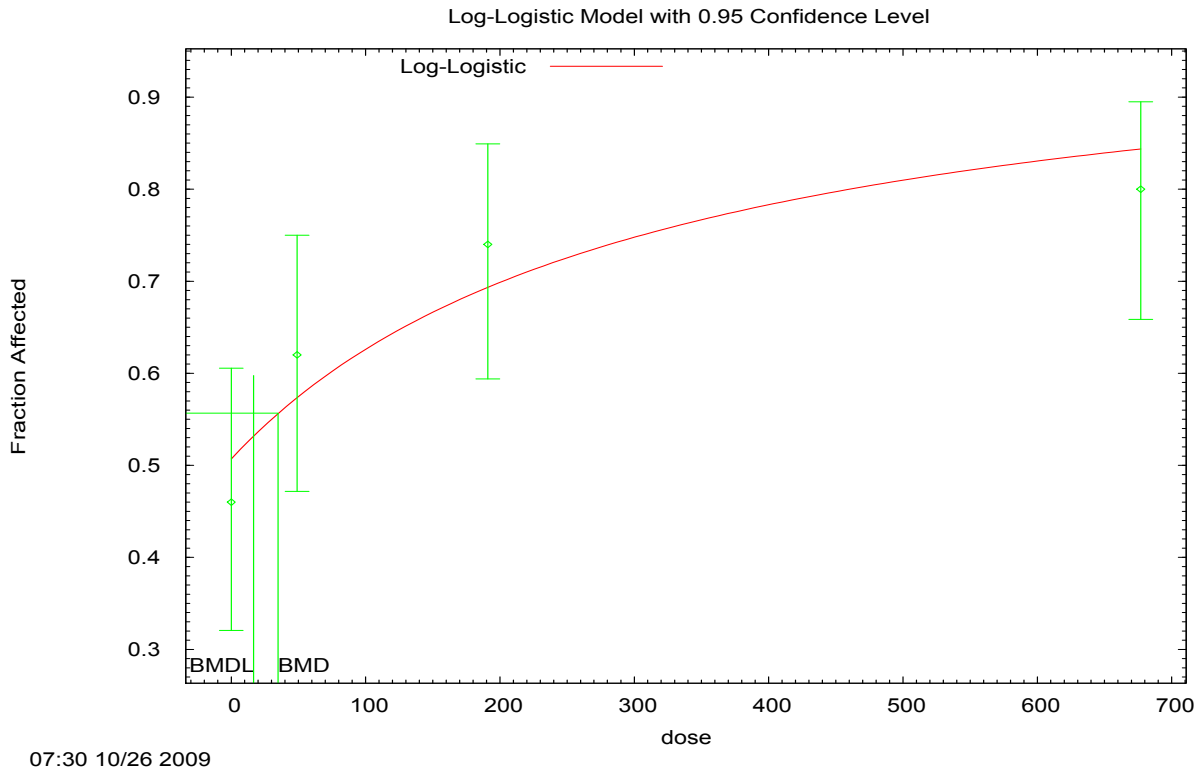
Source: Used with permission from Elsevier, Ltd., Kano et al. (2009, 594539).

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-15.

Table D-15. BMDS dose-response modeling results for the combined incidence of hepatic adenomas and carcinomas in male BDF1 mice (Kano et al., 2009, 594539)

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, 594539).

Figure D-14. LogLogistic BMD model for the combined incidence of hepatic adenomas and carcinomas in male BDF1 mice.

```
================================================================
Logistic Model. (Version: 2.12; Date: 05/16/2008)
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_mmouse_hepato_adcar_Lnl-BMR10-
Restrict.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_kano2009_mmouse_hepato_adcar_Lnl-BMR10-
Restrict.plt
Thu Nov 12 09:09:36 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 = 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 = 0.46
intercept = -5.58909
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	background	intercept
background	1	-0.69
intercept	-0.69	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.507468	*	*	*
intercept	-5.74623	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-121.373	4			
Fitted model	-122.419	2	2.09225	2	0.3513
Reduced model	-128.859	1	14.9718	3	0.001841

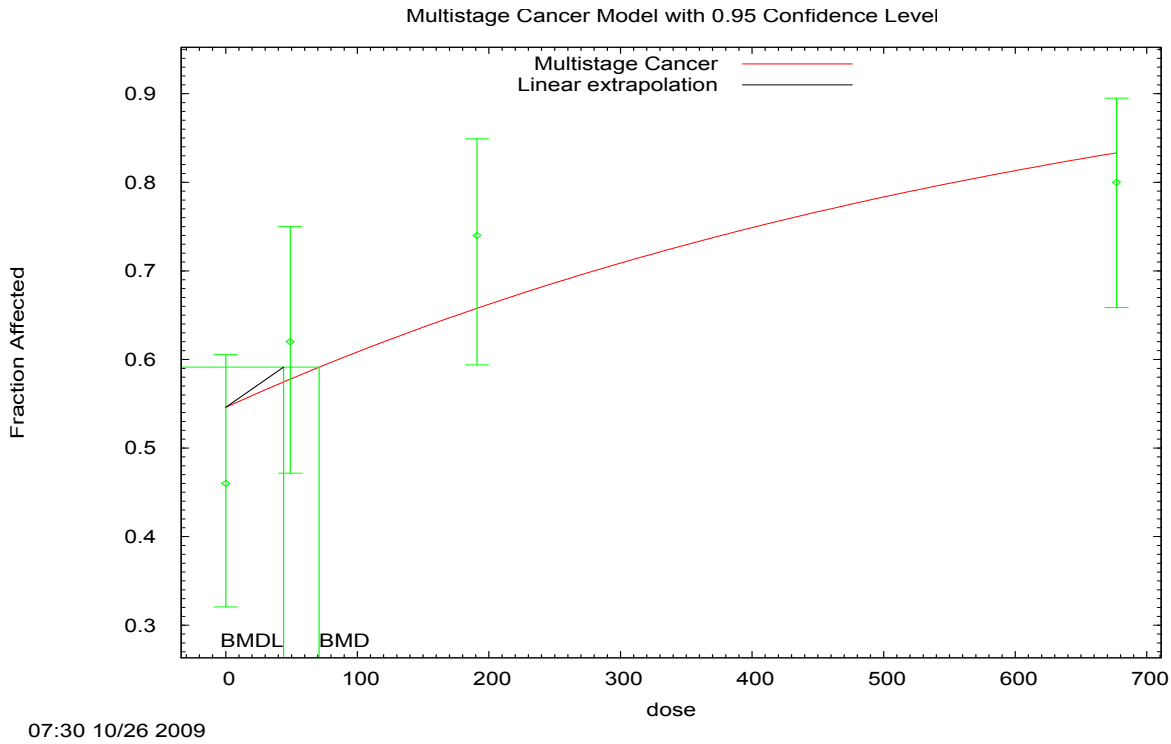
AIC: 248.839

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.5075	25.373	23.000	50	-0.671
49.0000	0.5741	28.707	31.000	50	0.656
191.0000	0.6941	34.706	37.000	50	0.704
677.0000	0.8443	42.214	40.000	50	-0.863

Chi^2 = 2.12 d.f. = 2 P-value = 0.3461

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 34.7787
BMDL = 16.5976



Source: Used with permission from Elsevier, Ltd., Kano et al. (2009, 594539).

Figure D-15. Multistage BMD model (1 degree) for the combined incidence of hepatic adenomas and carcinomas in male BDF1 mice.

```
=====================================================================
Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
Input Data File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mmouse_hepato_adcar_Msc-BMR10-1poly.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_kano2009_mmouse_hepato_adcar_Msc-BMR10-1poly.plt
Mon Oct 26 08:30:50 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 =     0.573756
Beta(1) =   0.00123152

Asymptotic Correlation Matrix of Parameter Estimates
            Background      Beta(1)
Background       1           -0.58
Beta(1)        -0.58            1


Parameter Estimates

                                                        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 (Kociba et al., 1974, 062929; NCI, 1978, 062935) were evaluated for comparison with the Kano et al. (2009, 594539) 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-16.

Table D-16. Summary of BMDS dose-response modeling estimates associated with liver and nasal tumor incidence data resulting from chronic oral exposure to 1,4-dioxane in rats and mice

Endpoint	Model selection criterion	Model Type	AIC	*p*-value	BMD_{10} mg/kg-day	$BMDL_{10}$ mg/kg-day	$BMD_{10\ HED}$ mg/kg-day	$BMDL_{10\ HED}$ mg/kg-day
Kociba et al., (1974, 062929) 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, 062935) 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, 062935) 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, 062935) 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, 062935) 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., 1974, 062929)

The incidence data for hepatocellular carcinoma and nasal squamous cell carcinoma are presented in Table D-17. The predicted $BMD_{10\ HED}$ and $BMDL_{10\ HED}$ values are also presented in Tables D-18 and D-19 for hepatocellular carcinomas and nasal squamous cell carcinomas, respectively.

Table D-17. Incidence of hepatocellular carcinoma and nasal squamous cell carcinoma in male and female Sherman rats (combined) (Kociba et al., 1974, 062929) treated with 1,4-dioxane in the drinking water for 2 years

Animal Dose (mg/kg-day) (average of male and female dose)	Incidence of hepatocellular carcinoma[a]	Incidence of nasal squamous cell carcinoma[a]
0	1/106[b]	0/106[c]
14	0/110	0/110
121	1/106	0/106
1307	10/66[d]	3/66[d]

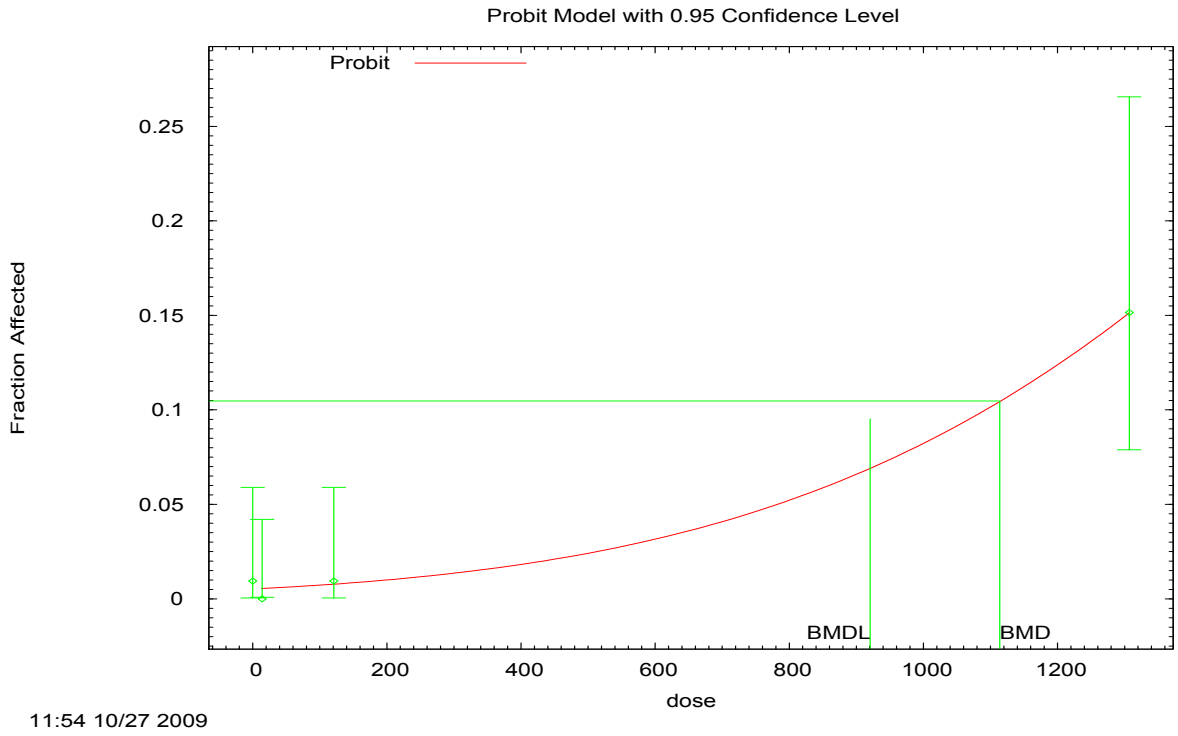
[a]Rats surviving until 12 months on study.
[b]$p < 0.001$; positive dose-related trend (Cochran-Armitage test).
[c]$p < 0.01$; positive dose-related trend (Cochran-Armitage test).
[d]$p < 0.001$; Fisher's Exact test.

Source: Used with permission from Elsevier, Ltd., Kociba et al. (1974, 062929).

Table D-18. BMDS dose-response modeling results for the incidence of hepatocellular carcinoma in male and female Sherman rats (combined) (Kociba et al., 1974, 062929) 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. (1974, 062929).

Figure D-16. Probit BMD model for the incidence of hepatocellular carcinoma in male and female Sherman rats exposed to 1,4-dioxane in drinking water.

```
=====================================================================
Probit Model. (Version: 3.1;  Date: 05/16/2008)
Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\pro_kociba_mf_rat_hepato_car_Prb-
BMR10.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\pro_kociba_mf_rat_hepato_car_Prb-BMR10.plt
Tue Oct 27 12:54:14 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

Initial (and Specified) Parameter Values
background =       0   Specified
intercept =     -2.62034
```

slope = 0.0012323

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	-0.82
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

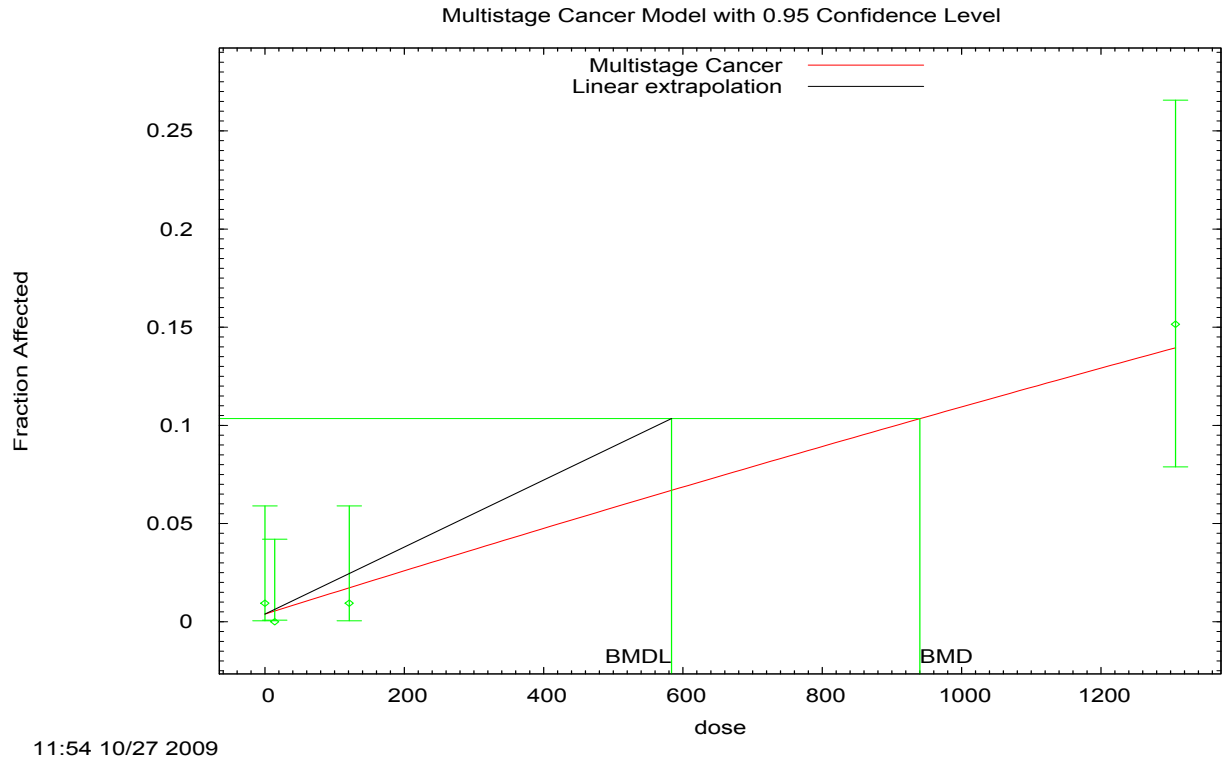
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0052	0.555	1.000	106	0.598
14.0000	0.0055	0.604	0.000	110	-0.779
121.0000	0.0078	0.827	1.000	106	0.191
1307.0000	0.1517	10.014	10.000	66	-0.005

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 = 1113.94
BMDL = 920.616



Source: Used with permission from Elsevier, Ltd., Kociba et al. (1974, 062929).

Figure D-17. Multistage BMD model (1 degree) for the incidence of hepatocellular carcinoma in male and female Sherman rats exposed to 1,4-dioxane in drinking water.

```
=================================================================
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

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0039	0.410	1.000	106	0.923
14.0000	0.0054	0.597	0.000	110	-0.775
121.0000	0.0173	1.832	1.000	106	-0.620
1307.0000	0.1396	9.213	10.000	66	0.279

Chi^2 = 1.92 d.f. = 2 P-value = 0.3838

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 940.124
BMDL = 583.576
BMDU = 1685.88

Taken together, (583.576, 1685.88) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.000171357

Table D-19. BMDS dose-response modeling results for the incidence of nasal squamous cell carcinoma in male and female Sherman rats (combined) (Kociba et al., 1974, 062929) 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	1572.09	1305.86	0	410.37	340.87
Logistic	28.4078	1	1363.46	1306.67	0	355.91	341.09
LogLogistic	28.4078	1	1464.77	1306.06	0	382.35	340.93
LogProbit[b]	28.4078	1	1644.38	1305.49	0	429.24	340.78
Multistage-Cancer (1 degree)	27.3521	0.9163	3464.76	1525.36	0.272	904.42	398.17
Multistage-Cancer (2 degree)	26.4929	0.9977	1980.96	1314.37	0.025	517.10	343.10
Multistage-Cancer (3 degree)[c]	26.4156	0.9999	1717.16	1306.29	0.002	448.24	340.99
Probit	28.4078	1	1419.14	1306.44	0	370.44	341.03
Weibull	28.4078	1	1461.48	1306.11	0	381.50	340.94
Quantal-Linear	27.3521	0.9163	3464.76	1525.35	0.272	904.42	398.17
Dichotomous-Hill	30.4078	0.9997	1465.77	1319.19	5.53×10^{-7}	382.62	344.35

[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.

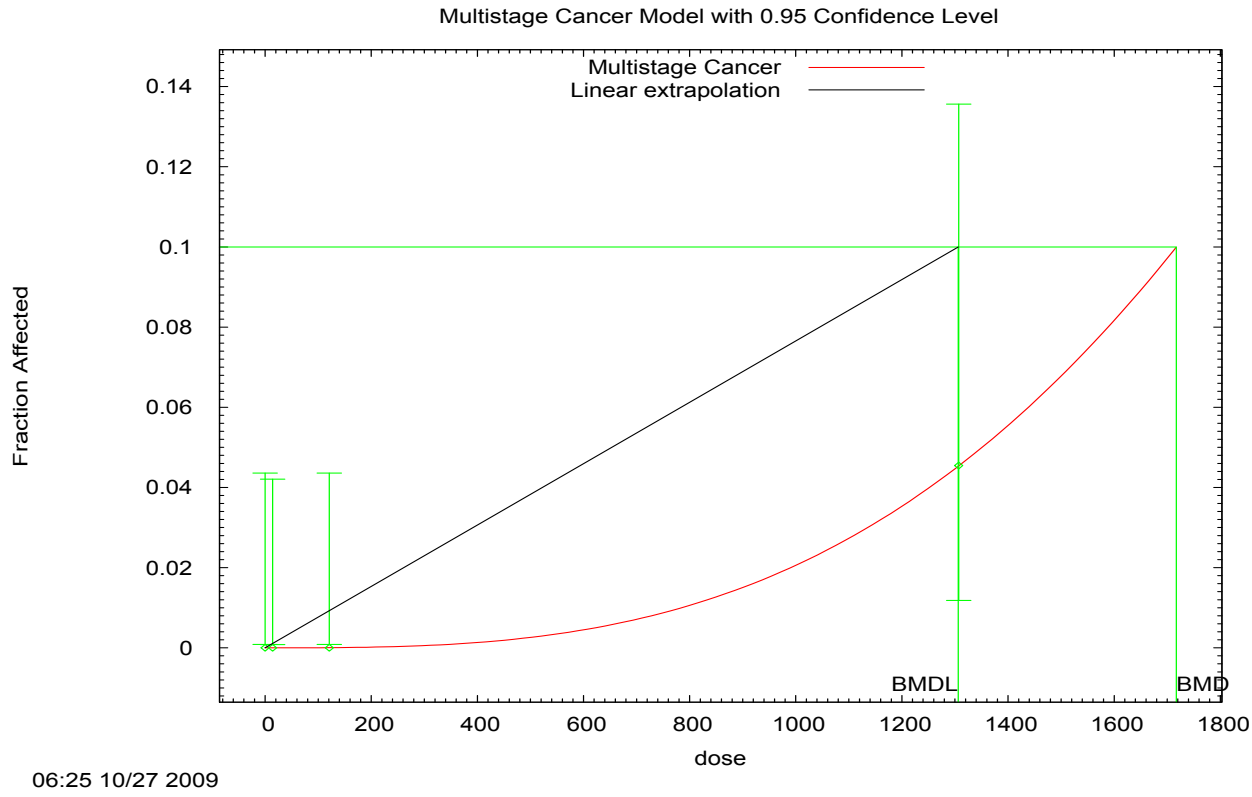


Figure D-18. Multistage BMD model (3 degree) for the incidence of nasal squamous cell carcinoma in male and female Sherman rats exposed to 1,4-dioxane in drinking water.

```
=================================================================
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 =       1717.16
            BMDL =       1306.29
            BMDU =       8354.46

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, 062935)

The incidence data for hepatocellular adenoma (female rats) and nasal squamous cell carcinoma (male and female rats) are presented in Table D-20. 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 Tables D-21 through D-23.

Table D-20. Incidence of nasal cavity squamous cell carcinoma and hepatocellular adenoma in Osborne-Mendel rats (NCI, 1978, 062935) 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]

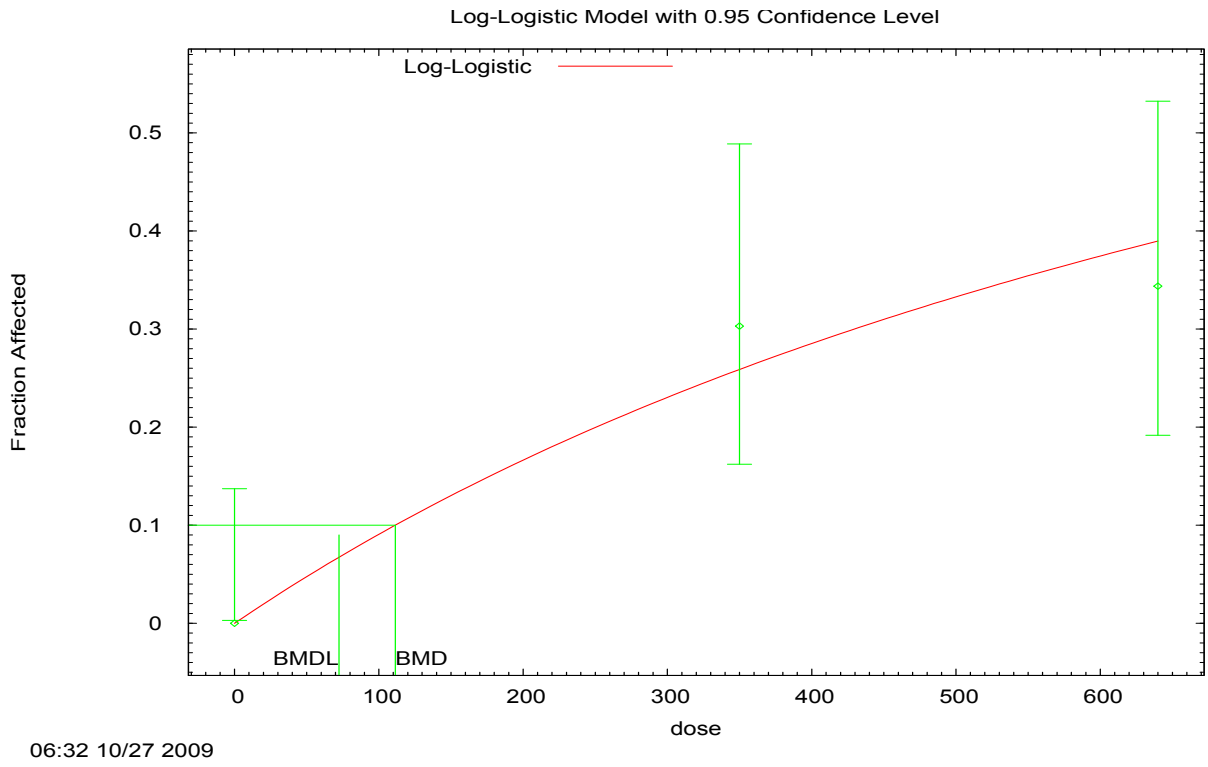
[a]Tumor incidence values were adjusted for mortality (animals surviving to 52 weeks, presented in text of NCI, 1978, 062935).
[b]Group not included in statistical analysis by NCI (1978, 062935) because the dose group was started a year earlier without appropriate controls.
[c]$p \leq 0.001$; positive dose-related trend (Cochran-Armitage test).
[d]$p \leq 0.001$; Fisher's Exact test.

Source: NCI (1978, 062935).

Table D-21. BMDS dose-response modeling results for the incidence of hepatocellular adenoma in female Osborne-Mendel rats (NCI, 1978, 062935) 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, 062935).

Figure D-19. LogLogistic BMD model for the incidence of hepatocellular adenoma in female Osborne-Mendel rats exposed to 1,4-dioxane in drinking water.

```
================================================================
Logistic Model. (Version: 2.12; Date: 05/16/2008)
Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_nci_frat_hepato_ad_Lnl-BMR10-
Restrict.(d)
Gnuplot Plotting File:  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_nci_frat_hepato_ad_Lnl-
BMR10-Restrict.plt
Tue Oct 27 07:32:13 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 = -6.62889
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -background -slope have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	intercept
intercept	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	*	*	*
intercept	-6.91086	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-40.8343	3			
Fitted model	-41.141	1	0.613564	2	0.7358
Reduced model	-50.4308	1	19.1932	2	<.0001

AIC: 84.2821

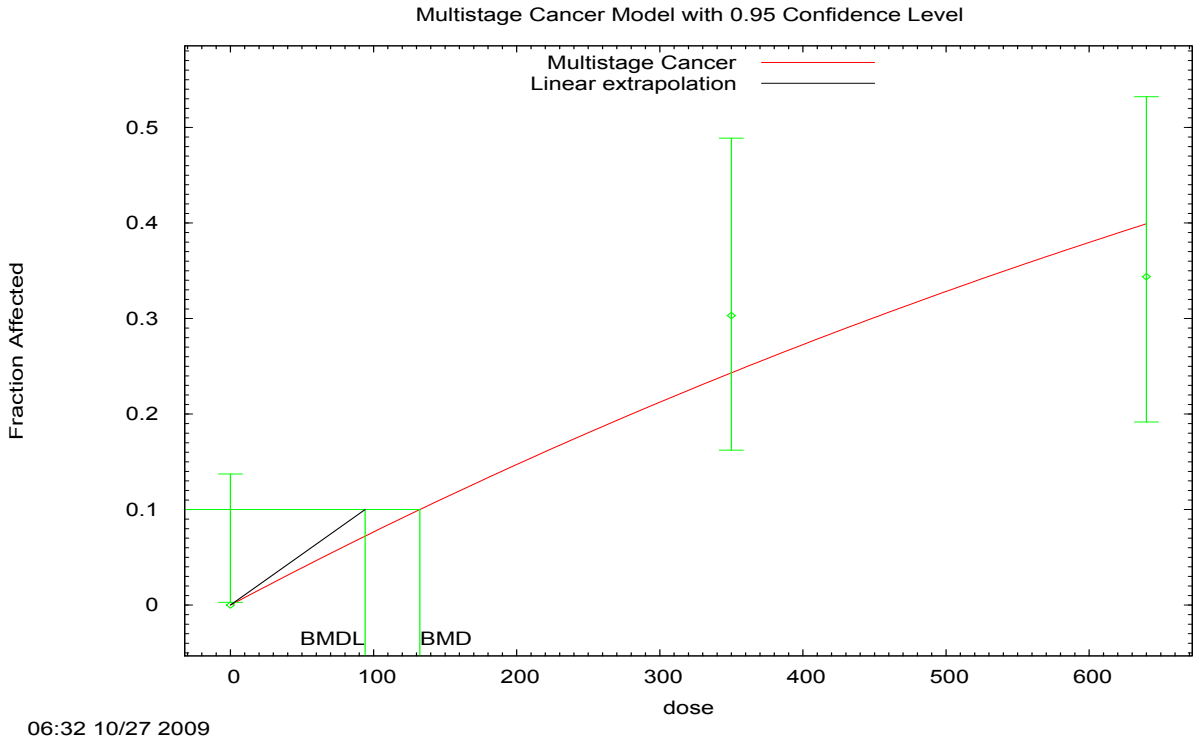
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	31	0.000
350.0000	0.2587	8.536	10.000	33	0.582
640.0000	0.3895	12.464	11.000	32	-0.531

Chi^2 = 0.62 d.f. = 2 P-value = 0.7333

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 111.457
BMDL = 72.4092



Source: NCI (1978, 062935).

Figure D-20. Multistage BMD model (1 degree) for the incidence of hepatocellular adenoma in female Osborne-Mendel rats exposed to 1,4-dioxane in drinking water.

```
=================================================================
Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_frat_hepato_ad_Msc-BMR10-
1poly.(d)
Gnuplot Plotting File:  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_frat_hepato_ad_Msc-
BMR10-1poly.plt
Tue Oct 27 07:32:16 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 = 0.0385912
Beta(1) = 0.000670869
Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Background have been estimated at a boundary point, or have been specified by the user, and do not appear in the correlation matrix)

	Beta(1)
Beta(1)	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0	*	*	*
Beta(1)	0.00079602	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-40.8343	3			
Fitted model	-41.3486	1	1.02868	2	0.5979
Reduced model	-50.4308	1	19.1932	2	<.0001

AIC: 84.6972

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	31	0.000
350.0000	0.2432	8.024	10.000	33	0.802
640.0000	0.3992	12.774	11.000	32	-0.640

Chi^2 = 1.05 d.f. = 2 P-value = 0.5908

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 132.359
BMDL = 94.0591
BMDU = 194.33

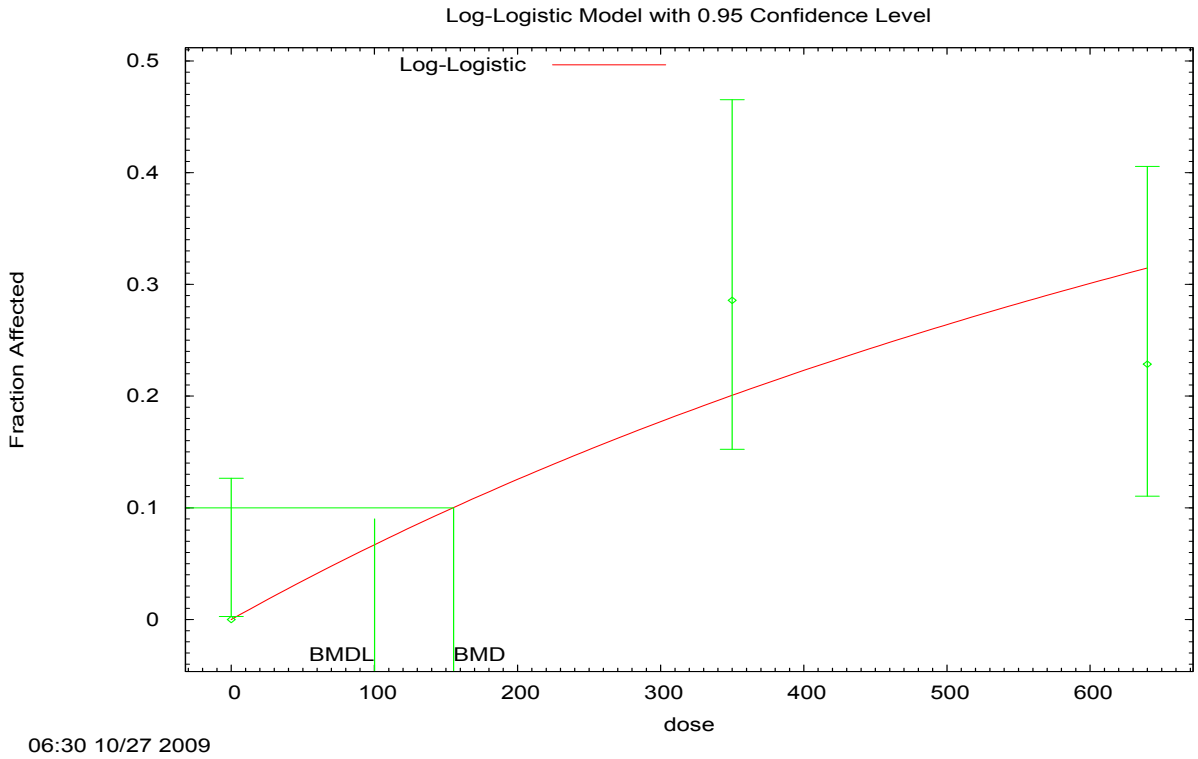
Taken together, (94.0591, 194.33) is a 90% two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.00106316

Table D-22. BMDS dose-response modeling results for the incidence of nasal cavity squamous cell carcinoma in female Osborne-Mendel rats (NCI, 1978, 062935) 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, 062935).

Figure D-21. LogLogistic BMD model for the incidence of nasal cavity squamous cell carcinoma in female Osborne-Mendel rats exposed to 1,4-dioxane in drinking water.

```
================================================================
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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

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 = -6.64005
slope = 1

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

	intercept
intercept	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0	*	*	*
intercept	-7.24274	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-39.7535	3			
Fitted model	-41.1117	1	2.71651	2	0.2571
Reduced model	-47.9161	1	16.3252	2	0.0002851

AIC: 84.2235

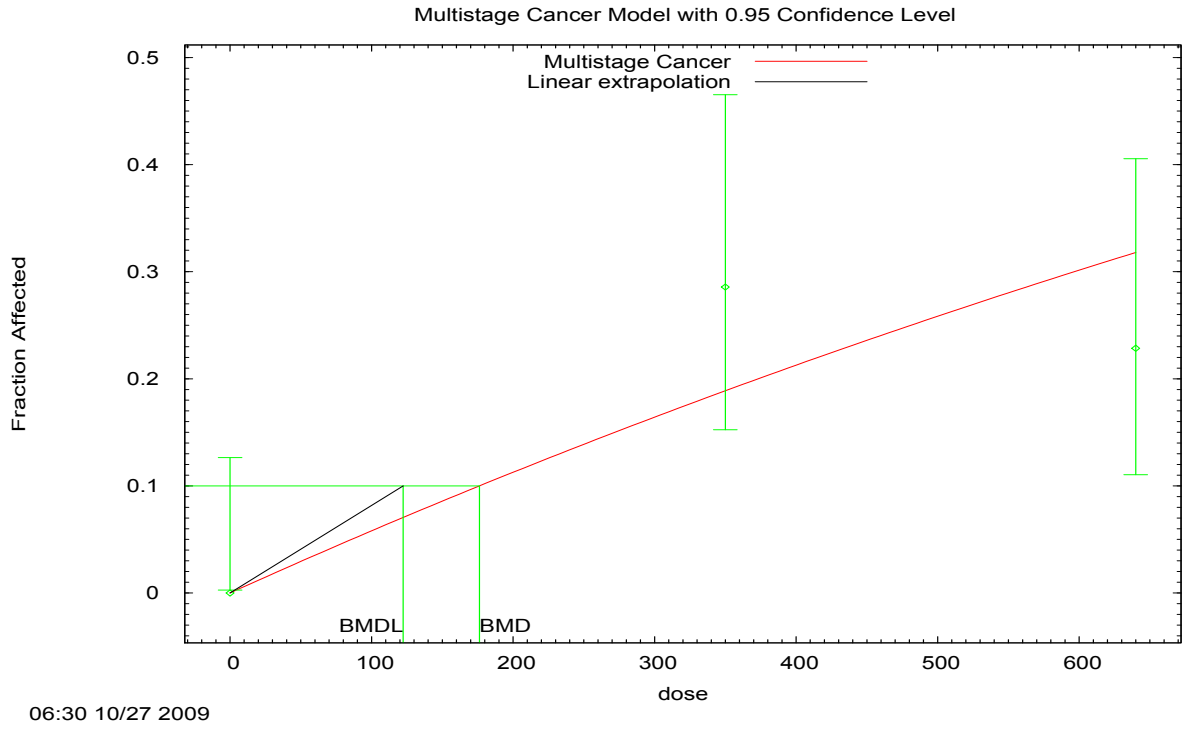
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0000	0.000	0.000	34	0.000
350.0000	0.2002	7.008	10.000	35	1.264
640.0000	0.3140	10.992	8.000	35	-1.090

Chi^2 = 2.78 d.f. = 2 P-value = 0.2486

Benchmark Dose Computation

Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 155.324
BMDL = 100.081



Source: NCI (1978, 062935).

Figure D-22. Multistage BMD model (1 degree) for the incidence of nasal cavity squamous cell carcinoma in female Osborne-Mendel rats exposed to 1,4-dioxane in drinking water.

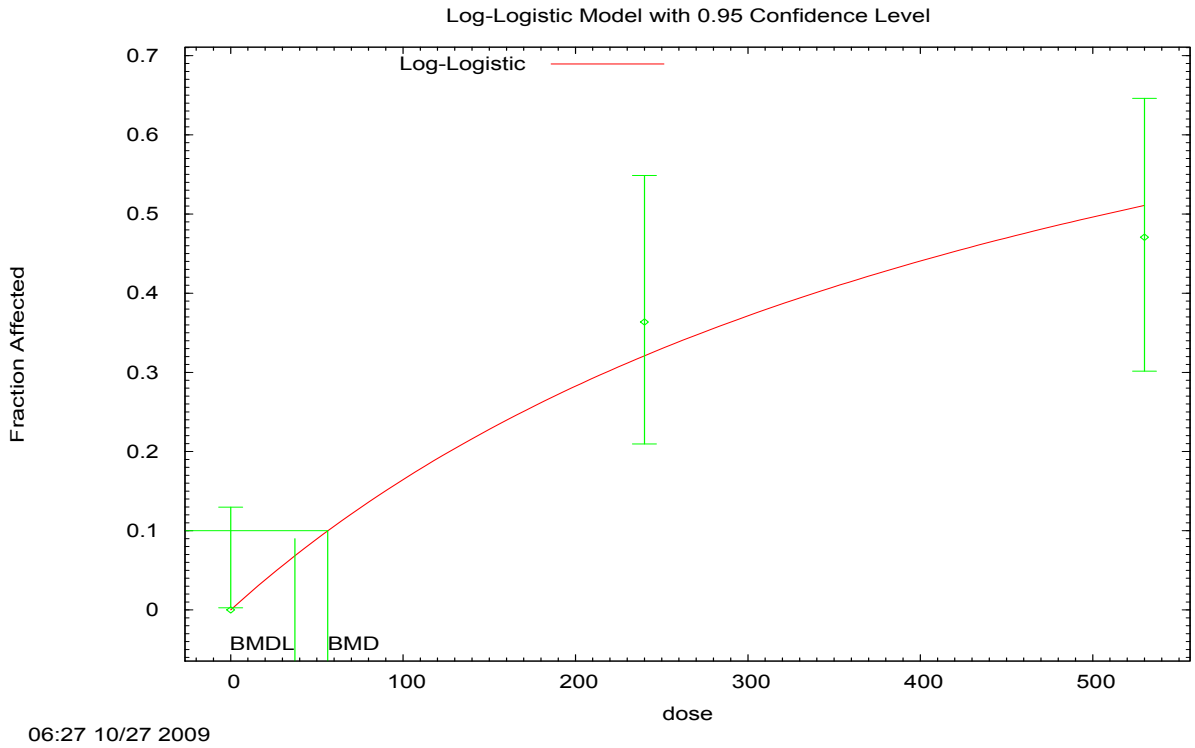
```
================================================================
Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_frat_nasal_car_Msc-BMR10-
1poly.(d)
Gnuplot Plotting File:  L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_frat_nasal_car_Msc-
BMR10-1poly.plt
Tue Oct 27 07:30:12 2009
================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

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 =    0.0569154
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-23. BMDS dose-response modeling results for the incidence of nasal cavity squamous cell carcinoma in male Osborne-Mendel rats (NCI, 1978, 062935) 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, 062935).

Figure D-23. LogLogistic BMD model for the incidence of nasal cavity squamous cell carcinoma in male Osborne-Mendel rats exposed to 1,4-dioxane in drinking water.

```
================================================================================
Logistic Model. (Version: 2.12; Date: 05/16/2008)
Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\lnl_nci_mrat_nasal_car_Lnl-BMR10-
Restrict.(d)
Gnuplot Plotting File:  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

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval 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

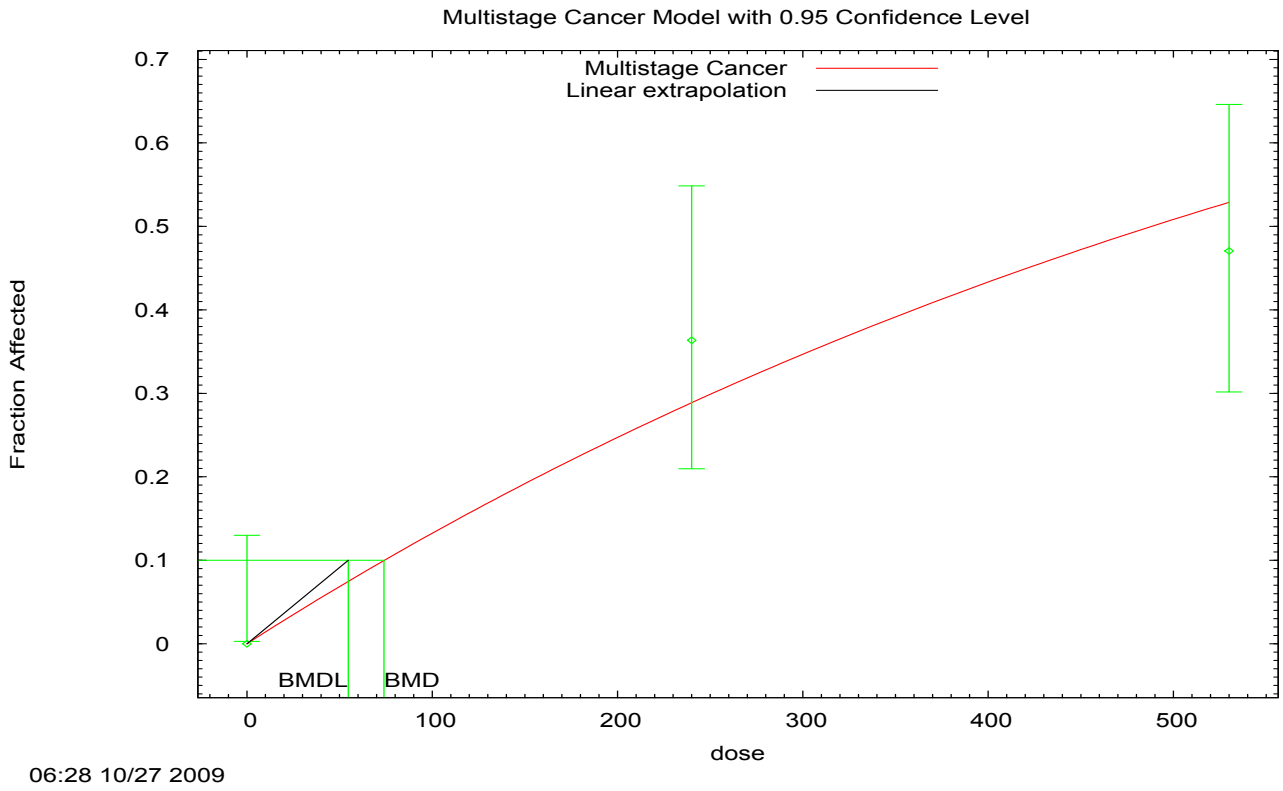
Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled 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, 062935).

Figure D-24. Multistage BMD model (1 degree) for the incidence of nasal cavity squamous cell carcinoma in male Osborne-Mendel rats exposed to 1,4-dioxane in drinking water.

```
================================================================
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, 062935)

The incidence data for hepatocellular adenoma or carcinoma in male and female mice are presented in Table D-24. The 2-degree polynomial model (betas restricted $\geq$ 0) was the lowest degree polynomial that provided an adequate fit to the female mouse data (Figure D-25), while the gamma model provided the best fit to the male mouse data (Figure D-26). The results of the BMDS modeling for the entire suite of models are presented in Tables D-25 and D-26 for the female and male data, respectively.

Table D-24. Incidence of hepatocellular adenoma or carcinoma in male and female B6C3F$_1$ mice (NCI, 1978, 062935) 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]

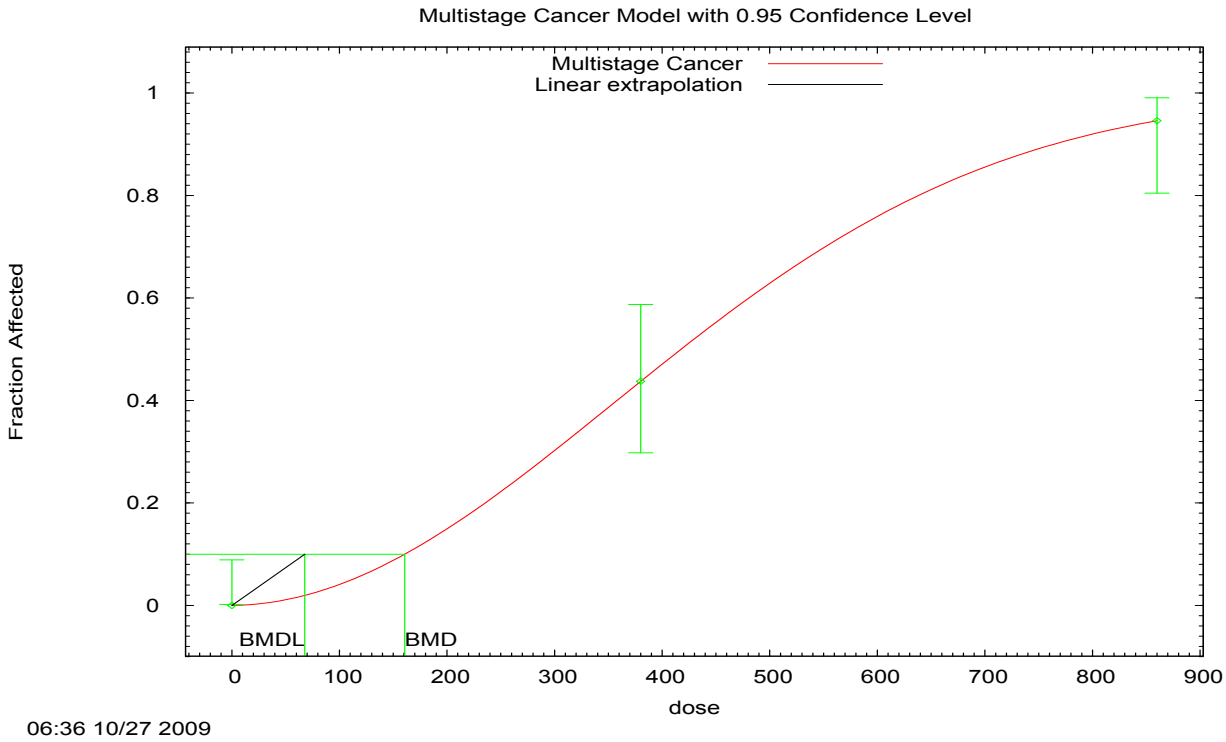
[a]Tumor incidence values were not adjusted for mortality.
[b]$p < 0.001$, positive dose-related trend (Cochran-Armitage test).
[c]$p < 0.001$ by Fisher's Exact test pair-wise comparison with controls.
[d]$p = 0.014$.

Source: NCI (1978, 062935).

Table D-25. BMDS dose-response modeling results for the combined incidence of hepatocellular adenoma or carcinoma in female B6C3F$_1$ mice (NCI, 1978, 062935) 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, 062935).

Figure D-25. 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.

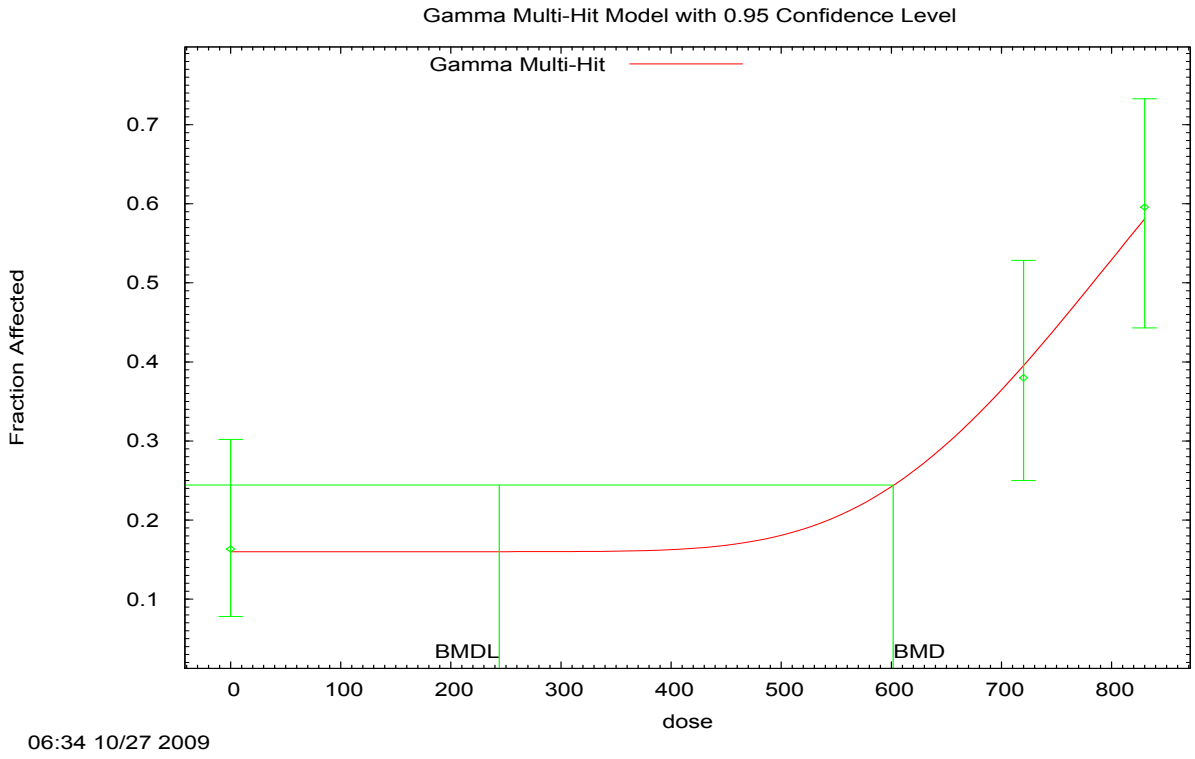
```
================================================================================
Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
Input Data File: L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_fmouse_hepato_adcar_Msc-
BMR10-2poly.(d)
Gnuplot Plotting File:
L:\Priv\NCEA_HPAG\14Dioxane\BMDS\msc_nci_fmouse_hepato_adcar_Msc-BMR10-2poly.plt
Tue Oct 27 07:36:26 2009
================================================================================
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

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
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-26. BMDS dose-response modeling results for the combined incidence of hepatocellular adenoma or carcinoma in male B6C3F$_1$ mice (NCI, 1978, 062935) 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, 062935).

Figure D-26. Gamma BMD model for the incidence of hepatocellular adenoma or carcinoma in male B6C3F$_1$ mice exposed to 1,4-dioxane in drinking water.

```
================================================================
Gamma Model. (Version: 2.13;  Date: 05/16/2008)
Input Data File: 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
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

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 =         0.17
Slope =          0.000671886
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	-0.52
Slope	-0.52	1

Parameter Estimates

			95.0% Wald Confidence Interval	
Variable	Estimate	Std. Err.	Lower Conf. Limit	Upper Conf. Limit
Background	0.160326	0.0510618	0.060247	0.260405
Slope	0.0213093	0.000971596	0.019405	0.0232136
Power	18	NA		

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	-86.7213	3			
Fitted model	-86.7693	2	0.096042	1	0.7566
Reduced model	-96.715	1	19.9875	2	<.0001

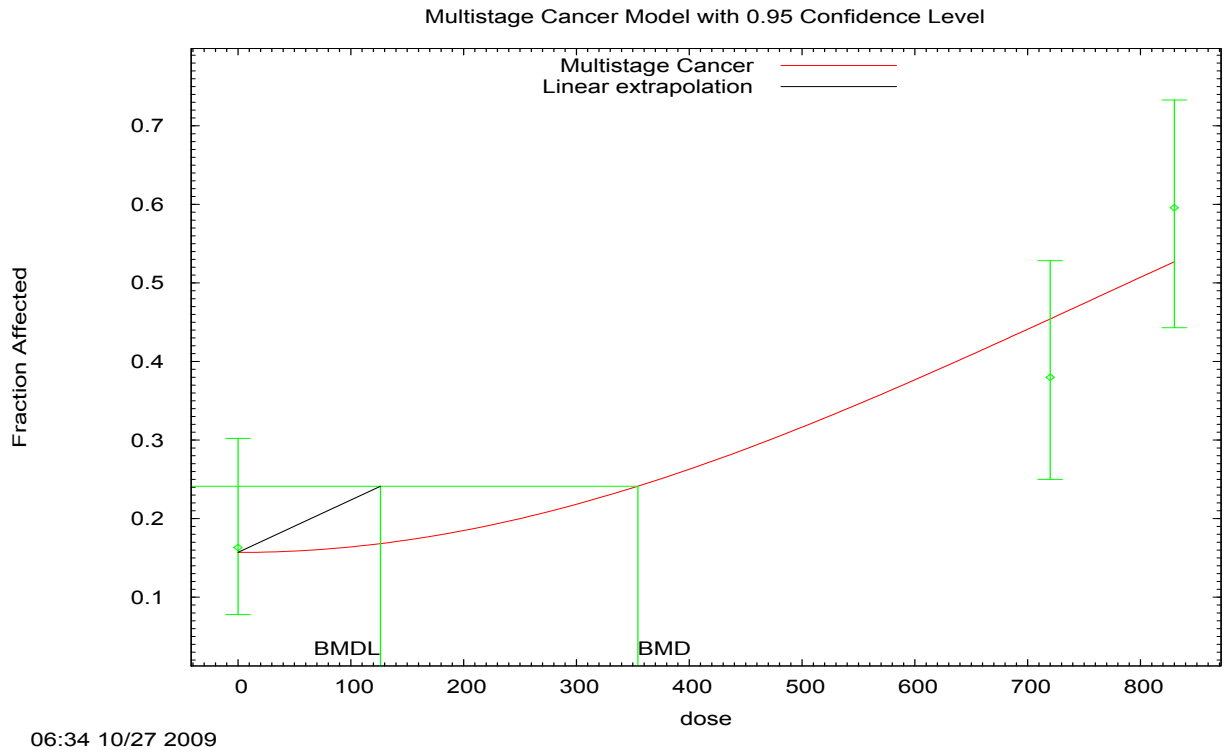
AIC: 177.539

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1603	7.856	8.000	49	0.056
720.0000	0.3961	19.806	19.000	50	-0.233
830.0000	0.5817	27.339	28.000	47	0.196

Chi^2 = 0.10 d.f. = 1 P-value = 0.7571

Benchmark Dose Computation
Specified effect = 0.1
Risk Type = Extra risk
Confidence level = 0.95
BMD = 601.692
BMDL = 243.917



Source: NCI (1978, 062935).

Figure D-27. 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., 1994, 196120), once as a detailed laboratory report (JBRC, 1998, 196240), and once as a published manuscript (Kano et al., 2009, 594539). After the External Peer Review draft of the *Toxicological Review of 1,4-Dioxane* (U.S. EPA, 2009, 628630) had been released, the Kano et al. (2009, 594539) 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 (JBRC, 1998, 196240; Kano et al., 2009, 594539; Yamazaki et al., 1994, 196120). Tables E-1 and E-2 show the histological nonneoplastic findings provided for male and female F344 rats, respectively. Tables E-3 and E-4 show the histological neoplastic findings provided for male and female F344 rats, respectively. Tables E-5 and E-6 show the histological nonneoplastic findings provided for male and female F344 rats, respectively. Tables E-7 and E-8 show the histological neoplastic findings provided for male and female Crj:BDF1 mice, respectively.

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

Table E-1. Nonneoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in male F344 rats

		Yamazaki et al. (1994)[a]				JBRC (1998)				Kano et al. (2009)			
		Drinking water concentration (ppm)											
		0	200	1,000	5,000	0	200	1,000	5,000	0	200	1,000	5,000
		Calculated Dose (Intake [mg/kg-day])b,c											
		Not reported				Control (0)	8-24 (16)	41-121 (81)	209-586 (398)	0	11±1	55±3	274±18
Nasal respiratory epithelium; nuclear enlargement	All animals	Not reported				0/50	0/50	0/50	26/50	0/50	0/50	0/50	26/50[e]
	Sacrificed animals	Not reported				0/40	0/45	0/35	12/22[e]	Not reported			
Nasal respiratory epithelium; squamous cell metaplasia	All animals	0/50	0/50	0/50	31/50	0/50	0/50	0/50	31/50	0/50	0/50	0/50	31/50[e]
	Sacrificed animals	Not reported				0/40	0/45	0/35	15/22[e]	Not reported			
Nasal respiratory epithelium; squamous cell hyperplasia	All animals	0/50	0/50	0/50	2/50	0/50	0/50	0/50	2/50	0/50	0/50	0/50	2/50
	Sacrificed animals	Not reported				0/40	0/45	0/35	1/22	Not reported			
Nasal gland; proliferation	All animals	0/50	0/50	0/50	5/50	Not reported				Not reported			
	Sacrificed animals	Not reported				Not reported				Not reported			
Nasal olfactory epithelium; nuclear enlargement	All animals	Not reported				0/50	0/50	5/50	38/50	0/50	0/50	5/50	38/50[e]
	Sacrificed animals	Not reported				0/40	0/45	4/35	20/22[e]	Not reported			
Nasal olfactory epithelium; respiratory metaplasia	All animals	Not reported				12/50	11/50	20/50	43/50	Not reported			
	Sacrificed animals	Not reported				10/40	11/45	17/35	22/22[e]	Not reported			
Nasal olfactory epithelium; atrophy	All animals	Not reported				0/50	0/50	0/50	36/50	Not reported			
	Sacrificed animals	Not reported				0/40	0/45	0/35	17/22[e]	Not reported			
Lamina propria; hydropic change	All animals	Not reported				0/50	0/50	0/50	46/50	Not reported			
	Sacrificed animals	Not reported				0/40	0/45	0/35	20/22[e]	Not reported			
Lamina propria; sclerosis	All animals	Not reported				0/50	0/50	1/50	44/50	Not reported			
	Sacrificed animals	Not reported				0/40	0/45	1/35	20/22[e]	Not reported			
Nasal cavity; adhesion	All animals	Not reported				0/50	0/50	0/50	48/50	Not reported			
	Sacrificed animals	Not reported				0/40	0/45	0/35	21/22[e]	Not reported			
Nasal cavity; inflammation	All animals	Not reported				0/50	0/50	0/50	13/50	Not reported			
	Sacrificed animals	Not reported				0/40	0/45	0/35	7/22[e]	Not reported			
Hyperplasia; liver	All animals	3/50	2/10	10/50	24/50	3/50	2/50	10/50	24/50	Not reported			
	Sacrificed animals	Not reported				3/40	2/45	9/35f	12/22[e]	Not reported			

		Yamazaki et al. (1994)[a]	JBRC (1998)	Kano et al. (2009)
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	All animals	Not reported	3/50 3/50 9/50 8/50	3/50 3/50 9/50 8/50
	Sacrificed animals	Not reported	3/40 3/45 9/35[f] 7/22[e]	Not reported
Acidophilic cell foci; liver	All animals	Not reported	Not reported	12/50 8/50 7/50 5/50
	Sacrificed animals	Not reported	Not reported	Not reported
Basophilic cell foci; liver	All animals	Not reported	7/50 11/50 6/50 16/50	7/50 11/50 8/50 16/50[f]
	Sacrificed animals	Not reported	7/40 11/45 6/35 8/22[f]	Not reported
Mixed-cell foci; liver	All animals	Not reported	2/50 8/50 14/50 13/50	2/50 8/50 14/50e 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. (1994). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.

[b]JBRC (1998, 196240) 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, 2009, 628630) .

[c]Kano et al. (2009, 594539) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in this final document (U.S. EPA, 2010, 625580).

[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-2. Nonneoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in female F344 rats

		Yamazaki et al. (1994)[a]	JBRC (1998)	Kano et al. (2009)
		Drinking water concentration (ppm)		
		0 200 1,000 5,000	**0 200 1,000 5,000**	**0 200 1,000 5,000**
		Calculated Dose (Intake [mg/kg-day])b,c		
		Not reported	**Control (0) 12-29 (21) 56-149 (103) 307-720 (514)**	**0 18±3 83±14 429±69**
Nasal respiratory epithelium; nuclear enlargement	All animals	Not reported	0/50 0/50 0/50 13/50	0/50 0/50 0/50 13/50[e]
	Sacrificed animals	Not reported	0/38 0/37 0/38 7/24[e]	Not reported
Nasal respiratory epithelium; squamous cell metaplasia	All animals	0/50 0/50 0/50 35/50	0/50 0/50 0/50 35/50	0/50 0/50 0/50 35/50[e]
	Sacrificed animals	Not reported	0/38 0/37 0/38 18/24[e]	Not reported
Nasal respiratory epithelium; squamous cell hyperplasia	All animals	0/50 0/50 0/50 5/50	0/50 0/50 0/50 5/50	0/50 0/50 0/50 5/50
	Sacrificed animals	Not reported	0/38 0/37 0/38 4/24[f]	Not reported
Nasal gland;	All animals	0/50 0/50 0/50 11/50	0/50 0/50 0/50 11/50	Not reported

		Yamazaki et al. (1994)[a]	JBRC (1998)	Kano et al. (2009)
proliferation	Sacrificed animals	Not reported	0/38 0/37 0/38 8/24[e]	Not reported
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	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	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	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	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	All animals	Not reported	1/50 1/50 3/50 11/50	1/50 1/50 3/50 11/50[f]
	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

	Yamazaki et al. (1994)[a]	JBRC (1998)	Kano et al. (2009)

[a]Dose rates (mg/kg-day) were not provided in Yamazaki et al. (1994). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.
[b]JBRC (1998, 196240) 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, 2009, 628630) .
[c]Kano et al. (2009, 594539) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in this final document (U.S. EPA, 2010, 625580).
[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-3. Neoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in male F344 rats

		Yamazaki et al. (1994)[a]				JBRC (1998)				Kano et al. (2009)			
		Drinking water concentration (ppm)											
		0	200	1,000	5,000	0	200	1,000	5,000	0	200	1,000	5,000
		Calculated Dose (Intake [mg/kg-day])[b,c]											
		Not reported				Control (0)	8-24 (16)	41-121 (81)	209-586 (398)	0	11±1	55±3	274±18
Nasal cavity													
Squamous cell carcinoma	All animals	0/50	0/50	0/50	3/50	0/50	0/50	0/50	3/50[e]	0/50	0/50	0/50	3/50[e]
	Sacrificed animals	Not reported				Not reported				Not reported			
Sarcoma NOS	All animals	0/50	0/50	0/50	2/50	0/50	0/50	0/50	2/50	0/50	0/50	0/50	2/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Rabdomyosarcoma	All animals	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Esthesioneuroepithelioma	All animals	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Liver													
Hepatocellular adenoma	All animals	0/50	2/50	4/50	24/50	0/50	2/50	4/49	24/50[d,e]	3/50	4/50	7/50	32/50[d,e]
	Sacrificed animals	Not reported				Not reported				Not reported			
Hepatocellular carcinoma	All animals	0/50	0/50	0/50	14/50	0/50	0/50	0/49	14/50[d,e]	0/50	0/50	0/50	14/50[d,e]
	Sacrificed animals	Not reported				Not reported				Not reported			

		Yamazaki et al. (1994)[a]	JBRC (1998)	Kano et al. (2009)
Hepatocellular adenoma or carcinoma	All animals	Not reported	0/50 2/50 4/49 33/50[d,e]	3/50 4/50 7/50 39/50[d,e]
	Sacrificed animals	Not reported	Not reported	Not reported
Tumors at other sites				
Peritoneum mesothelioma	All animals	2/50 2/50 5/50 28/50	2/50 2/50 5/50 28/50[d,e]	2/50 2/50 5/50 28/50[d,e]
	Sacrificed animals	Not reported	Not reported	Not reported
Subcutis fibroma	All animals	5/50 3/50 5/50 12/50	5/50 3/50 5/50 12/50[e]	5/50 3/50 5/50 12/50[e]
	Sacrificed animals	Not reported	Not reported	Not reported
Mammary gland fibroadenoma	All animals	1/50 1/50 0/50 4/50	1/50 1/50 0/50 4/50[e]	1/50 1/50 0/50 4/50[e]
	Sacrificed animals	Not reported	Not reported	Not reported
Mammary gland adenoma	All animals	0/50 0/50 0/50 0/50	Not reported	0/50 1/50 2/50 2/50
	Sacrificed animals	Not reported	Not reported	Not reported
Mammary gland fibroadenoma or adenoma	All animals	Not reported	Not reported	1/50 2/50 2/50 6/50[e]
	Sacrificed animals	Not reported	Not reported	Not reported

[a]Dose rates (mg/kg-day) were not provided in Yamazaki et al. (1994). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.
[b]JBRC (1998, 196240) 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, 2009, 628630) .
[c]Kano et al. (2009, 594539) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in this final document (U.S. EPA, 2010, 625580).
[d]$p \leq 0.01$ by Fisher's Exact test.
[e]Significantly increased by Peto test for trend $p < 0.01$.

Table E-4. Neoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in female F344 rats

		Yamazaki et al. (1994)[a]	JBRC (1998)	Kano et al. (2009)
		Drinking water concentration (ppm)		
		0 200 1,000 5,000	**0 200 1,000 5,000**	**0 200 1,000 5,000**
		Calculated Dose (Intake [mg/kg-day])b,c		
		Not Reported	**Control (0) 12-29 (21) 56-149 (103) 307-720 (514)**	**0 18±3 83±14 429±69**
Nasal cavity				
Squamous cell carcinoma	All animals	0/50 0/50 0/50 7/50	0/50 0/50 0/50 7/50[d,f]	0/50 0/50 0/50 7/50[e,f]
	Sacrificed animals	Not reported	Not reported	Not reported
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

		Yamazaki et al. (1994)[a]				JBRC (1998)				Kano et al. (2009)			
Rabdomyosarcoma	All animals	0/50	0/50	0/50	0/50	Not reported				0/50	0/50	0/50	0/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Esthesioneuroepithelioma	All animals	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Liver													
Hepatocellular adenoma	All animals	1/50	0/50	5/50	38/50	1/50	0/50	5/50	38/50[e,f]	3/50	1/50	6/50	48/50[e,f]
	Sacrificed animals	Not reported				Not reported				Not reported			
Hepatocellular carcinoma	All animals	0/50	0/50	0/50	10/50	1/50	0/50	0/50	10/50[e,f]	0/50	0/50	0/50	10/50[e,f]
	Sacrificed animals	Not reported				Not reported				Not reported			
Hepatocellular adenoma or carcinoma	All animals	Not reported				1/50	0/50	5/50	40/50[e,f]	3/50	1/50	6/50	48/50[e,f]
	Sacrificed animals	Not reported				Not reported				Not reported			
Tumors at other sites													
Peritoneum mesothelioma	All animals	1/50	0/50	0/50	0/50	Not reported				1/50	0/50	0/50	0/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Subcutis fibroma	All animals	0/50	2/50	1/50	0/50	Not reported				0/50	2/50	1/50	0/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Mammary gland fibroadenoma	All animals	3/50	2/50	1/50	3/50	Not reported				3/50	2/50	1/50	3/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Mammary gland adenoma	All animals	6/50	7/50	10/50	16/50	6/50	7/50	10/50	16/50[d,f]	6/50	7/50	10/50	16/50[d,f]
	Sacrificed animals	Not reported				Not reported				Not reported			
Mammary gland fibroadenoma or adenoma	All animals	Not reported				Not reported				8/50	8/50	11/50	18/50[d,f]
	Sacrificed animals	Not reported				Not reported				Not reported			

[a]Dose rates (mg/kg-day) were not provided in Yamazaki et al. (1994). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.
[b]JBRC (1998, 196240) 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, 2009, 628630) .
[c]Kano et al. (2009, 594539) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in this final document (U.S. EPA, 2010, 625580).
[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 $p < 0.01$.

Table E-5. Nonneoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in male Crj:BDF1 mice

		Yamazaki et al. (1994)	JBRC (1998)[d]				Kano et al. (2009)			
		Drinking water concentration (ppm)								
		0 500 2,000 8,000	0	500	2,000	8,000	0	500	2,000	8,000
		Calculated Dose (Intake [mg/kg-day])b,c								
		Not reported	Control 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			

		Yamazaki et al. (1994)	JBRC (1998)[d]				Kano et al. (2009)
Kidney proximal tubule; nuclear enlargement	All animals	Not reported	0/50	0/50	0/50	39/50	Not reported
	Sacrificed animals	Not reported	0/31	0/33	0/25	22/26[e]	Not reported
Testis; mineralization	All animals	Not reported	40/50	42/50	38/50	34/50	Not reported
	Sacrificed animals	Not reported	28/31	30/33	24/25[f]	21/26[f]	Not reported

[a]Dose rates (mg/kg-day) were not provided in Yamazaki et al. (1994). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.
[b]JBRC (1998, 196240) 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, 2009, 628630) .
[c]Kano et al. (2009, 594539) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in this final document (U.S. EPA, 2010, 625580).
[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-6. Nonneoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in female Crj:BDF1 mice

		Yamazaki et al. (1994)[a]				JBRC (1998)[b]				Kano et al. (2009)			
		Drinking water concentration (ppm)											
		0	500	2,000	8,000	0	500	2,000	8,000	0	500	2,000	8,000
		Calculated Dose (Intake [mg/kg-day])[b,c]											
		Not reported				Control 0	45-109 (77)	192-454 (323)	759-1374 (1066)	0	66 ± 10	278 ± 40	964 ± 88
Nasal respiratory epithelium; Nuclear enlargement	All animals	Not reported				0/50	0/50	0/50	41/50	0/50	0/50	0/50	41/50[e]
	Sacrificed animals	Not reported				0/29	0/29	0/17	5/5[e]	Not reported			
Nasal olfactory epithelium; Nuclear enlargement	All animals	Not reported				0/50	0/50	41/50	33/50	0/50	0/50	41/50[e]	33/50[e]
	Sacrificed animals	Not reported				0/29	0/29	17/17[e]	1/5	Not reported			
Nasal respiratory epithelium; Atrophy	All animals	Not reported				0/50	0/50	0/50	26/50	Not reported			
	Sacrificed animals	Not reported				0/29	0/29	0/17	1/5	Not reported			
Nasal olfactory epithelium; Atrophy	All animals	Not reported				0/50	0/50	1/50	42/50	Not reported			
	Sacrificed animals	Not reported				0/29	0/29	0/17	5/5[e]	Not reported			
Nasal cavity; Inflammation	All animals	Not reported				2/50	0/50	7/50	42/50	Not reported			
	Sacrificed animals	Not reported				0/29	0/29	5/17[e]	5/5[e]	Not reported			
Tracheal epithelium; Atrophy	All animals	Not reported				0/50	0/50	2/50	49/50	Not reported			
	Sacrificed animals	Not reported				0/29	0/29	1/17	5/5[e]	Not reported			
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. (1994, 196120). 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, 196240) report.
[c]JBRC (1998, 196240) 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, 2009, 628630) .
[d]Kano et al. (2009, 594539) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in this final document (U.S. EPA, 2010, 625580).
[e]$p \leq 0.01$ by chi-square test.

Table E-7. Neoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in male Crj:BDF1 mice

		Yamazaki et al. (1994)[a]				JBRC (1998)				Kano et al. (2009)			
		Drinking water concentration (ppm)											
		0	500	2,000	8,000	0	500	2,000	8,000	0	500	2,000	8,000
		Calculated Dose (Intake [mg/kg-day])[b,c]											
		Not reported				Control 0	37-94 (66)	144-358 (251)	451-1086 (768)	0	49±5	191±21	677±74
Nasal cavity													
Esthesioneuroepithelioma	All Animals	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Adenocarcinoma	All Animals	0/50	0/50	0/50	0/50	Not reported				0/50	0/50	0/50	0/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Liver													
Hepatocellular adenomas	All Animals	7/50	16/50	22/50	8/50	7/50	16/50	22/50[e]	8/50	9/50	17/50	23/50[e]	11/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Hepatocellular carcinomas	All Animals	15/50	20/50	23/50	36/50	15/50	20/50	23/50	36/50[d,e]	15/50	20/50	23/50	36/50[e,f]
	Sacrificed animals	Not reported				Not reported				Not reported			
Either adenoma or carcinoma	All Animals	Not reported				21/50	31/50	37/50	39/50[d,e]	23/50	31/50	37/50[d]	40/50[e,f]
	Sacrificed animals	Not reported				Not reported				Not reported			

[a]Dose rates (mg/kg-day) were not provided in Yamazaki et al. (1994). Drinking water concentrations of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.
[b]JBRC (1998, 196240) 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, 2009, 628630) .
[c]Kano et al. (2009, 594539) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in this final document (U.S. EPA, 2010, 625580).
[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-8. Neoplastic lesions: Comparison of histological findings reported for the 2-year JBRC drinking water study in female Crj:BDF1 mice

		Yamazaki et al. (1994)[a]				JBRC (1998)				Kano et al. (2009)			
		Drinking water concentration (ppm)											
		0	500	2,000	8,000	0	500	2,000	8,000	0	500	2,000	8,000
		Calculated Dose (Intake [mg/kg-day])[b,c]											
		Not reported				Control 0	45-109 (77)	192-454 (323)	759-1374 (1066)	0	66 ± 10	278 ± 40	964 ± 88
Nasal Cavity													
Esthesioneruoepithelioma	All animals	0/50	0/50	0/50	0/50	Not reported				0/50	0/50	0/50	0/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Adenocarcinoma	All animals	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50	0/50	0/50	0/50	1/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Liver													
Hepatocellular adenomas	All animals	4/50	30/50	20/50	2/50	4/50	30/50[d]	20/50[d]	2/50[e]	5/50	31/50[d]	20/50[d]	3/50
	Sacrificed animals	Not reported				Not reported				Not reported			
Hepatocellular carcinomas	All animals	0/50	6/50	30/50	45/50	0/50	6/50[f]	30/50[d]	45/50[d,g]	0/50	6/50[f]	30/50[d]	45/50[d,g]
	Sacrificed animals	Not reported				Not reported				Not reported			
Either adenoma or carcinoma	All animals	Not reported				4/50	34/50[d]	41/50[d]	46/50[d,g]	5/50	35/50[d]	41/50[d]	46/50[d,g]
	Sacrificed animals	Not reported				Not reported				Not reported			

[a]Dose rates (mg/kg-day) were not provided in Yamazaki et al. (1994, 196120). Drinking water concentrations (ppm) of 1,4-dioxane were used to identify the dose groups. Statistical test results were not reported.
[b]JBRC (1998, 196240) 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, 2009, 628630) .
[c]Kano et al. (2009, 594539) reported a mean intake dose for each group ± standard deviation. The mean shown in this table was used in this final document (U.S. EPA, 2010, 625580).
[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 $p < 0.01$