EPA/635/R-11/081C
www.epa.gov/iris

TOXICOLOGICAL REVIEW

OF

n-BUTANOL

(CAS No. 71-36-3)

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

June 2011

NOTICE

U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document is a preliminary draft for review purposes only. This information is distributed solely for the purpose of pre-dissemination peer review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

CONTENTS - TOXICOLOGICAL REVIEW OF n-BUTANOL (CAS No. 71-36-3)

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

ACGIH	American Conference of Governmental Industrial Hygienists
ACh	acetylcholine
AChE	acetylcholinesterase
ACSL	Advanced Continuous Simulation Language
ADH	alcohol dehydrogenase
AIC	Akaike's Information Criterion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
AST	aspartate aminotransferase
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
AUC	area under the curve
BMD	benchmark dose
BMDL	95% lower bound on BMD
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
CASRN	Chemical Abstracts Service Registry Number
CI	confidence interval
CNS	central nervous system
CO_2	carbon dioxide
DF	degrees of freedom
ECF	extracellular fluid
ER-α	estrogen receptor α
FEL	frank effect level
FID	flame ionization detection
GABA	γ-aminobutyric acid
GC	gas chromatography
GD	gestation day
GGT	gamma glutamyltransferase
GLDH	glutamate dehydrogenase
Glu	glutamate
GPT	glutamate pyruvate transaminase
GSH	reduced glutathione
GSSG	oxidized glutathione
HEC	human equivalent concentration
HEK	human embryonic kidney
5-HT	5-hydroxytryptamine
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IUR	Inventory Update Reporting
i.v.	intravenous
LDH	lactate dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level

MANOVA	multiple analysis of variance
MDA	malondialdehyde
MOE	Ontario Ministry of the Environment
4-MP	4-methylpyrazole
nACh	nicotinic acetylcholine
NADPH	nicotinamide adenine dinucleotide phosphate
NMDA	*N*-methyl-D-aspartate
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
PA	phosphatidic acid
PBPK	physiologically based pharmacokinetic
PBTK	physiologically based toxicokinetic
PKC	protein kinase C
PLD	phospholipase D
PMA	phorbol 12-myristate 13-acetate
PND	postnatal day
POD	point of departure
QSAR	quantitative structure-activity relationship
RBC	red blood cell
RfC	reference concentration
RfD	reference dose
RT-PCR	real time polymerase chain reaction
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SEM	standard error of the mean
TSCA	Toxic Substance Control Act
TSCATS	Toxic Substance Control Act Test Submission
UF	uncertainty factor
U.S. EPA	U.S. Environmental Protection Agency
WHO	World Health Organization

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 n-butanol. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of n-butanol.

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

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

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER/AUTHOR

Ambuja Bale
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

AUTHORS

Alan Sasso
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

James A. Weaver
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

CONTRIBUTORS

Karen Hogan
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Chester Rodriguez
Office of Chemical Safety and Pollution Prevention
U.S. Environmental Protection Agency
Washington, DC

Paul Schlosser
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Research Triangle Park, NC

Hui-Min Yang
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

CONTRACTOR SUPPORT

Heather Carlson-Lynch
Michael Lumpkin

Gary Diamond
Daniel Plewak
Julie Stickney
Chemical, Biological and Environmental Center
SRC, Inc.
7502 Round Pond Road
North Syracuse, NY 13212

INTERNAL EPA REVIEWERS

John Cowden
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Research Triangle Park, NC

Ted Berner
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Philip J. Bushnell
National Health and Environmental Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC

Barbara Glenn
National Center for Environmental Assessment
U.S. Environmental Protection Agency
Washington, DC

Karl Jensen
National Health and Environmental Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC

Ginger Moser
National Health and Environmental Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC

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 n-butanol. IRIS Summaries may include oral reference dose (RfD) and inhalation reference concentration (RfC) values for chronic and other exposure durations, and a carcinogenicity assessment.

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

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

Development of these hazard identification and dose-response assessments for n-butanol has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983). U.S. Environmental Protection Agency (U.S. EPA) Guidelines and Risk Assessment Forum Technical Panel Reports that may have been used in the development of this assessment include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of*

Inhalation Dosimetry (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000a), *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b), *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c), *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005b), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 2006a), and *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006b).

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 February 2011.

2. CHEMICAL AND PHYSICAL INFORMATION

n-Butanol is a flammable, colorless liquid with a vinous odor (O'Neil, 2006; Lewis, 2001). Physical and chemical properties of n-butanol are shown below in Table 2-1. n-Butanol is a natural component of many foods and is formed during the fermentation of carbohydrates (HSDB, 2009). Commercially, n-butanol is obtained via n-butyraldehyde formed from the Oxo reaction of propylene (Billig, 2001). According to company reports provided to U.S. EPA under the Inventory Update Reporting (IUR) program, the production volume of n-butanol in the United States during 2006 was >1 billion pounds (U.S. EPA, 2009). Commercial derivatives of n-butanol include n-butyl acrylate, methacrylate, butyl glycol ethers, 2-butoxyethanol, and butyl acetate (Billig, 2001). n-Butanol is used as a direct solvent in paints, surface coatings, lacquers, thinners, pharmaceutical formulations, waxes, and resins (Billig, 2001). It is also used to make plasticizer esters and mono-, di-, and tributylamines (Billig, 2001). More recently, n-butanol has been used as an oxygenate in fuels (see http://www.epa.gov/oust/oxygenat/index.htm).

Table 2-1. Physical and chemical properties of n-butanol

Chemical name	n-Butanol	
Synonym(s)	n-Butyl alcohol; butyl alcohol; 1-butyl alcohol; 1-butanol; 1-hydroxybutane; butyl hydroxide; butyric alcohol; propyl carbinol; hemostyp; methylolpropane; propylmethanol; n-butan-1-ol	ChemID Plus, 2009
Structure		ChemID Plus, 2009
Chemical formula	$C_4H_{10}O$	ChemID Plus, 2009
CASRN	71-36-3	ChemID Plus, 2009
Molecular weight	74.121	Lide, 2008
Form	Colorless liquid	Lewis, 2001
Odor	Vinous; similar to fusel oil	O'Neil, 2006; Lewis, 2001
Melting point	-88.6°C	Lide, 2008
Boiling point	117.73°C	Lide, 2008
Odor threshold Air Water	 0.83 ppm 7.1 ppm	 Amoore and Hautala, 1983 Amoore and Hautala, 1983
Density	0.8095 g/cm^3 at 20°C	Lide, 2008
Log octanol-water partition coefficient (log K_{ow})	0.88	Hansch et al., 1995
Solubility: Water Organic solvents	 6.32×10^4 mg/L at 25°C Soluble in benzene, miscible in ethanol and diethyl ether, very soluble in acetone	 Tewari et al., 1982 Lide, 2008
Vapor pressure	6.70 mm Hg at 25°C	Boublik et al., 1984
Henry's law constant	8.81×10^{-6} atm-m^3/mol	Buttery et al., 1969
Flash point	2–3°C	Billig, 2001
Auto ignition temperature	365°C	Lewis, 2001
Explosive limits in air	1.4–11.2%	Billig, 2001
Conversion factors ppm to mg/m^3 mg/m^3 to ppm	 1 ppm = 3.08 mg/m^3 1 mg/m^3 = 0.32 ppm	 Calculated Calculated

n-Butanol is expected to have high mobility in soil and is not expected to bind to organic matter and sediments in water (HSDB, 2009). n-Butanol in soil and water is expected to be removed through volatilization into the atmosphere and through biodegradation (HSDB, 2009). The environmental fate assessment indicates that n-butanol will be completely removed from soil and water within days to weeks (HSDB, 2009). In the atmosphere, n-butanol is expected to be broken down by reaction with photochemically-produced hydroxyl radicals with a calculated half-life of 46 hours (HSDB, 2009).

3. TOXICOKINETICS

Toxicokinetic studies of n-butanol in humans and experimental animals are described below. n-Butanol is readily absorbed following oral administration (80%; DiVincenzo and Hamilton, 1979a), moderately absorbed following an inhalation exposure (50%; DiVincenzo and Hamilton, 1979a; Astrand et al., 1976) and poorly absorbed following a dermal exposure (1%; Boman and Maibach, 2000). Once absorbed, n-butanol is rapidly distributed to many tissues including the liver, kidney, lung, brain and heart (Kaneko et al., 1994; DiVincenzo and Hamilton, 1979a). n-Butanol is rapidly metabolized to butyric aldehyde by alcohol dehydrogenase (ADH) and further to n-butyric acid by aldehyde dehydrogenase (ECETOC, 2003). n-Butanol is also oxidized by cytochrome P450 in rat liver (Albano et al., 1991). Excretion of n-butanol is primarily as CO_2 in exhaled breath with minor amounts eliminated in the urine (2.6–5.1%) and feces (0.6–1.1%) (DiVincenzo and Hamilton, 1979a). A physiologically based toxicokinetic (PBTK) model has been developed (Teeguarden et al., 2005) to describe blood kinetics for n-butyl acetate and its metabolites, n-butanol and n-butyric acid, in rats and humans.

3.1. ABSORPTION

3.1.1. Oral Absorption

DiVincenzo and Hamilton (1979a) administered gavage doses of 4.5, 45, or 450 mg/kg [^{14}C]-n-butanol in corn oil to male CD (Sprague-Dawley) rats (2–4/group) that had fasted for 16 hours. After administration, expired air, urine, and feces were collected for 24 hours. 78.3–83.3% of the amount administered was recovered as expired $^{14}CO_2$ by 24 hours post exposure. A smaller fraction, approximately 0.20–0.56% of the administered radioactivity, was recovered as the unchanged compound in expired air at 24 hours post exposure. Urine and fecal elimination accounted for approximately 2.6–5.0 and 0.6–1.1% of the administered radioactivity, respectively, at 24 hours post exposure. Thus, up to 89% of the administered n-butanol was absorbed and measured in breath or urine. The extent of absorption was not dose-dependent.

DiVincenzo and Hamilton (1979a) also collected blood samples from three male Sprague-Dawley rats at 0.5, 1, 2, 4, and 8 hours after gavage dosing with 450 mg/kg n-butanol. A peak plasma concentration of 70.9 µg/mL was measured 1 hour after gavage dosing.

3.1.2. Respiratory Tract Absorption

Astrand et al. (1976) exposed 12 human volunteers (all male) to 300 or 600 mg/m^3 (100 and 200 ppm) n-butanol in air for four 30-minute periods of rest or exercise. The concentration of n-butanol in inspiratory air was continuously measured. Expiratory air was collected and was analyzed for n-butanol. The amount of n-butanol absorbed was calculated as

the difference between the total quantities in inspiratory and expiratory air. Arterial and venous blood was collected and analyzed for n-butanol concentration. At rest, 46–48% of n-butanol was taken up by inhalation, while 36–41% was absorbed during exercise (intensity estimated at 50–150 watts) and was independent of exposure concentration. Measured arterial blood concentrations after 30 minutes of exposure ranged from 0.5 to 1.3 mg/kg

DiVincenzo and Hamilton (1979a) exposed four male beagle dogs to 50 ppm (150 mg/m^3) n-butanol vapor for 6 hours in a whole-body chamber. Venous blood was collected at regular intervals. Based on breath concentrations, the uptake curve indicates that approximately 55% of the inhaled vapor was absorbed through the lungs. Expired air concentrations of 22 ppm (67 mg/m^3) n-butanol were essentially unchanged during the 6 hour exposure.

Swiercz et al. (1995) exposed 32 male Wistar rats (4/exposure duration group) to 100 ppm (300 mg/m^3) n-butanol for up to 7 hours. Blood samples were obtained from separate groups of four rats prior to exposure and at hourly intervals from 1 to 7 hours and analyzed for n-butanol. The concentration of n-butanol in blood increased rapidly with inhalation exposure, reaching near steady-state concentrations of approximately 2.2 µmol/dm^3 (20 mg/kg) within 1 hour of exposure. Mean blood n-butanol levels changed little throughout the remaining exposure period, with blood from rats measured at 4 hours of exposure exhibiting a maximum n-butanol concentration of 2.8 µmol/dm^3 (26 mg/kg).

3.1.3. Dermal Absorption

Boman and Maibach (2000) conducted ex vivo studies using male and female human thigh skin samples obtained at autopsy in a 3-mL flow-through penetration/evaporation cell. Skin was exposed to neat n-butanol under either unventilated or ventilated conditions with various air flow rates. Steady state absorption was not attained during the 24-hour exposure time studied for either the unventilated or ventilated exposures. In the unventilated experiment, the absorption of neat butanol through the skin samples varied between 2.2 and 9.4%; the absorption during ventilation (i.e., allowing for n-butanol evaporation from the skin) resulted in ≤ 1% absorption.

Scheuplein and Blank (1973) conducted ex vivo studies using human abdominal skin samples obtained at autopsy to determine permeability rates for n-butanol in an aqueous solution. Sheets of dermis or epidermis were fitted into a permeability cell in which one side of the sample was exposed to neat n-butanol or n-butanol in an aqueous solution (2.54 cm^2 exposure area), while the other was exposed to water, from which aliquots were drawn to measure the flux of n-butanol across the skin. In aqueous solution, the permeability rates of the epidermis and dermis were 2.5×10^3 and 30.0×10^3 cm/hour, respectively. For neat n-butanol, the permeability rates were 0.06×10^3 and 1.0×10^3 cm/hour for the epidermis and dermis, respectively.

Using skin preparations from nude mice and glass diffusion cells, Behl et al. (1984) measured permeability coefficients of 3.7×10^3 cm/hour for dorsal skin and 23.7×10^3 cm/hour for abdominal skin. Under hydrated conditions using skin preparations from male Sprague-Dawley rats, Behl et al. (1983) determined the n-butanol permeability coefficient to be increased by approximately 20–25% through the first 5 hours of hydration, and remained at this value (6.4×10^3 cm/hour) through the end of the experiment at 80 hours.

DelTerzo et al. (1986) estimated an in vitro permeability rate for n-butanol in nude rat skin. Rat skin was sandwiched between 3 mL saline filled compartments. [^{14}C]-n-Butanol was introduced into the donor side of the cell, and samples were removed from both sides of the cell at predetermined intervals to measure the flux of n-butanol across the skin. From these measurements, a permeability rate of 14.2×10^3 cm/hour was calculated.

DiVincenzo and Hamilton (1979a) exposed young male beagle dogs to [^{14}C]-n-butanol using a sealed glass absorption cell secured on the shaved thorax for 60 minutes. Expired air was collected continuously using an oral endotracheal tube and urine was collected continuously by a urethral catheter. An identical amount of [^{14}C]-n-butanol was administered intravenously to other dogs. Radioactivity excreted in the urine and exhaled breath of dermally and intravenously treated dogs was compared to determine the rate of systemic uptake by dermal absorption. Assuming that the metabolic fate and tissue disposition is identical after intravenous (i.v.) or skin administration, the calculated absorption rate was 8.8 µg minute^{-1} cm^{-2}.

Boman et al. (1995) conducted studies to evaluate the percutaneous absorption of n-butanol during intermittent exposure in guinea pigs. Groups of 5–16 animals were exposed to neat n-butanol either continuously for 4 hours or for 1 minute at 30-minute intervals over 4 hours for a total of eight intermittent exposures. At the end of 4 hours, the continuously exposed animals exhibited maximum blood n-butanol levels that were twofold higher (25.63 µM) than the levels in intermittently exposed animals (12.43 µM).

3.2. DISTRIBUTION

In vitro determination of tissue:blood partition coefficients for n-butanol suggest that it will distribute throughout the body, as calculated values were very close to 1, ranging from 0.78 to 1.08 for muscle, brain, kidney, liver, and fat (Kaneko et al., 1994). DiVincenzo and Hamilton (1979a) reported the distribution of n-butanol in male CD (Sprague-Dawley) rats gavaged with a single dose of 450 mg/kg [^{14}C]-n-butanol in corn oil after fasting for 16 hours. Blood samples were collected from three rats at 0.5, 1, 2, 4, and 8 hours after gavage dosing. Groups of four animals were sacrificed at 4, 8, or 24 hours post exposure and liver, kidney, heart, brain, lung, and adrenal glands were collected and analyzed for radioactivity. The systemic distribution was rapid, as plasma concentration of n-butanol rose from 40 µg/mL at 30 minutes post exposure to a peak at 1 hour of 70.9 µg/mL, followed by a rapid decline to <10 µg/mL at 2 hours. n-Butanol was not detected in the plasma 4 hours after gavage dosing. Peak levels of

[^{14}C]-radioactivity in rat tissues occurred from 4 to 8 hours post exposure. The highest percentages of administered [^{14}C]-radioactivity (constituting parent compound and metabolites) were recovered at 4 hours in the kidney (0.24%) and heart (0.05%), and 8 hours in the liver (3.88%), blood (0.74%), lung (0.12%), fat (0.09%), brain (0.04%), and adrenal glands (0.009%). Twenty-four hours after gavage doses of 4.5, 45, or 450 mg [^{14}C]-n-butanol/kg, approximately 12.1–16.3% of the administered radioactivity remained in the carcass. Because radioactivity was measured instead of specific compounds, these data do not inform as to the distribution of n-butanol or specific metabolites.

3.3. METABOLISM

n-Butanol is primarily metabolized to butyric aldehyde by alcohol dehydrogenase (ADH) (and to a minor extent by cytochrome P450) and further to n-butyric acid by aldehyde dehydrogenase (ECETOC, 2003). Further oxidation of n-butyric acid produces CO_2. A minor conjugation pathway exists resulting in n-butanol-O-glucuronide or n-butanol-O-sulfate, which are excreted in the urine (Figure 3-1). In addition to exogenous sources of n-butanol, this alcohol is readily and rapidly metabolized from n-butyl acetate as shown in Figure 3-1.

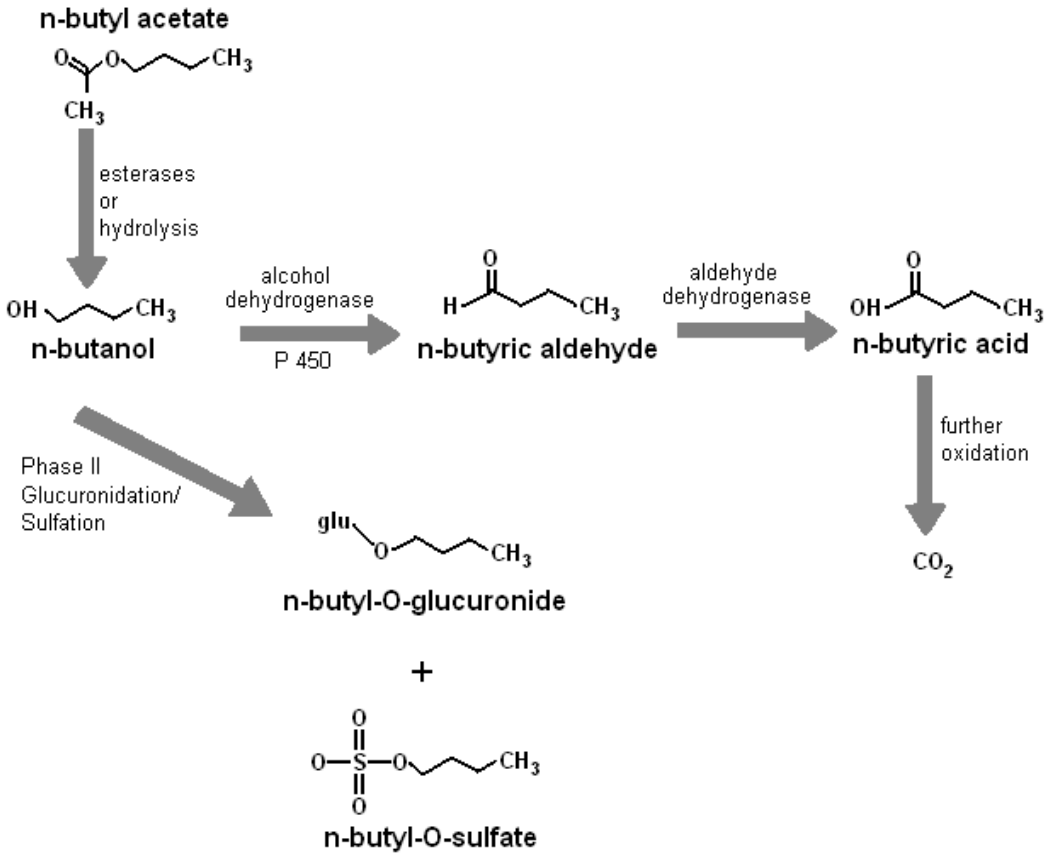


Figure 3-1 Metabolism of n-Butanol.

Deisinger and English (2001) administered i.v. doses of 0.28 mmol/kg (21 mg/kg) of n-butyl acetate, n-butanol, or 0.028 mmol/kg (2.1 mg/kg) n-butyric acid to groups of adult male Sprague-Dawley rats. In addition, they administered an i.v. infusion of approximately 0.28 mmol/kg (21 mg/kg) n-butyric acid over 3 minutes. Blood samples were collected and analyzed for each of the butyl series compounds. Metabolism of n-butanol to n-butyric acid was very rapid with the peaks for both compounds (in the case of n-butyl acetate injection) measured within 1 minute of each other.

Poet et al. (2003a, unpublished and as presented and cited in Teeguarden et al., 2005) exposed male Sprague-Dawley rats to n-butanol in closed chambers having initial concentrations of approximately 2,000 ppm (6,000 mg/m^3). Blood was collected throughout the exposure and analyzed for n-butanol and n-butyric acid. Blood n-butanol levels remained at peak values (0.1 mM) from about 0.2 to 0.6 hours, suggesting that n-butanol metabolism was saturated during this period. n-Butyric acid levels peaked (0.009 mM) at about 0.6 hours and were typically an order of magnitude lower than n-butanol levels throughout the exposure.

In gavage studies in CD (Sprague-Dawley) rats dosed with 450 mg [^{14}C]-n-butanol/kg body weight, 83.3% of the administered radioactivity was metabolized via oxidative pathways, appearing in exhaled breath as carbon dioxide (CO_2) by 24 hours post exposure (DiVincenzo and Hamilton, 1979). Phase II glucuronidation and sulfation of n-butanol resulted in 4.4% of administered dose being excreted in the urine. Of this fraction, about 75% was in the form of n-butyl-o-sulfate (44%) or n-butyl-o-glucuronide (30%). Less than 1% of was not metabolized and was eliminated in the exhaled breath as unchanged compound. The study authors reported similar metabolism and excretion patterns at lower doses (4.5 and 45 mg/kg body weight), but did not provide any additional quantitative details.

Metabolism of n-butanol by ADH from the perfusate of isolated rat liver was evaluated and an apparent Michaelis-Menten K_m (affinity constant) and V_{max} (maximum reaction velocity) were reported to be 0.86×10^{-3} M and 0.077 mmol/minute, respectively (Auty and Branch, 1976). When simultaneous exposures of n-butanol and ethanol were given, the V_{max} for n-butanol remain unchanged, but the K_m value increased to 1.2×10^{-3} M, indicating that ethanol competitively inhibits n-butanol metabolism.

The in vitro metabolism of n-butanol by rat hepatic microsomes has been studied by Teschke et al. (1974) and Cederbaum et al. (1979, 1978). Teschke et al. (1974) showed that hepatic microsomes catalyzed the oxidation of n-butanol to its aldehyde in a reaction requiring molecular oxygen and nicotinamide adenine dinucleotide phosphate (NADPH). This reaction was inhibited by carbon monoxide. Cederbaum et al. (1979) observed that hydrogen peroxide (H_2O_2) added to microsomal preparations stimulated the oxidation of n-butanol. Indirect evidence was further provided by the observation that azide, which prevents the decomposition of H_2O_2 by catalase, stimulated the oxidation of n-butanol. Thiourea, a compound that reacts with hydroxyl radicals, inhibited NADPH-dependent microsomal oxidation of n-butanol to a

similar extent, in both the absence and presence of the catalase inhibitor azide (Cederbaum et al. 1979).

Carlson and Olson (1995) evaluated the metabolism of n-butanol by rat hepatic and pulmonary cytosolic preparations measured with regard to ADH activity as influenced by pH and substrate concentration. The values are given in Table 3-1. At pH 10, the hepatic K_m value was about fourfold lower than at pH 7.2. V_{max} and K_m values could not be calculated for ADH activity in lung cytosol at pH 7.2 or 9.0 because activity was negligible under these conditions. These data suggest that oxidation of n-butanol in the lung may not contribute significantly to systemic metabolism.

Table 3-1. n-Butanol metabolism by hepatic or pulmonary ADH in male rats

pH	V_{max} (nmol/min/mg protein)	K_m (mM)
Hepatic		
7.2	13.2 ± 1.5	0.159 ± 0.085
9.0	15.9 ± 6.9	0.136 ± 0.051
10.0	9.2 ± 1.1	0.045 ± 0.013
Pulmonary		
7.2	ND	ND
9.0	ND	ND
10.0	20.2 ± 2.1	19.4 ± 7.4

ND = not determined due to low activity

Source: Carlson and Olson (1995).

Winer (1958) measured the initial reaction velocities of 19 alcohols as substrates for purified horse liver ADH. n-Butanol had the highest initial velocity of 215 moles/L/minute/mol ADH, which was 1.5-fold faster than the oxidation of ethanol (135 moles/L/minute/mol ADH).

Albano et al. (1991) investigated the role of cytochrome P450 2E1 in oxidizing n-butanol in vitro via a free radical intermediate. These authors incubated Sprague-Dawley rat liver microsomes with n-butanol in the presence of NADPH, desferrioxamine methane-sulphonate, and the spin trapping agent, 4-pyridyl-1-oxide-t-butyl nitrone, resulting in the detection of a free radical intermediate tentatively identified as the 1-hydroxylbutyl radical. The formation of the radical was strictly dependent of the presence of NADPH and oxygen and not influenced by the addition of mannitol. In reconstituted membrane vesicles, ethanol-inducible cytochrome P450 2E1 was twice as active as phenobarbital-inducible P450 2B1 in producing n-butanol free radicals. Indeed, n-butanol oxidation in rat liver microsomes was induced to a significantly greater (~25%) extent by pretreatment of the animals with ethanol than with phenobarbital. This suggests a greater affinity of n-butanol for cytochrome P450 2E1 compared to cytochrome P450

2B1 (Gadberry and Carlson, 1994). This inducible metabolism, however, was not observed in rat lung microsomes, indicating that P450-mediated oxidation of n-butanol in this tissue may be of minor consequence. Although cytochrome P450-mediated n-butanol oxidation has been demonstrated in rat liver, it is not clear to what extent this oxidation reaction has on systemic disposition of n-butanol.

Deters et al. (1998a) demonstrated that metabolism of n-butanol by ADH was not a necessary step in the liver toxicity of this compound. 4-Methylpyrazole (4-MP) was used to inhibit ADH in isolated perfused rat livers (from male Wistar rats) exposed to 130.2 mmol/L n-butanol. n-Butanol caused leakage of glutamate pyruvate transaminase (GPT), lactate dehydrogenase (LDH), and glutamate dehydrogenase (GLDH) into the perfusate and decreased oxygen consumption, perfusion flow, and adenosine triphosphate (ATP) concentration, both in the presence and absence of 4-MP. This study indicates that the hepatotoxicity of n-butanol is not related to metabolism by ADH. This study is further described in Section 4.5.5 (Mechanistic Studies Evaluating Liver and Metabolic Effects).

3.4. ELIMINATION

Deisinger and English (2001) administered i.v. doses of 0.28 mmol/kg (21 mg/kg) of n-butyl acetate, n-butanol, or 0.028 mmol/kg (2.1 mg/kg) n-butyric acid to groups of adult male Sprague-Dawley rats. In addition, they administered an i.v. infusion of approximately 0.28 mmol/kg (21 mg/kg) n-butyric acid over 3 minutes. Blood samples were collected and analyzed for each of the butyl series compounds. n-Butanol exhibited biphasic elimination and approached background levels by 18 minutes.

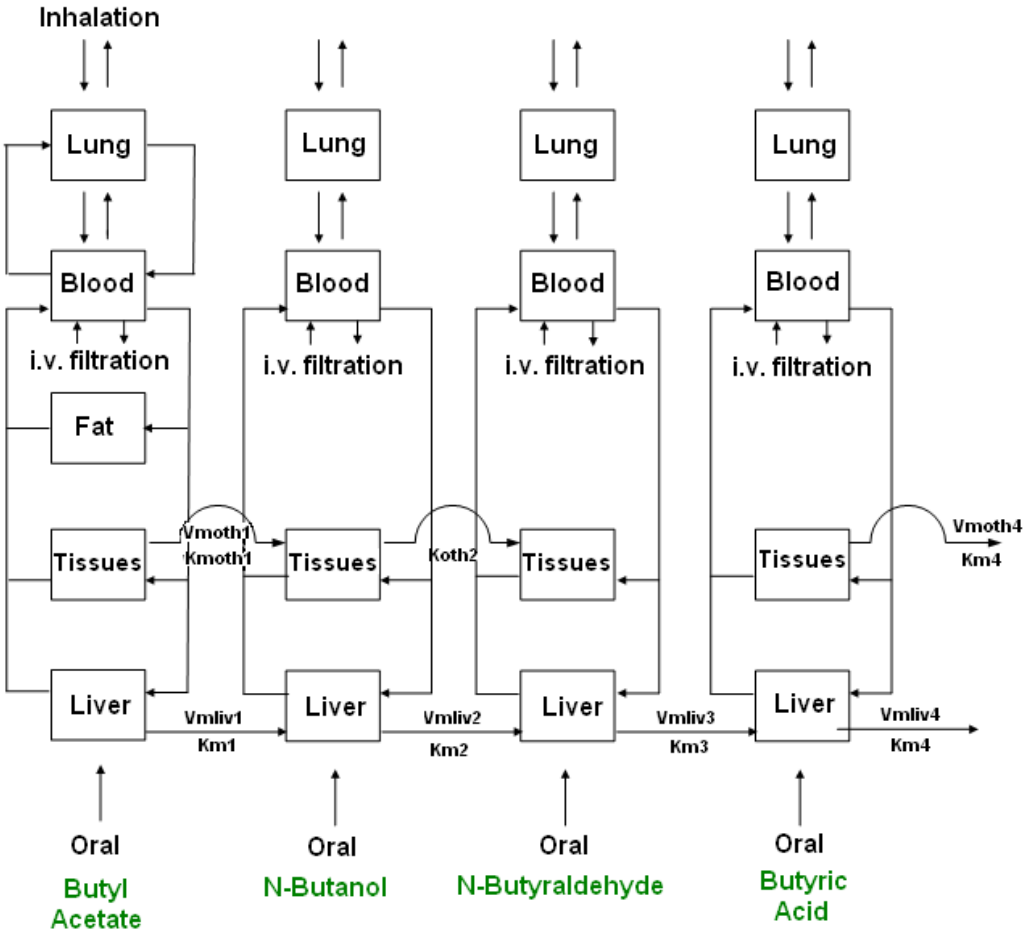
Astrand et al. (1976) exposed 12 volunteers (all male) to 300 or 600 mg/m^3 (100 or 200 ppm) n-butanol in air for four 30-minute periods of rest or exercise. By 30 minutes after cessation of exposure, the peak blood n-butanol level of 0.17 mM had diminished to near zero.

DiVincenzo and Hamilton (1979a) administered [^{14}C]-n-butanol in corn oil by gavage to male CD (Sprague-Dawley) rats at doses of 4.5, 45, or 450 mg/kg body weight. n-Butanol from a 450 mg/kg dose disappeared from the plasma rapidly, decreasing from a peak concentration of 70 µg/mL at 1 hour to <10 µg/mL at 2 hours and was below the limit of detection after 4 hours. Within 24 hours post exposure, excretion of the administered radioactivity was primarily as CO_2 in exhaled breath (78.3–83.3%). Of the remainder, 2.6–5.1% was eliminated in the urine, 0.6–1.1% in the feces, and <1% of the administered radioactivity was exhaled as unchanged compound.

3.5. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

A physiologically based pharmacokinetic (PBPK) model for n-butyl acetate and its butyl metabolites (including n-butanol) was developed by Barton et al. (2000) and then later refined by Teeguarden et al. (2005). The model simulates the kinetics of n-butyl acetate, n-butanol, n-

butyraldehyde, and n-butyric acid in the rat or human following i.v or inhalation exposures to n-butyl acetate or n-butanol. The PBPK model refined by Teeguarden et al. (2005) was updated from ACSL to ACSLXTREME (Advanced Continuous Simulation Language, Aegis, Inc., Huntsville, AL) by U.S. EPA. Details of the model evaluation are presented in Appendix C. A schematic of the Teeguarden et al. (2005) model is shown in Figure 3-2.



Source: Teeguarden et al. (2005).

Figure 3-2. Schematic of the PBPK model for the butyl series (including n-butanol) in rats and humans.

The model is comprised of four similar submodels for each component of the butyl series. The submodels are linked to one another via passage of the metabolite formed in the compartments for liver, blood, or extra-hepatic tissues to the respective compartments of the submodel for that metabolite. For example, the amount of n-butanol formed from n-butyl acetate in the liver is passed along to the liver compartment for the n-butanol submodel. The overall structure of linked submodels for the butyl series is based on the structure developed by Barton et al. (2000) for families of metabolically-related compounds.

Each submodel includes compartments for lung, arterial and venous blood, liver, and the remaining perfused tissues. The submodel for n-butyl acetate also includes a compartment for fat. All compartments are well mixed and flow limited, which assumes that compounds are homogeneously distributed in each compartment, and that the equilibration of chemical concentration between the blood and tissues occurs sufficiently fast so that changes in concentration at the blood:tissue interface are limited by the blood flow rather than rate of diffusion into the tissues. The proportion of chemical transferred from blood to tissue (and vice versa) at any given moment was estimated by in vitro derived equilibrium partition coefficients. To determine the fraction of inhaled n-butyl acetate or n-butanol that bypasses extraction by the upper respiratory tract (because of the water-soluble nature of these compounds) and absorbed into the blood, bioavailability constants (FA1 and FA2) were estimated from in vivo data (discussed below) and multiplied by the inhaled concentration. Metabolism of each of the butyl series compounds in the liver, as well as n-butyl acetate and n-butyric acid in the other perfused tissues compartment, is described as Michaelis-Menten saturable processes defined by a maximum reaction rate (V_{max}), scaled to body weight to the ¾ power (mmol/hour/$kg^{0.75}$), and an affinity constant (K_m, mM). First-order metabolism of n-butanol in the other perfused tissues is scaled to body weight to the ¼ power (K_{other}, $kg^{0.25}$/hour) and was required to attain fit of the model to experimental data. Elimination of n-butyl acetate, n-butanol, and n-butyric acid via the urine (filtration) is represented as a first-order process, scaled to body weight to the ¼ power ($kg^{0.25}$/hour).

Physiological model parameters such as cardiac and pulmonary ventilation flow rates, relative blood perfusion of various tissue compartments, and tissue volumes (as fraction of body mass) for rats and humans were obtained from the literature (Teeguarden et al., 2005) and are shown in Table 3-2. Urinary elimination rate constants for rats were estimated based on literature values. Urinary clearance of 2-butanol was reported to be up to 14% in different species (Dietz et al., 1981). Thus, a value of 30 $hour^{-1}$, scaled to ¼ power of body weight, was used for the first-order n-butanol urinary elimination rate constant. In the absence of urinary excretion data for n-butyric acid, it was assumed to be <3.5%, as observed for isobutyric acid in the rat (DiVincenzo and Hamilton, 1979b). Human values were allometrically scaled from rat values. Rat values for the inhalation bioavailability factors were fitted to in vivo time-course

data for the blood levels of n-butyl acetate (Poet et al., 2003a) and n-butanol (Poet et al., 2003b). The human value for n-butyl acetate was assumed to be equal to the rat, while the value for n-butanol (59% of alveolar ventilation) was reported by Astrand et al. (1976).

Table 3-2. Physiological parameters for a PBPK model for the n-butyl series in the rat and human (adapted from Teeguarden et al., 2005).

Parameter	Abbreviation	Value		Reference
		Rat	Human	
Body weight (kg)	BW	0.3	70.0	Study specific
Alveolar ventilation ($L/hr/kg^{0.75}$)	QPC	14.0	8.678	Brown et al., 1997
Cardiac output ($L/hr/kg^{0.75}$)	QCC	14.0	12.892	Brown et al., 1997
Blood flow (percentage of cardiac output)				
Fat	QFatC	0.07	0.052	Brown et al., 1997
Liver	QLivC	0.175	0.227	Brown et al., 1997
Tissue volume (percentage of body weight)				
Arterial blood	VABC	0.022	0.0257	Benareggi and Rowland, 1991 (as cited in Teeguarden et al., 2005)
Fat	VFatC	0.07	0.214	Brown et al., 1997
Liver	VLivC	0.037	0.026	Brown et al., 1997
Lung	VLungC	0.005	0.008	Brown et al., 1997
Venous blood	VVBC	0.045	0.0514	Bernareggi and Rowland, 1991 (as cited in Teeguarden et al., 2005
Other tissues	VOthC	0.751	0.5319	Brown et al., 1997
Urinary elimination rate ($kg^{0.25}$/hr)				
n-Butyl acetate	kFiltC1	0.074	Scaled[a]	Estimated
n-Butanol	kFiltC2	22.2	Scaled	Estimated based on Dietz et al., 1981
n-Butyric acid	kFiltC4	0.0074	Scaled	Estimated based on DiVincenzo and Hamilton, 1979b
Inhalation absorption (percentage of inhaled concentration)				
n-Butyl acetate	FA1	1.0	1.0	Rat, fitted; human = rat
n-Butanol	FA2	0.5	0.59	Rat, fitted; human = rat (Astrand et al., 1976)
n-Butyraldehyde	FA3	–	–	–
n-Butyric acid	FA4	–	–	–

[a]Allometrically-scaled rat value used for human.
Source: Teeguarden et al. (2005).

Metabolic parameter values for the model are given in Table 3-3. Metabolic constants (V_{max} and K_{other}) were estimated by visually fitting the model output to i.v. blood time-course data for each compound (Deisinger and English, 2001). Fitting of V_{max} for n-butyl acetate and its metabolites was preceded by first estimating V_{max} for the furthest downstream metabolite (n-butyric acid), and proceeding upstream to estimates V_{max} values for n-butyraldehyde, n-butanol, and n-butyl acetate, in that order. n-Butyraldehyde is not detected in blood and thus, metabolic constants for this compound were approximated by n-butyric acid concentration in blood following n-butanol or n-butyl acetate exposure. The affinity constants (K_m) for n-butanol, n-butyraldehyde, and n-butyric acid were previously selected by Barton et al. (2000). The in vitro affinity constant for metabolism of ethyl acetate in rats was used as a surrogate for n-butyl acetate in blood, liver, and other tissues. Metabolic constants for n-butanol metabolism were re-optimized to data and updated values are given in Table 3-3.

Table 3-3. Metabolic parameters for a PBPK model for the n-butyl series in the rat and human[a]

Parameter	Abbreviation	Value	Notes
n-Butyl acetate			
Maximum metabolic rate in blood	VMBloodC1	600.0	Fitted
Affinity constant in blood	KMBlood1	100.0	K_m for ethyl acetate in rat
Maximum metabolic rate in liver	VMLivC1	38.7	Fitted
Affinity constant in liver	KMLiv1	1.0	K_m for ethyl acetate in rat
Maximum metabolic rate in other tissues	VMOthC1	6,000.0	Fitted
Affinity constant in other tissues	KMOth1	100.0	K_m for ethyl acetate in rat
n-Butanol[b]			
Maximum metabolic rate in liver	VMLivC2	2.17/0.62	Fitted (rat/human, respectively)
Affinity constant in liver	KMLiv2	0.16	Fitted
First-order metabolism in other tissues	KOthC2	4.0/20.1	Fitted (rat/human, respectively)
n-Butyraldehde			
Maximum metabolic rate in liver	VMLivC3	17.78	Fitted
Affinity constant in liver	KMLiv3	0.1	Estimated (Barton et al., 2000)
n-Butyric acid			
Maximum metabolic rate in liver	VMLivC4	1.4	Fitted
Affinity constant in liver	KMLiv4	0.1	Estimated (Barton et al., 2000)
Maximum metabolic rate in other tissues	VMOthC4	3.0	Fitted
Affinity constant in other tissues	KMOth4	0.1	Estimated (Barton et al., 2000)

[a]Rat values were derived as described; human values were allometrically scaled from rat values as ¾ or ¼ power of body weight. Units: V_{max} (mmol/hr/$kg^{0.75}$); K_m (mM); K_{other} ($kg^{0.25}$/hr).

[b]Values for n-Butanol re-optimized. Details are presented in Appendix C.

Source: Teeguarden et al. (2005).

Blood:air and tissue:blood equilibrium partition coefficients (Table 3-4) for n-butyl acetate and n-butanol were calculated by Kaneko et al. (1994); values for n-butyraldehyde and n-butyric acid were assumed to be identical to those for n-butanol. The values for lung and other

perfused tissues:blood were based on the Kaneko et al. (1994) muscle:blood value. Human values were assumed to be equivalent to rat values.

Table 3-4. Equilibrium partition coefficients for a PBPK model for the n-butyl series in the rat and human[a]

Parameter	Abbreviation	n-Butyl Acetate	n-Butanol	n-Butyraldehyde	n-Butyric acid
Blood:air	PB	89.4	1,160.0	1,160.0	1,160.0
Fat:blood	PFat	17.0	–	–	–
Liver:blood	PLiv	3.14	1.08	1.08	1.08
Lung:blood	PLung	1.76	0.78	0.78	0.78
Other perfused tissues:blood	POth	1.76	0.78	0.78	0.78

[a]Calculated from Kaneko et al. (1994).

Source: Teeguarden et al. (2005).

The model was evaluated against experimental data for i.v. exposures in the rat (Deisinger and English, 2001), closed-chamber inhalation exposures in the rat (Poet et al., 2003a), and inhalation exposure in human volunteers (Astrand at al., 1976). Low residual errors between model and data were observed (particularly for rat i.v. data of n-butanol), suggesting high confidence in the estimation/choice of metabolic and urinary elimination parameter values. For the human model, the simulated blood levels were within two standard errors (SE) of the observations and were always less than twofold higher. The profile of the simulated elimination of n-butanol from the blood was similar to observations. Further discussion regarding the model fit and parameter optimization can be found in Appendix C.

Overall, the human and rat models in Teeguarden et al (2005) were developed for inhalation exposures. The internal dose metric available for extrapolation between species is limited to n-butanol levels in blood. Only n-butanol blood concentration data are available for both rats and humans. The human model performance has not been verified for other metabolites. Further, the liver is the only defined organ system (other than blood) for which the model may provide quantitative internal dosimetry. The model cannot be used to explicitly extrapolate internal doses for other target organs (i.e., central nervous system [CNS], reproductive organs, and upper respiratory system). However, for species differences in toxicity due primarily to n-butanol metabolic differences, resulting in differences in systemic levels of n-butanol or metabolites, the model is useful to quantitatively reduce uncertainty in the determining human equivalent concentrations (HECs) of critical rat exposures.

4. HAZARD IDENTIFICATION

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

4.1.1. Oral Exposure

Bunc et al. (2006) described the case of a 47-year-old man who apparently ingested n-butanol in a suicide attempt. Initial symptoms of sleepiness, headache, and abdominal pain later progressed to vomiting and coma. The patient was transported to a hospital comatose, where his presenting symptoms included tachycardia, hypotension, shallow breathing, hypotonic muscles, absent myotatic reflexes, and aromatic odor. Echocardiogram and chest x-ray results were normal. A brain CT scan performed when the patient arrived at the hospital showed a distorted and narrower fourth ventricle and a hypodense area; an additional scan performed 10 hours later showed no changes. The patient regained consciousness approximately 16 hours after admission. Initial (days 1–2) blood laboratory tests conducted on the patient showed elevated erythrocytes and leukocytes, elevated creatinine, myoglobin, and creatine kinase; these changes were resolved by day 6 after arrival at the hospital. The cause of his symptoms was determined only after analysis of his stomach contents and urine, which showed the presence of n-butanol. No information on the dose of n-butanol ingested was provided in the report and no follow-up information was available.

4.1.2. Inhalation Exposure

4.1.2.1. *Occupational Studies*

Four studies reported health findings in workers occupationally exposed to n-butanol.. Details of these studies including study design, exposure, and notable effects are presented in Table 4-1. Occupational health findings associated with n-butanol exposure include eye irritation, hearing loss, dermatitis, and other systemic effects. All of these studies have limitations that prevent their use in establishing dose response relationships. For example, xposure to other solvents was reported in Tabershaw et al. (1944) and Cogan and Grant (1945) and effect levels for n-butanol were not reported in Velazquez et al. (1969) and Sterner et al. (1949).

Table 4-1. Occupational Studies with n-butanol exposure -

Reference(s)	No Subjects	Study Design	Exposure	Findings	Strengths and limitations
Tabershaw et al., 1944	Not reported	Eye irritation, dermatitis, and systemic effects were evaluated in workers from six industrial facilities involved in the manufacture of raincoats and other waterproof materials, in which n-butanol was the primary solvent used	5-14 ppm (15-42 mg/m^3) and 20-65 ppm (61-197 mg/m^3)	Eye irritation reported at a concentration ranging from 20 to 65 ppm (61-197 mg/m^3) in 1 out of 6 facilities with n-butanol only	No details of the worker populations were provided. Other solvents exposures reported in 4 out of 6 facilities
Velazquez et al., 1969	11 butanol + noise (72-78 db), 47 noise only (90-110 db)	Audiologic effects of n-butanol exposed workers were compared with workers from another factory exposed to higher noise levels but not n-butanol or other solvents associated with hearing loss.	80 ppm (242 mg/m^3) as measured by gas chromatography (GC) in the work room at a cellulose acetate ribbon factory	Hearing loss was observed in 9/11 workers exposed to n-butanol and in 23/47 workers without exposure to n-butanol but with exposure to industrial noise.	The effect levels and sampling protocol for measurement of n-butanol was not described
Cogan and Grant, 1945	75 female workers	Eye examinations were performed on symptomatic workers employed at a facility reported in the Tabershaw study	15 -100 ppm (46-303 mg/m^3) ethanol and diacetone alcohol (concentrations unknown)	28/35 workers exposed to n-butanol exhibited evidence of coreal inflammation and no unexposed workers showed signs of inflammation	Exposure to multiple solvents. No effect levels were reported
Sterner et al., 1949	16 male workers	Physical examinations of workers exposed to n-butanol during coating of photographic paper over a 10-year period were conducted.	Initial concentrations of n-butanol averaged 200 ppm (606 mg/m^3), but decreased to 100 ppm (303 mg/m^3) over the course of the study.	Workers exposed to n-butanol concentrations averaging ≥ 200ppm (606 mg/m^3) were reported to experience corneal edema and mild edema of the conjuctiva	Only 4 subjects remained in the study at 10 years

A number of other occupational studies examined health endpoints in worker populations such as painters or chemical/petrochemical workers who were exposed to mixtures of solvents that included n-butanol. Table 4-2 lists each of the studies and provides a brief description. All of the studies in Table 4-2 suffer from design limitations that preclude their use in establishing causal associations or dose-response relationships. Several of the studies (Chen et al., 1999; Jang et al., 1999; Akhmadeyeva, 1993; Bleecker et al., 1991; Murata et al., 1991) did not report any quantitative estimates of exposure to individual compounds. In the three studies that quantified exposure to individual compounds and included health effect information (Tucek et al., 2002; Triebig et al., 1992; Angerer and Wulf, 1985), exposure to n-butanol was small compared with exposure to other solvents such as xylenes, ethylbenzene, toluene, and/or acrylates.

Table 4-2. Studies of human exposure to mixtures including n-butanol

Reference(s)	No. subjects	Study type	Exposure	Findings	Strengths and limitations
Angerer and Wulf, 1985	31 exposed and 31 controls	Study compared hematology findings in varnish workers from six different workplaces (within two plants) with those of matched controls. Exposure to n-butanol was reported at only one workplace, with eight workers.	Air contaminants at the only workplace with butanol exposure (1.2 ± 0.8 mL/m^3) included o-, m-, and p-xylenes (averaging 3.4, 11.7, and 4.3 mL/m^3 respectively) and ethylbenzene (7.5 mL/m^3).	Hematology results pooled across the six locations suggested statistically significant reductions in erythrocyte count and hemoglobin content, increased lymphocyte count, and reduced (not statistically significantly) segmented granulocyte count.	Exposure to multiple solvents. n-Butanol was not detected in blood of workers at the workplace where it was measured in air; however, xylenes and ethylbenzene were detected in blood.
Bleecker et al., 1991	187 exposed	Cross-sectional study compared neurobehavioral findings in paint manufacturing workers across quartiles of total hydrocarbon exposure.	Exposures included toluene, xylene, aliphatic and aromatic hydrocarbons, methyl ethyl ketone, other hydrocarbons, alcohols (including n-butanol), ketones, and esters.	Statistically significant correlation (by linear regression analysis) between increasing exposure to mixed organic solvents and impairment of performance on several neuropsychological test outcomes, as well as vibration threshold.	Exposure to multiple solvents. Exposure measured as "lifetime average weighted exposure" to total hydrocarbons. No quantitative information on individual compounds was provided.
Murata et al., 1991	11 exposed and 11 controls	Study compared neurological findings in workers with solvent exposure with age-matched controls.	Only one of the workers reported exposure to n-butanol; this individual also reported exposure to xylene, methanol, and toluene	Coefficient of variation in electrocardiographic RR-intervals was statistically significantly reduced, and the distribution of nerve conduction velocities were slowed in exposed workers compared with controls.	No quantitative measures of exposure reported.

Table 4-2. Studies of human exposure to mixtures including n-butanol

Reference(s)	No. subjects	Study type	Exposure	Findings	Strengths and limitations
Triebig et al., 1992	83 exposed and 42 controls	Study compared subjective symptoms of neurotoxic endpoints in spray painters with controls.	Spray painters were from 10 different work sites; air concentrations of several solvents were reported for each work site. Four sites had n-butanol measurements (5.7, 1.5, 5.4, and >300 mg/m^3); the number of spray painters at each site was not reported. At the sites with n-butanol exposure, there was co-exposure to other solvents including toluene, xylene, ethylbenzene, styrene, acetone, butylacetate, methyl isobutylketone, ethyl acetate, n-heptane, and/or decane.	No difference in the prevalence of subjective symptoms of neurotoxicity between painters and controls.	Exposure to multiple solvents. Data pooled across all work sites, including those that had n-butanol exposure and those that did not.
Akhmadeyeva, 1993	347 exposed and 1,526 controls	Study compared health, physical development, and immunologic and psychochemical indices in blood of newborns of petrochemical workers with those of control infants.	Workplace air contaminants reportedly included aromatic hydrocarbons, isopropylbenzene, ethanol, and butanol.	Newborns of workers had higher frequency of delayed intrauterine development (24.2 vs. 13.7% in controls; $p < 0.01$). Other differences between study and control newborns included hematological and immunological effects.	Exposure to multiple solvents. Brief report; no information on study design. No quantitative measure of exposure; no quantitative information on effects other than intrauterine developmental delay.

Table 4-2. Studies of human exposure to mixtures including n-butanol

Reference(s)	No. subjects	Study type	Exposure	Findings	Strengths and limitations
Chen et al., 1999	309 exposed (mortality study) 260 exposed and 539 controls (neuro-psychology study)	Mortality study and cross-sectional study of self-reported neuro-psychological symptoms in dockyard painters and unexposed controls. Mortality analysis included 309 painters comprising 3,690 person-yrs at risk. Cross-sectional study of neuropsychological symptoms included 260 surviving painters and 539 unexposed controls.	According to the authors, primary solvents used at dockyard were white spirit, xylene, trimethylbenzene, n-butanol, trichloroethylene, naphtha, and cumene; exposures to other compounds were also reported to be likely.	Neither proportional mortality nor standardized mortality ratios for cancers or diseases of the CNS, circulatory system, or respiratory system were increased in painters. Cross-sectional study suggested increased prevalence of neuropsychological symptoms in painters compared with controls; relative risk for these symptoms increased with yrs of exposure.	Exposure to multiple solvents. Exposures characterized only by yrs as a dockyard painter; no quantitative measures of exposure.
Jang et al., 1999	674 exposed and 191 controls	Study compared neuropsychological effects (measured via questionnaire and function tests) in shipyard workers exposed to solvents and unexposed controls.	Primary solvents were xylene, ethyl-benzene, trimethylbenzene, toluene, 2-ethoxyethanol, and 2-ethoxyethyl acetate (constituted 85% of total exposure "intensity"). n-Butanol reported as one of several other solvents detected.	Rate of abnormal neuropsychological diagnosis (primarily executive dysfunction, attention dysfunction, memory disorder, or functional disorder) was >4 times higher in the exposed workers compared with controls (9.3 vs. 2.1% in controls, $p < 0.01$). Rate of abnormal diagnosis exhibited dose-response relationship with cumulative exposure.	Exposure to multiple solvents. Exposure quantified as "exposure intensity", a metric that incorporated environmental (total solvent concentrations) and/or biological measures (urinary metabolites of the primary solvents) as well as duration in certain job categories.

Table 4-2. Studies of human exposure to mixtures including n-butanol

Reference(s)	No. subjects	Study type	Exposure	Findings	Strengths and limitations
Tucek et al., 2002	60 exposed and 60 controls	Prospective cohort study of workers at a chemical facility producing acrylic acid. Study examined health effects including hematology, serum chemistry, serum immunity and tumor markers, spirometry, and cytogenetic analysis of peripheral lymphocytes.	Exposures included acrylonitrile, n-butanol, butyl acrylate, ethyl acrylate, methyl acrylate, methyl methacrylate, toluene, and styrene. Measurements of the various solvents (measured using personal air sampling) were binned by concentration and reported using stacked bar charts; these charts suggested that >70% of n-butanol concentrations were <0.02 mg/m^3. Exposures to the other compounds were of a similar magnitude and generally lower than occupational health limits in the Czech Republic; butyl acrylate was most frequently (at 2.1% of measurements) measured at a level exceeding its limit.	Statistically significantly higher percentage of chromosomal aberrations in peripheral lymphocytes.	Exposure to multiple solvents.

4.1.2.2. *Controlled Acute Exposure Experiments*

There several controlled acute exposure studies that evaluated irritation and conditioned reflexes in humans during n-butanol exposure. The exposure ranges were highly variable from as low as 0.1 mg/m^3 (Baikov and Khachaturyan, 1973; duration unknown) to 12,000 mg/m^3 for a 2 minute interval exposure (Kjaerguard et al., 1997). Notable effects in the controlled acute exposure studies included increased eye, nose, and throat irritation (Nelson et al., 1943; Cometto-Muniz and Cain, 1995; Wysocki and Dalton, 1996; Kjaerguard et al., 1997; Hempel-Jorgensen et al., 1998; 1999; Podlekareva et al., 2002) and CNS effects including increased response latencies and inhibition of the blinking reflex (Baikov and Khachaturyan, 1973). Only one study (Baikov and Khachaturyan, 1973) examined toxicity effects of n-butanol beyond irritation effects. These studies are described more fully in Table 4-3.

Table 4-3. Controlled Acute Exposure Studies

Reference	Subjects	Study Design and Exposure	Results	Strengths and Limitations
Nelson et al., 1943	10 volunteers	Comparison of eye, throat and nasal irritation in subjects and controls. Concentrations ranging from 0-50 ppm (0 – 150 mg/m^3; groups not specified by authors) of n-butanol were administered for 3-5 minutes in an inhalation exposure chamber	Eye irritation at 50 (150 mg/m^3) Nose and throat irritation at 25 ppm (76 mg/m^3) Highest concentration acceptable for 8 hours as reported by the volunteers was <25 ppm (<76 mg/m^3)	Limited information on dose concentrations
Baikov and Khachaturyan, 1973	18 volunteers in groups of 3	Comparison of the odor threshold and effects of n-butanol (0.1-2.5 mg/m^3) on conditioned reflexes in subjects	Odor threshold determined to be 1.2 mg/m^3 Dark adaption slowed at 1.2 mg/m^3 Statistically significant increase in response latency at 0.7 mg/m^3 Statistically significant inhibition of the conditioned blinking reflex at 1.5 and 2.5 mg/m^3 Transient development of conditioned reflex described by shifts in electrocephalograms. (effective concentration not reported)	Lack of study design details; Study identified CNS effects associated with n-butanol.
Cometto-Muniz and Cain et al., 1995	5 males and 5 females	n-Butanol vapors (100, 33, 11, 3.7, etc. % v/v) were administered to eye or nostrils for 1-3 seconds, using squeezable bottles. Eye irritation and odor threshold responses were evaluated.	Odor threshold was reported as 20 ppm (61 mg/m^3) Eye irritation threshold was reported as 3000 ppm (9000 mg/m^3)	Limited statistical power due to the small number of subjects
Wysocki and Dalton, 1996	32 exposed workers 32 unexposed residents	Odor and irritation thresholds for n-butanol (99.8% pure; diluted to 26 concentrations ranging from 100% to 1.1802 x 10^{-10}% v/v) in acetone –exposed workers and naïve volunteers were evaluated.	Median odor detection and nasal irritation thresholds were 0.17 ppm (0.52 mg/m^3) and 2,042 ppm (6,190 mg/m^3), respectively. Acetone workers considered n-butanol to be nonirritating, while naïve subjects considered the exposure to have irritating properties	Included a group exposed to one other solvent (acetone)
Kjaerguard et al., 1997	6 men and 6 women	Eye irritant effects of n-butanol after local (eye only) exposure and whole-body exposure in volunteers were evaluated. Doses of n-butanol used in the study were 1500-12000 mg/m^3 for 2	Statistically significant association (by repeated-data multiple analysis of variance) between exposure concentration, general irritation, eye irritation, throat	Only irritation effects were evaluated; No systemic toxicity effects were examined.

Table 4-3. Controlled Acute Exposure Studies

Reference	Subjects	Study Design and Exposure	Results	Strengths and Limitations
		minute intervals (lowest to highest) in goggle experiments and 0, 2.5,5, and 10 mg/m^3 in chamber experiments.	irritation, odor irritation, nose irritation, air quality, and continuously-measured irritation by potentiometer Analysis suggests an interaction between individual threshold for eye irritation in the goggle experiment and several other measures of irritation (general irritation, eye irritation, and throat irritation.	
Hempel-Jorgensen et al., 1998; 1999	8 subjects	Evaluated eye irritation associated with eye-only exposure to n-butanol n-butanol at (0, 99, 314, or 990 ppm (0, 300, 952, or 3,000 mg/$m^3$for periods of 15 or 60 minutes) and the results of conjunctival hyperemia evaluations using photographs of eyes before and after exposure, and conjunctival fluid cytology measured by optical microscopy. One eye was exposed to n-butanol, and the other to CO_2	The average irritation intensity was estimated for each second of the 60-minute exposure and plotted to show the time course of irritation Eye irritation intensity did not differ among the various concentrations of n-butanol and exposure to clean air The mean conjunctival hyperemia score in subjects exposed to 990 ppm (3,000 mg/m^3) n-butanol was statistically significantly higher (1.40 ± 0.76 on a scale from -2 to +2) than the score after exposure to clean air (0.48 ± 0.96); Conjunctival cytology was not affected by exposure to n-butanol at any concentration.	n-Butanol exposures in the study were not high enough to result in ocular irritation
Podlekareva et al., 2002	9 subjects	Compared physiological evidence of irritation after eye-only exposure to either n-butanol, CO_2, or clean air. n-butanol and CO_2 exposures were designed to stimulate the same perceived level of irritation (50% of maximal on a linear scale).	Eye exposure to n-butanol did not result in a significant change in tear film stability or conjunctival corrosion measures	Limited statistical power due to the small number of subjects

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

4.2.1. Oral

Research Triangle Institute (RTI; unpublished study, 1985) exposed groups of 30 male and 30 female rats with n-butanol (purity not reported, in deionized water) via daily gavage at doses of 0, 30, 125, or 500 mg/kg-day for 13 weeks. Daily observations for mortality and clinical signs, and weekly measurements of body weight and food consumption were made. Ophthalmology (specific tests not reported) was evaluated before exposure and during the final week of exposure. Blood and urine were collected from rats (10 rats/sex/group) at the end of 6 weeks of exposure and at terminal sacrifice (at the end of 13 weeks of exposure) for assessment of hematology (hematocrit, hemoglobin, red blood cell [RBC], mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, total and differential leukocyte count, platelet count), clinical chemistry (alkaline phosphatase [ALP], blood urea nitrogen [BUN], alanine aminotransferase [ALT], aspartate aminotransferase [AST], glucose, total protein, albumin, total bilirubin, electrolytes, inorganic phosphate, CO_2, total cholesterol, and creatinine), and urinalysis parameters (pH, specific gravity, glucose, protein, ketones, bilirubin, urobilinogen, and sediment microscopy). All animals were subjected to gross necropsy. Organ weights (brain, heart, liver, spleen, kidneys, adrenals, thyroid with parathyroid, testes with epididymides, and ovaries) were recorded for animals sacrificed after 13 weeks but not those sacrificed after 6 weeks of exposure. Comprehensive histopathologic examination (> 30 tissues) was performed on control and high-dose animals; in addition, the liver, kidneys, heart, and gross lesions were examined in the low- and mid-dose groups. The available copy of the unpublished report was lacking data tables and appendices. The original microfiche for the Toxic Substances Control Act Test Submission (TSCATS) also lacked the data tables and appendices; thus, the summary provided herein is based on the text of the report.

No n-butanol-treatment related death occurred in these rats. Three animals (one mid-dose and two high-dose animals) died prior to sacrifice due to gavage errors (RTI; unpublished, 1985). There were no statistically significant changes in body weight, food consumption, or ophthalmoscopic findings.

Clinical signs of toxicity, specifically hypoactivity and ataxia, were observed in high-dose animals but not in animals at lower doses. These signs were first observed during week 8, infrequently through week 10, and reached maximum incidence (29% for hypoactivity and 32% for ataxia) during weeks 11–13 (no other details provided). The study authors reported that these effects were evident 2–3 minutes after dosing and lasted for < 1 hour.

At the interim sacrifice, hematology analysis showed reduced hematocrit, erythrocyte count, and hemoglobin content (5% lower than controls) in 500 mg/kg-day females. No hematological changes were evident in males at interim sacrifice or in either sex at terminal sacrifice. There were higher absolute neutrophil count in mid-dose males at interim evaluation

and higher relative segmented neutrophil count and lower relative lymphocyte count in low-dose females at final evaluation. The study authors also reported the following statistically significant serum chemistry and urinalysis changes that were described as "small", occurring in one sex and at one evaluation only, and without a dose-response relationship: lower cholesterol in high-dose males at interim evaluation; and higher urine pH in low-dose males at the interim evaluation and in low-dose females at final evaluation. High-dose males exhibited a 14% increase in average thyroid weight compared with controls; no other statistically significant organ weight changes were reported. The report indicated that there were no treatment-related gross or microscopic pathology findings; however, the lack of data tables precluded independent review of the data.

The study authors did not identify effect levels, but indicated that no treatment-related effects were seen at 30 or 125 mg/kg-day. Based on the available information, EPA identified a LOAEL 500 mg/kg-day based on neurotoxic signs (hypoactivity and ataxia) and reduced hematocrit, erythrocyte count, and hemoglobin content in females. The NOAEL is 125 mg/kg-day.

Groups of 15 male Wistar rats (5 weeks of age at study commencement) were exposed to n-butanol (purity not reported) in drinking water (0 or 6.9% v/v; approximate saturation limit) for up to 3 months (Wakabayashi et al., 1991). This exposure concentration corresponds to an estimated dose of 8,200 mg/kg-day[1] based on default estimates of body weight (0.217 kg) and water intake (32 mL/day) (U.S. EPA, 1988) and a chemical density of 810 mg/mL for n-butanol (HSDB, 2009). Groups of 3–4 animals were sacrificed at various intervals; the first sacrifice occurred after 1 week of exposure. Toxicological evaluation was limited to electron microscopy of liver sections. Animals exposed to n-butanol and other alcohols reportedly exhibited poor appetite, weakness, and loss of body weight (no data provided). The study authors reported that there were no ultrastructural changes to hepatic mitochondria after n-butanol exposure up to 1 month; however, after 2 months of exposure, hepatic mitochondria were observed to be smaller and with poorly developed cristae. After 3 months of exposure, enlarged mitochondria with little or no cristae, and cup-shaped or elongated mitochondria were also observed. Other hepatic effects noted by the authors were proliferation of smooth endoplasmic reticulum and increased numbers of lysosomes and microbodies. The study authors proposed two possible mechanisms for the effects on mitochondria: fusion of adjacent mitochondria and suppression of the process of mitochondrial division via perturbation of protein synthesis.

EPA did not identify a NOAEL or LOAEL for this study due to study design limitations (single high concentration used, only ultrastructural changes in the liver were examined, and the incidence and severity in exposed or control groups were not reported). This study was designed to investigate whether mitochondrial effects observed with exposure to ethanol are also evident after exposure to other alcohols.

[1]Calculated as follows: 6.9% n-butanol = 0.069 mL/mL water × 810 mg/mL × 32 mL water/day × (1/0.217 kg) = 8,200 mg/kg-day.

Munoz et al. (1991, 1990) exposed groups of three male Wistar rats (initial weight 200–300 g) to unmodified drinking water or n-butanol in drinking water for 4 months in a study focused on examining effects on protein synthesis in the brain. The exposure concentration of n-butanol (purity not specified) was gradually increased from 1% (v/v) during the 1st week to 2% during week 2 and 4% for the remainder of the treatment period until sacrifice after 4 months. This exposure regimen corresponds to a time-weighted average concentration of 3.7%, resulting in an estimated dose of 4,400 mg/kg-day[2] based on default estimates of body weight (0.217 kg) and water intake (32 mL/day) (U.S. EPA, 1988) and a chemical density of 810 mg/mL for n-butanol (HSDB, 2009). Toxicological evaluations were limited to mortality, body weight, selected serum chemistry parameters (urea, glucose, creatinine, triglycerides, total proteins, albumin, and ALP), and in vitro protein synthesis in a cell-free translation system using brain extract. There were no mortalities during the study. Body weight gain for rats exposed to n-butanol for 100 days was 46% of the body weight gain observed in control rats. The authors reported that treated rats exhibited "signs of severe pathology" in several tissues, especially liver and kidney, but data were not provided and there was no information to indicate whether these organs were examined grossly or microscopically. Serum chemistry findings were reported without statistical analysis; however, group sizes were small (3/group). Treated rats had statistically significantly higher total protein (29% higher than controls) and albumin levels (28% higher). While not statistically significant, when compared with controls, the triglyceride level was higher (almost twofold) and ALP level was lower (20% of control) in treated rats. The study authors did not identify effect levels, and the information provided was inadequate for the identification of effect levels.

4.2.2. Inhalation

Korsak et al. (1994) exposed male Wistar rats (12/exposure group, 24 controls) to n-butanol (purity not specified) vapor concentrations of 0, 50, or 100 ppm (0, 154, or 308 mg/m^3) 6 hours/day, 5 days/week, for 3 months. Exposure concentrations, which were generated by heating the liquid solvent in a washer followed by dilution in air, were subsequently measured at 30-minute intervals using GC/flame ionization detection (FID). Body weights were recorded weekly during the study. Terminal body weight and organ weights (heart, lungs, liver, spleen, kidneys, adrenals, and testes) were recorded. Rotarod performance tests, designed to assess neuromuscular function, were performed on rats before the initiation of n-butanol exposure, and monthly during the exposure period. Rats were placed on a horizontal wooden rod that rotated at a rate of 12 revolutions/minute and was placed at a height of 20 cm above the floor. Prior to n-butanol exposure, rats were trained on the rotarod task for 10 days and received a shock from the floor after falling to prevent voluntary jumps from the rod. Therefore, learned avoidance

[2]Calculated as follows: 3.7% n-butanol = 0.037 mL/mL water × 810 mg/mL × 32 mL water/day × (1/0.217 kg) = 4,400 mg/kg-day.

behavior (from the negative enforcement of the shock from the floor) in addition to neuromuscular function was evaluated in the rotarod task as designed by the authors.

Hot-plate behavior (latency of the paw-lick response) was tested after the 3-month exposure period. Blood samples were collected from rat tail before the exposure and 1 week prior to the termination of exposure and used for hematological evaluations (hematocrit, hemoglobin, erythrocyte count [RBC], and total and differential leukocyte counts). Clinical biochemistry studies (ALT, AST, sorbitol dehydrogenase [SDH], ALP, total protein, albumin, glucose, electrolytes) were performed on serum samples taken at sacrifice 24 hours after the last exposure. The livers were homogenized to analyze total microsomal cytochrome P450 (CYP) content, aniline p-hydroxylase activity, triglyceride content, and malondialdehyde (MDA) content.

All rats survived and there were no clinical abnormalities observed in these rats (Korsak et al., 1994). Mean body weights of rats exposed to n-butanol were higher than controls (5–6%) during the first 2 months of the exposure period; during the 3rd month, average weights were higher (up to 7%), but not statistically significantly different from controls. There were no significant differences in absolute or relative organ weights. Table 4-4 shows changes in hematology and lipid peroxidation parameters. Compared to controls, the exposed groups exhibited decreased erythrocyte counts (5 and 16% lower in the 154 and 308 mg/m^3 groups, respectively); the difference was statistically significant in the 308 mg/m^3 group only. Statistically significantly decreased hemoglobin levels (10% lower than controls) were noted in both exposure groups, but hematocrit was not changed. There were increased leukocyte counts (25 and 57% higher in the 154 and 308 mg/m^3 group, respectively); the difference was statistically significant in the 308 mg/m^3 group, and was beyond the normal range of variability ($16.5 \times 10^3/nm^3$ in exposed rats, compared with a range of $1.96–8.25 \times 10^3/nm^3$; Giknis and Clifford, 2008). The leukocyte differential counts were not statistically significantly different among exposure and control groups, except for an increase (77% higher than controls), in the percentage of eosinophils in the 308 mg/m^3 group; this increase was well beyond the normal range (13.8%, compared with 0–2% for untreated male rats < 6 months of age; Wolford et al., 1986). There were no statistically significant exposure-related changes in any measured serum chemistry parameters. Statistically significantly increased lipid peroxidation, as measured by increased MDA in hepatic microsomes, was noted in both exposure groups (16 and 30% higher than controls in the 154 and 308 mg/m^3 groups, respectively). Hepatic total CYP content, microsomal aniline p-hydroxylase activity, and liver triglycerides were not affected by exposure to n-butanol. There were dose- and duration-related increases in the percentage of rotarod test failures, indicating impaired neuromuscular function and learned avoidance behavior, in the rats. The rotarod data were presented in Korsak et al. (1994) in a graphical format and the failure rates were approximately 2–15% in the 154 mg/m^3 group and 15–33% in the 308 mg/m^3 group when measured over the 3 month period and are presented in Table 4-5. The increased failure rate in

the 308 mg/m^3 group was statistically significant during the 2nd and 3rd months of exposure; the changes in the low exposure group were not statistically significant at any time point. Pain sensitivity (assessed by hot-plate behavior) was not affected in the rats exposed to n-butanol at these concentrations. EPA identified aNOAEL and LOAEL of 154 and 308 mg/m^3, respectively, based on impaired neuromuscular function. Decreased hemoglobin and increased lipid peroxidation were both observed at the NOAEL; however, the changes at that concentration were not biologically significant.

Table 4-4. Hematological effects in rats exposed to n-butanol by inhalation for 3 months

	Control	154 mg/m^3 (50 ppm)	308 mg/m^3 (100 ppm)
Number of animals	24	12	12
Hemoglobin (g/dL)	15.9 ± 0.4[a]	14.2 ± 0.8[b]	14.1 ± 0.7[b]
Erythrocytes (× 10^6/nm^3)	9.97 ± 0.02	9.45 ± 0.05	8.35 ± 0.06[b]
Leukocytes (× 10^3/nm^3)	10.5 ± 0.13	13.1 ± 0.26	16.5 ± 0.27[b]
Eosinophils (%)	7.8 ± 2.3	11.5 ± 4.6	13.8 ± 4.6[c]
MDA (nmol/mg microsomal protein equivalent to g liver)	40.41 ± 2.10	46.68 ± 2.16[c]	52.45 ± 2.88[c]

[a]Mean ± standard deviation (SD).
[b]Significantly different from control at $p < 0.01$.
[c]Significantly different from control at $p < 0.05$.

Source: Korsak et al. (1994).

Table 4-5. Changes in neurobehavioral function as measured by the rotorod test in rats exposed to n-butanol by inhalation over 3 months

Exposure Group	Number of animals	Percentage of Rotarod Failures[a]		
		Month 1	Month 2	Month 3
Control	24	0	0	0
154 mg/m^3(50 ppm)	12	2	10	15
308 mg/m^3(100 ppm)	12	15	25[b]	33[b]

[a] Percentage of rotarod failures were derived from data approximations.
[b]Significantly different from control at $p < 0.05$.

Source: Korsak et al. (1994).

Smyth and Smyth (1928) performed three experiments in which guinea pigs (at least three/group, sex unspecified) were exposed to n-butanol (purity not reported) vapor at a concentration of 100 ppm (300 mg/m^3) every day for 2 weeks (hours/day were not specified) and then for 4 hours/day, 6 days/week, for durations ranging from 1 to 2.5 months. The three groups were not exposed concurrently. There were two control groups: one sham-treated and one untreated, three animals per group (sex unspecified). The guinea pigs were weighed weekly.

Blood counts and urinalysis (parameters not reported) were assessed prior to exposure and at biweekly intervals thereafter. The schedule for and tests performed at sacrifice were not described. The study authors reported that there were no deaths among the first group treated for 64 exposures, but there was a decrease in erythrocyte count as well as lymphocytosis. Of this group of three animals, two were reported to exhibit hemorrhagic areas in the lungs and transient albuminuria. The second group tested at this concentration developed severe skin infections after the 30th exposure, and two died during the 38th exposure. These animals also reportedly exhibited decreased erythrocytes and hemoglobin along with increased total leukocytes (data not provided). The surviving guinea pig gained weight and had a "decidedly improved blood picture" (details not provided) at sacrifice. The authors indicated that all three animals had "toxic degeneration" of the livers and kidneys (no further information given). The third experiment was continued for 28 exposures, and the authors reported similar changes in hematology (decreased erythrocytes and absolute and relative lymphocytosis) as well as central liver and marked renal degeneration (characterized by cloudy swelling of convoluted tubules, marked degeneration of tubules). Data on weight, hematology, and specific gravity of urine were reported for each of the three treated animals, but control data were not provided. In the two control groups, one animal each died of skin infection.

Rumyantsev et al. (1979; published in Russian and translated for this review) rats and mice were exposed to butanol (isomer and purity not reported) via inhalation at concentrations of 0, 0.8, 6.6, or 40 mg/m^3 for 4 months (frequency not reported). This study did not provide other details of the study design (for example, sex, strain, or number per group, or toxicological evaluations performed) or any quantitative information on the effects observed. The only effect information provided in the report was a list of effects at each exposure concentration; the affected species was not reported in most cases. Increased thyroid gland activity reportedly occurred in all experimental groups. At 6.6 and 40 mg/m^3, narcosis induced by hexanal administration was shortened, there was an increase in the conditioned reflex activity and the pituitary-adrenal system was characterized as disrupted. Other changes were noted such as a loss of CNS summation capacity (generally an electrophysiological measurement of nerve response), a reduced eosinophilic response following administration of adrenocorticotropic hormone, and reduced demand of oxygen in what was termed the "cold test." Finally, increased blood cholinesterase activity was observed in rats at $\geq$ 6.6 mg/m^3. The authors characterized the 6.6 mg/m^3 concentration as a threshold value and could be considered a LOAEL and 0.8 mg/m^3 is the NOAEL for the study. Although the authors provide effect levels for the study, there is no information on the study design, no data for the toxicological effects are available, and statistical information is not provided for the various endpoints. As a result, EPA considers the information reported in the publication as inadequate for the purpose of determining effect levels.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

4.3.1. Oral Studies

Sitarek et al. (1994) exposed female Wistar Imp:DAK rats (11–17/group) to n-butanol (purity not reported) in drinking water at concentrations of 0.24, 0.8, and 4%, estimated as 300, 1,000, and 5,000 mg/kg-day by the authors. A control group of 16 rats received plain tap water. Treatment was initiated at 10 weeks of age and continued for 8 weeks prior to mating. For 14 days prior to exposure and during weeks 4–5 and 7–8 of the premating exposure period, vaginal smears were collected daily for assessment of estrus cycle. The females were then mated to untreated males for up to 3 weeks. Gestation day (GD) 0 was defined as the day of sperm detection in vaginal smears. The females were exposed to n-butanol continuously through the mating and gestation periods. Weight gain, food consumption, and water intake were measured weekly in females that did not become pregnant and on GDs 3, 7, 10, and 17 in pregnant females. Upon sacrifice on GD 20, uterine contents (numbers of live and dead fetuses, numbers of early and late resorption sites) were evaluated. Body weight and crown-rump length of live fetuses were recorded. All live fetuses were examined for external malformations; half were then prepared for skeletal examination and the remainder for visceral examination. Statistical analysis of malformations was performed using either the fetus or the litter as the statistical unit.

All of the animals survived and treatment with n-butanol did not affect food or water consumption or body weight of female rats during the premating period (Sitarek et al., 1994). Mean daily intake of food and water by high-dose dams was lower than controls (10 and 9%, respectively), but the difference was not statistically significant. Mean body weight gain was 5% higher in high-dose dams compared with controls; the difference was not statistically significant. There were no differences in estrus cycle duration or duration of individual stages of the cycle. The study authors reported that hemoglobin concentration and hematocrit, as well as absolute and relative organ weights were not different between exposed and control dams; however, detailed data were not provided. Treatment with n-butanol did not affect the pregnancy rate, number of corpora lutea or total implants, number of litters with resorptions, or numbers of pre- or post implantation losses/litter. A slight increase in the number of early resorptions/litter was observed (two-fold increase over control at the high-dose), but the difference from control was not statistically significant.

Table 4-6. Selected fetal developmental changes in offspring of rats exposed to n-butanol in drinking water for 8 weeks before mating and during gestation[a]

Parameter	n-Butanol dose in mg/kg-d (% in water)			
	0	300 (0.24%)	1,000 (0.8%)	5,000 (4.0%)
Fetal body weight[b] (g)	3.2 ± 0.2	3.2 ± 0.3	3.2 ± 0.2	3.2 ± 0.3
Fetal crown-rump length[b] (cm)	4.0 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.8 ± 0.1[c]
No. of litters examined for visceral variations	12	14	12	9
Percentage of litters with dilation of	8	64[c]	83[c]	100[c]
Subarachnoid space (%)	0	14[c]	25[c]	78[c]
Lateral ventricle and/or third ventricle of the brain (%)	8	57[c]	67[c]	78[c]
Unilateral renal pelvis (%)	0	0	42[c]	0
Bilateral renal pelvis (%)	0	0	25[c]	0
Percentage of litters with congenital defects	0	0	33[c]	22[c]
External hydrocephalus (%)	0	0	17[c]	0
Internal hydrocephalus (%)	0	0	25[c]	22[c]
No. of litters examined for skeletal variations	12	14	12	9
Percentage of litters with delayed ossification	67	50	58	67
Percentage of litters with congenital defect	0	7[c]	0	11[c]
14th rib (%)	0	0	0	11[c]
Wavy ribs (%)	0	7[c]	0	0

[a] Fetal and litter data was provided by authors; only litter data is presented
[b]Mean ± SD.
[c]Significantly different from control at $p < 0.05$.

Source: Sitarek et al. (1994).

Table 4-6 shows the percentages of fetuses and litters with malformations. Mean fetal body weight was not affected by treatment, but mean fetal crown-rump length was statistically significantly decreased in offspring of high-dose dams compared with controls (5%). Dose-related increases in the incidences of litters with visceral malformations were observed; the difference from control was statistically significant at all doses (Sitarek et al., 1994). The predominant visceral malformation was dilation of the lateral and/or third ventricle of the brain, affecting from 57 to 78% of litters in treated animals and only 8% of control litters. The incidence of litters with dilation of the subarachnoid space was also statistically significantly increased in a dose-related manner; the percentages in treated groups were 14–78% of litters, compared with 0% in controls. Dilation of the renal pelvis was observed only at the mid-dose (25–42% of litters and 0% in other groups). The percentages of litters with internal hydrocephalus was statistically significantly increased at the mid- and high-doses (25 and 22%, respectively, compared with 0% in controls), while the percentage of litters with external hydrocephalus was statistically significantly increased at the mid-dose only (17% compared with 0% in controls). The authors reported that the percentage of fetuses with delayed ossification

was increased at the high-dose; however, the percentage of litters affected was not different between controls and high-dose animals. The percentage of litters with an extra (14th) rib was statistically significantly higher in the group exposed to 5,000 mg/kg-day (11 vs. 0% in controls); this occurred in two fetuses of a single litter at this dose. The authors did not determine effect levels. EPA determined that the low-dose (300 mg/kg-day, or 0.24% in water) is a LOAEL based on increased incidences of visceral malformations (dilation of the lateral and/or third ventricle of the brain and of the subarachnoid space) and a NOAEL was not identified.

Ema et al. (2005) also investigated the developmental effects (brain malformations and variations) of n-butanol in Sprague-Dawley rats. Pregnant (sperm positive) Sprague-Dawley (Crj:CD(SD)IGS) rats (20/group) were exposed to n-butanol (99.9% pure) in drinking water at concentrations of 0, 0.2, 1.0, or 5.0% on GDs 0–20. The authors estimated daily doses of 0, 316 ± 39, 1,454 ± 186, or 5,654 ± 1,402 mg/kg-day based on measured body weight and water consumption. Maternal evaluations included recording of clinical signs, body weights, and food and water intake. Dams were sacrificed on GD 20 for assessment of numbers of corpora lutea, implantation sites, resorptions, and live and dead fetuses; placental weight was also recorded. Live offspring were sexed, weighed, measured (crown-rump), and examined for external and oral malformations. Half of the fetuses were prepared for examination of internal malformations and the remainder for skeletal malformations. The litter was used as the statistical unit for analysis of the data. Treatment-related findings are summarized in Table 4-7.

None of the animals in any group died. As shown in Table 4-7, maternal body weight gain in the group exposed to 5,654 mg/kg-day (5% n-butanol in water) was statistically significantly decreased during GDs 0–7 (45% of controls) and GDs 0–20 (10% less than controls). Food consumption was statistically significantly decreased compared to controls in mid- and high-dose dams (8 and 20% lower than controls, respectively) throughout pregnancy. During GDs 0–7, water consumption was statistically significantly decreased at the mid- and high-doses (9 and 38% lower than controls), and was decreased throughout gestation in the high-dose dams (28% lower than controls over GDs 0–20).

There were no statistically significant differences between exposed and control rats in placental weight or numbers of corpora lutea, implantations, pre- or post implantation losses, resorptions, or live or dead fetuses (Ema et al., 2005). The sex ratio of offspring was not different among the groups. Fetal body weight was statistically significantly reduced in both male (8% lower than controls) and female (10%) offspring of dams exposed to 5,654 mg/kg-day (5% n-butanol). Body weights were decreased in the mid- and low-dose fetuses; however, the difference from control was not statistically significant. The crown-rump length of offspring was not affected by treatment.

Table 4-7. Selected changes in rats exposed to n-butanol in drinking water on GDs 0–20

Parameter	n-Butanol dose in mg/kg-d (% in water)			
	0	**316 (0.2%)**	**1,454 (1.0%)**	**5,654 (5.0%)**
Maternal body weight gain on GDs 0–7 (g)[a]	44 ± 7	45 ± 7	40 ± 6	20 ± 28[b]
Maternal body weight gain on GDs 0–20 (g)[a]	162 ± 19	168 ± 16	165 ± 15	146 ± 16[b]
Maternal food consumption GDs 0–20 (g)[a]	548 ± 38	548 ± 46	503 ± 34[b]	441 ± 34[b]
Maternal water consumption GDs 0–20 (mL)[a]	930 ± 105	983 ± 126	890 ± 106	669 ± 182[b]
Male fetal body weight (g)[a]	4.18 ± 0.27	4.00 ± 0.24	4.04 ± 0.25	3.83 ± 0.18[b]
Female fetal body weight (g)[a]	3.97 ± 0.25	3.86 ± 0.20	3.83 ± 0.16	3.59 ± 0.17[b]
Skeletal examination[c]				
Total no. of litters examined	20	20	20	20
Total no. of litters with variations	11	12	17	20[b]
Short supernumerary ribs	10	9	16	19[b]
Degree of ossification				
No. of forepaw proximal phalanges	1.6 ± 1.3	1.6 ± 0.9	1.2 ± 1.1	0.3 ± 0.4[b]
Internal examination[c]				
Total no. of litters examined	20	20	20	20
Thymic remnant in neck	4	5	8	8

[a]Mean ± SD.
[b]Significantly different from control at $p < 0.01$ (litter as unit of statistical analysis).
[c] Fetal and litter data was provided by authors; only litter data is presented

Source: Ema et al. (2005).

The incidences of external, oral, and visceral malformations were not increased by treatment with n-butanol (Ema et al., 2005). At the highest dose, a statistically significant increase (20/20 vs. 11/20 litters in controls) in the incidence of litters with skeletal variations (primarily short supernumerary ribs) was observed, as well as a decrease in the degree of ossification (number of forepaw proximal phalanges was 0.3 ± 0.4 vs. 1.6 ± 1.3 in controls). The study authors suggested that the skeletal effects may have been related to growth retardation in the high-dose group. Although there was no statistically significant increase in the incidence of litters with internal malformations, the incidence of litters with thymic remnant in the neck increased with dose (see Table 4-7) and the incidence at the high-dose was twice that of controls (8/20 litters vs. 4/20 litters in controls).

The study authors did not identify a LOAEL for this study (Ema et al., 2005); however, they did indicate that the NOAEL for dams and fetuses was 1,454 mg/kg-day (1%). EPA has determined that the high-dose (5,654 mg/kg-day, or 5% in water) is a LOAEL for developmental effects (decreased fetal body weight and increased incidence of skeletal variations). The NOAEL for the fetal effects is 1,454 mg/kg-day (1%).

McLain (2008) summarized a teratogenicity study by Bariliak et al. (1991) of n-butanol and other alcohols administered by gavage. This study was published in Russian with a brief English summary and was not translated for this review.According to McLain (2008), groups 10–16 white rats (weighing 160–180 g, strain not specified) were given various alcohols (methanol, ethanol, n-butanol, nonanol, and decanol; purity not given) by gavage (1 mL of 40% solution in water) from GDs 1 through 15. Controls (20 rats) were given water alone during the same gestational treatment period. Without information on the body weights of the pregnant rats, estimating a dose associated with the administered solution of n-butanol is uncertain. Assuming a body weight of 0.250 kg and chemical density of 810 mg/mL, a dose of 1,300 mg/kg-day[3] was estimated. At sacrifice on GD 20, the numbers of corpora lutea and live and dead fetuses were counted. The review did not discuss any maternal evaluations. Parameters reported in the secondary source include: fertility index (description not provided), number of implantations, percent pre- and post implantation losses, and number of live fetuses. In addition, ADH activity was measured in livers excised from selected fetuses (1–2/litter). According to McLain (2008), these measurements were performed daily on fetuses from GDs 16 to 21 and on postnatal days (PNDs) 1, 3, and 20.

Results of the study, as reported by McLain (2008), are shown in Table 4-8. According to the review, treatment with n-butanol resulted in statistically significant increases in the percents of pre- and post implantation losses and in total fetal deaths. A decrease in the fertility index of treated animals was reported in the review (6.5 vs. 9.7% in controls; statistical analysis not reported). In addition, ADH activity in fetal livers, which was at its highest level on GD 20, was reduced 77.6% at this measurement in offspring of dams exposed to n-butanol. No other information was provided in the review or in the English summary in the publication. Effect levels were not provided in the review. Given the lack of information from which to reliably estimate dose, as well as reliance on a secondary source for information, EPA has not determined effect levels for this study.

[3]Calculated assuming % v/v in water as follows: 40% n-butanol (in 1 mL) = 0.4 mL/day × 810 mg/mL × (1/0.250 kg) = 1,300 mg/kg-day.

Table 4-8. Teratogenic observations in rats exposed to n-butanol by gavage during GDs 1–15

	Historical control	Control	1 mL of 40% n-butanol solution (dose estimated to be ~1,600 mg/kg-d)
Number of animals	362	20	10
Index of fertility	9.6	9.7	6.5
Number of corpora lutea	3,684	207	106
Number of implantations	3,668	203	83
Fetal deaths (%)	10.5 ± 0.5[a]	6.3 ± 1.7	38.7 ± 4.7[b]
Pre implantation	5.6 ± 0.4	2.0 ± 1.0	21.7 ± 4.0[b]
Post implantation	5.2 ± 0.4	4.4 ± 1.4	21.7 ± 4.4[b]
Number of live fetuses	3,476	194	65

[a]Mean ± SD.
[b]According to the review, "all indices of the embryotoxic activity in the experimental groups are reliably higher than control ($p < 0.001$)".

Sources: Bariliak et al. (1991) as reported in McLain (2008).

4.3.2. Inhalation Studies

Nelson et al. (1989a) studied the developmental toxicity of n-butanol (≥ 99% pure) and other butanol isomers in rats exposed by inhalation. Groups of 15–20 sperm-positive female Sprague-Dawley rats were whole-body exposed to n-butanol vapor at nominal concentrations of 0, 3,500, 6,000, or 8,000 ppm (0, 11,000, 18,000, or 24,000 mg/m^3), 7 hours/day on GDs 1–19. Test concentrations were selected on the basis of pilot testing. In the pilot experiment, exposure to 27,000 mg/m^3 (9,000 ppm) n-butanol was lethal to two of six nonpregnant rats within 2 days, and exposure to 24,000 mg/m^3 (8,000 ppm) resulted in narcosis in half of tested dams. In the main study, dams were evaluated weekly for food and water intake, and body weight was measured on GDs 0, 7, 14, and 20. After sacrifice on GD 20, the numbers of corpora lutea, resorptions (early, middle, or late), and live fetuses were recorded. Fetuses were weighed, sexed, and examined for external malformations. Half of the fetuses were examined for skeletal malformations and the other half for visceral malformations.

In the highest exposure concentration of n-butanol, 2/18 dams died prior to sacrifice (Nelson et al., 1989a). There were no deaths in the low- or mid-exposure groups. The body weights of dams in the 24,000 mg/m^3 group were approximately 17% less than controls at termination (based on visual inspection of data presented graphically), although this difference was not statistically significant when the authors adjusted for multiple comparisons using the Bonferroni technique. However, the body weights were slightly higher than controls in 11,000 and 18,000 mg/m^3 groups. Food consumption was lower in dams exposed to the mid- and high concentrations (11–19% lower than controls at 18,000 mg/m^3 and 10–17% lower at 24,000 mg/m^3); the difference from controls was statistically significant during all 3 weeks of treatment

at the mid concentration and during the 1st week only at the high concentration. Water intake was reportedly higher in treated groups than in controls, but the difference was not statistically significant (data not reported). Exposure to n-butanol had no effect on number of corpora lutea, resorptions or live fetuses/litter, or sex ratio. External malformations were not observed in any group. Statistically significant concentration-related reductions in body weight were observed in male and female fetuses at the mid- and high-exposure levels (12 and 24–27% lower than controls in the 18,000 and 24,000 mg/m^3 groups, respectively). The percent of fetuses having normal skeletal development was statistically significantly lower at 24,000 mg/m^3 n-butanol. The authors indicated that rudimentary cervical ribs were the primary skeletal malformation observed with n-butanol exposure but incidence data for the different exposures was not provided by the authors. Statistical analysis of the incidences of litters with skeletal or visceral malformations or variations was not reported in the study. Based on Fisher's exact tests performed for this review, the incidence of litters with skeletal malformations was increased at all concentrations, and the incidence of litters with visceral malformations was statistically significantly increased at the highest concentration. Table 4-9 reports selected findings from the study. The authors did not identify effect levels. determined that lowest concentration tested (11,000 mg/m^3 or 3,500 ppm) was associated with an increase in the incidence of litters with skeletal variations and is considered a LOAEL.

Table 4-9. Selected changes in rats exposed to n-butanol via inhalation on GDs 1–19

Parameter	n-Butanol exposure in mg/m^3 (ppm)			
	0	11,000 (3,500 ppm)	18,000 (6,000 ppm)	24,000 (8,000 ppm)
Mortality	0/17	0/15	0/18	2/18
Number pregnant/number bred	15/17	13/15	18/18	15/16
Maternal food consumption during wk 1 (g)	124 ± 15[a]	142 ± 17	102 ± 24[b]	103 ± 24[b]
Maternal food consumption during wk 2 (g)	134 ± 21	142 ± 16	108 ± 12[b]	118 ± 17
Maternal food consumption during wk 3 (g)	124 ± 16	133 ± 16	110 ± 9[b]	111 ± 17
Male fetal body weight (g)	3.4 ± 0.31	3.4 ± 0.18	3.0 ± 0.31[b]	2.6 ± 0.25[b]
Female fetal body weight (g)	3.3 ± 0.27	3.2 ± 0.18	2.9 ± 0.30[b]	2.4 ± 0.23[b]
Fetuses (litters) with skeletal malformations	0/102 (0/15)[c]	5/85 (4/12)[d]	8/129 (5/18)[d]	16/98 (9/15)[d]
Fetuses (litters) with skeletal variations	43/102 (14/15)	24/85 (11/12)	52/129 (17/18)	75/98 (-)[e]
Percent of fetuses with normal skeletal development	100 ± 0	94 ± 3	94 ± 3	85 ± 4[b]
Fetuses (litters) with visceral malformations	0/106 (0/15)	0/97 (0/13)	2/134 (2/18)	8/96 (4/15)[d]
Fetuses (litters) with visceral variations	7/106 (4/15)	8/97 (6/13)	6/134 (4/18)	19/96 (8/15)
Percent of fetuses with normal visceral development	100 ± 0	100 ± 0	99 ± 1	92 ± 4

[a]Mean ± SD.
[b]Significantly different from control at $p < 0.05$.
[c]Affected fetuses/fetuses examined (affected litters/litters examined).
[d]Significantly different from control at $p < 0.05$ by Fisher's exact test performed for by EPA.
[e]The study reported that 25 of 15 litters had skeletal variations. This was assumed to be a reporting error.

Source: Nelson et al. (1989a).

Nelson et al. (1989b) evaluated behavioral teratology in young rats following in utero or paternal inhalation exposure to n-butanol vapor. Groups of 15 pregnant female Sprague-Dawley rats were exposed to 0, 3,000, or 6,000 ppm (0, 9,000, or 18,000 mg/m^3) n-butanol for 7 hours/day on GDs 1–19 (termed "maternal exposure group" by the authors). Groups of 18 male Sprague-Dawley rats were exposed to the same concentrations of n-butanol for 7 hours/day for 6 weeks and then mated to non-butanol exposed females (termed "paternal exposure group" by the authors). On the day of birth (PND 0), offspring were culled to four males and four females per litter and fostered to untreated controls. Individual pup weights were recorded weekly for 5 weeks. On PND 10, one male and one female pup/group were randomly assigned to one of four testing groups. There were three groups assigned to behavioral testing for neuromotor

coordination (ascent on a wire mesh screen on PNDs 10, 12, and 14 and rotarod, not mentioned in the methods section), activity (open field activity and photoelectrically-monitored activity on PNDs 16–18, 30–32, 44–46, and 58–60, and running wheel activity on PNDs 32 and 33), and learning (avoidance conditioning, with separate groups tested beginning PNDs 34 and 60 and operant conditioning beginning PND 40). The fourth group was used for brain neurotransmitter analysis. On PND 21, brains were removed from 10 pups/group (one male and one female/litter) and dissected into four regions (cerebrum, cerebellum, midbrain, and brainstem), these samples were used to measure the levels of total proteins and neurotransmitters, including acetylcholine (ACh), dopamine, norepinephrine, serotonin, met-enkephalin, B-endorphin, and substance P. The study authors indicated that, since exposures to the two concentrations of n-butanol were separated by about 5 months, results could be compared to the contemporaneous controls, but comparisons between the two concentrations would not be appropriate.

Analysis of exposure concentrations showed consistency with target exposures (method of analysis not reported); measured concentrations were 3,010 ± 50 and 6,000 ± 80 ppm (9824 ± 163 and 19580 ± 261 mg/m^3, respectively; Nelson et al., 1990, 1989b). The study authors reported that n-butanol exposure did not affect pregnancy rate in any exposure groups. There were no behavioral changes in the offsping in term of their performance in ascent test, rotarod performance, open field performance, or operant conditioning. In offspring of 18,000 mg/m^3 paternal exposure group, the time receiving shock and the total number of times that rats crossed from one side of the cage to the other were both statistically significantly increased over controls. Monitoring of photoelectric activity showed statistically significantly lower counts in female offspring of to the 9,000 mg/m^3 paternal exposure group but not in offspring of the high concentration paternal exposure group (data not shown, and magnitude of change not reported). Tests for avoidance conditioning showed that male offspring of the 9,000 mg/m^3 paternal exposure group required statistically significantly fewer trials to reach criterion than controls; no statistically significant change was observed at the high concentration. Analysis of neurotransmitter concentrations in the brains of offspring revealed statistically significant increases in the overall concentration of serotonin (mean ± SEs were 14.48 ± 2.38 vs. 7.802 ± 1.48 in controls; units not reported) and dopamine (0.715 ± 0.127 vs. 0.515 ± 0.095 in controls; units not reported) in offspring of the 18,000 mg/m^3 paternal exposure group. There were no other statistically significant changes in neurotransmitter concentrations associated with exposure to n-butanol. The study authors also indicated that the changes in both the neurobehavioral tests and neurotransmitter concentrations observed in the animals exposed to n-butanol were within the range of control data from their laboratory. The authors did not define effect levels. EPA determined that the highest concentration (18,000 mg/m^3, or 6,000 ppm) tested in this study is a NOAEL for neurobehavioral effects in offspring after parental inhalation exposure to n-butanol.

In a study examining potential testicular toxicity, Cameron et al. (1985) exposed groups of five male Sprague-Dawley rats to n-butanol at a concentration of 50 ppm (150 mg/m^3) 6 hours/day for 1 day or 1 week. Testicular effects were assessed through the measurement of serum concentrations of testosterone, luteinizing hormone (LH), and corticosterone in blood samples collected at sacrifice at the end of exposure or 18 hours after the end of exposure following both the 1-day and 1-week exposures. In rats sacrificed after 1 day of exposure, statistically significant reductions in testosterone concentrations were observed; the decreases were 37 ± 8 and 52 ± 22% of controls when measured immediately after and 18 hours after exposure, respectively. In rats sacrificed after 1 week of exposure, testosterone levels were decreased (73–83% of controls), but the difference from controls was not statistically significant. Serum concentrations of LH were higher than controls (115–124% of controls) in animals treated with n-butanol for 1 day, but lower (78–98% of controls) in those treated for 1 week; none of the differences were statistically significant. Rats treated with n-butanol for 1 day had statistically significantly higher serum corticosterone than controls (143% of control); however, rats treated for 1 week had lower corticosterone (85% of control, not statistically significantly different from control).

4.4. OTHER DURATION- OR ENDPOINT-SPECIFIC STUDIES

4.4.1. Acute and Short-term (≤ 30 Days) Studies

Several studies determined LD_{50} values for n-butanol following acute and short-term exposure. Among rats, rabbits, hamsters, and mice, oral LD_{50}s ranged from 1,200 mg/kg to 4,360 mg/kg (Rumyantsev, 1979; Dubina and Maksimov, 1976; Munch, 1972; Purchase, 1969; Jenner et al., 1964; Smyth et al., 1951; McOmie and Anderson, 1949; Munch and Schwartze, 1925). Behavioral toxicities following or during an acute exposure included narcosis and necropsies indicated that there was necrosis of the liver and kidney as well as general organ congestion (Munch, 1972; Purchase, 1969; Jenner et al., 1964; McOmie and Anderson, 1949; Munch and Schwartze, 1925).

General short-term studies for n-butanol primarily evaluated effects on narcosis and pathological effects in the liver and kidney. Detailed summaries for these studies are provided in Table 4-10.

Table 4-10. Summary of Short-term Studies

Species/Sex	Dose	Duration	Effect Observed	Reference
Mice	24,624 mg/m^3 inhalation	130 hours	Narcosis and reversible fatty infiltration of the liver and kidneys	Weese, 1928

Mice	22 mg/L (22,000 mg/m^3) 27 mg/L (27,000 mg/m^3) inhalation	12 hours 22.6 hours	At 22 mg/L for 12 hours 2/10 animals died and 6/10 lost righting reflex. At 27 mg/L for 22.6 hours 4/10 mice died. Irritation was reported at 15 min, somnolence at 3 hours, anesthesia at 6 hours and death at 12 hours in saturated room air (concentration unknown).	McOmie and Anderson, 1949
Mice/male	0 mg/m^3 0.78 ± 0.05 mg/m^3 6.6 ± 0.39 mg/m^3 40 ± 42 mg/m^3	30 days	Statistically significant decrease in hexenal induced sleep duration at both the mid and high n-butanol exposure levels.	Kolenikov, 1975
Rats/male	Various doses (not reported)	60 min	The threshold at which no change in rectal temperature was observed 60 minutes after dosing was reported as 2.5 nM/kg	Mohler and Gordon, 1991
Cats	200 mg/kg i.v.	Not reported	Dose was lethal	Macht, 1920

4.4.2. Liver Toxicity

Videla (1982) administered a single oral dose of 560 mg/kg n-butanol (purity not reported) to groups of four male Wistar rats. Levels of reduced glutathione (GSH), diene conjugates, proteins, and ADH activity in the liver were measured 6 hours after dosing. Based on visual inspection of data presented graphically, levels of GSH were lower, and diene conjugates were higher in animals given n-butanol compared with saline-treated controls; however, the differences were small and were not statistically significant. ADH activity in n-butanol treated rat livers was about 41% of that in ethanol-treated rat livers; however, information on controls was not reported.

Shehata and Saad (1978) evaluated the effect of oral exposure to alcohols on vitamin content in the livers of rats. Groups of six male albino rats were given daily gavage doses of 0, 1, or 2 mL/kg (810 and 1,620 mg/kg) n-butanol for 7 consecutive days. The animals were sacrificed 2 hours after the last dose and the liver was assayed for content of thiamine, riboflavin, pyridoxine, niacin, and pantothenic acid. At both doses of n-butanol, the liver content of all five vitamins was statistically significantly lower than the corresponding control value. The decreases, which were dose-related, ranged between 25 and 49% at the high-dose.

4.4.3. Immunotoxicity

Strubelt and Zetler (1980) tested the antiinflammatory effects of n-butanol and other alcohols. Groups of eight male Wistar rats were given single gavage doses of 0, 600, or

1,200 mg/kg n-butanol (analytical purity not further specified), followed 15 minutes later with an injection of 0.1 mL of 1% carageenan in the right hind paw. Inflammation of the paw was measured as mean difference from pretreatment paw volume after 2, 3, 4, 5, and 5 hours following injection. Exposure to n-butanol resulted in reduced inflammation, as shown by a statistically significant inhibition of the increase in paw volume associated with carageenan injection (19 and 15% smaller mean increase in paw volume compared with controls in the low- and high-dose groups, respectively). Similar inhibition of inflammation was observed with other alcohols. In a separate experiment, groups of 12–16 male Wistar rats were treated with single gavage doses of 0, 300, 600, or 1,200 mg/kg followed by injection of 0.2 mL of 1% carageenan into the pleural cavity. After a period of 4 hours, the rats were sacrificed and chest cavity was opened. The volume of exudate was recorded, and leukocyte count and osmolality of the exudate were measured; blood osmolality was also measured. Exposure to n-butanol at ≥ 600 mg/kg resulted in statistically significantly reduced volume of pleural exudate (53 and 13% of control volume at 600 and 1,200 mg/kg, respectively) and decreased leukocyte count (51 and 9% of control count, respectively), indicating a reduction in the inflammatory response to carageenan. The osmolality of pleural exudates was not affected by exposure. Significant changes in blood osmolality were observed at the low- and high-doses, but not the mid-dose. The blood osmolality changes were not consistent with dose; the low-dose resulted in a decrease in osmolality, while the high-dose resulted in an increase. This study suggested that oral exposure to n-butanol results in anti-inflammatory effects.

4.4.4. Neurotoxicity

Wallgren (1960) tested the behavior of 5-month-old rats (strain not reported, groups of 5–7/sex) exposed to a single oral dose of 0.0163 mol/kg n-butanol (1,210 mg/kg). Beginning 20 minutes after dosing, the animals were subjected to six "tilted plane" tests at 20-minute intervals. In this test, the angle at which a rat slides after being placed head up on a tilted plane was recorded. Results were reported as lowest and mean percentage of control performance; for n-butanol, the lowest value in the six tests corresponded to 55.8 ± 1.7% of control and the mean of the six tests was 73.3 ± 9.2% of control. Statistical analysis was not reported.

Maickel and Nash (1985) treated groups of 23–25 male Swiss-Cox mice to oral doses of 500, 1,000, or 2,000 mg/kg n-butanol and assessed body temperature (via rectal thermometer) and rotarod performance in groups of five mice each at 10, 20, 40, 80, and 120 minutes after treatment. Blood was collected for analysis of n-butanol at the same time points. Treatment with n-butanol resulted in dose-dependent decreases in body temperature that persisted through 40 minutes after exposure in all groups and through 80 minutes in the mid- and high-dose groups. n-Butanol doses of 1,000 and 2,000 mg/kg resulted in dose-related decreases in rotarod performance (65 and 25% of baseline, respectively, 10 minutes after exposure, based on visual inspection of data presented graphically). Rotarod performance gradually improved over time

until it was 100% of baseline by 80 minutes (at 1,000 mg/kg) or 120 minutes (at 2,000 mg/kg) after dosing. Exposure to n-butanol at 500 mg/kg did not affect rotarod performance.

In a short-term study of a series of industrial solvents, De Ceaurriz et al. (1983) exposed groups of 10 male Swiss OF1 mice for 4 hours to n-butanol (98.5% pure) concentrations of 0, 470, 548, 844, or 965 ppm (0, 1,420, 1,660, 2,560, or 2,930 mg/m^3). Vapors were generated using either an injector with added heat to increase vaporization or by bubbling air through a vial containing the test material (the authors did not specify which method was used for n-butanol). After exposure, each animal was tested in a "behavioral despair" swimming test, in which it was immersed in water in a glass cylinder and observed for duration of immobility (passive floating). A concentration-related, statistically significant decrease in the duration of immobility during the first 3 minutes of testing (i.e., prolongation of escape-directed activity) was observed at all of the exposure concentrations; the percent decrease from control was 38, 47, 60, and 70% in the low through high-exposure groups. The concentration of n-butanol resulting in 50% decrease in immobility was reported to be 617 ppm (95% CI 547–681), or 1,870 mg/m^3. According to the authors, a decrease in immobility in this test is characteristic of compounds with antidepressant activity.

In a study designed to investigate the potential ototoxicity of solvent exposure, Crofton et al. (1994) exposed groups of 10 adult male Long-Evans rats via whole-body inhalation to n-butanol vapor concentrations of 0 or 4,000 ppm (12126 mg/m^3), 6 hours/day for 5 days. Auditory function was tested via reflex modification audiometry 5–8 weeks after exposure. There was no effect of n-butanol exposure on the auditory threshold of rats through the frequency range that was tested (0.4–40 kHz).

Frantik et al. (1994) tested the acute neurotoxicity of n-butanol in male albino SPF Wistar rats and in female mice (H strain). Groups of four male rats were exposed (whole-body) to n-butanol vapors for 4 hours, while groups of 16 female mice were exposed for 2 hours. The tested concentrations were not reported, as the focus of the analysis was to identify isoeffective concentrations for the different solvents. Within a minute after the exposure period ended, the investigators applied a short electrical impulse through electrodes in the ears of the tested animals to generate a tonic extension of hindlimbs in rats and in mice. The only n-butanol effect information presented by the authors was the estimated concentration evoking a 30% depression in these parameters (3,500 ppm [10,000 mg/m^3] in rats and 2,400 ppm [7,300 mg/m^3] in mice).

A series of studies (Korsak and Rydzynski, 1994; Korsak et al., 1993) evaluated the acute neurobehavioral effects of n-butanol in rats exposed via inhalation. In the first study (Korsak et al., 1993), groups of male Wistar rats (10/group) were exposed for 4 hours to various concentrations of n-butanol. Immediately after exposure, the rats were tested for rotarod performance, and an hour after exposure ended, spontaneous motor activity was assessed. The authors estimated the medial effective concentrations (EC_{50}) for the rotarod performance to be 6,531 ppm (19,800 mg/m^3). In the tests of spontaneous motor activity, low concentrations of

n-butanol were associated with increased activity (compared with control animals), while higher concentrations reduced activity back to control levels (based on data presented graphically).

The second study (Korsak and Rydzynski, 1994) also exposed groups of 10 male Wistar rats to various concentrations of n-butanol for 4 hours, and tested rotarod performance immediately after exposure. An hour after exposure ended, pain sensitivity (measured as latency to paw lick response on a hot plate) was assessed. The authors estimated the EC_{50} value for disturbance of rotarod performance to be 7,559 ppm (22,920 mg/m^3) in this study; this is similar to the value (6,531 ppm) estimated by Korsak et al. (1993). Hot plate behavior was also affected by exposure. The EC_{50} estimated by Korsak and Rydzynski (1994) for decreased sensitivity to pain was 5,901 ppm (17,890 mg/m^3) (95% CI 4,841–7,232 ppm).

In a study using i.p. administration of n-butanol, McCreery and Hunt (1978) compared the acute neurotoxic potencies of 62 compounds in an attempt to correlate potency with physical-chemical parameter such as the membrane/buffer partition coefficient. Male Sprague-Dawley rats (number/group not specified) received a single i.p. injection of the compound in various doses. Neurotoxic potency was assessed after dosing (duration of observation not reported), and characterized as the dose that caused pronounced impairment of gait (ataxia) and motor incoordination without causing the abdomen or pelvis to drop (the authors termed this dose the ED_3). The ED_3 for n-butanol was 5.4 mmol/kg (400 mg/kg); by comparison, the ED_3 values for the related alcohols ethanol and methanol were 32.6 and 109 mmol/kg, respectively. Neurotoxic potency showed a strong inverse correlation with membrane:buffer partition coefficient in alcohols up to a certain carbon number (C8 for straight-chain alcohols).

4.4.5. Pulmonary Toxicity

Kristiansen et al. (1988) evaluated the sensory irritation response in mice exposed to n-butanol vapors by assessing the reflexive decrease in respiratory rate that accompanies pulmonary irritation. Three different exposures (normal inhalation, inhalation via tracheal cannula, and i.p. injection) were tested in order to explore whether effects on respiration resulted from stimulation of receptors on the trigeminal (in the nasal mucosa) or vagal (in the lower respiratory tract) nerves, or from depression of the CNS. Groups of four male Ssc:CF-1 mice were used; a control group was exposed to clean air. For the inhalation (normal and cannulated) exposures, the mice were exposed to 0, 435, 2,500, 5,600, or 9,200 ppm (normal; or 0, 1,319, 7,579, 16,976, or 27,890 mg/m^3) or 4,630, 5,400, 7,335, or 9,250 ppm (cannulated; or 14,036, 16,370, 22,236, or 28,041 mg/m^3) of n-butanol (99.5% pure) for 30 minutes followed by a 20-minute recovery period. Respiratory rate and relative tidal volume were measured continuously using a pressure transducer attached to a body plethysmograph into which each animal was placed, with its head inserted into the exposure chamber. Doses of 1.6, 4.1, 12.1, and 20.2 mg/mouse were administered to the groups exposed via i.p. injection immediately prior to respiratory measurements for 50 minutes. The animals were observed for escape attempts as a

measure of CNS depression. The relationship between percent decrease in respiratory rate and the log of the exposure concentration was plotted and used to estimate the concentrations of n-butanol that would result in a 0 or 50% reduction in respiration rate (RD_0 and RD_{50}, respectively).

The experiments showed that normal inhalation of n-butanol resulted in a concentration-related decrease in respiratory rate, with the maximum depression occurring in the first minute (Kristiansen et al., 1988). The authors estimated RD_0 and RD_{50} values of 233 and 11,696 ppm (706 and 35,456 mg/m^3), respectively, for the first minute of inhalation exposure. After the first minute, there was evidence of desensitization. The response after the first minute showed concentration-dependent patterns; at concentrations below 3,000 ppm (9,094 mg/m^3), the response leveled off, while above 3,000 ppm (9,094 mg/m^3), a secondary depression occurred. The authors suggested that the secondary response occurred as a result of stimulation of receptors in the lower respiratory tract, as a similar response occurred in the cannulated animals. No evidence of CNS depression was evident in any of the inhalation-exposed groups; however, the decrease in respiratory rate seen with i.p.-exposed rats indicated CNS-mediated effects, as this group had no direct exposure to the respiratory tract. Comparison between results in the cannulated and i.p.-exposed groups showed a stronger response in the cannulated group, leading the authors to conclude that both CNS and lung receptors were involved in the secondary response.

Korsak and colleagues (Korsak and Rydzynski, 1994; Korsak et al., 1993) also evaluated respiratory irritation in mice exposed via inhalation. In the first study, groups of male Balb/C mice (8–10/group) were exposed for 4 hours to various concentrations of n-butanol. Respiratory rate was measured by plethysmograph before exposure, for 6 minutes during exposure, and for 6 minutes after the end of exposure. Exposure to n-butanol resulted in a concentration-dependent decrease in respiratory rate, with maximum depression occurring during the 1st minute of exposure. The authors estimated the RD_{50} value to be 3,008 ppm (9,119 mg/m^3). The second study (Korsak and Rydzynski, 1994) used the same protocol and estimated an RD_{50} value of 4,300 ppm (13,000 mg/m^3), which compares favorably with the value estimated by Korsak et al. (1993).

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

4.5.1. Genotoxicity Studies

A series of genotoxicity assays of n-butanol in both bacterial and mammalian systems have produced primarily negative results (see Table 4-11). n-Butanol did not increase reverse mutations in *Salmonella typhimurium* (strains TA98, TA100, A1535, and TA1537) in the presence or absence of microsomal enzyme activation (i.e., liver S9 preparations) (Jung et al., 1992; Nakamura et al., 1987; Connor et al., 1985; McCann et al., 1975). Obe and Ristow (1977)

did not observe an increase in the frequency of sister chromatid exchanges in the Chinese hamster ovary cell, and no increase in the frequency of micronuclei was observed in Chinese hamster cells (V79) treated with a concentration of 50 µL/mL of n-butanol (Lasne et al., 1984). When V79 cells were tested at a concentration of 0.1 M n-butanol, an increased frequency of polyploidy cells was observed, but survival at this concentration was only 36% (Onfelt, 1987). Yoshiyama et al. (1973) showed that n-butanol selectively suppresses the initiation of DNA replication in irradiated *Escherichia coli* (W2252) cells. n-Butanol was also inhibited the incorporation of uridine in HeLa cells (Obe et al., 1977), suggesting inhibition of RNA synthesis. Using a cell-free system with calf thymus DNA, Obe et al. (1977) showed that n-butanol concentrations of 1–4% inhibited RNA transcription in a dose-dependent fashion.

Table 4-11. Genotoxicity studies of n-butanol in vitro

Test system	Endpoint	Test conditions	Results[a]		Dose[b]	Reference
			Without activation	With activation[a]		
S. typhimurium TA98, TA100	Reverse mutation	Plate incorporation assay	–	–	2,000 µg/plate	Connor et al. (1985)
S. typhimurium TA102		Plate incorporation assay	–	–	5,000 µg/plate	Muller et al. (1993); Jung et al. (1992)
S. typhimurium TA100, TA1535, TA1537, TA98		Plate incorporation assay	–	–	10 µg/plate	McCann et al. (1975)
S. typhimurium TA1535/pSK 1002	*Umu* gene expression	*Umu* test	–	–	27,000 µg/mL	Nakamura et al. (1987)
E. coli (W2252)	Inhibition of DNA replication	[^{14}C]-amino acids added to *E. coli* cells prior to treatment with n-butanol; radioactive nucleic acids and proteins were then fractionized.	+	ND	0.8%	Yoshiyama et al. (1973)
Chinese hamster lung cells (V79)	Micronuclei	48-hr incubation	–	ND	50 µL/mL	Lasne et al. (1984)
Chinese hamster lung cells (V79)	Polyploidy	Incubated with chemical for 3 hrs; harvested and examined 26 hrs later	+	ND	0.1 M	Onfelt (1987)
Chinese hamster ovary cells (CHO)	Sister chromatid exchanges	Treated 1 time/d for 7 d	–	ND	0.1% (v/v)	Obe and Ristow (1977)

Table 4-11. Genotoxicity studies of n-butanol in vitro

Test system	Endpoint	Test conditions	Results[a]		Dose[b]	Reference
			Without activation	With activation[a]		
HeLa cells	RNA synthesis; inhibition of uridine incorporation	Treated with increasing doses for 5 min prior to treatment with [^{3}H]-uridine, then incubated for 30 min and precipitated.	+	ND	0.25%	Obe et al. (1977)
Cell-free system containing calf thymus DNA	RNA synthesis; inhibition of uridine incorporation	RNA polymerase added to system after n-butanol; transcription terminated 30 min later.	+	ND	1%	Obe et al. (1977)

[a]Exogenous metabolic activation used.
[b]Lowest effective dose for positive results or highest dose tested for negative or equivocal results.
+ = positive, − = negative, ND = no data

There was only one in vivo test of n-butanol genotoxicity. Bloom (1982) injected n-butanol into the inner shell membrane of chick embryos to assess the frequency of sister chromatid exchanges and chromosomal breaks. When tested at a concentration of 10 µL, no increases in the frequency of sister chromatid exchanges or chromosomal breakages were observed.

4.5.2. Mechanistic Studies Evaluating Carcinogenic Parameters

Etique et al. (2004) cultured human breast cancer cells (MCF-7) for 24 hours prior to treatment with 0.1% n-butanol for 6 days. Cell proliferation was evaluated, and P450-aromatase activity (which is involved in estrogen synthesis and has been shown to be increased in breast cancer) and estrogen receptor α (ER-α) mRNA expression were measured using semiquantitative real time polymerase chain reaction (RT-PCR). In addition, ER-α protein content was analyzed using Western blot analysis. Exposure to n-butanol resulted in a statistically significant decrease in cell proliferation (38% less than controls); n-Butanol did not modify the ER-α protein content.

4.5.3. Mechanistic Studies Evaluating Neurological Effects

Lovinger et al (1989) demonstrated that n-butanol dose-dependently inhibited the N-methyl-D-aspartate (NMDA) glutamate receptor in mouse hippocampal neurons. Following Lovinger et al (1989), there were many characterization studies of the potential molecular sites of action for n-alcohols in the central nervous system. Table 4-12 summarizes and provides experimental details on the electrophysiological studies conducted with n-butanol on individual

neuronal ligand-gated ion channels expressed in *in vitro* cell lines (*Xenopus* oocyte; human embryonic kidney cells) or mixed neurotransmitter systems in primary neuronal cell cultures. It was generally found that n-butanol dose-dependently and reversibly inhibited glutamate receptor (NMDA, Kainate, and α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate [AMPA]) function (Akinshola, 2001; Peoples and Weight, 1999; Dildy-Mayfield et al., 1996; Lovinger et al., 1989) and potentiated the function of inhibitory systems such as glycine and gamma-aminobutyric acid (GABA) receptors (Mascia et al., 2000; Peoples and Weight, 1999; Ye et al., 1998; Dildy-Mayfield et al., 1996; Nakahiro et al., 1991). Three different studies (Zuo et al., 2003; Zuo et al., 2001; Godden et al., 2001) examined the effects of n-butanol on nAChRs and it could be concluded that n-butanol may act as a partial agonist on this receptor class. A few studies (Rusch et al., 2007; Stevens et al., 2005; Zhou et al., 1998) reported that n-butanol (11 – 22 mM) potentiated the serotonin (or 5-HT [5 hydroxytryptamine]) current. N-butanol was reported to inhibit the function of voltage gated potassium channels (Harris et al., 2003; Shahidullah et al., 2003). However, when a residue in the S6 segment of the potassium channel was mutated from a proline (P) to an alanine (A) [P410A], the n-butanol potentiated channel function (Bhattacharji et al., 2006). Studies with rat brain membranes indicated that n-butanol interacted with delta-specific, but not kappa, opioid receptor binding sites (Hiller et al., 1984) and n-butanol increased membrane fluidity and decreased membrane ATPase activity (Edelfors and Ravn-Jonsen, 1990).

Table 4-12. Study details for mechanistic data and other studies in support of the mode of action for neurological effects

Reference	Test system	Number of samples/ group	Dose or concentration range	Study protocol	Result
Studies of effects on ligand-gated ion channels					
Lovinger et al. (1989)	Mouse hippocampal neurons	4 neurons/test	0.01–25 mM	Neurons were co-exposed to 50 uM NMDA and n-butanol. Whole-cell patch clamp techniques were used to measure the elicited current from the treatments.	n-Butanol dose dependently inhibited the NMDA-glutamate receptor response in the mouse hippocampal neuron. The inhibition was reversible following a 2 minute washout period.
Nakahiro et al. (1991)	Primary cultures of rat dorsal root ganglion neurons	3–9 Neurons/ test	1–30 mM	Neurons were exposed to n-butanol and GABA or after GABA current had reached steady state, and the effect on current was measured using whole cell patch-clamp technique.	Initial peak current (nondesensitized) evoked by GABA was increased with increasing n-butanol concentration; no current induced in the absence of GABA. Potency of current enhancement was positively correlated with carbon chain length and membrane:buffer partition coefficient for n-alcohols. In contrast, n-butanol at 30 mM inhibited the desensitized steady-state current induced by GABA.
Dildy-Mayfield et al. (1996)	*X. laevis* oocytes expressing recombinant $GABA_A$, NMDA, AMPA, or kainite receptors	4–12 Oocytes/ test	5–50 mM	Cultured oocytes expressing $GABA_A$, NMDA, AMPA, or kainite receptors were exposed to alcohols and effects on current were measured using the two-electrode voltage clamp technique.	n-Butanol potentiated the function of $GABA_A$ receptors composed of $\alpha_1\beta_1$ or $\alpha_1\beta_1\gamma_{2L}$ subunits, but inhibited the responses to NMDA, AMPA, and kainite.
Ye et al. (1998)	*X. laevis* oocytes or HEK-293 cells expressing wild type or mutant glycine Rα1 or GABA ρ1 receptors or chimeric glycine Rα1/GABA ρ1 receptor	4–13 Oocytes/ test	10–20 mM	Cells were exposed to n-butanol and the effect on current was measured using whole cell patch-clamp technique.	n-Butanol exhibited a dose-related potentiation of glycine-induced currents in cells expressing wild type glycine receptors and a dose-related inhibition of submaximal GABA-induced currents in cells expressing wild type GABA ρ1 subunits. In cells expressing chimeric receptors, n-butanol potentiated GABA-induced currents and slightly inhibited glycine-induced currents.

Table 4-12. Study details for mechanistic data and other studies in support of the mode of action for neurological effects

Reference	Test system	Number of samples/ group	Dose or concentration range	Study protocol	Result
Zhou et al. (1998)	neuroblastoma (NCB-20) cells	4–11 Cells/ test	20 mM	Cells were exposed to n-butanol and 5-HT and the effect on current was measured using whole cell patch-clamp technique.	n-Butanol exposure increased the initial slope, the rise time, and the measured desensitization rate of low concentrations of 5-HT (1–2 µM). It also decreased the measured desensitization rate of current evoked by 10 µM 5-HT, and increased the relative amplitude of steady-state to peak current evoked by 2 or 10 µM 5-HT.
Peoples and Weight (1999)	Primary cultures of hippocampal neurons from 15 to 17-d-old fetal mice	4–7 Neurons/ test	~0.0013–0.125 M	Neurons were exposed to n-butanol with GABA or NMDA and effects on current were measured using the two-electrode voltage clamp technique.	n-Butanol enhanced the $GABA_A$- activated ion currents and inhibited NMDA-activated currents. Anesthetic potency of n-butanol and other short-chain n-alcohols was more closely related to NMDA-modulated effects than $GABA_A$-modulated effects
Mascia et al. (2000)	*X. laevis* oocytes expressing human α1(wild type) and α1(S267Q) (anesthetic-resistant mutant) glycine receptor subunits	3–9 Oocytes/ test	6.6–66 mM	Cultured oocytes expressing human α1and α1(S267Q) glycine receptor were exposed to n-butanol, and glycine, and effects on glycine receptor function was measured using the two-electrode voltage clamp technique.	n-Butanol potentiated the glycine response in oocytes expressing wild type (α1) glycine receptors, and modestly inhibited the response in those expressing the anesthetic-resistant mutant (α1[S267Q]). Mortality of oocytes (2/5) was observed at the highest tested concentration.
Akinshola (2001)	*X. laevis* oocytes expressing recombinant AMPA GluR1 and GluR3 receptor subunits	5–8 Oocytes/ test	1–1,000 mM	Cultured oocytes expressing glutamate GluR1 or GluR3 receptor subunits were treated with various concentrations of alcohols, and inhibition of kainite-activated ion-currents was measured using the two-electrode voltage clamp technique.	n-Butanol exhibited dose-related inhibition of kainite-activated currents in oocytes expressing recombinant AMPA GluR1 and GluR3 receptor subunits.
Godden et al. (2001)	*X. laevis* oocytes expressing human nACh receptor subunits α_2, α_4, and β_4	3–16 Oocytes/ test	0.0001–0.1 M	Cultured oocytes expressing nACh receptor subunits α_2, α_4, and β_4 were bathed in alcohols with ACh substrate, and effect current was measured using the two-electrode voltage clamp technique.	n-Butanol potentiated the function of nACh receptors composed of $\alpha_2\beta_4$ subunits, but had no effect on receptors composed of the $\alpha_4\beta_4$ combination. Molecular volume of alcohols was shown to correlate with potency of effect on the receptors.

Table 4-12. Study details for mechanistic data and other studies in support of the mode of action for neurological effects

Reference	Test system	Number of samples/ group	Dose or concentration range	Study protocol	Result
Zuo et al. (2001)	HEK-293 expressing the α4β2 subunit of neuronal nACh receptors	6 Cells/test	1–300 mM	Cells were exposed to n-butanol with ACh and the effect on current was measured using whole cell patch-clamp technique.	n-Butanol (1 and 100 mM) exhibited a dose-dependent inhibition on the effect of 30 µM ACh, and potentiated the effect at an n-butanol concentration of 300 mM. At a concentration of 30 mM, n-butanol slightly potentiated the effect of 10 µM ACh.
Zuo et al. (2003)	HEK-293 expressing the α4β2 subunit of neuronal nACh receptors	6 Cells/test	1–300 mM	Cells were exposed to n-butanol and ACh and the effect on current was measured using whole cell patch-clamp technique.	Prolonged exposure to n-butanol resulted in small currents blocked by the ACh channel blocker mecamylamine or the receptor blocker dihydro-β-erythroidine, indicating that n-butanol acted as a partial agonist. At a high concentration (300 mM), n-butanol potentiated the effects of low ACh concentrations (≤30 µM) and inhibited the effects of high ACh (100–3,000 µM). A low concentration of n-butanol (10 mM) inhibited effects of ACh (10–3,000 µM).
Stevens et al. (2005)	*X. laevis* oocytes expressing 5-HT_{3A} or 5-HT_{3AB} receptors	At least three oocytes/test	11 and 22 mM	Cultured oocytes expressing 5-HT_{3A} or 5-HT_{3AB} receptors were exposed to n-butanol and dopamine, and effects on current were measured using the two-electrode voltage clamp technique.	n-Butanol enhanced the currents induced by low concentrations of 5-HT (~EC_{10}) and caused a slight decrease in the EC_{50} for 5-HT. In cells expressing HT_{3A}, the potentiation was much higher than in cells expressing the heteromeric 5-HT_{3AB} receptor.
Rusch et al. (2007)	*X. laevis* oocytes expressing 5-HT_{3A} or 5-HT_{3AB} receptors	5–10 Oocytes/ test	21.6 mM	Cultured oocytes expressing serotonin 5-HT_{3A} or 5-HT_{3AB} receptors were exposed to n-butanol and dopamine, and effects on current were measured using the two-electrode voltage clamp technique.	n-Butanol strongly potentiated the peak currents elicited by dopamine in a dose-dependent fashion in oocytes expressing the 5-HT_{3A} receptor, and modestly potentiated the currents in those expressing the 5-HT_{3AB}. Coexposure to n-butanol and octanol did not modify the potentiation compared with n-butanol alone.
Other mechanistic studies of neurological effects					
Hiller et al. (1984)	Isolated rat brain membrane	Experiments conducted in triplicate	0.5%	Effect of n-butanol on binding opioid binding to kappa site tested by blocking mu and delta sites with appropriate ligands. Kinetic studies were made to assess possible mechanism of selective inhibition of delta sites.	n-Butanol did not inhibit kappa opioid binding sites, but selectively inhibited binding to the delta sites. Kinetic studies indicated that the inhibition is a result of a decrease in affinity of the binding site (increased dissociation rate) for the ligand.

Table 4-12. Study details for mechanistic data and other studies in support of the mode of action for neurological effects

Reference	Test system	Number of samples/ group	Dose or concentration range	Study protocol	Result
Edelfors and Ravn-Jonsen (1990)	Rat brain synaptosomal membranes	Experiments conducted in triplicate	12.5, 25, and 50% of saturation (solubility reported to be 912 mmol/L)	Membranes were bathed in n-butanol and the membrane Ca2+/Mg2+ ATPase activity was measured spectrophoto-metrically. Membrane fluidity was measured as incorporation of fluorescent 1,6-diphenyl-1,3,5-hexatriene.	n-Butanol decreased the membrane ATPase activity in a concentration-dependent. Similarly, membrane fluidity was increased with increasing n-butanol concentration.
Bhattacharji et al. (2006); Harris et al. (2003); Shahidullah et al. (2003	*X. laevis* oocytes expressing wild-type and selectively mutated Shaw2 voltage-gated K^+ channels from *Drosophila melanogaster*	3–5 Oocytes/ test	1–100 mM	Cultured oocytes expressing wild-type and mutant (generated by S5 alanine-scanning mutagenesis) Shaw2 K^+ channels were exposed to n-butanol, and the effect on current was measured using the two-electrode voltage clamp, whole cell patch-clamp, or fast-concentration clamp technique.	n-Butanol inhibited the wild-type K+ channels, and several of the mutations modulated the inhibition associated with n-butanol. Mutation at a key site (P410A) in the S6 segment of the channel changed the n-butanol response from inhibition into potentiation. Studies were designed to explore molecular features of the binding site on the channel.

AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; ATPase = adenosine triphosphatase; 5-HT = 5-hydroxytryptamine; nACh = nicotinic acetylcholine; NMDA = *N*-methyl-D-aspartate

4.5.4. Mechanistic Studies Evaluating Neurodevelopmental Effects

Kotter and colleagues (Kotter et al., 2000; Kotter and Klein, 1999) conducted a series of studies comparing the effects of ethanol and butanols on the proliferation of neonatal rat brain astroglial cells. In experiments on astrocyte cell proliferation rates, the effects of alcohols on proliferation were assessed by measuring [^{3}H]-thymidine incorporation after treatment of the cell cultures with alcohol and a mitogenic agent. Using fetal calf serum as the mitogen, Kotter and Klein (1999) treated astrocyte cells with n-butanol at concentrations of 0.1, 0.3, or 1.0%. Compared with cells treated with fetal calf serum alone, cell proliferation was inhibited by ~40 and 70%, at 0.1 and 0.3% n-butanol (respectively); proliferation was completely inhibited at 1% n-butanol. Similar results were observed using platelet-derived growth factor and endothelin 1 (Kotter and Klein, 1999).

Kotter et al (2000) also measured astrocyte cell proliferation, but used phorbol ester as the mitogen. At concentrations of 0.1 and 0.3%, n-butanol inhibited proliferation of astroglial cells by ~50 and nearly 100% (based on visual inspection of data presented graphically), respectively, compared with cells treated with phorbol ester alone. The potency of inhibition of n-butanol was much stronger than that of ethanol; at 0.3% ethanol, cell proliferation was inhibited by only ~40%, compared with nearly 100% inhibition at the same concentration of n-butanol.

In two sets of experiments on the activation of astroglial phospholipase D (PLD), phospholipids in the cells were labeled by incubating the cells with [^{3}H]-glycerol for 24 hours prior to treatment with a mitogen and alcohol (Kotter et al., 2000; Kotter and Klein, 1999). The cultures were exposed to ethanol or butanols (0.1–1.0%) for 5 minutes prior to isolation of phospholipids and quantification of phosphatidic acide (PA) and phosphatidylcholine. When fetal calf serum was used as the mitogen (Kotter and Klein, 1999), treatment with n-butanol statistically significantly increased the formation of phosphatidylbutanol and decreased the formation of PA (~30–40% at 0.3% n-butanol based on visual inspection of data presented graphically). As with the effect on cell proliferation, n-butanol exhibited a stronger effect on PA formation than ethanol at the same concentration (~15% decrease in PA formation at 0.3% ethanol). Similar results were observed when phorbol ester was used as the mitogen (Kotter et al., 2000).

Kotter et al. (2000) also measured protein kinase C (PKC) activity after alcohol exposure; rat brain PKC was incubated with lipid micelles, phorbol ester, phosphatidylserine and alcohol for 5 minutes, followed by the addition of [^{32}P]-ATP. PKC activity was statistically significantly reduced by both ethanol and n-butanol at a concentration of 0.3% (average of nine experiments showed ~95 and 85%, respectively, of activity in the absence of the alcohols).

These studies demonstrate that, like ethanol, n-butanol inhibits the proliferation of astroglial cells, probably by disrupting the PLD signaling pathway by formation of phosphatidyl-

butanols and concomitant reduction in PA formation. In all of the experiments reported, n-butanol exerted a stronger inhibitory effect than ethanol at the same concentration.

4.5.5. Mechanistic Studies Evaluating Liver Effects

Carlson (1994a, b) showed that n-butanol can esterify fatty acids in vivo (in rats) and in vitro (in the homogenized liver, lung, and pancreas of rats and rabbits). In the in vivo experiment, n-butanol was administered via i.p. injection (1.0 mL/kg or 810 mg/kg) to male Sprague-Dawley rats, and the animals were sacrificed for analysis of butyl palmitate, butyl stearate, and butyl oleate (butyl esters of fatty acids) in the liver 1 or 6 hours after dosing. Butyl palmitate and butyl stearate were detected in the livers of all 12 rats sacrificed after 1 hour, while butyl oleate was detected in only 5 out of 12 rats. Of the four rats sacrificed after 6 hours, butyl palmitate was detected in all, butyl stearate was detected in three, and butyl oleate was not detected in any. In the in vitro study, homogenized liver, lung, and pancreas from rats and rabbits were incubated with [^{14}C]-oleic acid (0.4 mM) and 0.2 M n-butanol for 45 minutes. Esterified oleic acid was isolated from the mixture and radioactivity in the isolate was measured. In rat liver, lung, and pancreas, the esterified oleic acid content was 271.8, 58.3, and 2,170 nmol/g tissue/hour, respectively. Lower levels were observed in rabbit liver, lung, and pancreas: 45.8, 33.5, and 487 nmol esterified [^{14}C]-oleic acid/g tissue/hour, respectively. These studies demonstrate that n-butanol, like ethanol, can esterify fatty acids.

McKarns et al. (1997) observed a correlation between the octanol:water partition coefficient, a measure of hydrophobicity, and release of LDH from rat liver epithelial cells treated in vitro with short-chain aliphatic alcohols. Cultures of WB rat hepatic epithelial cells were incubated with one of several alcohols (including n-butanol, 99.5% pure) for 1-hour durations at various concentrations. The concentration of LDH in the medium was assessed at the conclusion of exposure as a measure hepatotoxicity. n-Butanol was tested at concentrations between 0.05 and 0.2 M to establish a concentration-response curve for the purpose of estimating the LDH_{50} (concentration eliciting a 50% increase in LDH over control values) and EC_{50} (concentration eliciting 50% of maximum LDH release). LDH_{50} and EC_{50} were plotted against previously reported partition coefficients. The LDH_{50} and EC_{50} for release of LDH by n-butanol were both 0.16 M. The authors observed a strong positive correlation between release of LDH and octanol:water partitioning ($r = 0.993$) and derived QSAR equations for both LDH_{50} and EC_{50} that were nearly identical. The study authors postulated that the observed relationship implies that the alcohols' ability to disrupt membrane integrity is nonspecific, as it is not affected by molecular shape.

Deters et al. (1998a) demonstrated that, unlike ethanol, metabolism of n-butanol by ADH was not a necessary step in the hepatotoxic action of this compound. The study authors used 4-MP to inhibit ADH in isolated perfused rat livers (from male Wistar rats) exposed to various alcohols at 130.2 mmol/L. Liver damage was assessed by measuring leakage of GPT, LDH, and

GLDH into the perfusate and by measuring oxygen consumption, bile secretion, perfusion flow, and concentrations of ATP and GSH in the liver. Exposure to n-butanol alone resulted in a statistically significant change from control for all of the above parameters with the exception of GSH content of the liver. Exposure of the isolated livers to ethanol in the presence of 4-MP resulted in little or no evidence of liver injury; bile secretion was diminished, but other parameters were attenuated by 4-MP. In contrast, there were no differences between the effects observed in livers treated with n-butanol with or without 4-MP in the perfusate. This study demonstrates that the hepatotoxicity of n-butanol may not be related to metabolism by ADH.

Deters et al. (1998b) examined whether glycine, which had previously been shown to reduce hypoxia-related effects on the liver, would mitigate the hepatotoxic effects of n-butanol and several other liver toxicants. Using the isolated perfused liver system described earlier, and the same measures of hepatotoxicity, the study authors tested n-butanol with and without glycine. Exposure of the isolated livers to n-butanol resulted in statistically significantly increased release of GPT and LDH into the perfusate, decreased bile flow, decreased oxygen consumption and hepatic ATP content, decreased GSH in the liver, and increased levels of MDA in the liver. Deters et al. (1998b) observed that inclusion of glycine reduced the release of GPT and LDH into the perfusate; levels with glycine were similar to those observed in the control systems. In addition, when glycine was included, the oxygen consumption in the liver was increased compared with the n-butanol treatment without glycine; oxygen consumption in the livers treated with both n-butanol and glycine was similar to that of controls. Finally, glycine modulated the effect of n-butanol on GSH and MDA content in the liver; levels of both were similar to controls in the livers treated with n-butanol in the presence of glycine. Glycine did not affect the inhibition of bile flow induced by n-butanol.

Strubelt et al. (1999) assessed evidence of injury to perfused livers treated with 23 aliphatic alcohols including n-butanol. Liver injury was assessed by measuring release of GPT, LDH, and GLDH into the perfusate; oxygen consumption, bile flow, and perfusion flow; ATP, GSH, oxidized glutathione (GSSG), and MDA in the liver; and lactate and pyruvate concentrations in the perfusate. The alcohol exposures all used a concentration of 65.1 mmol/L added to the perfusate for 120 minutes. Exposure of perfused livers to n-butanol resulted in statistically significantly increased GPT and LDH (more than eightfold higher than controls for each) in the perfusate. The study authors reported that the potency of effects on LDH, GPT, and GLDH was strongly correlated with carbon chain length among straight-chain aliphatic alcohols ($r = 0.82–0.87$). In addition, n-butanol exposure resulted in decreased bile flow and perfusion flow (2 and 57% of control values, respectively), and an increase in the lactate/pyruvate ratio (increased lactate and decreased pyruvate). Treatment with n-butanol did not result in statistically significant changes in ATP, GSH, GSSG, or MDA content of the livers.

4.5.6. Other Mechanistic Studies

Krill et al. (1993) examined the effects of n-butanol, n-propanol, and isopropanol on thermotropic behavior of the stratum corneum of hairless mice and a model multilamellar vesicle system. The study was designed to examine the mechanisms for alcohol-related enhancement of lipophilic solute permeation across the skin. The model vesicle system was prepared from the phospholipids distearoylphosphatidylcholine and distearoylphosphatidic acid. Stratum corneum sheets were removed from the abdomen of male hairless mice (strain SKH-HR-1). Fourier transform infrared spectroscopy was used to measure effects of the perdeuterated alcohols on stratum corneum lipid alkyl chain packing, mobility, and conformational order. Exposure of hairless mouse stratum corneum to 3% n-butanol resulted in increased lipid chain freedom of motion above 45°C and decreased the alkyl chain freedom of motion below 45°C, but did not alter the stratum corneum lipid interchain interactions or gel-gel phase transition. The study authors concluded that their experiments were consistent with the hypothesis that alcohols disrupt the polar head plane and increase the interfacial area of lipids, which enhances penetration of some solutes across the stratum corneum.

Kowalczyk et al. (1996) exposed two-cell preimplantation mouse embryos in culture to 0, 0.05, 0.1, 0.2, or 1.0% (w/v) n-butanol for 24 hours and followed the development of the embryos after 5 days. The number of embryos reaching blastocyst stage by day 5 was statistically significantly reduced at all concentrations of n-butanol. The reductions, which ranged from ~2 to 35% of control values, were not dose-dependent; the maximum reduction occurred at the lowest concentration. This result was the opposite of that observed with ethanol, which accelerates embryogenesis (increased number of blastocytes). In a separate experiment, 8-cell mouse morulae were pretreated with fluo-3-acetoxymethyl ester (a fluorescent indicator for intracellular calcium) and then exposed to 0.1 or 1.0% n-butanol. Fluorescence intensity was measured before and immediately after exposure and used to calculate calcium concentration. n-Butanol exposure statistically significantly increased the intracellular calcium concentration of the morulae at the high concentration but not at the low concentration (~sixfold increase at 1%). The study authors concluded that the increased release of calcium was a result of substantial disruption of membrane integrity and was a possible cause of the embryotoxicity observed in the first experiment.

Gastaldi et al. (1991) exposed small intestinal microvillous vesicles obtained from adult Wistar rats to butanol in vitro to assess the effects on vesicular morphology. The study did not specify whether n-butanol or t-butanol was used. The vesicles were incubated for 30 minutes with butanol at a concentration of 3% (v/v). After exposure, the vesicles were fixed for morphometric analysis (parameters were diameter of the area-equivalent circle, minimum to maximum diameter ratio [shape factor], and vesicular volume) by electron microscopy. A statistically significant increase in mean vesicular volume was observed with butanol exposure, and the study authors attributed this change to the fluidizing effect of the alcohol.

Gordon et al. (1995) compared the effects of several n-alcohols (methanol, ethanol, n-butanol, and 1-propanol) on isolated cannulated rat intracerebral arterioles (ex vivo). Concentrations of 1–100 mM of the n-alcohols were tested, and change in arteriolar diameter was measured. n-Butanol exposure resulted in dose-related increases in vessel diameter (vasodilation) ranging from ~15% (at 1 mM) to ~50% (at 100 mM) increase over the basal diameter.

Arsov et al. (2005) exposed erythrocyte ghosts prepared from bovine blood to n-butanol in an effort to determine the mechanism of n-butanol-induced inhibition of erythrocyte acetylcholinesterase (AChE) at high concentrations. AChE activity was measured with and without different concentrations of n-butanol (0.07, 0.15, 0.22, and 0.37 M) and at different temperatures. At 0.07 M and a temperature of ≥ 310°K, there was no inhibition of AChE; at higher concentrations, n-butanol inhibited AChE activity in the temperature range tested (up to 320°K). In experiments in which the n-butanol was removed by dialysis after the exposure, reversibility of AChE inhibition was shown at 0.22 M n-butanol, but at 0.37 M, AChE inhibition was still evident after the alcohol was removed. The study authors used the experimental results, along with published information, in kinetics studies to estimate the number of molecules that bind to the AChE enzyme. They concluded that, since the activity equation that best fit the data was the inverse of a second order polynomial, two n-butanol molecules likely bind to AChE.

Ding and Badwey (1994) examined the effects of n-butanol on four uncharacterized protein kinases that are activated by the chemoattractant fMet-Leu-Phe. When exposed to this chemoattractant, neutrophils exhibit shape changes, chemotaxis, degranulation, and release of superoxide. The study authors measured the release of superoxide from cultured guinea pig peritoneal neutrophils treated with 20–70 mM n-butanol and fMet-Leu-Phe (1 μm). n-Butanol exposure resulted in a concentration-related inhibition of superoxide release (from about 30% inhibition at 20 mM to about 90% inhibition at 70 mM compared with cells not exposed to n-butanol). Addition of four β-phorbol 12-myristate 13-acetate (PMA), a PKC activator, to cells treated with 55 mM n-butanol restimulated the release of superoxide, counteracting the inhibitory effects of n-butanol. Without PMA, n-butanol at 55 mM resulted in 74% inhibition of superoxide release, while the same concentration with PMA resulted in only 27% inhibition. While this study was primarily aimed at elucidating the mechanism of action of the novel protein kinases, it does suggest that n-butanol exposure may interfere with immune cell signaling. The potential impact on immune function, however, is uncertain.

4.6. SYNTHESIS OF MAJOR NONCANCER EFFECTS

4.6.1. Oral

Information on the effects of n-butanol in humans from oral exposure is limited to one case report from a suicide attempt (Bunc et al., 2006) where neurological, gastrointestinal, and

cardiovascular symptoms were noted. In animal studies, there are short-term, subchronic, reproductive and developmental toxicity studies that reported effects.

Among rats, rabbits, hamsters, and mice, oral LD_{50}s ranged from 1,200 mg/kg to 4,360 mg/kg (Rumyantsev, 1979; Dubina and Maksimov, 1976; Munch, 1972; Purchase, 1969; Jenner et al., 1964; Smyth et al., 1951; McOmie and Anderson, 1949; Munch and Schwartze, 1925). Behavioral toxicities following or during an acute exposure included narcosis and necropsies indicated that there was necrosis of the liver and kidney as well as general organ congestion (Munch, 1972; Purchase, 1969; Jenner et al., 1964; McOmie and Anderson, 1949; Munch and Schwartze, 1925).

Short term study evaluations reported neurotoxic, hepatoxic, and antiinflammatory effects. Acute oral dosages of n-butanol resulted in impaired performance in the tilted plane test at 1,210 mg/kg in rats (Wallgren, 1960) and dose-related decreases in rotarod performance in Swiss-Cox mice orally dosed with 1,000 and 2,000 mg/kg (Maickel and Nash, 1985). Liver toxicity was also noted in a study where decreased thiamine, riboflavin, pyroxidine, niacin, and panthothenic acid content was noted in rats orally gavaged with 1 or 2 mL/kg (810 or 1,620 mg/kg) n-butanol for 7 days (Shehata and Saad, 1978). In rats, oral doses of $\geq$ 600 mg/kg resulted in a reduction of inflammatory response when treated with carageenan, a pro-inflammatory agent (Strubelt and Zetler, 1980).

CNS effects including hypoactivity and ataxia (RTI; unpublished, 1985) and hepatic and renal effects (Munoz et al., 1991; Wakabayashi et al., 1991; Munoz et al., 1990) were reported in three subchronic studies. Table 4-11 summarizes these studies. In the 13 week oral gavage study in rats (RTI; unpublished, 1985), the 500 mg/kg-day dose (highest in the study) resulted in hypoactivity and ataxia that lasted for up to one hour after dosing. Statistically significant decreases in the hematocrit, erythrocyte count, and hemoglobin content were also reported for females dosed with 500 mg/kg-day and there was a statistically significant increase (~14%) in thyroid weight in males dosed with 500 mg/kg-day. Male rats exposed to 4,400 mg/kg-day for 4 months in a drinking water study (Munoz et al.,1991; 1990) were reported to have severe pathological changes in the liver and kidney. Similarly, hepatic effects such as proliferation of smooth endoplasmic reticulum and enlarged mitochondria in a 3 month drinking water study with male rats exposed to 8,200 mg/kg-day (Wakabayashi et al., 1991).

There were two oral developmental toxicity studies, Sitarek et al. (1994) and Ema et al., (2005), that are summarized in Table 4-13. Sitarek et al (1994) reported an increased incidence of visceral malformations including malformations in the brain (dilation of the lateral and/or third ventricle of the brain and/or arachnoid space) and kidney (renal pelvis) in the offspring of female Wistar Imp:DAK rats treated with n-butanol in drinking water at a dose of 300 mg/kg-day administered for 8 weeks prior to mating and throughout mating and gestation. Maternal effects were not observed at doses up to 5,000 mg/kg-day by Sitarek et al. (1994). In another developmental study, Ema et al. (2005) did not observe an increased incidence of any

malformation in Sprague-Dawley rats exposed to n-butanol in drinking water throughout gestation at doses up to 5,654 mg/kg-day. Maternal and fetal toxicity, manifested as body weight reductions, were observed at the high-dose in this study (5,654 mg/kg-day) (Ema et al., 2005). The discrepancy between the results of Ema et al. (2005) and Sitarek et al. (1994) may be due to differences in study design, particularly rat strain used and exposure regimens. In addition, dilation of the subarachnoid spaces and the ventricles in the brain are precursors of hydrocephalus (Raimondi, 1994). Sitarek et al. (1994) reported internal hydrocephalus in the fetuses from the 1,000 and 5,000 mg/kg-day dose groups along with the observed increased incidence of visceral malformations including malformations in the brain (dilation of the lateral and/or third ventricle of the brain and/or arachnoid space) at ≥ 300 mg/kg-day. Observation of hydrocephaly and dilation of neuronal structures (subarachnoid space and lateral ventricle) as reported in Sitarek et al (1994) is also consistent with effects observed with ethanol (Sakata-Haga et al., 2004). Therefore, the discrepancies in results between the developmental toxicity studies of Sitarek et al (1994) and Ema et al (2005) is likely due to differences in strain sensitivity.

Overall, the most sensitive toxicological effect was reported in Sitarek et al (1994) where increased developmental visceral malformations were observed in rat pups at the 300 mg/kg-day dose. At comparable exposures, neurotoxicological effects were observed in adult rats at 500 mg/kg-day and no effects were observed at 125 mg/kg-day (RTI; unpublished, 1985).

Table 4-13. Summary of oral noncancer dose-response information for n-butanol

Species and study type	Exposure	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)	Responses at the LOAEL	Comments	Reference
Subchronic or chronic studies						
Rat strain not specified 30/sex/dose Gavage	0, 20, 125, or 500 mg/kg-d daily for 13 wks	125	500	Clinical signs (hypoactivity and ataxia) and effects on hematology (reduced hematocrit, erythrocyte count, and hemoglobin content)		RTI (1985), unpublished
Wistar rat 15 males/group drinking water	0 or ~8,200 mg/kg-d for up to 3 mo	Could not be determined	Could not be determined	Structural changes of hepatic mitochondria	Data not reported. Evaluation limited to clinical signs, body weight, and electron microscopy of liver slices.	Wakabayashi et al. (1991)
Wistar rat 3 males/group drinking water	0 or 4,400 mg/kg-d, 7 d/wk for 4 mo	Could not be determined	Could not be determined	Unspecified liver and kidney damage; serum chemistry changes	Pathology data not reported. Evaluations limited to mortality, body weight, serum chemistry, and unspecified pathology evaluations.	Munoz et al. (1991, 1990)
Reproductive and developmental studies						
Wistar Imp:DAK rat 11–17 females/ group drinking water	0, 300, 1,000, or 5,000 mg/kg-d for 8 wks prior to mating, through mating and gestation	5,000 (maternal) NA (developmental)	NA (maternal) 300 (developmental)	Increased incidences of malformations (dilation of the lateral and/or third ventricle of the brain and the subarachnoid space)	No maternal effects.	Sitarek et al. (1994)
Sprague-Dawley rat 20 females/group drinking water	0, 316, 1,454, or 5,654 mg/kg-d on GDs 0–20	1,452 (maternal and developmental)	5,654 (maternal and developmental)	Decreased body weight gain, food consumption, and water consumption (maternal); decreased fetal body weight (developmental)		Ema et al. (2005)

4.6.2. Inhalation

The inhalation database contains a few acute human exposure experiments and occupational health studies. Controlled human exposure experiments have demonstrated that acute exposures to n-butanol vapors can exert an irritant effect on the eyes, nose, and throat under acute exposure conditions of up to 90 minutes (Kjaerguard et al., 1997; Nelson et al., 1943; Wysocki et al., 1996, as cited in McLain, 2008 and ACGIH, 2002; Cometto-Muniz and Cain, 1995). A number of occupational health studies have also been conducted which examined neurological and neurobehavioral effects in workers who were exposed to mixtures of solvents including n-butanol (see Table 4-2). However, exposure to n-butanol in these studies was low compared to other solvents, and a number of the other solvents are known to exert neurotoxic effects. Therefore, it is not possible to conclusively attribute any of the effects to n-butanol exposure. Table 4-14 summarizes the available human occupational data.

1

Table 4-14. Summary of inhalation noncancer dose-response information for n-butanol

Species and study type (n/sex/group)	Exposure	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Responses at the LOAEL	Comments	Reference
Human occupational health studies						
35 rubber factory workers; gender not specified	15–300 mg/m^3	15–42	46–200	Eye irritation	LOAEL identified for factory with coexposure to diacetone alcohol.	Cogan et al. (1945); Tabershaw et al. (1944)
16 male photographic paper factory workers	300 or 600 mg/m^3	300	600	Eye irritation	Irritation not observed after n-butanol concentration reduced from 600 to 300 mg/m^3.	Sterner et al. (1949)
11 exposed cellulose acetate factory workers; sex not specified 47 workers in referent group with similar age and duration of employment	240 mg/m^3	NA	240	Hearing loss, chronic bronchitis	Sampling protocol for n-butanol was not described.	Velazquez et al. (1969)
Subchronic or chronic animal studies						
Wistar rat 12 male/exposure; 24 controls	0, 154, or 308 mg/m^3 6 hr/d, 5 d/wk for 3 months	154	308	Impaired rotarod performance; increased leukocyte count	Histopathology not evaluated.	Korsak et al. (1994)
Guinea pig 3 group; sex unspecified	0, 300 mg/m^3 3 months	N/A	300	Decreased erythrocyte count and hemoglobin. Histopathological changes in liver and kidney	Data and methodological details are not provided for the observed effects.	Smyth and Smyth (1928)
Mice and rats No details provided for number/exposure, sex, or methods	0, 0.8, 6.6, 40 mg/m^3 4 months	0.8	6.6	Mice: Increased thyroid gland activity. Disturbances of pituitary-adrenal system. Rats: Increased blood cholinesterase activity.	Only list of effects were provided in the report. No detailed information was provided on the analyses of the data.	Rumyantsev et al. (1979)

Table 4-14. Summary of inhalation noncancer dose-response information for n-butanol

Species and study type (n/sex/group)	Exposure	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Responses at the LOAEL	Comments	Reference
Reproductive/developmental animal studies						
Sprague-Dawley rat;15–20 females/ group	0, 11,000, 18,000, or 24,000 mg/m^3 7 hrs/d on GDs 1–19	11,000 (maternal) NA (developmental)	18,000 (maternal) 11,000 (developmental)	Reduced food consumption (maternal) Increased incidence of litters with skeletal malformations (developmental)	Maternal narcosis and mortality were observed at 24,000 mg/m^3 (FEL).	Nelson et al. (1990, 1989a)
Sprague-Dawley rat; 15–20 females and 18 males/group	0, 9,000, 18,000 mg/m^3 7 h/d on GDs 1–19 (females) or for 6 wks prior to mating with nonexposed females (males)	18,000	NA	None.	No neurobehavioral effects in offspring, regardless of whether mothers or fathers exposed.	Nelson et al. (1990, 1989b)

Three studies of n-butanol exposure in rats are available including one subchronic toxicity study of male rats in which histopathology was not assessed (Korsak et al., 1994), a developmental toxicity study (Nelson et al., 1989a), and a neurodevelopmental toxicity study (Nelson et al., 1989b). In addition, several short-term inhalation exposure studies have characterized toxicities associated with n-butanol.

Specifically, fatty infiltration of liver and kidney were observed in mice exposed to 24,624 mg/m^3 for 130 hours (Weese, 1928). Neurotoxic effects such as decreased duration of immobility were reported at exposures of 1420 mg/m^3 and higher for 4 hours in mice (DeCeaurriz et al., 1983), and decreased responding (Frantik et al., 1994) in rats and mice following a 2-4 hour exposure. Respiration rates declined in Ssc:CF-1 mice exposed to exposure of 435 ppm (1,319 mg/m^3) and higher for 30 minutes (Kristiansen et al., 1988) as well as in balb/C mice with a 50% respiratory decline following 4 hour exposures to 3,008 ppm (9119 mg/m^3; Korsak et al., 1993) or 4,300 ppm (13036 mg/m^3; Korsak and Rydzynski, 1994).

There are a few subchronic inhalation toxicity studies evaluating the effects of n-butanol (Korsak et al., 1994; Rumanystev et al., 1979; Smyth and Smyth, 1928). Hematological effects, primarily decreased erythrocyte count, were noted in all three subchronic studies. The lowest exposure at which hematological changes were observed was at 6.6 mg/m^3 in rats where increased blood cholinesterase activity was noted (Rumyantsev et al., 1979). However, it is unclear if the hematological changes are biologically relevant. The other primary toxicological effect observed in the subchronic exposure studies was deficits in neurobehavioral performance. Korsak et al (1994) reported a statistically significant increase in the failure rate for the rotarod test in rats exposed to 308 mg/m^3 n-butanol for 3 months.

Nelson et al (1989a, b) evaluated the developmental and neurodevelopmental toxicity potential of n-butanol in rats. Increased incidence of litters with skeletal variation was observed at exposures of 3,500 ppm (11,000 mg/m^3) and increased levels of serotonin and dopamine were observed in offspring from male rats exposed to 5,000 ppm (18,000 mg/m^3) without changes in neurobehavioral function.

The most sensitive effects in humans identified from a sparse literature were eye, nose, and throat irritation following a 90 minute chamber exposure to 10 mg/m^3 n-butanol (Kjaerguard et al., 1997). In animals, hematological changes were observed at exposures as low as 6.6 mg/m^3 for 4 months (Rumanystev et al., 1979), but it is unknown if these changes were biologically significant. The statistically significant change in neurological function following a subchronic exposure to 308 mg/m^3 n-butanol in rats (Korsak et al., 1994) is the most sensitive biologically significant endpoint from inhalation following exposure durations that are subchronic or longer.

4.6.3. Mode of Action Information for Noncancer Endpoints

The mode of action for noncancer endpoints following n-butanol exposure is unknown. However, because of its structural relationship to ethanol, n-butanol has been tested in a large number of studies examining possible mechanisms for alcohol-induced neurotoxicity. One proposed mechanism is that alcohols including n-butanol produced the observed neurological changes by disrupting the lipid bilayer. A few studies have shown that n-butanol, like other alcohols, can disrupt membrane integrity (Kowalczyk et al., 1996; Krill et al., 1993; Gastaldi et al., 1991). Other studies have found that n-butanol inhibits the excitatory glutamate receptor function (Akinshola, 2001; Peoples and Weight, 1999; Dildy-Mayfield et al., 1996; Lovinger et al., 1989) and potentiates inhibitory receptors such as glycine and gamma-aminobutyric acid (GABA) (Mascia et al., 2000; Peoples and Weight, 1999; Ye et al., 1998; Dildy-Mayfield et al., 1996; Nakahiro et al., 1991). The modulatory action of n-butanol inhibiting the excitatory glutamate receptors and potentiating the inhibitory GABA and glycine receptors is supportive of the observed neurobehavioral changes (e.g. CNS depressant profile) associated with n-butanol exposure in humans (Seitz, 1972; Baikov and Khachaturyan, 1972; Tabershaw et al., 1944; Nelson et al., 1943) and animals (Korsak et al., 1994; RTI; unpublished, 1985). Application of n-butanol (11 – 22 mM) also potentiated the serotonin (or 5-HT [5 hydroxytryptamine]) current (Rusch et al., 2007; Stevens et al., 2005; Zhou et al., 1998). $5HT_3$ receptors are known to regulate dopamine release and increased dopamine levels are associated with reward mechanisms. Nelson et al (1990; 1989b) found that pups exposed to n-butanol *in utero* had statistically significant increases in brain levels of dopamine and serotonin which could be indirectly linked to n-butanol effects on the $5HT_3$ channel. Collectively, these studies suggest possible modes of action, via effects on these ion channels, for anesthetic effects and possibly other subtle neurological effects of n-butanol.

In addition, studies have shown that n-butanol inhibits rat brain astroglial cell proliferation by disrupting the PLD signaling pathway (Kotter et al., 2000; Kotter and Klein, 1999). n-Butanol was shown to be a good substrate for transphosphatidylation, resulting in the formation of phosphatidylbutanol and concomitantly decreasing the formation of the second messenger PA. Importantly, the authors demonstrated that n-butanol is substantially more potent than ethanol in the inhibition of astroglial cell proliferation, which has been postulated as a mode of action for ethanol-induced microencephaly and mental retardation observed in cases of fetal alcohol syndrome.

There is more limited information on possible modes of n-butanol-induced liver effects. Carlson (1994a, b) showed that n-butanol can esterify fatty acids in the liver and several other organs; fatty aid ethyl esters observed after ethanol exposure have been postulated to play a role in damage to several organs. Deters et al. (1998a) demonstrated that metabolism of n-butanol by ADH was not a necessary step in the hepatotoxic action of this compound. The same authors observed mitigation of several, but not all in vitro measures of hepatotoxicity when glycine was added to isolated perfused liver systems

along with n-butanol (Deters et al., 1998b); glycine had previously been shown to reduce hypoxia-related effects on the liver.

4.7. EVALUATION OF CARCINOGENICITY

4.7.1. Summary of Overall Weight-of-Evidence

Under the EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is *inadequate information to assess the carcinogenic potential* of n-butanol. None of the available human occupational health studies evaluated cancer endpoints. One occupational health study of workers exposed to n-butanol and several other solvents while employed as dockyard painters (Chen et al., 1999) examined mortality rates due to cancer; neither proportional mortality nor standardized mortality due to cancers were increased in the painters relative to controls. There are no chronic animal studies of n-butanol exposure through any exposure route, and the available subchronic studies did not report any cancer endpoints. The genotoxicity database for n-butanol is limited; available information suggests that n-butanol does not induce mutations in *S. typhimurium* with or without exogenous metabolic activation (Muller et al., 1993; Jung et al., 1992; Nakamura et al., 1987; Connor et al., 1985; McCann et al., 1975), and does not increase the frequency of micronuclei or sister chromatid exchanges in Chinese hamster cells (Lasne et al., 1984; Obe et al., 1977) or in chick embryos in culture (Bloom, 1982). Studies of DNA and RNA replication have shown that n-butanol can exert an inhibitory effect on these processes (Obe and Ristow, 1977; Yoshiyama et al., 1973).

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

There are no studies of age-related differences in susceptibility to the toxic effects of n-butanol. In a developmental toxicity study of oral exposure to n-butanol before and during gestation using Wistar Imp:DAK rats, increased incidences of some malformations (primarily dilation of the subarachnoid space and/or the lateral or third ventricle of the brain) were reported (Sitarek et al., 1994), suggesting that the developing fetus may be particularly susceptible to the effects of n-butanol exposure. However, in a study using a different strain of rat (Sprague-Dawley) and a exposure scenario (gestation only), Ema et al., 2005) did not observe neurodevelopmental effects. Differences in strain susceptibility and exposure regimen may address this discrepancy (see Section 5.1.1.). Inhalation studies of n-butanol (Nelson et al., 1989a) reported maternal toxicity (reduced food consumption and/or body weight gain) at concentrations resulting in fetal effects (primarily reduced body weight). A single study of behavioral teratology in offspring of rats exposed to n-butanol (Nelson et al., 1989b) did not observe treatment-related neurobehavioral changes.

Studies have demonstrated lifestage specific differences in activitiy in the enzymes responsible for metabolism of n-butanol. Specifically, in humans, there is evidence of differential expression for

three types of alcohol dehydrogenase (type I) enzymes: ADH1A, ADH1B, and ADH1C over different lifestages (Smith et al., 1971). Expression of the ADH enzymes was examined in the liver, lung, and kidney in 222 humans from 9 weeks gestation to up to 20 years of age. In the liver, it was reported that at 11 weeks of gestation ADH1A was preferentially expressed and ADH1B and ADH1C were nondetectable. However, in adults, ADH1A is no longer detectable and there is equal expression of ADH1B and ADH1C. ADH expression was considerably lower in the lung and kidney and expression did not change with the lifestages. Studies in mice, rats, and guinea pigs have also reported significantly lower alcohol and aldehyde dehydrogenase activity in fetal animals (ranging from 10 – 40% of adult activity) in comparison to the adults (Timms and Holmes, 1981; Card et al., 1989; Lindahl, 1977; Boleda et al., 1992). The lower enzymatic activity of alcohol and aldehyde dehydrogenase during development and childhood could potentially impact the metabolic capacity of n-butanol and may make this population more susceptible to this chemical agent.

4.8.2. Possible Gender Differences

There are no studies of gender differences in susceptibility to the toxic effects of n-butanol, and available information does not suggest gender differences.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

Data on the health effects of oral exposure to n-butanol in humans is limited to one case report from a suicide attempt (Bunc et al., 2006) in which neurological, gastrointestinal, and cardiovascular symptoms were noted. This case study is not suitable for use in deriving an RfD. The animal toxicological database for subchronic, chronic, and reproductive or developmental studies of n-butanol consists of three subchronic studies, Munoz et al. (1991; 1990), Wakabayashi et al. (1991), and RTI (1985), and two developmental toxicity studies, Ema et al. (2005) and Sitarek et al. (1994) where female reproductive parameters were also evaluated.

Figure 5-1 is an exposure-response array for oral exposure to n-butanol. Overall, the most sensitive toxicological effect was reported in the developmental toxicity study by Sitarek et al (1994), in which increased incidences of developmental visceral malformations were observed in rat litters at ≥ 300 mg/kg-day. Sitarek et al. (1994) reported increased incidences of developmental visceral malformations (dilation of the lateral ventricle, dilation of subarachnoid space in the brain and dilation of the renal pelvis) in the litters of female Wistar Imp:DAK rats treated with n-butanol in drinking water at doses of 300, 1,000, and 5,000 mg/kg-day administered for 8 weeks prior to mating and then continued through mating and gestation.

Neurotoxicological effects (hypoactivity and ataxia) were also observed in adult rats at a similar dose of 500 mg/kg-day (RTI; unpublished, 1985). However, these neurotoxicological effects were not considered as sensitive as the developmental visceral malformations because the changes were transient and not observed within an hour of exposure as noted by the study authors. Additionally, the reported LOAEL was higher for the neurotoxicological effects observed in RTI (1985) than for the developmental effects noted in Sitarek et al (1994).

In a second developmental toxicity study, Ema et al. (2005) did not observe an increased incidence of any malformation in the offspring of Sprague-Dawley rats exposed to n-butanol in drinking water throughout gestation (GD 0-20) at doses of 316, 1,454, and 5,654 mg/kg-day. However, maternal and fetal toxicity, manifested as body weight reductions, were observed at the high dose in this study (5,654 mg/kg-day) (Ema et al., 2005). The discrepancy between the developmental studies is uncertain, but may be due to differences in experimental protocols particularly strain differences and exposure regimens (see Section 5.3.).

Sitarek et al (1994) was selected as the principal study because it was a well-designed developmental toxicity study with a sufficient number of dams and litters per dose group. Three dose groups and an untreated control group were included in the study, and there was extensive evaluation of fetal parameters in the litters. There was a statistically significant increase in the incidence of litters with developmental visceral malformations at all dose groups (lateral ventricle and subarachnoid space in the brain; unilateral and bilateral renal pelvis) (Table 5-1). With respect to the malformations (dilation) observed in the brain, this effect may be irreversible since there is a link between lateral ventricular dilation and neurodevelopmental delay, which was reported in a retrospective epidemiology study of fetal ventriculomegaly in 29,000 pregnancies (Weichert et al., 2010). In addition, dilation of the subarachnoid spaces and the ventricles in the brain are observed in hydrocephalus (Raimondi, 1994), and Sitarek et al. (1994) also reported internal hydrocephalus in the fetuses from the 1,000 and 5,000 mg/kg-day dose groups. Observation of hydrocephaly and dilation of neuronal structures (subarachnoid space and lateral ventricle) as reported in Sitarek et al (1994) is also consistent with ethanol (Sakata-Haga et al., 2004). The functional significance of dilation of the lateral ventricles and subarachnoid space appears to be related to the rate and severity of the dilation, as well as the developmental stage at which it occurs (Weichert et al., 2010; Del Bigio, 2001). Sitarek et al. (1994) did not provide any indication of the severity of the dilation of the lateral ventricles and subarachnoid space in the affected fetuses or litters, and the only information provided by the investigators was a characterization of the changes at the mid and high doses, 1,000 and 5,000 mg/kg-day, respectively, as frank hydrocephalus. In the absence of severity characterization, and given the observation of hydrocephalus at higher doses, the increased litter incidences of visceral malformations (dilation of subarachnoid space, lateral ventricle, unilateral and bilateral renal pelvis) that were observed in the low-dose group (300 mg/kg-day) were considered to be biologically significant and were selected as the critical effect.

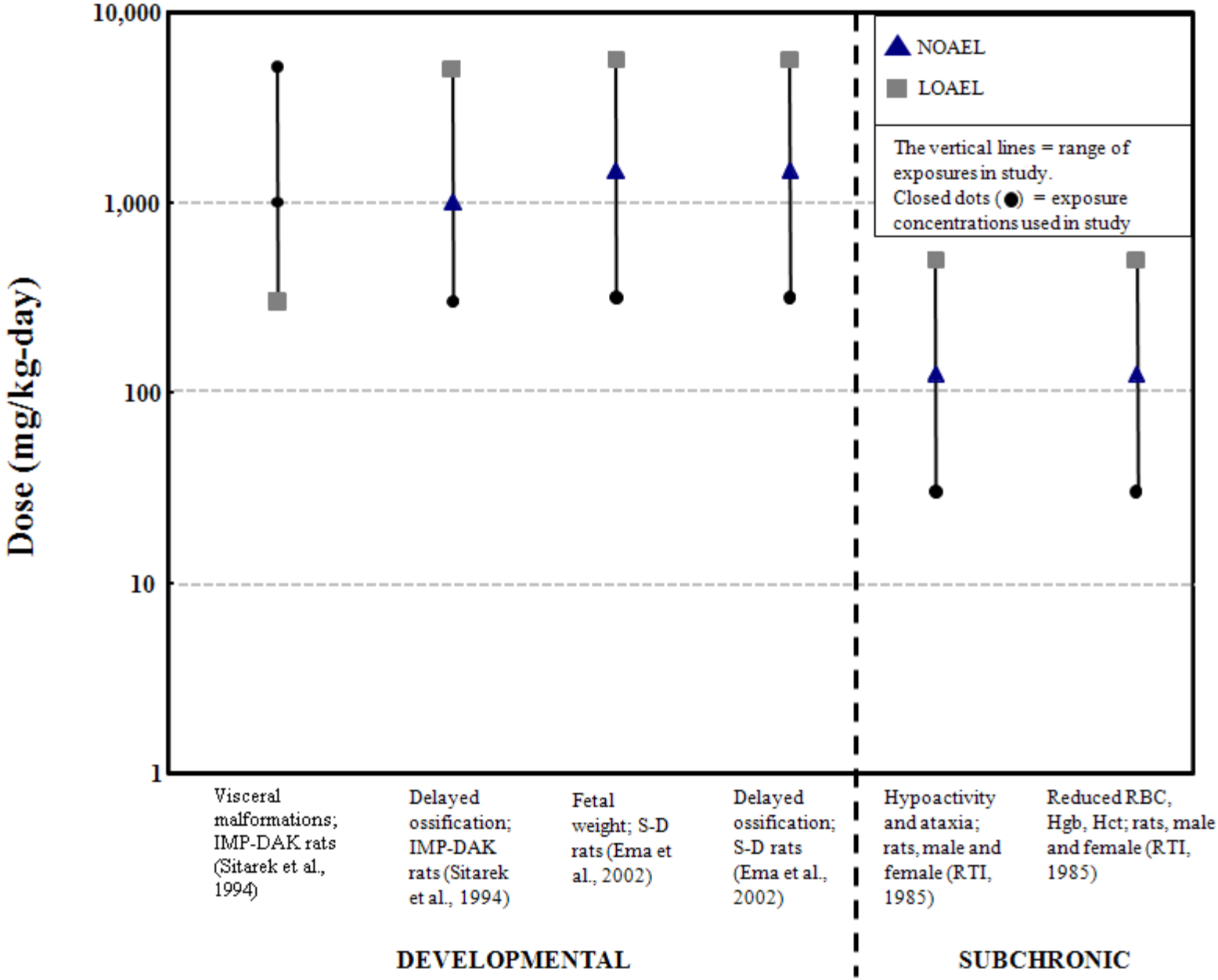


Figure 5-1. Exposure-response array for oral exposure to n-butanol.

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

A PBPK model for n-butanol is available (Teeguarden et al., 2005); however, this model was not suitable for use in deriving the RfD. The principal study used for the RfD derivation (Sitarek et al., 1994) exposed rats before and during gestation, and fetal effects were the most sensitive endpoint. The available PBPK model does not simulate oral exposure, precluding estimation of the relevant internal dose metric for this study.

BMD modeling was conducted using the EPA's benchmark dose software (BMDS, version 2.1.1.) to analyze the developmental visceral malformation associated with exposure to n-butanol (see Appendix B for modeling details). The software was used to calculate potential PODs for deriving the RfD by estimating the effective dose at a specified level of response (BMD_x) and its 95% lower bound ($BMDL_x$). Since internal doses could not be estimated using a PBPK model, administered doses were used to derive a BMDL.

In the study by Sitarek et al. (1994), a LOAEL of 300 mg/kg-day was identified by EPA based on increased litter incidences of visceral malformations (dilation of subarachnoid space, lateral ventricle, unilateral and bilateral renal pelvis). A NOAEL was not identified for this study. These teratogenic effects could not be modeled using nested developmental toxicity dose-response models, as these models require individual (i.e., litter-specific) animal data which were not reported by Sitarek et al. (1994). The incidences of litters affected with visceral malformations were reported in the study (see Table 5-1). Although individual data were provided for dilation of the parameters in the brain (lateral ventricle and subarachnoid space) and was more sensitive that the kidney parameters (unilateral and bilateral renal pelvis), the combined data for the dilation incidences in the brain would provide a better estimate of the overall developmental toxicity associated with n-butanol since dilation of subarachnoid space and the lateral ventricle are reported precursors of hydrocephalus (Raimondi, 1994). However, combined incidences of dilation in the fetal brain via the subarachnoid space or lateral ventricle could not be separated from the kidney endpoints since the incidences were not reported independent of each other. For example, in the 500 mg/kg-day group it was noted that 2 out of 14 litters had dilation of the subarachnoid space, 8 out of 14 had dilation of the lateral ventricle but 9 out of 14 litters were scored as having overall visceral malformations per litter (see Table 5-1). Therefore, overall incidence of visceral malformations was used for BMD modeling to provide a better dose response estimate of developmental toxicity associated with n-butanol.

Table 5-1. Incidences of rat litters with dilation of the subarachnoid space or dilation of the lateral ventricle/third ventricle of the brain

Incidence of affected litters	n-Butanol dose in mg/kg-d			
	0	300	1,000	5,000
Litters with visceral malformations (dilation) – overall	1/12 (8%)	9/14 (64%)	10/12 (83%)	9/9 (100%)
Dilation of subarachnoid space	0/12 (0%)	2/14 (14%)	3/12 (25%)	7/9 (78%)
Dilation of lateral ventricle and/or third ventricle of the brain	1/12 (8%)	8/14 (57%)	8/12 (67%)	7/9 (78%)
Dilation of unilateral renal pelvis	0/12 (0%)	0/14 (0%)	5/12 (42%)	0/9 (0%)
Dilation of bilateral renal pelvis	0/12 (0%)	0/14 (0%)	3/12 (25%)	0/9 (0%)

Source: Sitarek et al. (1994).

All available dichotomous models in the EPA Benchmark Dose Software (BMDS, v. 2.1.1) were fit to the datasets for the increased incidences of visceral malformations (dilation of subarachnoid space, lateral ventricle, unilateral and bilateral renal pelvis). Table 5-2 summarizes the BMD modeling results for the overall observed dilation. A benchmark response (BMR) of 10% was selected from which to derive the point of departure (POD), because the endpoints were characterized in terms of affected litters for reproductive and developmental studies when data are available which characterize the incidence of effects within litters. Since this level of reporting was not available, a nested analysis was not possible. The overall incidence of affected offspring for these endpoints (not shown here) in the control and low dose groups indicated that not all offspring in each affected litter were similarly affected, so using a BMR of 5% extra risk on the basis of affected litters corresponded to a lower overall incidence in the affected offspring. Thus, a BMR of 10% extra risk among affected litters was employed, in order to approximate better a 5% extra risk in affected offspring, while recognizing the litter as the experimental unit for appropriate estimation of confidence intervals. The log-probit model was selected because it provided the best fit to the data based on: (1) having the lowest Akaike's Information Criterion (AIC), and (2) visual inspection of the plot of observed versus expected values across the fitted models. Appendix B contains a more detailed description of the modeling and results, along with graphs of the selected best-fit models.

Table 5-2. Summary of BMD modeling of results based on Sitarek et al. (1994) developmental toxicity data – Dilation in the brain and kidney

BMD Models	AIC	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)
Gamma	41.16	48	31
Logistic	44.28	115	74
Log-logistic	42.49	36	10
Log-probit	**40.94**	**79**	**48**
Multistage (1)	41.16	48	31
Probit	44.42	118	81
Weibull	41.16	48	31

The $BMDL_{10}$ of 48 mg/kg-day for increased litter incidences of visceral malformations (dilation of subarachnoid space, lateral ventricle, unilateral and bilateral renal pelvis) was selected as the POD for derivation of the chronic RfD for n-butanol.

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

To derive the RfD, the $BMDL_{10}$ of 48 mg/kg-day for increased incidence of visceral malformations was divided by a total UF of 300. The UF of 300 comprises component factors of 10 for interindividual variability, 10 for interspecies extrapolation, and 3 for database uncertainty, as described below.

An UF of 10 was selected to account for interspecies variability in extrapolation from laboratory animals (rats) to humans because information is not available to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans.

An UF of 10 was selected to account for intraspecies variability in susceptibility to n-butanol, as quantitative information for evaluating toxicokinetic and toxicodynamic differences among humans are not available.

An UF of 3 was selected to account for deficiencies in the database, specifically the lack of a multigeneration reproductive toxicity study. The toxicological database for n-butanol includes one unpublished subchronic gavage study, two subchronic drinking water studies evaluating limited toxicological endpoints, and two developmental toxicity studies. One of the developmental toxicity studies, Sitarek et al. (1994), included 8 weeks of premating exposure and evaluation of estrous cyclicity and a number of fertility endpoints; however, this study was not a multi-generation reproductive toxicity study and does not abrogate all concerns with potential reproductive toxicity.

An UF for extrapolation from a subchronic study to chronic exposure was not applied, because the POD is from a developmental toxicity study where the critical effect appears to be more dependent on the exposure concentration during a susceptible lifestage than on the duration of exposure.

An UF for extrapolation from a LOAEL to a NOAEL was not applied because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of 10% change in the incidence of dilation of the lateral ventricle was selected based on the assumption that it represents a minimal biologically significant change.

The RfD of 0.2 mg/kg-day for n-butanol was calculated as follows:

$$\begin{aligned} \text{RfD} &= \text{BMDL}_{10} \div \text{UF} \\ &= 48 \text{ mg/kg-day} \div 300 \\ &= 0.2 \text{ mg/kg-day} \end{aligned}$$

5.1.5. Previous RfD Assessment

The previous RfD for n-butanol was posted to the IRIS database on September 1, 1990. An RfD of 1×10^{-1} mg/kg-day was derived based on hypoactivity and ataxia observed in the unpublished subchronic study by RTI (1985). A cumulative UF of 1,000 (including 10-fold each for interindividual variability, interspecies uncertainty, and extrapolation from a subchronic study) was applied to the NOAEL of 125 mg/kg-day to derive the RfD. Both of the oral developmental toxicity studies of n-butanol (Ema et al., 2005; Sitarek et al., 1994) were published after the previous RfD was posted on IRIS.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect—with Rationale and Justification

Information on the health effects of humans from inhalation exposure to n-butanol consists of a few acute human exposure and occupational health studies. Controlled human exposure experiments have demonstrated that n-butanol vapors can exert an irritant effect on the eyes, nose, and throat under acute exposure conditions at air concentrations $\geq$ 10 mg/m^3 (Kjaerguard et al., 1997; Nelson et al., 1943). Occupational health studies in which the primary exposure was to n-butanol reported effects including eye irritation (at 46 to 200 mg/m^3) (Cogan et al., 1945; Tabershaw et al., 1944) and hearing loss (at 240 mg/m^3) (Velazquez et al., 1969). Lack of methodological detail prevents evaluation of the adequacy of the exposure assessments used in all of these studies (Sterner et al., 1949; Cogan et al., 1945; Tabershaw et al., 1944). The eye irritation effects reported by Cogan and colleagues may also have been caused by other chemicals, however, prevalence also was reported among workers in other plants where n-butanol was the only solvent exposure (Tabershaw et al., 1944). The high prevalence of hearing loss observed among n-butanol exposed workers is compelling (Velazquez et al., 1969), but the study has limitations which present uncertainty for its use in the quantification of risk, including the small number of exposed workers (affects precision of the prevalence estimate) and lack of methodological detail (e.g., sampling protocol, selection of referent group).

The animal toxicological database for inhalation exposure to n-butanol includes three subchronic exposure studies (Korsak et al., 1994; Rumanystev et al., 1979; Smyth and Smyth, 1928), a developmental toxicity study (Nelson et al., 1989a), and a neurodevelopmental study (Nelson et al., 1989b). There are no chronic exposure studies or experiments evaluating reproductive toxicity.

Several short-term inhalation exposure studies that have characterized toxicities associated with n-butanol are also available. Fatty infiltration was observed in the liver and kidney in mice exposed to n-butanol air concentrations of 24,624 mg/m^3 for 130 hours (Weese, 1928). Neurotoxic effects, such as decreased response (Frantik et al., 1994) were reported in rats and mice following a 2-4 hour exposure. Respiration rates declined in Ssc:CF-1 mice exposed to air concentrations of 435 ppm and higher for 30 minutes (Kristiansen et al., 1988), as well as in balb/C mice, who experienced a 50% respiratory decline following 4-hour exposures to air concentrations of 3,008 ppm (Korsak et al., 1993) or 4,300 ppm (Korsak and Rydzynski, 1994).

In the three subchronic studies, hematological effects, primarily decreased erythrocyte counts, were noted (Korsak et al., 1994; Rumanystev et al., 1979; Smyth and Smyth, 1928). The lowest concentration at which hematological changes were observed was at 6.6 mg/m^3 in rats where increased blood cholinesterase activity was seen (Rumyantsev et al., 1979). However, it is unclear if the hematological changes observed following exposure to n-butanol are biologically relevant. The other primary toxicological effect observed in the subchronic exposure studies was deficits in neurobehavioral performance. Korsak et al (1994) reported a statistically significant

increase in the failure rate for the rotarod test in rats exposed to 308 mg/m^3 n-butanol for 3 months. Of the three subchronic toxicity studies, only Korsak et al (1994) reported the observed raw data. The other two studies (Rumanystev et al., 1979; Smyth and Smyth, 1928) only indicated statistically significant effects without reporting the raw data. Therefore, these two studies were not amenable to dose-response analysis.

Nelson et al. (1989a,b) evaluated the potential developmental and neurodevelopmental toxicity of n-butanol in rats. Increased incidence of litters with skeletal variation was observed at concentrations of 3,500 ppm (11,000 mg/m^3) and increased levels of serotonin and dopamine were observed in offspring from male rats exposed to 5,000 ppm (18,000 mg/m^3) without changes in neurobehavioral function.

Figure 5-2 is an exposure-response array for inhalation exposure to n-butanol for select animal and human studies employing repeated exposure and reporting the most sensitive and/or relevant toxicological effects. The most sensitive effects were reported in the human occupational studies (Velazquez et al., 1969; Sterner et al., 1949) and in an animal subchronic inhalation study (Korsak et al., 1994). The Nelson et al. (1989a,b) developmental toxicity studies identified effects at concentrations several orders of magnitude higher. As mentioned earlier, although sensitive effects were noted in the human studies, the uncertainties presented by the lack of detail in reporting the assessment of exposure and sparse discussion of study design preclude their use as principal studies.

Korsak et al (1994) was selected as the principal study for derivation of the RfC because it was a well-designed study and reported sensitive toxicological effects. Additionally, there was evaluation of the body and organ weights, measurement of biochemical and hematological parameters, and performance of neurological testing. In the study, two statistically significant effects, decreased hemoglobin and increased lipid peroxidation, were reported at the lowest concentration of 154 mg/m^3. The decreased hemoglobin levels were within historical levels reported for male Wistar rats (Giknis and Clifford, 2008) and hematocrit levels were not changed. The biological relevance of the observed increased hepatic lipid peroxidation is unknown because other hepatic endpoints were not affected in this study.

At the next highest concentration, 308 mg/m^3, there were statistically significant increases in white blood cell counts and impairments in learned avoidance behavior (due to negative reinforcement for falling off the rod prior to n-butanol exposure) and neuromuscular function, as measured by the rotarod test (Korsak et al., 1994). Although the increased white blood cell counts were outside of the historical range reported for male Wistar rats (Giknis and Clifford, 2008), other associated effects, such as immunotoxicological effects, were not tested in this study, and have not been observed in other studies, except for in an acute anti-inflammatory study in Wistar rats, where the WBC count decreased with n-butanol administration (Strubelt and Zetler, 1980).

Impaired learned avoidance behavior and neuromuscular function, as measured by decreased rotarod performance, was selected as the critical effect for RfC derivation. The selection of this critical effect is supported by other neurotoxic effects (e.g., decreased immobility in the swim despair test and decreased response) observed in acute animal studies at concentrations ≥1,420 mg/m^3 (Frantik et al., 1994; DeCeaurriz et al., 1983), as well as the neurotoxic effects, ataxia and hearing impairment, observed in occupationally-exposed humans (Velazquez et al., 1969), and vertigo (Seitz, 1972).

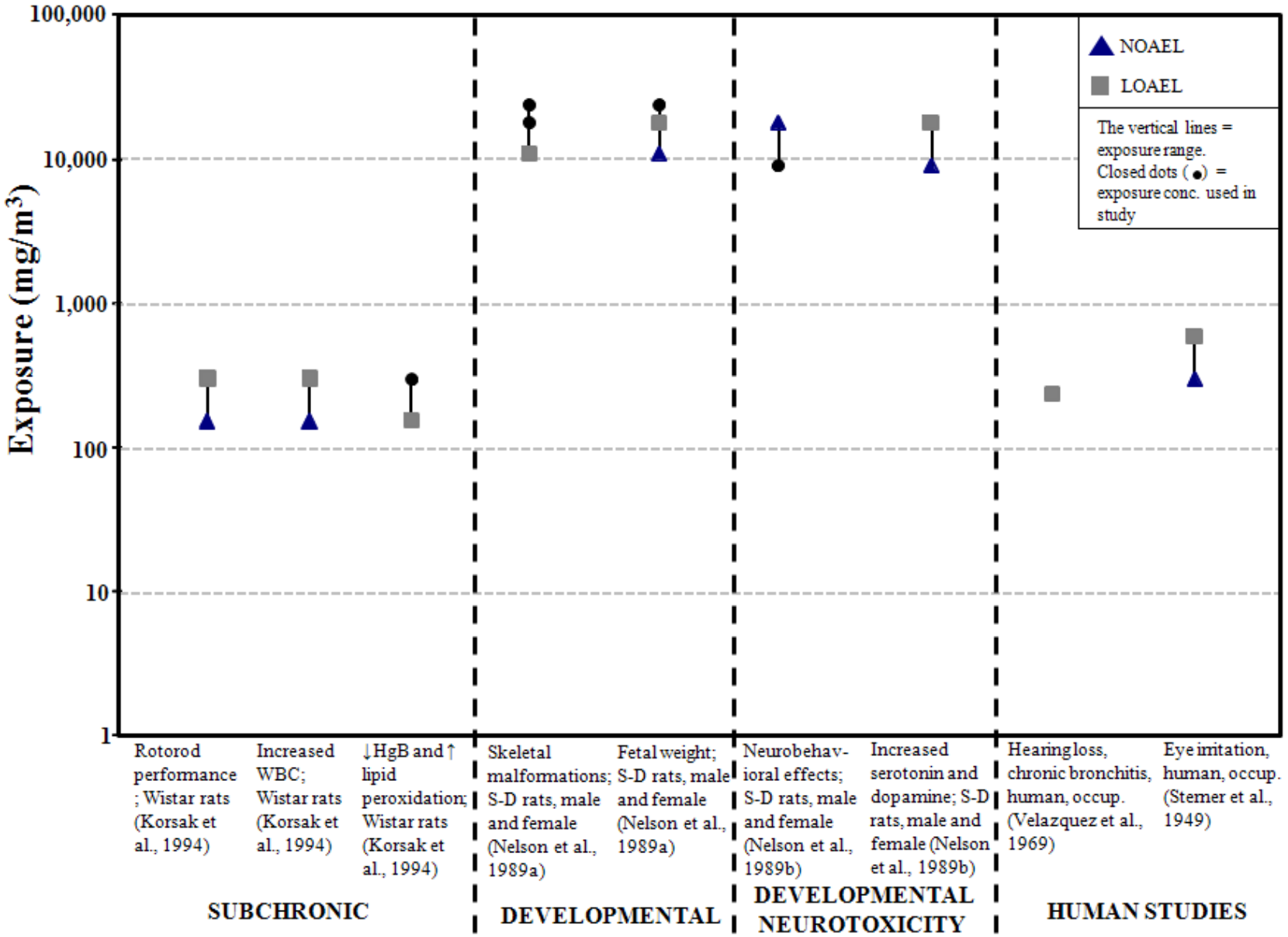


Figure 5-2. Exposure response array for inhalation exposure to n-butanol.

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

A LOAEL of 308 mg/m^3 and a NOAEL of 154 mg/m^3 were identified for impaired neurobehavioral function (as assayed by rotarod performance) in rats exposed for 3 months in Korsak et al. (1994). Korsak et al. (1994) reported the data on rotarod performance graphically and without any estimate of variability within groups; thus, these data were not amenable to BMD modeling. Instead, the rat NOAEL of 154 mg/m^3 was selected as the POD for RfC derivation.

Interspecies extrapolation (i.e., rat-to-human) of n-butanol inhalation dosimetry was accomplished using a rat and human PBPK model originally described in Teeguarden et al. (2005) with modifications described in Appendix C. The rat PBPK model was used to estimate the value for the internal dose metric, represented as the area under the curve (AUC) for n-butanol concentration in arterial blood, corresponding to the NOAEL for motor incoordination in a 90-day rat study (Korsak et al., 1994). The human PBPK model was used to estimate the continuous human inhalation exposure (mg/m^3) that would result from the value of the internal dose metric corresponding to the NOAEL in the rat. Details of the model code and parameters that were selected are presented in Section 3.5 and Appendix C.

The internal dose metric for the neurobehavioral effects of n-butanol is uncertain based on the currently available data. The PBPK model for n-butanol (Teeguarden et al., 2005), with modifications, simulates n-butanol concentrations in blood, liver, and a lumped compartment representing other tissues. The model does not simulate concentrations of n-butanol in the brain. In the absence of dose-metric-specific data, parent compound concentration in the blood is generally selected as a surrogate since it represents the circulating matrix for distribution in the body. Therefore, blood n-butanol concentration was selected as the dose metric for neurobehavioral effects in animals. Korsak et al. (1994) observed progressive increases in severity of the motor incoordination with increasing exposure duration (1–3 months) at a constant exposure concentration, as well as with increasing exposure concentration. These observations suggest that the toxic response is a function of both n-butanol blood concentration and the duration of internal exposure to blood n-butanol. Therefore, arterial blood AUC was selected as the dose metric for dosimetry extrapolation to humans.

Korsak et al. (1994) exposed rats (average body weight of 0.392 kg as measured in the study) for 6 hours/day, 5 days/week, for a period of 3 months. This exposure was simulated in the rat PBPK model as a 90-day exposure of a 0.392 kg rat to 50 ppm (154 mg/m^3, the NOAEL) for 6 hours/day, 5 day/week. The internal dose metric (model variable: $AUCAB2_{24}$) was calculated as the integral of the time profile for the arterial concentration of n-butanol (model variable: CAB2) divided by the simulation time (t = 90 days × 24 hours/day = 2,160 hours), multiplied by 24 hours:

$$AUCAB2_{24} = \left(\frac{\int_0^t CAB2}{t}\right) \cdot 24\,hr$$

Conceptually, the internal dose metric ($AUCAB2_{24}$) represents the average 24-hour AUC for arterial n-butanol concentration for the 2,160-hour exposure duration. The value for $AUCAB2_{24}$ achieved pseudo steady state behavior after 2,000 hours (i.e., ±1% of the final value). The human exposure was simulated as a continuous exposure (24 hours/day, 7 days/week) for a 70-kg human. The simulated exposure duration was 1,000 hours. An exposure duration exceeding 500 hours was sufficient to achieve steady state levels (i.e., > 99.99% stability of the value for $AUCAB2_{24}$). Values for the rat NOAEL, $AUCAB2_{24}$, and human equivalent NOAEL concentration (59 mg/m^3) calculated from the PBPK model are shown in Table 5-4.

Table 5-4. PBPK model calculation of HECs of the NOAEL based on rotarod performance in male rats exposed to n-butanol for 3 months

Rat NOAEL mg/m^3 (ppm)	Rat $AUCAB2_{24}$[a] mM	Human $AUCAB2_{24}$ mM	Human NOAEL (HEC) mg/m^3 (ppm)
154 (50)	0.01282	0.01282	59 (19.21)

[a]Area under the blood concentration vs. time curve for rats estimated using rat PBPK model and exposure to NOAEL concentration of 50 ppm for 6 hours/day, 5 days/week for 3 months (Korsak et al., 1994).

5.2.3. RfC Derivation—Including Application of Uncertainty Factors (UFs)

The $NOAEL_{HEC}$ of 59 mg/m^3 for impaired motor coordination and obtained from PBPK modeling was used as the POD for derivation of the RfC for n-butanol. The RfC was calculated by dividing the POD by a total UF of 1,000, comprised of 3 for interspecies extrapolation, 10 for interindividual variability, 10 for extrapolation from subchronic to chronic exposure, and 3 for database uncertainty, as described below.

An UF of 3 was applied for interspecies extrapolation. The interspecies UF comprises two areas of uncertainty: toxicokinetic and toxicodynamic differences. Use of PBPK modeling to convert the rat exposure concentration to a HEC accounts for toxicokinetic differences between rats and humans and reduces uncertainty associated with cross-species extrapolation. A factor of 3 is retained to account for interspecies variability in extrapolating from laboratory animals (rats) to humans because information is not available to quantitatively assess toxicodynamic differences between animals and humans.

An UF of 10 was selected to account for intraspecies variability in susceptibility to n-butanol, as quantitative information for evaluating toxicokinetic and toxicodynamic differences among humans are not available.

An UF of 10 was selected to account for extrapolation from a subchronic exposure duration study to a chronic RfD, as data for evaluating response after chronic exposure are not available. The study selected as the principal study was a 3-month study by Korsak et al. (1994), a study duration that falls well short of a standard lifetime study.

An UF of 3 was selected to account for deficiencies in the database, specifically the lack of a multigeneration reproductive toxicity study. The toxicological database for inhaled n-butanol includes several acute human experiments, occupational health studies, subchronic toxicity studies, and developmental and neurodevelopmental toxicity studies.

An UF for extrapolation from a LOAEL to a NOAEL was not applied because a NOAEL was used as the POD.

The RfC of 0.06 mg/m^3 for n-butanol was calculated as follows:

$$\begin{aligned} \text{RfC} &= \text{NOAEL}_{\text{HEC}} \div \text{UF} \\ &= 59\ \text{mg/m}^3 \div 1{,}000 \\ &= 0.06\ \text{mg/m}^3 \end{aligned}$$

5.2.5. Previous RfC Assessment

An inhalation RfC for n-butanol was not previously available on IRIS.

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

The following discussion identifies uncertainties associated with the quantification of the RfD and RfC for n-butanol. Following EPA practices and guidance (U.S. EPA, 1994b), the uncertainty factor approach was applied to the identified PODs to derive an RfD and an RfC (see Sections 5.1.3 and 5.2.3). Factors accounting for uncertainties associated with a number of steps in the analyses were adopted to account for extrapolating from an animal study to human exposure, a diverse human population of varying susceptibilities, extrapolation from subchronic to chronic exposure duration, and database deficiencies.

The oral toxicity database for n-butanol contains one subchronic gavage study in rats (RTI; unpublished, 1985), two subchronic drinking water studies in rats (Munoz et al., 1991, 1990; Wakabayashi et al., 1991), and two developmental toxicity studies in rats (Ema et al., 2005; Sitarek et al., 1994). Toxicity associated with oral exposure is predominantly associated with neurodevelopmental toxicity. Effects observed after oral exposure to n-butanol include teratogenicity, clinical signs of CNS effects, and hematology changes.

The toxicological database for inhaled n-butanol includes several acute human experiments, a few occupational health studies, a subchronic study in rats (Korsak et al., 1994), and developmental and neurodevelopmental toxicity studies in rats (Nelson et al., 1989a, b). Neurotoxicity is the predominant effect associated with inhalation exposure to n-butanol. Critical data gaps have been identified and uncertainties associated with data deficiencies are more fully discussed below.

The RfD was derived based on the increased incidence of overall visceral malformations in a developmental toxicity study of n-butanol (Sitarek et al., 1994). Uncertainty exists in the discrepancy between the results of the developmental studies by Sitarek et al. (1994) and Ema et al. (2005). Specifically, Sitarek et al. (1994) observed developmental effects on the brain at low doses ($\geq$ 300 mg/kg-day), but Ema et al. (2005) did not observe any developmental malformations at any dose. The discrepancy in these results may be due to differences in study design, particularly rat strain used and exposure regimens. Data support a strain differences between Wistar and Sprague Dawley rats in developmental effects on the brain. For example, one study evaluated strain differences for developmental brain effects in Sprague-Dawley and Wistar rats following exposure to aspirin (Gupta et al., 2003). Gupta et al. (2003) reported that the Wistar pups had a significantly higher rate of hydrocephaly than the Sprague-Dawley pups. In addition, Ema et al. (2005) utilized a gestation-ony exposure regimen compared to Sitarek et al. (1994) which exposed rats prior to mating, during mating, and throughout gestation.

Uncertainty also exists in the selection of the appropriate BMR for use in the BMD modeling of the critical effect (i.e., increased incidence of dilation of the lateral ventricle of the brain in Wistar IMP:Dak rat litters) to estimate the POD. Since the results of Sitarek et al. (1994) were reported in a way that the litter membership of individual pups could not be

determined, a nested BMD modeling approach could not be used and therefore, a litter-based BMD modeling approach was employed. In the absence of specific information to identify a change in the litter incidence of dilation of the lateral ventricle that represents a biologically significant change, a BMR of 10% was selected, which is consistent with the default BMR of 10% for quantal data (U.S. EPA, 2000).

The RfC was derived based on neurobehavioral effects in rats exposed to n-butanol via inhalation for 3 months (Korsak et al., 1994). With regards to the study design, it is not known when the behavioral testing following n-butanol exposure. Therefore, the extent of n-butanol internally (whether cleared or not) is unknown. It is likely that the measures are reflective of persistent effects of n-butanol based on the increased deterioration of the rotarod performance over the 3 months but still represents an uncertainty. BMD modeling could not be performed using these data due to the lack of reporting by the investigators of within-group variability estimates. By definition, the identification of a NOAEL or LOAEL is restricted to the particular doses or exposure concentrations used in a study, and thus, this type of analysis lacks characterization of the entire dose-response curve for the effect of interest. As a result, a POD based on a NOAEL or LOAEL is less informative than one derived from BMD modeling.

With respect to uncertainty associated with the RfC derivation, there is one human study, Velazquez et al. (1969), that reported hearing loss in 82% (9 out of 11) of the workers exposed to 240 mg/m^3 n-butanol for a duration ranging from 3 to 11 years in a cellulose acetate factory. Although human data is typically preferred for deriving reference values, there are limitations with this study, including the small number of exposed workers, which decreases the power of the study, and the lack of information with regards to exposure sampling and exposure duration, which precluded the selection of this study as the principal study.

An additional source of uncertainty in the derivation of both RfD and RfC is extrapolation from animals (rats) to humans. An effect and its magnitude associated with the concentration at the POD in rodents are extrapolated to human response. Pharmacokinetic models are useful in examining species differences in pharmacokinetic processing; however, dosimetric adjustment using pharmacokinetic modeling was not possible following oral exposure to n-butanol since the model does not contain an oral route of administration and incorporate the subsequent first pass effect. There are data gaps in pharmacokinetic parameters since there is no information on distribution of n-butanol to the fetus as well as no information of the elimination of n-butanol via breast milk which represents an uncertainty for both the RfD and RfC. For RfC derivation, a PBPK model was used to account for toxicokinetic differences between rats and humans, and the internal dose metric, blood n-butanol AUC, was selected. PBPK models attempt to simulate what occurs in vivo, and thus dose estimates from these models represent an additional source of uncertainty in the analysis. In the absence of specific data that could inform the selection of the appropriate internal dose metric, the blood AUC of the parent compound (i.e., n-butanol) was selected as the preferred dose metric. Additionally, there is uncertainty

related to the urinary clearance rate of n-butanol and its metabolites. Specifically, butyric acid, a metabolite of n-butanol, interacts with colonocytes and is incorporated into lipid membranes (Thibault et al., 2010). However, kinetic information is currently not available to address this uncertainty and thus urinary clearance of metabolites was not incorporated into the model. Another uncertainty with the model is the extent that n-butyric acid is metabolized to acetyl CoA and then to carbon dioxide and water or to acetoacetyl CoA, (produced by beta oxidation of the buttanoic acid).

Heterogeneity among humans with respect to the toxicokinetics and toxicodynamics of n-butanol represents another area of uncertainty in both the RfD and RfC derivations. n-Butanol is rapidly metabolized to butyric aldehyde by ADH and further to n-butyric acid by aldehyde dehydrogenase. Polymorphisms in the genes encoding ADH and aldehyde dehydrogenase exist in the human population and may contribute to variability in metabolism of n-butanol. One key genetic polymorphism is the ALDH2 (aldehyde dehydrogenase 2) allele that has been identified as a slow metabolizing variant of aldehyde dehdrogenase and has been more commonly found in East Asian populations (Dickson et al., 2006). Populations with low metabolizing capacity (slow alcohol and aldehyde dehydrogenases) are more likely to be susceptible to toxicities associated with n-butanol since there would be a longer half-life in these individuals. Another key polymorphism has been identified in the ADH3 (alcohol dehydrogenase type 3) gene where the gamma1 variant is found in individuals that are fast metabolizers and the gamma2 variant is found in individuals that are slow metabolizers (Hines et al., 2001). The polymorphisms in the n-butanol metabolizing enzymes indicate that there may be a great deal of variability in the effects associated with exposure in humans.

In the absence of n-butanol-specific data to adequately characterize such genetic variability among humans, a factor of 10 was used to account for this uncertainty.

Data gaps in the toxicological database for n-butanol include: lack of chronic toxicity studies; lack of toxicity data in a laboratory species other than rats; limitations in the available subchronic studies (e.g., the oral study by RTI [1985] is unpublished and lacks data tables and appendices, and the inhalation study by Korsak et al. [1994] did not include histopathology evaluation); lack of a multigeneration reproductive toxicity study; and lack of an oral neurodevelopmental toxicity study. Critical endpoints for both the RfD and RfC were neurological, suggesting that further evaluation of the neurotoxic and neurodevelopmental effects of n-butanol would be beneficial. There was a lack of postnatal observations in the developmental toxicity studies since offspring were sacrificed at the end of the gestational period (Sitarek et al., 1994; Nelson et al., 1989a; Ema et al., 2005). Epidemiology studies of n-butanol exposure evaluating neurological and otological endpoints and using documented, quantified assessments of long-term exposure also would contribute to a reduction in uncertainty.

5.4. CANCER ASSESSMENT

Under the EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is *inadequate information to assess the carcinogenic potential* of n-butanol. None of the available occupational health studies of n-butanol evaluated cancer endpoints. One occupational health study of workers exposed to n-butanol, and several other solvents, while employed as dockyard painters examined mortality rates due to cancer (Chen et al., 1999); however, neither proportional mortality nor standardized mortality due to cancers were increased in the painters relative to controls. There are no chronic animal studies of n-butanol exposure via any exposure route, and the subchronic studies did not report any cancer endpoints. The genotoxicity database for n-butanol is limited; available information suggests that n-butanol does not induce mutations in *S. typhimurium* with or without exogenous metabolic activation (Muller et al., 1993; Jung et al., 1992; Nakamura et al., 1987; Connor et al., 1985; McCann et al., 1975), and does not increase the frequency of micronuclei or sister chromatid exchanges in Chinese hamster ovary cells (Lasne et al., 1984; Obe et al., 1977) or in chick embryos in culture (Bloom, 1982). Studies of DNA and RNA replication have shown that n-butanol can exert an inhibitory effect on these processes (Obe and Ristow, 1977; Yoshiyama et al., 1973).

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

6.1. HUMAN HAZARD POTENTIAL

n-Butanol (CASRN 71-36-3) is used as a direct solvent in paints, surface coatings, lacquers, thinners, pharmaceutical formulations, waxes, and resins. It is also used to make plasticizer esters and mono-, di-, and tributylamines and oxygenate for gasoline. Commercial derivatives of n-butanol include n-butyl acrylate, methacrylate, butyl glycol ethers, 2-butoxy-ethanol, and butyl acetate.

Toxicokinetic studies of n-butanol have been conducted in humans and experimental animals. Absorption via inhalation has been demonstrated in humans (Astrand et al., 1976), and absorption via the oral, dermal, and inhalation routes was demonstrated in laboratory animals (Poet et al., 2003a, b; Deisinger and English, 2001; Boman et al., 1995; Swiercz et al., 1995; DiVincenzo and Hamilton, 1979a). Only limited information is available regarding tissue distribution of n-butanol in animal studies; after gavage dosing of rats with radiolabelled n-butanol, the liver had the largest percentage of radioactivity (DiVincenzo and Hamilton, 1979a). n-Butanol is rapidly metabolized to butyric aldehyde by ADH and further to n-butyric acid, also known as butanoic acid, by aldehyde dehydrogenase (ECETOC, 2003). n-Butanol is also oxidized by cytochrome P450 in rat liver (Albano et al., 1991). n-Butanol is excreted primarily as CO_2 in exhaled breath with minor amounts eliminated in the urine and feces (DiVincenzo and Hamilton, 1979a). A PBPK model has been developed (Teeguarden et al., 2005) to describe blood kinetics for n-butyl acetate and its metabolites, n-butanol and n-butyric acid, in rats and humans exposed via inhalation.

Information on noncancer effects in humans orally exposed to n-butanol is limited to one case report from a suicide attempt (Bunc et al., 2006) in which neurological, gastrointestinal, and cardiovascular symptoms were noted. The database on animal toxicity indicates that developmental visceral malformations (dilation of the lateral ventricle and subarachnoid space in the brain; dilation in renal pelvis in the kidney) are the most sensitive toxicological endpoint associated with oral n-butanol administration (Sitarek et al., 1994). Increased incidences of brain malformations (dilation of the lateral and/or third ventricle of the brain) were observed in the pups of female Wistar Imp:DAK rats treated with n-butanol in drinking water at doses of 300, 1,000, and 5,000 mg/kg-day administered for 8 weeks prior to mating and then continuously through mating and gestation. Neurotoxicity (hypoactivity and ataxia) in adult rats was also observed in a 13week oral gavage study at 500 mg/kg-day (RTI; unpublished, 1985). Other observed effects included hematological changes and increased thyroid weight at 500 mg/kg-day (RTI; unpublished, 1985), and pathological changes in the liver and kidney at an exposure of 4,400 mg/kg-day in male rats (Munoz et al. 1991; 1990).

Controlled human exposure experiments have demonstrated that n-butanol vapors can exert an irritant effect on the eyes, nose, and throat under acute exposure conditions at air concentrations ≥ 10 mg/m^3 (Kjaerguard et al., 1997; Nelson et al., 1943). Occupational health studies in which the primary exposure was to n-butanol reported effects including eye irritation (at 46 to 200 mg/m^3) (Cogan et al., 1945; Tabershaw et al., 1944) and hearing loss (at 240 mg/m^3) (Velazquez et al., 1969). These studies are limited by uncertainties arising from a lack of detail in methods description for exposure assessments and other details of study design.

The CNS is the most sensitive target following a repeated inhalation exposure to n-butanol in animals. Korsak et al (1994) reported a statistically significant increase in the failure rate for the rotarod test in rats exposed to 308 mg/m^3 n-butanol for 3 months. Nelson et al (1989a, b) evaluated the developmental and neurodevelopmental toxicity potential of n-butanol in rats. Increased incidence of litters with skeletal variation was observed at exposures of 3,500 ppm (11,000 mg/m^3) and increased levels of serotonin and dopamine were observed in offspring from male rats exposed to 5,000 ppm (18,000 mg/m^3) without changes in neurobehavioral function. Several short-term inhalation exposure studies have reported fatty infiltration of liver and kidney in mice exposed to 24,624 mg/m^3 (Weese, 1928), neurotoxic effects at exposures of 1,420 mg/m^3 in mice and rats (Frantik et al., 1994; DeCeaurriz et al., 1983), and decreased respiration rates in mice (Korsak and Rydzynski, 1994; Korsak et al., 1993; Kristiansen et al., 1988).

None of the available human occupational health studies in which the primary exposure was to n-butanol evaluated cancer endpoints. One occupational health study of workers exposed to n-butanol, as well as several other solvents, while employed as dockyard painters examined mortality rates due to cancer (Chen et al., 1999); however, neither proportional mortality nor standardized mortality due to cancers were increased in the painters relative to controls. There are no chronic animal studies of n-butanol exposure via any exposure route, and the available subchronic studies did not report any cancer endpoints. Therefore, under the EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is *inadequate information to assess the carcinogenic potential* of n-butanol.

6.2. DOSE RESPONSE

6.2.1. Oral Noncancer

From the available data on oral exposure to n-butanol, it was determined that neurodevelopmental changes were the most sensitive noncancer endpoint. The developmental toxicity study in Wistar Imp:DAK rats (Sitarek et al.,1994) was selected as the principal study for RfD derivation and the increased incidence developmental visceral malformations (dilation in the fetal brain [lateral ventricle and subarachnoid space] and kidney [renal pelvis]) was selected as the critical effect.

An RfD of 0.2 mg/kg-day was derived. The RfD derivation involved fitting all of the available dichotomous models in BMDS, version 2.1.1, to the litter incidence of overall increased visceral malformations (dilation). A BMR of 10% was selected because even though the principal study had a nested design, litter-specific results were not reported by the investigators which prevented the conduct of a nested analysis. Thus, a BMR of 10% was employed, which is generally the default BMR for quantal data (U.S. EPA, 2000). Dose-response modeling of the incidence of litters with overall dilation yielded a $BMDL_{10}$ of 48 mg/kg-day, which was used as the POD. A composite UF of 300 (10 to account for interspecies uncertainty, 10 to account for human variability, 3 for database uncertainty) was applied to the POD to derive an RfD of 0.2 mg/kg-day.

Confidence in the principal study (Sitarek et al., 1994) is medium. This developmental toxicity study evaluated exposure in three dose groups and a control, using group sizes of 11–17 female rats exposed for 8 weeks prior to mating as well as during mating and gestation. The study evaluated body weight, clinical signs, and estrous cyclicity in dams, as well as litter parameters and teratogenicity endpoints. Confidence in the database is low-to-medium. Oral toxicity studies of n-butanol include an unpublished subchronic study that evaluated comprehensive endpoints but lacked data tables and appendices, two published subchronic studies evaluating limited endpoints, and two developmental toxicity studies. All of the available animal studies were conducted in rats. The database is lacking chronic toxicity studies, toxicity data in a laboratory species other than rats, and a multigeneration reproductive toxicity study. Neurodevelopmental effects should also be further evaluated in light of the effects on brain development in the Sitarek et al. (1994) developmental toxicity study. Overall confidence in the RfD is low-to-medium.

6.2.2. Inhalation Noncancer

The CNS is the most sensitive target for noncancer toxicity in rats following repeated inhalation exposure to n-butanol. The most sensitive endpoint was impairments in learned avoidance behavior and neuromuscular function, represented by decreased rotarod performance, in rats exposed to n-butanol for 3 months (Korsak et al., 1994). Korsak et al. (1994) was selected as the principal study for derivation of the RfC because it was a relatively well-designed study, evaluated lower exposure effects, and provided a sensitive endpoint of neurologic impairment. Decreased rotarod performance was selected as the critical effect.

An RfC of 0.06 mg/m^3 was derived based on the observed critical effect of neuromuscular impairment in the principal study. The data for this endpoint were not amenable to BMD modeling due to the absence of within-group variability estimates for rotarod performance, so a NOAEL identified from this study (154 mg/m^3) was selected as the POD. PBPK modeling using a modification of the model published by Teeguarden et al. (2005) was used to estimate a $NOAEL_{HEC}$ concentration of 59 mg/m^3 based on arterial n-butanol blood

concentration (AUC) as the internal dose metric. A composite UF of 1000 (3 to account for interspecies toxicodynamic uncertainty, 10 to account for human variability, 10 to account for uncertainity of subchronic to chronic extrapolation, 3 for database uncertainty) was applied to the POD to derive an RfC of 0.06 mg/m^3.

Confidence in the principal study (Korsak et al., 1994) is low-to-medium. This subchronic study evaluated exposure in two dose groups and a sham-treated control, using group sizes of 12 male rats. The study evaluated body weight, hematology, clinical chemistry, neurotoxicity endpoints (rotarod performance and hot-plate response), and selected organ weights, but did not evaluate histopathology. Confidence in the database is low-to-medium. Inhalation toxicity studies of n-butanol include the subchronic study used as the basis for the RfC as well as developmental toxicity and developmental neurotoxicity studies, all conducted in rats. The database is lacking chronic toxicity studies, toxicity data in a laboratory species other than rats, and a multigeneration reproductive toxicity study. Neurological effects were the most sensitive endpoint in the available subchronic study; however, the study evaluated only two measures of neurotoxicity (rotarod performance and hot-plate response). Overall confidence in the RfC is low-to-medium.

6.2.3. Cancer - Oral and Inhalation

Under the EPA *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), there is *inadequate information to assess the carcinogenic potential* of n-butanol. Cancer endpoints were not evaluated in any of the human occupational health studies. There are no chronic animal studies of n-butanol exposure via any exposure route, and the subchronic studies did not report any cancer endpoints. The genotoxicity database is limited and results primarily support that n-butanol does not induce mutations in *S. typhimurium* with or without exogenous metabolic activation (Muller et al., 1993; Jung et al., 1992; Nakamura et al., 1987; Connor et al., 1985; McCann et al., 1975), and does not increase the frequency of micronuclei or sister chromatid exchanges (Lasne et al., 1984; Bloom, 1982; Obe et al., 1977).

7. REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). (2002) n-Butanol. In: Documentation of the threshold limit values and biological exposure indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Akhmadeyeva, EN. (1993) Health of newborns of workers in the petroleum-chemical industries. Reprod Toxicol 7(5):491–492.

Akinshola, BE. (2001) Straight-chain alcohols exhibit a cutoff in potency for the inhibition of recombinant glutamate receptor subunits. Br J Pharmacol 133:651–658.

Albano, E; Tomasi, A; Persson, J; et al. (1991) Role of ethanol-inducible cytochrome P450 (P450IIE1) in catalysing the free radical activation of aliphatic alcohols. Biochem Pharmacol 41(12):1895–1902.

Amoore, JE; Hautala, E. (1983) Odor as an aid to chemical safety: odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. J Appl Toxicol 3(6):272–290.

Andjus, PR; Bataveljić, D; Vanhoutte, G; et al. (2009) In vivo morphological changes in animal models of amyotrophic lateral sclerosis and Alzheimer's-like disease: MRI approach. Anat Rec 292:1882–1892.

Angerer, J; Wulf, H. (1985) Occupational chronic exposure to organic solvents. XI. Alkylbenzene exposure of varnish workers: effects on hematopoetic system. Int Arch Occup Environ Health 56:307–321.

Anonymous. (1987) Final report of the safety assessment of n-butyl alcohol. J Am Coll Toxicol 6(3):403–424.

Arsov, Z; Zorko, M; Schara, M. (2005) Inhibition of erythrocyte acetylcholinesterase by n-butanol at high concentrations. Arch Biochem Biophys 437:78–84.

Ashley, DL; Prah, JD. (1997) Time dependence of blood concentrations during and after exposure to a mixture of volatile organic compounds. Arch Environ Health 52(1):26–33.

Astrand, I; Ovrum, P; Lindqvist, T; et al. (1976) Exposure to butyl alcohol: uptake and distribution in man. Scand J Work Environ Health 2(3):165–175.

Auty, RM; Branch, RA. (1976) The elimination of ethyl, n-propyl, n-butyl and iso-amyl alcohols by the isolated perfused rat liver. J Pharmacol Exp Ther 197:669–674.

Baikov, BK; Khachaturyan, MK. (1973) Hygienic evaluation of the reflex action on the body of low concentrations of butyl alcohol entering the atmosphere. Gig Sanit 38(12):7–11. (Russian)

Bariliak, IR; Korkach, VI; Spitkovskaia, LD. (1991) The embroyotoxic effects of monohydric alcohols. Ontogenez 22(1):71–75. (Russian)

Barton, HA; Deisinger, PJ; English, JC; et al. (2000) Family approach for estimating reference concentrations/doses for series of related organic chemicals. Toxicol Sci 54:251–261.

Behl, CR; El-Sayed, AA; Flynn, GL. (1983) Hydration and percutaneous absorption. IV: Influence of hydration on n-alkanol permeation through rat skin; comparison with hairless and Swiss mice. J Pharm Sci 72(1):79–82.

Behl, CR; Flynn, GL; Kurihara, T; et al. (1984) Age and anatomical site influences on alkanol permetion of skin of the male hairless mouse. J Soc Cosmet Chem 35:237–252.

Bhattacharji, A; Kaplan, B; Harris, T; et al. (2006) The concerted contribution of the S4-S5 linker and the S6 segment to the modulation of a Kv channel by 1-alkanols. Mol Pharmacol 70(5):1542–1554.

Billig E. 2001. Butyl alcohols. In: Kirk-Othmer encyclopedia of chemical technology. Available online at http://mrw.interscience.wiley.com/emrw/9780471238966/kirk/article/butybill.a01/current/pdf (accessed August 17, 2009).

Birley. AJ; James, MR; Dickson, PA; Montgomery, GW; Heath, AC; Martin, NG; Whitfield, JB (2009). ADH single nucleotide polymorphism associations with alcohol metabolism in vivo. Hum Mol Genet 18(8): 1533-42.

Bleecker, ML; Bolla, KI; Agnew, J; et al. (1991) Dose-related subclinical neurobehavioral effects of chronic exposure to low levels of organic solvents. Am J Ind Med 19(6):715–728.

Bloom, SE. (1982) Detection of sister chromatid exchanges in vivo using avian embryos. In: Hsu, TC; ed. Cytogenic assays of environmental mutagens. Totowa, NJ: Allanheld, Osmun & Co.; pp. 137–159.

Boleda, MD; Farrés, J; Guerri, C; Parés, X (1992). Alcohol dehydrogenase isoenzymes in rat development. Effect of maternal ethanol consumption. Biochem Pharmacol. 43(7):1555-61.

Boman, A; Maibach, HI. (2000) Influence of evaporation and solvent mixtures on the absorption of toluene and n-butanol in human skin in vitro. Ann Occup Hyg 44(2):125–135.

Boman, A; Hagelthorn, G; Magnusson, K. (1995) Percutaneous absorption of organic solvents during intermittent exposure in guinea pigs. Acta Derm Venereol 75:114–119.

Boublik, T; Fried, V; Hala, E. (1984) The vapor pressures of pure substances: selected values of the temperature dependence of the vapour pressures of some pure substances in the normal and low pressure region. Vol. 17. Amsterdam, Netherlands: Elsevier Science Publications.

Brown, RP; Delp, MD; Lindstedt, SL; et al. (1997) Physiological parameter values for physiologically based pharmacokinetic models. Toxicol Ind Health 13(4):407–484.

Bunc, M; Pezdir, T; Mozina, H; et al. (2006) Butanol ingestion in an airport hangar. Hum Exp Toxicol 25(4):195–197.

Buttery, RG; Ling, LC; Guadagni, DG. (1969) Volatilities of aldehydes, ketones, and esters in dilute water solution. J Agric Food Chem 17(2):385–389.

Cameron, AM; Zahlsen, K; Haug, E; et al. (1985) Circulating steroids in male rats following inhalation of n-alcohols. Arch Toxicol Suppl 8:422–424.

Card, SE; Tompkins, SF; Brien, JF (1989). Ontogeny of the activity of alcohol dehydrogenase and aldehyde dehydrogenases in the liver and placenta of the guinea pig. Biochem Pharmacol. 38(15):2535-41.

Carlson, GP. (1994a) In vitro esterification of fatty acids by various alcohols in rats and rabbits. Toxicol Lett 70:57–61.

Carlson, GP. (1994b) Formation of esterified fatty acids in rats administered 1-butanol and 1-pentanol. Res Commun Mol Pathol Pharmacol 86(1):111–117.

Carlson, GP; Olson, RMC. (1995) Comparison of the metabolism of alcohols by rat hepatic and pulmonary alcohol dehydrogenase. Biochem Mol Biol Int 37(1):65–71.

Cater, BR; Cook, MW; Gangolli, SD; et al. (1977) Studies on dibutyl phthalate-induced testicular atrophy in the rat: Effect on zinc metabolism. Toxicol Appl Pharmacol 41:609–618.

Cederbaum, AI. (1978) The effect of calcium on the oxidation of acetaldehyde by rat liver mitochondria. Life Sci 22(1):111–125.

Cederbaum, AI; Dicker, E; Rubin, E; et al. (1979) Effect of thiourea on microsomal oxidation of alcohols and associated microsomal functions. Biochemistry 18(7):1187–1191.

ChemIDplus. (2009) N-Butanol. ChemIDplus. Bethesda, MD: U.S. National Library of Medicine. Available online at http://sis.nlm.nih.gov/chemical.html (accessed on August 14, 2009).

Chen, R; Dick, F; Seaton, A. (1999) Health effects of solvent exposure among dockyard painters: mortality and neuropsychological symptoms. Occup Environ Med 56(6):383–387.

Chvapil, M; Zahradnik, R; Cmuchalova, B. (1962) Influence of alcohols and potassium salts of xanthogenic acids on various biological objects. Arch Int Pharmacodyn Ther 135:330–343.

Cogan, DG; Mort, W; Grant, MD. (1945) An unusual type of keratitis associated with exposure to n-butyl alcohol (butanol). Arch Ophthalmol 33:106–108.

Cometto-Muniz, JE; Cain, WS. (1995) Relative sensitivity of the ocular trigeminal, nasal trigeminal and olfactory systems to airborne chemicals. Chem Senses 20(2):191–198.

Connor, TH; Theiss, JC; Hanna, HA; et al. (1985) Genotoxicity of organic chemicals frequently found in the air of mobile homes. Toxicol Lett 25:33–40.

Crofton, KM; Lassiter, TL; Rebert, CS. (1994) Solvent-induced toxicity in rats: an atypical selective mid-frequency hearing deficit. Hear Res 80:25–30.

David, R; Tyler, T; Ouellette, R; et al. (1998) Evaluation of subchronic neurotoxicity of n-butyl acetate vapor. Neurotoxicology 19:809–822.

David, RM; Tyler, TR; Ouellette, R; et al. (2001) Evaluation of subchronic toxicity of n-butyl acetate vapor. Food Chem Toxicol 39:877–886.

Deisinger, PJ; English, JC. (2001) Pharmacokinetics of n-butyl acetate and its metabolites in rats after intravenous administration. Rochester, NY: Toxicological Sciences Laboratory, Health and Environmental Laboratories, Eastman Kodak Company.

DelTerzo, S; Behl, CR; Nash, RA; et al. (1986) Evaluation of the nude rat as a model: effects of short-term freezing and alkyl chain length on the permeabilities of n-alkanois and water. J Soc Cosmet Chem 37:297–307.

Deters, M; Siegers, CP; Strubelt, O. (1998a) The influence of 4-methylpyrazole on the acute hepatotoxic actions of seven aliphatic alcohols. Res Commun Alcohol Subst Abuse 19(1-2):37–46.

Deters, M; Siegers, CP; Strubelt, O. (1998b) Influence of glycine on the damage induced in isolated perfused rat liver by five hepatotoxic agents. Toxicology 128:63–72.

Dietz, FK; Rodriguez-Giaxola, M; Traiger, GJ; et al. (1981) Pharmacokinetics of 2-butanol and its metabolites in the rat. J Pharmacokinet Biopharm 9(5):33–76.

Dildy-Mayfield, JE; Mihic, SJ; Liu, Y; et al. (1996) Actions of long chain alcohols on GABAA and glutamate receptors: relation to in vivo effects. Br J Pharmacol 118:378–384.

Ding, J; Badwey, JA. (1994) Wortmannin and 1-butanol block activation of a novel family of protein kinases in neutrophils. FEBS Lett 348:149–152.

DiVincenzo, GD; Hamilton, ML. (1979a) Fate of n-butanol in rats after oral administration and its uptake by dogs after inhalation or skin application. Toxicol Appl Pharmacol 48:317–325.

DiVincenzo, GD; Hamilton, ML. (1979b) Metabolic fate of [1-14C]isobutyric acid in the rat. Toxicol Appl Pharmacol 47(609–612).

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals). (2003) n-Butanol (CAS No. 71-36-3). JACC No. 41. Brussels, Belgium.

Edelfors, S; Ravn-Jonsen, A. (1990) The effects of alcohols in vitro on the nervous cell membrane measured by changes in the (Ca2+/Mg2+) ATPase activity and fluidity of the synaptosomal membrane. Pharmacol Toxicol 67(1):56–60.

Ema, M; Hara, H; Matsumoto, M; et al. (2005) Evaluation of developmental toxicity of 1-butanol given to rats in drinking water throughout pregnancy. Food Chem Toxicol 43:325–331.

Etique, N; Chardard, D; Chesnel, A; et al. (2004) Analysis of the effects of different alcohols on MCF-7 human breast cancer cells. Ann NY Acad Sci 1030:78–85.

Frantik, E; Hornychova, M; Horvath, M. (1994) Relative acute neurotoxicity of solvents: isoeffective air concentrations of 48 compounds evaluated in rats and mice. Environ Res 66(2):173–185.

Gadberry, MG; Carlson, GP. (1994) 2-Butanol metabolism by rat hepatic and pulmonary cytochromes P450. Toxicol Lett 74:203–209.

Gaillard, D; Derache, R. (1966) Action de quelques alcools aliphatiques sure la mobilisation de differentes fractions lipidiques chez la Rate. Food Cosmet Toxicol 4:515–520.

Gastaldi, G; Casirola, D; Ferrari, G; et al. (1991) The effect of ethanol and other alcohols on morphometric parameters of rat small intestinal microvillous vesicles. Eur J Basic Appl Histochem 35(2):185–193.

Giknis, MLA; Clifford, CB. (2008) Clinical laboratory parameters for Crl:WI(Han). Charles River, 1–14.

Godden, EL; Harris, RA; Dunwiddie, TV. (2001) Correlation between molecular volume and effects of n-alcohols on human neuronal nicotinic acetylcholine receptors expressed in Xenopus oocytes. J Pharmacol Exp Ther 296:716–722.

Gordon, EL; Nguyen, TS; Ngai, AC; et al. (1995) Differential effects of alcohols on intracerebral arterioles. Ethanol alone causes vasoconstriction. J Cereb Blood Flow Metab 15(3):532–538.

Grobin, AC; Matthews, DB; Devaud, LL; et al. (1998) The role of $GABA_A$ receptors in the acute and chronic effects of ethanol. Psychopharmacology 139:2–19.

Gupta U, Cook JC, Tassinari MS, Hurtt ME (2003). Comparison of developmental toxicology of aspirin (acetylsalicylic acid) in rats using selected dosing paradigms. Birth Defects Res B Dev Reprod Toxicol. 68:27-37.

Hansch, C; Leo, A; Hoekman, D. (1995) In: Heller, SR; ed. Exploring QSAR. Hydrophobic, electronic, and steric constants. Washington, DC: American Chemical Society; p. 10.

Harris, T; Graber, AR; Covarrubias, M. (2003) Allosteric modulation of a neuronal K+ channel by 1-alkanols is linked to key residue in the activation gate. J Cell Physiol 285:C788–C796.

Hempel-Jorgensen, A; Kjaergaard, SK; Molhave, L. (1998) Cytological changes and conjunctival hyperemia in relation to sensory eye irritation. Int Arch Occup Environ Health 71(4):225–235.

Hempel-Jorgensen, A; Hudnell, HK; Kjaergaard, SK; et al. (1999) Time course of sensory eye irritation in humans exposed to n-butanol and 1-octene. Arch Environ Health 54(2):86–94.

Hiller, JM; Angel, LM; Simon, EJ. (1984) Characterization of the selective inhibition of the delta subclass of opioid binding sites by alcohols. Mol Pharmacol 25(2):249–255.

Hines, LM; Stampfer, MJ; Ma, J; Gaziano, JM; Ridker, PM; Hankinson, SE; Sacks, F; Rimm, EB; Hunter, DJ (2001). Genetic variation in alcohol dehydrogenase and the beneficial effect of moderate alcohol consumption on myocardial infarction. N Engl J Med. 2001 Feb 22;344(8):549-55.

HSDB (Hazardous Substances Data Bank). (2009) N-Butyl alcohol. Hazardous Substances Data Bank. National Library of Medicine. Available online at http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~SoTFja:1 (accessed on August 16, 2009).

Jang, JY; Lee, SY; Kim, JI; et al. (1999) Application of biological monitoring to the quantitative exposure assessment for neuropsychological effect by chronic exposure to organic solvents. Int Arch Occup Environ Health 72(2):107–114.

Jenner, PM; Hagan, EC; Taylor, JM; et al. (1964) Food flavourings and compounds of related structure. I. Acute oral toxicity. Food Cosmet Toxicol 2:327–343.

Jung, R; Engelhart, G; Herbolt, B; et al. (1992) Collaborative study of mutagenicity with Salmonella typhimurium TA102. Mutat Res 278(4):265–270.

Kaneko, T; Wang, PY; Sato, A. (1994) Partition coefficients of some acetate esters and alcohols in water, blood, olive oil, and rat tissues. Occup Environ Med 51(1):68–72.

Kempton, MJ; Stahl, D; Williams, SCR; et al. (2010) Progressive lateral ventricular enlargement in schizophrenia: a meta-analysis of longitudinal MRI studies. Schizophr Res 120:54–62.

Kitagaki, H; Mori, E; Kazunari, I; et al. (1998) CSF spaces in idiopathic normal pressure hydrocephalus: morphology and volumetry. AJNR American Journal of Neuroradiology 19:1277–1284.

Kjaergaard, S; Molhave, L; Jorgensen, AH. (1997) Relation between local eye irritation testing and whole body irritation response using n-butanol as a model substance. U.S. Environmental Protection Agency, Office of Research and Development. EPA/600/A-97/014. PB193817.

Kolesnikov, PA. (1975) Adaptation to butyl alcohol. Gig I Sanit 5:104-105.

Korsak, Z; Rydzynski, K. (1994) Effects of acute combined inhalation exposure to n-butyl alcohol and n-butyl acetate in experimental animals. Int J Occup Environ Health 7(3):273–280.

Korsak, Z; Swiercz, R; Jedrychowski, R. (1993) Effects of acute combined exposure to n-butyl alcohol and m-xylene. Pol J Occup Med Environ Health 6(1):35–41.

Korsak, Z; Wisniewska-Knypl, J; Swiercz, R. (1994) Toxic effects of subchronic combined exposure to n-butyl alcohol and m-xylene in rats. Int J Occup Med Environ Health 7(2):155–166.

Kotter, K; Klein, J. (1999) Ethanol inhibits astroglial cell proliferation by disruption of phospholipase D-mediated signaling. J Neurochem 73(6):2517–2523.

Kotter, K; Jin, S; Klein, J. (2000) Inhibition of astroglial cell proliferation by alcohols: interference with the protein kinase C-phospholipase D signaling pathway. Int J Dev Neurosci 18(8):825–831.

Kowalczyk, CL; Stachecki, JJ; Schultz, JF; et al. (1996) Effects of alcohols on murine preimplantation development: relationship to relative membrane disordering potency. Alcohol Clin Exp Res 20(3):566–571.

Krill, SL; Knutson, K; Higuchi, WI. (1993) The influence of iso-propanol, n-propanol and n-butanol on stratum corneum lipid phase behavior. J Control Release 25:31–42.

Kristiansen, U; Vinggaard, AM; Nielsen, GD. (1988) The effects of n-butanol vapour on respiratory rate and tidal volume. Arch Toxicol 61:229–236.

Kulig, B; Alleva, E; Bignami, G; et al. (1996) Animal behavioral methods in neurotoxicity assessment: SGOMSEC Joint Report. Environ Health Perspect 104 (Suppl 2):193-204.

Lasne, C; Gu, ZW; Venegas, W; et al. (1984) The in vitro micronucleus assay for detection of cytogenetic effects induced by mutagen-carcinogens: Comparison with the in vitro sister-chromatid exchange assay. Mutat Res 130(4):273–282.

Lewis, RJ. (2000) Sax's dangerous properties of industrial materials. 10th ed. New York, NY: John Wiley & Sons, Inc.; p. 611.

Lide, DR; ed. (2008) CRC handbook of chemistry and physics. 88th ed. New York, NY: CRC Press; p. 3–74.

Lindahl, R (1977). Aldehyde dehydrogenase in 2-acetamidofluorene-induced rat hepatomas. Ontogeny and evidence that the new isoenzymes are not due to normal gene de-repression. Biochem J. 164(1):119-23.

Macht,DI. (1920) A toxicological study of some alcohols, with special reference to isomers. J Pharmacol Exp Ther 16:1-10.

Maickel, RP; McFadden, DP. (1979) Acute toxicology of butyl nitrites and butyl alcohols. Res Commun Chem Pathol Pharmacol 26:75–83.

Maickel, RP; Nash, JF. (1985) Differing effects of short-chain alcohols on body temperature and coordinated muscular activity in mice. Neuropharmacology 24(1):83–89.

McCann, J; Choi, E; Yamasaki, E; et al. (1975) Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Natl Acad Sci USA 72(12):5135–5139.

McCreery, MJ; Hunt, WA. (1978) Physico-chemical correlates of alcohol intoxication. Neuropharmacology 17:451–461.

McKarns, SC; Hansch, C; Caldwell, WS; et al. (1997) Correlation between hydrophobicity of short-chain aliphatic alcohols and their ability to alter plasma membrane integrity. Fundam Appl Toxicol 36:62–70.

McLain, VC. (2008) Final report of the addendum to the safety assessment of n-butyl alcohol as used in cosmetics. Int J Toxicol 27 (Suppl 2):53–69.

McOmie, WA; Anderson, HH. (1949) Comparative toxicologic effects of some isobutyl carbinols and ketones. Pharmacology 2:217–230.

MOE (Ministry of the Environment). (2007) Ontario Air Standards for n-butanol. Ontario's Ministry of the Environment. Available online at http://www.ene.gov.on.ca/envision/env_reg/er/documents/2007/PA05E0020-f.pdf (accessed April 22, 2009).

Mohler, FS; Gordon, CJ. (1991) Hypothermic effects of a homologous series of short-chain alcohols in rats. J Toxicol Environ Health 32(2):129–139.

Muller, W; Engelhart, G; Herbold, B; et al. (1993) Evaluation of mutagenicity testing with Salmonella typhimurium TA102 in three different laboratories. Environ Health Perspect 101(Suppl. 3):33–36.

Munch, JC. (1972) Aliphatic alcohols and alkyl esters: narcotic and lethal potencies to tadpoles and to rabbits. Ind Med 41:31–33.

Munch, JC; Schwartze, EW. (1925) Narcotic and toxic potency of aliphatic alcohols upon rabbits. J Lab Clin Med 10:985–996.

Munoz, R; Ferreras, JM; Iglesias, R; et al. (1990) Adaption of in vitro rat brain protein synthesis to long-term ingestion of n-butanol. Brain Res 517:330–332.

Munoz, R; Iglesias, R; Ferreras, JM; et al. (1991) Effect of long-term n-butanol ingestion on rat brain polypeptide synthesis directed by endogenous messengers. Cell Mol Biol 37(7):671–677.

Murata, K; Araki, S; Yokoyama, S; et al. (1991) Autonomic and peripheral nervous system dysfunction in workers exposed to mixed organic solvents. Int Arch Occup Environ Health 63(5):335–340.

Nakahiro, M; Arakawa, O; Narahashi, T. (1991) Modulation of gamma-aminobutyric acid receptor-channel complex by alcohols. J Pharmacol Exp Ther 259(1):235–240.

Nakamura, S; Oda, Y; Shimada, T; et al. (1987) SOS-inducing activity of chemical carcinogens and mutagens in Salmonella typhimurium TA1535/pSK1002: examination with 151 chemicals. Mutat Res 192:239–246.

Nelson, KW; Ege, JF; Ross, M; et al. (1943) Sensory response to certain industrial solvent vapors. J Ind Hyg Toxicol 25:282–285.

Nelson, BK; Brightwell, WS; Khan, A; et al. (1989a) Lack of selective developmental toxicity of three butanol isomers administered by inhalation to rats. Fundam Appl Toxicol 12:469–479.

Nelson, BK; Brightwell, WS; Robertson, SK; et al. (1989b) Behavioral teratology investigation of 1-butanol in rats. Neurotoxicol Teratol 11(3):313–315.

Nelson, BK; Brightwell, WS; Krieg, EFJ. (1990) Developmental toxicology of industrial alcohols: a summary of 13 alcohols administered by inhalation to rats. Toxicol Ind Health 6(3-4):373–387.

NRC (National Research Council). (1983) Risk assessment in the federal government: managing the process. Washington, DC: National Academy Press.

Obe, G; Ristow, H. (1977) Acetaldehyde, but not ethanol, induces sister chromatid exchanges in Chinese hamster cells in vitro. Mutat Res 56:211–213.

Obe, G; Ristow, HJ; Herha, J. (1977) Chromosomal damage by alcohol in vitro and in vivo. Adv Exp Med Biol 85A:47–70.

O'Neil, MJ; Heckelman, PE; Koch, CB; et al. (2006) The Merck index. 14th ed. Whitehouse Station, NJ: Merck & Co. Inc.; p. 253.

Onfelt, A. (1987) Spindle disturbances in mammalian cells. III. Toxicity, c-mitosis and aneuploidy with 22 different compounds. Specific and unspecific mechanisms. Mutat Res 182:135–154.

Palm, WM; Saczynski, JS; van der Grond, J; et al. (2009) Ventricular dilation. Association with gait and cognition. Ann Neurol 66:485–493.

Paola Mascia, M; Gong, DH; Eger, EI; et al. (2000) The anesthetic potency of propanol and butanol versus propanethiol and butanethiol in alpha1 wild type and alpha1(S267Q) glycine receptors. Anesth Analg 91:1289–1293.

Peoples, RW; Weight, FF. (1999) Differential alcohol modulation of GABAA and NMDA receptors. Neuroreport 10(1):97–101.

Podlekareva, D; Pan, Z; Kjaergaard, S; et al. (2002) Irritation of the human eye mucous membrane caused by airborne pollutants. Int Arch Occup Environ Health 75(5):359–364.

Poet, TS; Corley, RA; Woodstock, A. (2003a) Evaluation of the respiratory bioavailability and pharmacokinetics of a series of esters and alcohols in rats. Richland, WA: Batelle, Pacific Northwest National Laboratory.

Poet, TS; Creim, JA; Pierce, JT; et al. (2003b) Evaluation of the respiratory bioavailability and pharmacokinetics of butyl acetate using whole-body plethysmography and gas uptake inhalation. Richland, WA: Batelle, Pacific Northwest National Library.

Purchase, IFH. (1969) Studies in Kaffircorn malting and brewing. XXII. The acute toxicity of some fusel oils found in Bantu beer. S Afr Med J 43(25):795–798.

Raimondi AJ. (1994) A unifying theory for the definition and classification of hydrocephalus. Childs Nerv Syst 10:2–12.

Research Triangle Institute. (1985) Rat oral subchronic toxicity study. Submitted under TSCA Section 4; EPA Document No. 44-8630046; NTIS No. OTS0531032.

Rumyantsev, AP; Ostroumova, NA; Astapoval, SA; et al. (1976) Sanitary toxicological features of butyl alcohol under conditions of prolonged inhalation route entry. Gig Sanit 11:12–15. (Russian)

Rusch, D; Musset, B; Wulf, H; et al. (2007) Subunit-dependent modulation of the 5-hydroxytryptamine type 3 receptor open-close equilibrium by n-alcohols. J Pharmacol Exp Ther 321(3):1069–1074.

Saillenfait, AM; Gallissot, F; Sabate, JP; et al. (2007) Developmental toxic effects of ethylbenzene or toluene alone and in combination with butyl acetate in rats after inhalation exposure. J Appl Toxicol 27(1):32–42.

Sakata-Haga, H; Sawada, K; Ohnishi, T; et al. (2004) Hydrocephalus following prenatal exposure to ethanol. Acta Neuropathol (Berl) 108:393–398.

Scheuplein, RJ; Blank, IH. (1976) Mechanism of percutaneous absorption. IV. Penentration of nonelectrolytes (alcohols) from aqueous solutions and from pure liquids. J Invest Dermatol 60:286–296.

Seitz, B. (1972) Occurrence of serious vertigo after handling of butanol and isobutanol: three cases. Archives Mal Prof Med Trav Secur Soc 33:393–395. (French)

Shahidullah, M; Harris, T; Germann, MW; et al. (2003) Molecular features of an alcohol binding site in a neuronal potassium channel. Biochemistry 42:11243–11252.

Shehata, M; Saad, S. (1978) The effect of aliphatic alcohols on certain vitamins of the B-complex group in the liver of the rat. Pol J Pharmacol Pharm 30(1):35–38.

Sitarek, K; Berlinska, B; Baranski, B. (1994) Assessment of the effect of n-butanol given to female rats in drinking water on fertility and prenatal development of their offspring. Int J Occup Med Environ Health 7(4):365–370.

Smith, M; Hopkinson, DA; Harris, H (1971). Developmental changes and polymorphisms in human alcohol dehydrogenase. Ann Hum Genet 34:251-271.

Smyth, HF; Smyth, HFJ. (1928) Inhalation experiments with certain laquer solvents. J Ind Hyg 10:261–271.

Sterner, JH; Crouch, HC; Brockmyre, HF; et al. (1949) A ten-year study of butyl alcohol exposure. Am Ind Hyg Assoc Q 10(3):53–59.

Stevens, R; Rusch, D; Solt, K; et al. (2005) Modulation of human 5-hydroxytryptamine type 3AB receptors by volatile anesthetics and n-alcohols. J Pharmacol Exp Ther 314(1):338–345.

Strubelt, O; Zetler, G. (1980) Anti-inflammatory effect of ethanol and other alcohols on rat paw edema and pleurisy. Agents Actions 10(3):279–286.

Strubelt, O; Deters, M; Pentz, R; et al. (1999) The toxic and metabolic effects of 23 aliphatic alcohols in the isolated perfused rat live. Toxicol Sci 49:133–142.

Swiercz, R; Korsak, Z; Rydzynski, K. (1995) Kinetics of n-butyl alcohol and m-xylene in blood during single and combined inhalation exposure in rats. Int J Occup Med Environ Health 8(4):361–365.

Tabershaw, IR; Fahy, JP; Skinner, JB. (1944) Industrial exposure to butanol. J Ind Hyg Toxicol 26:328–330.

Teeguarden, JG; Deisinger, PJ; Poet, TS; et al. (2005) Derivation of a human equivalent concentration for n-butanol using a physiologically based pharmacokinetic model for n-butyl acetate and metabolites n-butanol and n-butyric acid. Toxicol Sci 85:429–446.

Teschke, R; Hasumura, Y; Lieber, CS. (1974) NADPH-dependent oxidation of methanol, ethanol, propanol and butanol by hepatic microsomes. Biochem Biophys Res Commun 60(2):851–857.

Tewari, YB; Miller, MM; Wasik, SP; et al. (1982) Aqueous solubility and octanol/water partition coefficient of organic compounds at 25.0C. J Chem Eng Data 27:451–454.

Tichy, M; Trcka, V; Roth, Z; et al. (1985) QSAR analysis and data extrapolation among mammals in a series of aliphatic alcohols. Environ Health Perspect 61:321–328.

Timms, GP; Holmes, RS. (1981) Genetics and ontogeny of aldehyde dehydrogenase isozymes in the mouse: evidence for a locus controlling the inducibility of the liver microsomal isozyme. Biochem Genet 19(11-12): 1223-36.

Triebig, G; Schaller, KH; Weltle, D. (1992) Neurotoxicity of solvent mixtures in spray painters. Int Arch Occup Environ Health 64(5):353–359.

Tucek, M; Tenglerova, M; Kvasnickova, M; et al. (2002) Effect of acrylate chemistry on human health. Int Arch Occup Environ Health 75(Suppl 1):S67–S72.

U.S. EPA (Environmental Protection Agency). (1986a) Guidelines for the health risk assessment of chemical mixtures. Federal Register 51(185):34014–34025. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1986b) Guidelines for mutagenicity risk assessment. Federal Register 51(185):34006–34012. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1988) Recommendations for and documentation of biological values for use in risk assessment. Prepared by the Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC; EPA 600/6-87/008. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1991) Guidelines for developmental toxicity risk assessment. Federal Register 56(234):63798-63826. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1994a) Interim policy for particle size and limit concentration issues in inhalation toxicity studies. Federal Register 59(206):53799. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1994b) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. Office of Research and Development, Washington, DC; EPA/600/8-90/066F. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1995) Use of the benchmark dose approach in health risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/R-94/007. Available online at http://cfpub.epa.gov/ncea/raf/recordisplay.cfm?deid=42601 (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1996) Guidelines for reproductive toxicity risk assessment. Federal Register 61(212):56274–56322. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (1998) Guidelines for neurotoxicity risk assessment. Federal Register 63(93):26926–26954. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2000a) Science policy council handbook: risk characterization. Office of Science Policy, Office of Research and Development, Washington, DC; EPA 100-B-00-002. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2000b) Benchmark dose technical guidance document. External review draft. Risk Assessment Forum, Washington, DC; EPA/630/R-00/001. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2000c) Supplementary guidance for conducting health risk assessment of chemical mixtures. Risk Assessment Forum, Washington, DC; EPA/630/R-00/002. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2002) A review of the reference dose and reference concentration processes. Risk Assessment Forum, Washington, DC; EPA/630/P-02/0002F. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2005a) Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/P-03/001B. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2005b) Supplemental guidance for assessing susceptibility from early-life exposure to carcinogens. Risk Assessment Forum, Washington, DC; EPA/630/R-03/003F. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2006a) Science policy council handbook: peer review. Third edition. Office of Science Policy, Office of Research and Development, Washington, DC; EPA/100/B-06/002. Available online at http://www.epa.gov/iris/backgr-d.htm (accessed January 15, 2009).

U.S. EPA (Environmental Protection Agency). (2006b) A Framework for Assessing Health Risk of Environmental Exposures to Children. National Center for Environmental Assessment, Washington, DC, EPA/600/R-05/093F. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=158363 (accessed January 15, 2009).

Velazquez, J; Escobar, R; Almaraz, A. (1969) Audiologic impairment due to n-butyl alcohol exposition. In: Proceedings of the XVI International Congress on Occupational Health, Tokyo, Japan, 22-27 September 1969 Japan Industrial Safety Association.

Videla, LA; Fernandez, V; de Marinis, A. (1982) Liver lipoperoxidative pressure and glutathione status following acetaldehyde and aliphatic alcohols pretreatments in the rat. Biochem Biophys Res Commun 104(3):965–970.

Wakabayashi, T; Adachi, K; Popinigis, J. (1991) Effects of alkyl alcohols and related chemicals on rat liver structure and function: I. Induction of two distinct types of megamitochondria. Acta Pathol Jpn 41(6):405–413.

Wallgren, H. (1960) Relative intoxicating effects on rats of ethyl, propyl and butyl alcohols. Acta Pharmacol Toxicol (Copenh) 16:217–222.

Weese, H. (1928) Comparative studies on the efficacy and toxicity of the vapours of low aliphatic alcohols. Naunyn-Schmiedebergs Arch Exp Pathol Pharmakol 135:118–130. (German)

Wieichert, J; Hartge, D; Knapp, M; et al. (2010) Prevalence, characteristics and perinatal outcome of fetal ventriculomegaly in 29,000 pregnancies followed at a single institution. Fetal Diagn Ther 27:142–148.

WHO (World Health Organization). (1987) Environmental health criteria 65. Butanols: Four isomers. United Nations Environment Programme. International Labour Organisation. World Health Organization. Available online at http://www.inchem.org/documents/ehc/ehc/ehc65.htm (accessed August 16, 2009).

Winer, AD. (1958) A note on the substrate specificity of horse liver alcohol dehydrogenase. Acta Chem Scand 12:1695–1696.

Wolford, ST; Schroer, RA; Gohs, FX; et al. (1986) Reference range data base for serum chemistry and hematology values in laboratory animals. J Toxicol Environ Health 18:161–188.

Ye, Q; Koltchine, V; Mihic, SJ; et al. (1998) Enhancement of glycine receptor function by ethanol is inversely correlated with molecular volume at position alpha267. J Biol Chem 273(6):3314–3319.

Yoshiyama, Y; Nagai, K; Some, H; et al. (1973) Selective inhibition by pantoyl lactone and butyl alcohol of the initiation of DNA replication in E. coli. Agric Biol Chem 37:1317–1320.

Zhou, Q; Verdoorn, TA; Lovinger, DM. (1998) Alcohols potentiate the function of 5-HT3 receptor-channels on NCB-20 neuroblastoma cells by favouring and stabilizing the open channel state. J Physiol 507(2):335–352.

Zuo, Y; Aistrup, GL; Marszalec, W; et al. (2001) Dual action of n-alcohols on neuronal nicrotinic acetylcholine receptors. Mol Pharmacol 60(4):700–711.

Zuo, Y; Yeh, JZ; Narahashi, T. (2003) Dual action of n-butanol on neuronal nicotinic α4β2 acetylcholine receptors. J Pharmacol Exp Ther 304(3):1143–1152.

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

(page intentionally left blank)

APPENDIX B. BENCHMARK DOSE CALCULATIONS

In the study by Sitarek et al. (1994), a LOAEL of 300 mg/kg-day was identified based on increased incidences of developmental visceral malformations (dilation of the lateral and/or third ventricle, subarachnoid spaces of the brain and dilation of renal pelvis) in the offspring of treated female rats. The teratogenic effects were not amenable to modeling with nested developmental toxicity dose-response models, as these models require knowledge of the litters to which individual animals belonged, and such data were not reported by Sitarek et al. (1994). However, the percentages of affected litters were reported in the study; these data (see Table B-1 below) were subjected to BMD modeling to identify possible PODs for RfD derivation.

Table B-1. Incidences of rat litters with visceral malformations

Incidence of affected litters	n-Butanol dose in mg/kg-d			
	0	**300**	**1,000**	**5,000**
Litters with visceral malformations (dilation) – overall	1/12 (8%)	9/14 (64%)	10/12 (83%)	9/9 (100%)
Dilation of subarachnoid space	0/12 (0%)	2/14 (14%)	3/12 (25%)	7/9 (78%)
Dilation of lateral ventricle and/or third ventricle of the brain	1/12 (8%)	8/14 (57%)	8/12 (67%)	7/9 (78%)
Dilation of unilateral renal pelvis	0/12 (0%)	0/14 (0%)	5/12 (42%)	0/9 (0%)
Dilation of bilateral renal pelvis	0/12 (0%)	0/14 (0%)	3/12 (25%)	0/9 (0%)

Source: Sitarek et al. (1994).

All available dichotomous models in the EPA BMDS (version 2.1.1) were fit to the litter data with overall observed visceral malformations. This data included a combined incidence of dilation amongst four measured parameters: subarachnoid space, lateral and/or third ventricle, unilateral renal pelvis, and bilateral renal pelvis. A BMR of 10% was selected, and the BMD and BMDL associated with this BMR were estimated using the best-fit model for each dataset. Detailed modeling results are shown in Table B-2. Figure B-1 shows the best-fitting models for this endpoint .

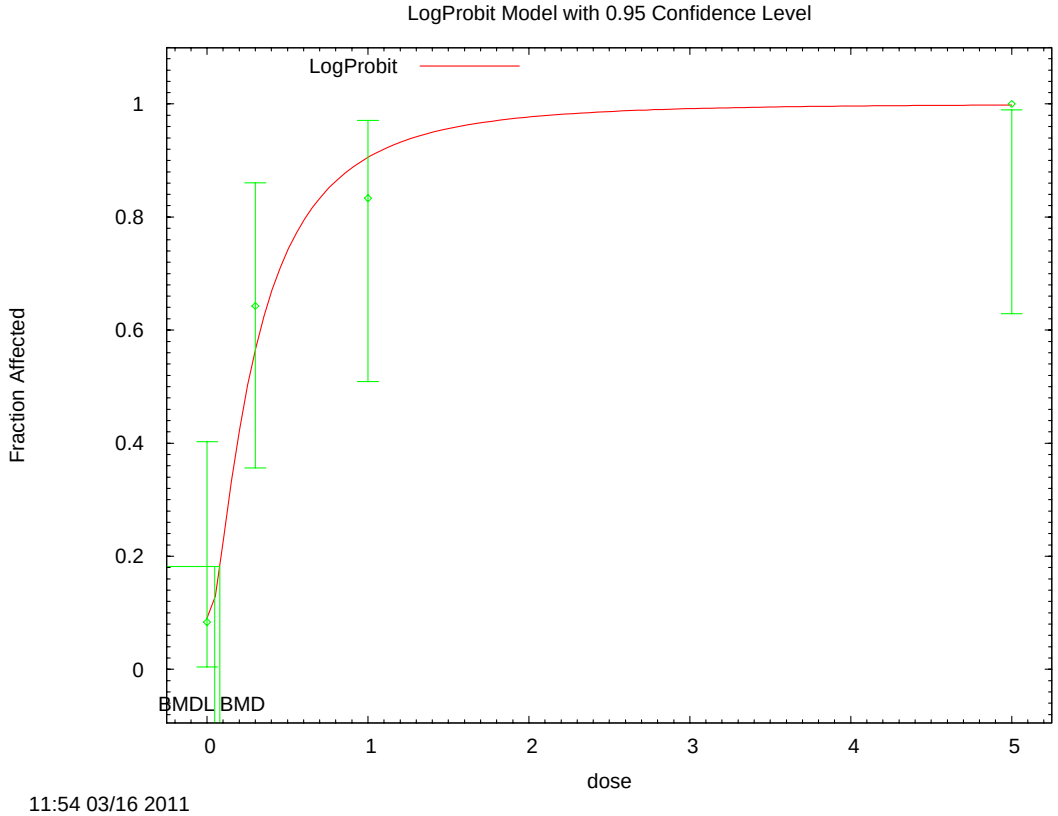
Of the models exhibiting adequate fit, a "best-fit" model was selected consistent with the EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000b) as follows. If the BMDL estimates from the models exhibiting adequate fit are "sufficiently close," then the model with the lowest AIC is used to estimate the BMDL from which the POD will be derived. Under the U.S. EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2000), the log-probit model results using the full dataset provide the best fit to the data and was the $BMDL_{10}$ from this model was used as the POD for RfD derivation.

Table B-2. BMD modeling results for the incidence of litters with visceral malformations (overall) after maternal exposure to n-butanol in drinking water.

Model	DF	χ^2	χ^2 Goodness of fit *p*-value[a]	Scaled residual of interest[b]	AIC	BMD_{10} (mg/kg-d)	$BMDL_{10}$ (mg/kg-d)
Gamma[c]	2	1.28	0.53	-0.20	41.16	48	31
Logistic	2	4.04	0.13	-1.24	44.28	115	74
Log-Logistic[d,e]	1	0.37	0.54	-0.01	42.49	36	10
Log-Probit[d]	**2**	**1.10**	**0.58**	**-0.09**	**40.94**	**79**	**48**
Multistage (1degree)[f]	2	1.28	0.53	-0.20	41.16	48	31
Probit	2	4.15	0.13	-1.24	44.42	118	81
Weibull[c]	2	1.28	0.53	-0.20	41.16	48	31

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Scaled residual at measured response closest to the BMR.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Best-fitting model; the only model with adequate fit.
[f]Betas restricted to ≥0.
Source: Sitarek et al. (1994).

Figure B-1. Fit of log-probit model to data on incidence of litters with visceral malformations (overall).



```
==========================================================================
         Probit Model. (Version: 3.1;  Date: 05/16/2008)
         Input Data File: C:\Usepa\BMDS21\Data\lnpSitarekSetting.(d)
         Gnuplot Plotting File:  C:\Usepa\BMDS21\Data\lnpSitarekSetting.plt
                                              Wed Mar 16 12:54:08 2011
 ==========================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = Background
               + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                  Default Initial (and Specified) Parameter Values
                     background =    0.0833333
                      intercept =     0.408347
                          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       0.0908047       0.0863715         -0.0784803            0.26009
      intercept         1.25846        0.309188           0.652459            1.86445
          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	-17.9734	4			
Fitted model	-18.4722	2	0.997729	2	0.6072
Reduced model	-31.2787	1	26.6106	3	<.0001

AIC: 40.9444

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0908	1.090	1.000	12	-0.090
0.3000	0.5652	7.912	9.000	14	0.586
1.0000	0.9053	10.864	10.000	12	-0.852
5.0000	0.9981	8.983	9.000	9	0.130

Chi^2 = 1.10 d.f. = 2 P-value = 0.5784

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 0.0788658

BMDL = 0.047882

APPENDIX C. n-BUTANOL PBPK MODEL

C.1. Model Code

```
PROGRAM BUTYL.CSL -- PBPK Model for Butyl Acetate and Its Butyl-Series Metabolites
! Modified version of BANew8I.csl
! Output units for this model are umoles and mL (or grams)

! Modified from BANew6.CSL -- November, 2001
! Last Modified:  Oct. 6, 1998
! Modified by:  Hugh A. Barton

! This version runs in kg as modified by Justin Teeguarden 6.9.99
! Validated 6.9.99 by Justin Teeguarden

! Modified 7.11.99 by Justin Teeguarden (of BANEW2) to include metabolism of
!   EtAcin other perfused tissues.  AUC of EtAc under Deisiv2 for this version
!   matches BANEW2.CSL with Metabolism in Other Perfused Tissues=0.

! Modified 6.20.00 by JGT changing the CEx1, CEX2, CEX3, CEX4 to use Free blood
!   concentration instead of lung concentration.

! Revised November 2001, Justin Teeguarden.  Removed URT for butyl acetate.
!   Added closed chamber for BuOH and FA (fractional uptake term) for Butyl
!   Acetate.  These changes were made to model the butyl acetate closed chamber
!   data from Batelle, Poet and Corely 2001.  Mass balance OK.  Without the URT,
!   the inhaled amount for the GrothInh2 simulation using *8.csl is the same as
!   *5.csl (the minor difference is due to an effective tstop which is a bit
!   different.)  This served as the validation for the removal of the URT.

! Alveolar Ventilation Rate Tables 1-7 QA'd 12.19.01 JGT

! NOTE:  All units are expressed as mL(g), hr, or umol in the derivative section

! This version has inhalation, IV and oral dosing.
! It uses a table command for the inhalation dose using the values from the
!   study in the Groth paper.

! Parameters followed by 1,2,3,4 stand for:
!   Submodel 1:  n-Butyl acetate
!   Submodel 2:  n-Butanol
!   Submodel 3:  n-Butyraldehyde
!   Submodel 4:  n-Butyric acid

!*************************************************************************
!Revised and imported into acslXtreme v 2.5.0.6 as "BUTYLAC.csl" on 04/23/2010 (G Diamond/SRC)
!Revisions are marked with "!" and "<GD>
!  Handling of exposure/dose parameters standardized to: inputs ppm/mg/kg --> convert to umol/L
or umol/kg -->
! inputs ppm/mg/kg --> convert to umol/L or umol/kg --> adjust for dose duration (e.g., < 1hr)
! --> apply timing pulse functions (i.e., on/off) --> Integration functions
!  CINT revised from function of CintC and TChng to a CONSTANT
!  TChng revised to route specific dose averaging time CONSTANTs (e.g. IVDAT, ORDAT)
!*************************************************************************
!  Modified on 02/25/2011 by AFS to create “clean” version:
!  Legacy code, and commented-out lines of code have been removed.  Some formatting changes
!  made to ensure proper word-wrap in documents.  Code was tested to ensure it properly ran
!*************************************************************************
```

```
1
2   INITIAL
3                                !<GD>
4          INTEGER   Rats, QPTable, Tube, INHTABLE  !<GD>
5     CONSTANT          BW = 0.30     ! Body weight (kg)
6     CONSTANT         QPC = 14000.0  ! Alveolar ventilation (mL/hr/kg**0.75)
7
8   ! Blood Flows (fraction of cardiac output)
9     CONSTANT         QCC = 14000.0  ! Cardiac output (mL/hr/kg**0.75)
10    CONSTANT       QFatC = 0.07     ! Fat
11    CONSTANT       QLivC = 0.175    ! Liver
12
13  ! Tissue Volumes (fraction of body weight)
14    CONSTANT        VABC = 0.022    ! Arterial blood
15    CONSTANT       VFatC = 0.07     ! Fat
16    CONSTANT       VLivC = 0.037    ! Liver
17    CONSTANT      VLungC = 0.005    ! Lung tissue
18    CONSTANT       VOthC = 0.751    ! Other perfused tissues
19    CONSTANT        VVBC = 0.045    ! Venous blood
20
21  ! Partition Coefficients
22  !  Butyl Acetate
23      CONSTANT       PB1 = 89.4     ! Blood:air
24      CONSTANT     PFat1 = 17.0     ! Fat:blood
25      CONSTANT     PLiv1 = 3.14     ! Liver:blood
26      CONSTANT    PLung1 = 1.76     ! Lung:blood
27      CONSTANT     POth1 = 1.76     ! Other perfused tissues:blood
28
29  !  n-Butanol
30      CONSTANT       PB2 = 1160.0   ! Blood:air
31      CONSTANT     PLiv2 = 1.08     ! Liver:blood
32      CONSTANT    PLung2 = 0.78     ! Lung:blood
33      CONSTANT     POth2 = 0.78     ! Other perfused tissues:blood
34
35  !  n-Butyraldehyde
36      CONSTANT       PB3 = 1160.0   ! Blood:air
37      CONSTANT     PLiv3 = 1.08     ! Liver:blood
38      CONSTANT    PLung3 = 0.78     ! Lung:blood
39      CONSTANT     POth3 = 0.78     ! Other perfused tissues:blood
40
41  !  Butyric Acid
42      CONSTANT       PB4 = 1160.0   ! Blood:air
43      CONSTANT     PLiv4 = 1.08     ! Liver:blood
44      CONSTANT    PLung4 = 0.78     ! Lung:blood
45      CONSTANT     POth4 = 0.78     ! Other perfused tissues:blood
46
47  ! Metabolism
48  !  Butyl Acetate
49      CONSTANT VMBloodC1 = 600000.0 ! Blood (umole/hr/kg**0.75)
50      CONSTANT  KMBlood1 = 100.0    ! Blood (umole/mL)
51      CONSTANT   VMLivC1 = 38700.0  ! Liver (umole/hr/kg**0.75)
52      CONSTANT    KMLiv1 = 1.0      ! Liver (umole/mL)
53      CONSTANT   VMOthC1 = 6.0e6    ! Other Perfused Tissues (umole/hr/kg**0.75)
54      CONSTANT    KMOth1 = 100.0    ! Other Perfused Tissues (umole/mL)
55
56  !  n-Butanol
57      CONSTANT   VMLivC2 = 2170.0   ! Liver (umole/hr/kg**0.75) <GD>
58      CONSTANT    KMLiv2 = 0.16      ! Liver (umole/mL) <GD>
```

```
      CONSTANT    kOthC2 = 4.0      ! First-order BuOH clearance (kg**0.25/hr) <GD>

!  n-Butyraldehyde
      CONSTANT   VMLivC3 = 17780.0  ! Liver (umole/hr/kg**0.75)
      CONSTANT    KMLiv3 = 0.1      ! Liver (umole/mL)

!  Butyric Acid
      CONSTANT   VMLivC4 = 1400.0   ! Liver (umole/hr/kg**0.75)
      CONSTANT    KMLiv4 = 0.1      ! Liver (umole/mL)
      CONSTANT   VMOthC4 = 3000.0   ! OPT (umole/hr/kg**0.75)
      CONSTANT    KMOth4 = 0.1      ! OPT (umole/mL)

! Filtration (blood-urine) from Venous Blood ((kg**0.25)/hr)
  CONSTANT     kFiltC1 = 7.401e-2 ! Butyl Acetate
  CONSTANT     kFiltC2 = 22.2     ! n-Butanol
  CONSTANT     kFiltC4 = 7.41e-2  ! Butyric Acid

! Fractional Inhalation Absorption - Butyl Acetate, Butanol
  CONSTANT         FA1 = 1.0
  CONSTANT         FA2 = 0.5
  CONSTANT         FA3 = 1.0
  CONSTANT         FA4 = 1.0

! Oral Absorption
  CONSTANT         kAC = 0.0      ! Absorption rate (kg**0.25/hr)

! Initial Amounts in Arterial Blood (i.e., integration start value)
  CONSTANT      AAB10C = 0.0      ! BuAc (mg/kg)
  CONSTANT      AAB20C = 0.0      ! BuOH (mg/kg)
  CONSTANT      AAB30C = 0.0      ! BuCHO (mg/kg)
  CONSTANT      AAB40C = 0.0      ! BuCOOH (mg/kg)

! Molecular Weights <GD>
        CONSTANT                              MW1 = 116.16         ! BuAc
        CONSTANT                              MW2 = 74.12           ! BuOH
        CONSTANT                              MW3 = 72.11           ! BuCHO
        CONSTANT                              MW4 = 88.11           ! BuCOOH

! Simulation Parameters
  CONSTANT       TStop = 0.5      ! Length of experiment (hr)
  CINTERVAL CINT = 1.                         !Communication interval (hr)
        !CINT = CIntC <GD>

! Intravenous Dosing Parameters
  CONSTANT     IVDose1 = 0.0      ! BuAc (mg/kg)
  CONSTANT     IVDose2 = 0.0      ! BuOH (mg/kg)
  CONSTANT     IVDose3 = 0.0      ! BuCHO (mg/kg)
  CONSTANT     IVDose4 = 0.0      ! BuCOOH (mg/kg)

        IVDUM1= IVDose1 * 1000 * BW/MW1   !BuAc (umol)  <GD>
        IVDUM2= IVDose2 * 1000 * BW/MW2   !BuOH  (umol) <GD>
        IVDUM3= IVDose3 * 1000 * BW/MW3   !BuCHO (umol) <GD>
        IVDUM4= IVDose4 * 1000 * BW/MW4   !BuCOOH (umol <GD>

CONSTANT IVDAT=1.  !IV injection averaging time (hr; for infusion, IVDAT = 1.) <GD>
CONSTANT IVDON=0.  !IV start time (hr) <GD>
CONSTANT IVDOFF=0.   !IV stop time (for single injection, IVDOFF must = IVDAT) <GD>
```

```
! Oral Dosing Parameters
  CONSTANT      PDose1 = 0.0      ! BuAc (mg/kg)
  CONSTANT      PDose2 = 0.0      ! BuOH (mg/kg)
  CONSTANT      PDose3 = 0.0      ! BuCOO (mg/kg)
  CONSTANT      PDose4 = 0.0      ! BuCOOH (mg/kg)

        ORDUM1 = PDose1 * 1000 * BW/MW1   !BuAc (umol)   <GD>
        ORDUM2 = PDose2 * 1000 * BW/MW2   !BuOH (umol)       <GD>
        ORDUM3 = PDose3 * 1000 * BW/MW3   !BuCOO (umol)     <GD>
        ORDUM4 = PDose4 * 1000 * BW/MW4   !BuCOOH (umol) <GD>

        CONSTANT ORDON=0.      !Exposure start (hr) <GD>
        CONSTANT ORDOFF=0.     !Exposure stop (hr) <GD>
        CONSTANT ORDPER1=20000.!Exposure pulse period (e.g. 24-hr day) <GD>
        CONSTANT ORDWID1=20000.!Exposure pulse 1 width (e.g. hr/day)) <GD>
        CONSTANT ORDPER2=20000.!Exposure pulse period (e.g. 168-hr wk) <GD>
        CONSTANT ORDWID2=20000.!Exposure pulse 1 width (120 hr/wk) <GD>
        CONSTANT ORDAT=1.0     !Dosing averaging time for single oral dose (e.g. gavage) <GD>

! Inhalation Dosing Paramters
  CONSTANT     INHTABLE = 0  ! Switch to use inhlation exposures from INHTABLE (=1)

  CONSTANT       CInh1 = 0.0      ! BuAc (ppm)
  CONSTANT       CInh2 = 0.0      ! BuOH (ppm)
  CONSTANT       CInh3 = 0.0      ! BuCOO (ppm)
  CONSTANT       CInh4 = 0.0      ! BuCOOH (ppm)
  CONSTANT     QPTable = 0                  ! Set to .T. for using observed MV data <GD>

CIUM1= CInh1/24450  !BuAc (umol/ml) <GD>
CIUM2= CInh2/24450  !BuOH (umol/ml) <GD>
CIUM3= CInh3/24450  !BuCOO (umol/ml) <GD>
CIUM4= CInh4/24450  !BuCOOH (umol/ml) <GD>

CONSTANT CIOFFPPM1=0.  !BuAc conc.  when inhalation exposure is off (ppm) <GD>
CONSTANT CIOFFPPM2=0.  !BuOH conc. when inhalation exposure is off (ppm) <GD>
CONSTANT CIOFFPPM3=0.  !BuCOO conc. when inhalation exposure is off (ppm) <GD>
CONSTANT CIOFFPPM4=0.  !BuCOOH conc.  when inhalation exposure is off (ppm) <GD>

CIOFF1= CIOFFPPM1/24450   !BuAc conc.  when inhalation exposure is off (umol/ml) <GD>
CIOFF2= CIOFFPPM2/24450   !BuOH conc.  when inhalation exposure is off (umol/ml) <GD>
CIOFF3= CIOFFPPM3/24450   !BuCOO conc. when inhalation exposure is off (umol/ml) <GD>
CIOFF4= CIOFFPPM4/24450   !BuCOOH conc. when inhalation exposure is off (umol/ml) <GD>

CONSTANT INHON=0.                    !Time inhalation exposure starts (hr) <GD>
CONSTANT INHOFF= 10.     !Time exposure stops (hr) <GD>
CONSTANT INHPER1=20000.  !Pulse period 1 for exposure (e.g., 24-hr day) <GD>
CONSTANT INHWID1=20000.  !Pulse width 1 (e.g. 6 hr/day) <GD>
CONSTANT INHPER2=20000.  !Pulse period 2 (e.g. 168-hr wk) <GD>
CONSTANT INHWID2=20000.  !Pulse width 2 (e.g.  120 hr/wk) <GD>

TABLE CINHTAB1,1,200/200*0.0,200*0.0/ !Call VentTableS2 from CINHTAB*.dat file <GD>
TABLE CINHTAB2,1,200/200*0.0,200*0.0/ !Call VentTableS2 from CINHTAB*.dat file <GD>
TABLE CINHTAB3,1,200/200*0.0,200*0.0/ !Call VentTableS2 from CINHTAB*.dat file <GD>
TABLE CINHTAB4,1,200/200*0.0,200*0.0/ !Call VentTableS2 from CINHTAB*.dat file <GD>
!Deleted Table values in CSL and replaced with m-files that fill tables <GD>
```

```
! Inhalation Dose for Closed Chamber
  CONSTANT    SCInPPM1 = 0.0      ! BuAc (ppm)
  CONSTANT    SCInPPM2 = 0.0      ! BuOH (ppm)
  CONSTANT    SCInPPM3 = 0.0      ! BuCHO (ppm)
  CONSTANT    SCInPPM4 = 0.0      ! BuCOOH (ppm)

       CICHUM1= SCInPPM1/24450  !BuAc (umol/ml) <GD>
       CICHUM2= SCInPPM2/24450  !BuOH (umol/ml) <GD>
       CICHUM3= SCInPPM3/24450  !BuCOO (umol/ml) <GD>
       CICHUM4= SCInPPM4/24450  !BuCOOH (umol/ml) <GD>

       ACh01 = 0.0  ;  ACh02 = 0.0  ;  ACh03 = 0.0  ;  ACh04 = 0.0 !<GD>
       ACh01 = VCh*CICHUM1 !BuAc (umol) <GD>
       ACh02 = VCh*CICHUM2    !BuOH (umol) <GD>
       ACh03 = VCh*CICHUM3    !BuCOO (umol) <GD>
       ACh04 = VCh*CICHUM4    !BuCOOH (umol) <GD>

  TABLE VentTable,1,200/200*0.0,200*0.0/     !Call VentTable from *.dat file <GD>
       !Deleted Table values <GD>

! Closed Chamber Parameters
! If a Plethysmorgraph tube is used, the given volume of the chamber should be
!  used directly without subtraction of the volume of the rat (number of rats x BW).
!  Set Tube=.T.
  CONSTANT        ClOn = 0.0         !Switch for closed chamber (=1), or open (=0)
  CONSTANT        VChC = 1.0e10   ! Volume of closed chamber (L)
  CONSTANT        Rats = 0                  ! Number of rats in chamber
  CONSTANT        Tube = 0                  !Switch to include Tube (=1)
  CONSTANT       LossT = 0.0      ! Time to switch to second chamber loss term

! Closed Chamber Parameters -- Loss Rates (/hr)
  CONSTANT     kLoss1R = 0.0      ! Rat body for n-Butyl acetate
  CONSTANT    kLoss1C1 = 0.0      ! Chamber for n-Butyl acetate
  CONSTANT    kLoss1C2 = 0.0      ! Chamber for n-Butyl acetate for 2nd period
  CONSTANT     kLoss2R = 0.0      ! Rat body for n-Butanol
  CONSTANT    kLoss2C1 = 0.0      ! Chamber for n-Butanol
  CONSTANT    kLoss2C2 = 0.0      ! Chamber for n-Butanol for 2nd period
  CONSTANT     kLoss3R = 0.0      ! Rat body for n-Butyraldehyde
  CONSTANT      kLoss3 = 0.0      ! Chamber for n-Butyraldehyde
  CONSTANT     kLoss4R = 0.0      ! Rat body for n-Butyric acid
  CONSTANT      kLoss4 = 0.0      ! Chamber for n-Butyric acid

! Blood Flows (mL/hr)
      QOthC = 1.0 - QFatC - QLivC       ! Other perfused tissues (fraction)

! Tissue Volumes (mL)
       VAB = VABC * BW * 1000.0        ! Arterial blood volume
      VFat = VFatC * BW * 1000.0       ! Fat
      VLiv = VLivC * BW * 1000.0       ! Liver
     VLung = VLungC * BW * 1000.0      ! Lung
      VOth = VOthC * BW * 1000.0       ! Other perfused tissues
       VVB = VVBC * BW * 1000.0        ! Venous blood
    VBlood = VAB + VVB                 ! Total blood
! Metabolism -- Butyl Acetate (umoles/hr)
     VMAB1 = VMBloodC1 * VAB/VBlood * (BW**0.75)      ! Arterial metabolism BuAc
     VMVB1 = VMBloodC1 * VVB/VBlood * (BW**0.75)      ! Venous metabolism BuAc
    VMLiv1 = VMLivC1 * (BW**0.75)                     ! Liver metabolism BuAc
           VMOth1 = VMOthC1 * (BW**0.75)      ! Other Perfused Tissue Metabolism EtAc

```

```
! Metabolism -- n-Butanol
    VMLiv2 = VMLivC2 * (BW**0.75)      ! Liver metabolism BuOH (umoles/hr)
     kOth2 = kOthC2 / (BW**0.25)       ! Other metabolism BuOH (/hr)

! Metabolism -- n-Butyraldehyde (umoles/hr)
    VMLiv3 = VMLivC3 * (BW**0.75)      ! Liver metabolism BuCHO

! Metabolism -- Butyric Acid (umoles/hr)
    VMLiv4 = VMLivC4 * (BW**0.75)      ! Liver metabolism BuCOOH
    VMOth4 = VMOthC4 * (BW**0.75)      ! OPT metabolism BuCOOH

! Filtration from Venous Blood (/hr)
    kFilt1 = kFiltC1 / (BW**0.25)      ! Kidney Filtration of BuAc
    kFilt2 = kFiltC2 / (BW**0.25)      ! Kidney Filtration of BuOH
    kFilt4 = kFiltC4 / (BW**0.25)      ! Kidney Filtration of BuCOOH

! Oral Absorption (/hr)
        kA = kAC / (BW**0.25)

! Initial Amounts in Arterial Blood (i.e. integration start value)
     AAB10 = ((AAB10C * 1000.0)/MW1) * BW     ! BuAc (umoles total)
     AAB20 = ((AAB20C * 1000.0)/MW2) * BW     ! BuOH (umoles total)
     AAB30 = ((AAB30C * 1000.0)/MW3) * BW     ! BuCHO (umoles total)
     AAB40 = ((AAB40C * 1000.0)/MW4) * BW     ! BuCOOH (umoles total)

! Initialize values
     ! CINT = CIntC
   DoseMod = 0.0
      kIV1 = 0.0  ;       kIV2 = 0.0  ;       kIV3 = 0.0  ;       kIV4 = 0.0
     Conc1 = 0.0  ;      Conc2 = 0.0  ;      Conc3 = 0.0  ;      Conc4 = 0.0
     Dose1 = 0.0  ;      Dose2 = 0.0  ;      Dose3 = 0.0  ;      Dose4 = 0.0
    XCInh1 = 0.0  ;     XCInh2 = 0.0  ;     XCInh3 = 0.0  ;     XCInh4 = 0.0
XSCInPPM1 = 0.0  ;  XSCInPPM2 = 0.0  ;  XSCInPPM3 = 0.0  ;  XSCInPPM4 = 0.0
  XPDose1 = 0.0  ;    XPDose2 = 0.0  ;    XPDose3 = 0.0  ;    XPDose4 = 0.0
  XAUCTB1 = 0.0  ;    XAUCTB2 = 0.0  ;    XAUCTB3 = 0.0  ;    XAUCTB4 = 0.0
   CVLiv1 = 0.0  ;     CVLiv2 = 0.0  ;     CVLiv3 = 0.0  ;     CVLiv4 = 0.0

     CCh1 = 0.0  ;   CCh2 = 0.0  ;   CCh3 = 0.0  ;   CCh4 = 0.0

! Closed Chamber Parameters
  IF (Tube .EQ. 0) THEN              !<GD>
      VCh = VChC*1000.0 - Rats*BW*1000.0         ! Volume adjusted to mL
  ELSE
      VCh = VChC*1000.0
  ENDIF

END     ! End of Initial

DYNAMIC
  ALGORITHM IALG = 2   ! Gear algorithm

DERIVATIVE
   Minutes = T * 60.0
   Seconds = Minutes * 60.0
```

```
PROCEDURAL  !<GD>
  IF (QPTable .EQ. 0) THEN
      QP = QPC*(BW**0.75)
      QC = QCC*(BW**0.75)
         ELSE
      QP = VentTable(Minutes)
      QC = QP
  ENDIF
  QFat = QFatC * QC     ! Fat
  QLiv = QLivC * QC     ! Liver
  QOth = QOthC * QC     ! Other perfused tissues
END

!IV Dosing Simulation

       IV1=IVDUM1/IVDAT  !BuAc (umol/hr) <GD>
       IV2=IVDUM2/IVDAT  !BuOH (umol/hr) <GD>
       IV3=IVDUM3/IVDAT  !BuCOO (umol/hr) <GD>
       IV4=IVDUM4/IVDAT  !BuCOOH (umol/hr) <GD>

       kIV1= RSW(T.LE.IVDOFF,IV1,0.)  !BuAC (umol/hr on/off) <GD>
       kIV2= RSW(T.LE.IVDOFF,IV2,0.)  !BuOH (umol/hr on/off) <GD>
       kIV3= RSW(T.LE.IVDOFF,IV3,0.)  !BuCOO(umol/hr on/off) <GD>
       kIV4= RSW(T.LE.IVDOFF,IV4,0.)  !BuCOOH (umol/hr on/off) <GD>

!Oral Dosing Simulation <GD>
       IF ((PDose1+PDose2+PDose3+PDose4) .GT. 0.)THEN
              ORAL= PULSE(ORDON,ORDPER1,ORDWID1)*PULSE(ORDON,ORDPER2,ORDWID2)
       ELSE
              ORAL=0.
       END IF

       ORAL1 = ORAL *ORDUM1/ORDAT    !BuAc (umol/hr)
       ORAL2=  ORAL *ORDUM2 /ORDAT   !BuOH(umol/hr)
       ORAL3 = ORAL *ORDUM3 /ORDAT   !BuCOO (umol/hr)
       ORAL4 = ORAL *ORDUM4/ORDAT     !BuCOOH (umol/hr)

       Dose1= RSW(T.LE.ORDOFF,ORAL1,0.)  !BuAc(umol/hr on/off)
       Dose2= RSW(T.LE.ORDOFF,ORAL2,0.)  !BuOH (umol/h on/off)
       Dose3= RSW(T.LE.ORDOFF,ORAL3,0.)  !BuCOO (umol/hr on/off)
       Dose4= RSW(T.LE.ORDOFF,ORAL4,0.)  !BuCOOH (umol/hr on/off)

!Inhalation Dosing Simulation <GD>

       IF ((CInh1+CInh2+CInh+CInh4) .GT. 0.)THEN
              CION= PULSE(INHON,INHPER1,INHWID1)*PULSE(INHON,INHPER2,INHWID2)
       ELSE
              CION=0.
       END IF

       CION1 = CION * CIUM1  !BuAc exposure (umol/ml)
       CION2 = CION * CIUM2  !BuOH exposure (umol/ml)
       CION3 = CION * CIUM3  !BuCOO exposure (umol/ml)
       CION4 = CION * CIUM4  !BuCOOH exposure (umol/ml)

       Conc11 = RSW(T.LE.AIROFF,CION1,CIOFF)
              !BuAc conc. in inhaled air (umol/L on/off)

```

```
        Conc22 = RSW(T.LE.AIROFF,CION2,CIOFF)
               !BuOH conc. in inhaled air (umol/L on/off)
        Conc33 = RSW(T.LE.AIROFF,CION3,CIOFF)
               !BuCOO conc. in inhaled air (umol/L on/off)
        Conc44 = RSW(T.LE.AIROFF,CION4,CIOFF)
               !BuCOOH conc. in inhaled air (umol/L on/off

 IF (INHTABLE .EQ. 1) THEN
        Conc1 = CINHTAB1(minutes)/24450.0
        Conc2 = CINHTAB2(minutes)/24450.0
        Conc3 = CINHTAB3(minutes)/24450.0
        Conc4 = CINHTAB4(minutes)/24450.0
ELSE
        Conc1 = Conc11  !BuAc conc. in inhaled air (umol/L on/off)
        Conc2 = Conc22  !BuOH conc. in inhaled air (umol/L on/off)
        Conc3 = Conc33  !BuCOO conc. in inhaled air (umol/L on/off)
        Conc4 = Conc44  !BuCOOH conc. in inhaled air (umol/L on/off
ENDIF

!Chamber Loss simulation
        kLoss1= RSW(T.LE.LossT,kLoss1C1,kLoss1C2)  !BuAC (umol/hr on/off) <GD>
        kLoss2= RSW(T.LE.LossT,kLoss2C1,kLoss2C2)  !BuAC (umol/hr on/off) <GD>

!************************   Butyl Acetate   ************************
! Arterial Blood (umoles)
     RAAB1 = (QC * (CVLung1 - CAB1)) - RMetAB1
      AAB1 = INTEG(RAAB1, AAB10)
      CAB1 = AAB1 / VAB
                      AUCAB1 = INTEG(CAB1, 0.0)

! Blood Metabolism (umoles)
   RMetAB1 = (VMAB1 * CAB1) / (KMBlood1 + CAB1)
    MetAB1 = INTEG(RMetAB1, 0.0)

! Fat (umole)
    RAFat1 = QFat * (CAB1 - CVFat1)
     AFat1 = INTEG(RAFat1, 0.0)
     CFat1 = AFat1 / VFat
    CVFat1 = CFat1 / PFat1

! Liver (umoles)
    RALiv1 = (QLiv * (CAB1 - CVLiv1)) - RMetLiv1 + RMR1
     ALiv1 = INTEG(RALiv1, 0.0)
     CLiv1 = ALiv1 / VLiv
    CVLiv1 = CLiv1 / PLiv1
   AUCLiv1 = INTEG(CLiv1, 0.0)

! Liver Metabolism (umoles)
  RMetLiv1 = (VMLiv1 * CVLiv1) / (KMLiv1 + CVLiv1)
   MetLiv1 = INTEG(RMetLiv1, 0.0)

! Amount remaining to be absorbed into liver from GI (umoles)
      RMR1 = kA * MR1
       MR1 = Dose1 - INTEG(RMR1, 0.0)
! Chamber concentration (umoles/mL)
     RACh1 = (Rats * QP * CEx1) - (FA1 * Rats * QP * CCh1) - (kLoss1 * ACh1)&
           - (kLoss1R * Rats * ACh1)
             ACh1 = INTEG(RACh1, ACh01)

```

```
      CCh1 = ((ACh1 / VCh) * ClOn) + (Conc1 * (1.0 - ClOn))
    CCPPM1 = CCh1 * 24450.0
    CLoss1 = INTEG(((kLoss1 * ACh1) + (kLoss1R * Rats * ACh1)), 0.0)

       IF (SCINPPM1 .GT. 0.) THEN
              CCPPM1PCT=100.*CCPPM1/SCINPPM1  !BuAc conc. as % of intitial <GD>
       ELSE
              CCPPM1PCT = 0.
       END IF

! Lungs
!  Amount in Lungs (umoles)
   RALung1 = (QP * ((FA1 * CCh1) - CEx1)) + (QC * (CVB1 - CVLung1))
    ALung1 = INTEG(RALung1, 0.0)
    CLung1 = ALung1 / VLung
   CVLung1 = CLung1 / PLung1

!  Amount Inhaled (umoles)
   RInhal1 = FA1 * QP * CCh1
   AInhal1 = INTEG(RInhal1, 0.0)
  AInhal1C = AInhal1 * Rats

!  Amount Exhaled (umoles)
      CEx1 = CvLung1 / PB1
     RAEx1 = QP * CEx1
      AEx1 = INTEG(RAEx1, 0.0)
     AEx1C = AEx1 * Rats

! Other Perfused Tissue Modified to Include Metabolism (umoles)
    RAOth1 = (QOth * (CAB1 - CVOth1)) - RMetOth1
     AOth1 = INTEG(RAOth1, 0.0)
     COth1 = AOth1 / VOth
    CVOth1 = COth1 / POth1
   AUCOth1 = INTEG(COth1, 0.0)

! Other Perfused Tissue Metabolism (umoles)
  RMetOth1 = (VMOth1 * CVOth1) / (KMOth1 + CVOth1)
   MetOth1 = INTEG(RMetOth1, 0.0)

! Venous Blood (umoles)
     RAVB1 = (QFat*CVFat1) + (QLiv*CVLiv1) + (QOth*CVOth1) + kIV1 &
           - (QC*CVB1) - RMetVB1 - RAFilt1
      AVB1 = INTEG(RAVB1, 0.0)
      CVB1 = AVB1 / VVB
     AUCB1 = INTEG(CVB1, 0.0)

!  Metabolism
   RMetVB1 = (VMVB1 * CVB1) / (KMBlood1 + CVB1)
    MetVB1 = INTEG(RMetVB1, 0.0)

!  Filtration
   RAFilt1 = kFilt1 * AVB1
    AFilt1 = INTEG(RAFilt1, 0.0)

! Average Blood (umoles)
      CTB1 = (AAB1 + AVB1) / (VAB + VVB)
    AUCTB1 = INTEG(CTB1, 0.0)
```

```
!*************************   n-Butanol   *************************
! Arterial Blood (umoles)
     RAAB2 = (QC * (CVLung2 - CAB2)) + RMetAB1
      AAB2 = INTEG(RAAB2, AAB20)
      CAB2 = AAB2 / VAB
               AUCAB2 = INTEG(CAB2, 0.0)
               IF (T>0.) THEN          !<GD>
                      AUCAB2_24=(AUCAB2/T)*24.  !AUC mM*24 hr <GD>
               ELSE                   !<GD>
                      AUCAB2_24=0.   !<GD>
               END IF

! Liver (umoles)
    RALiv2 = (QLiv * (CAB2 - CVLiv2)) + RMetLiv1 + RMR2 - RMetLiv2
     ALiv2 = INTEG(RALiv2, 0.0)
     CLiv2 = ALiv2 / VLiv
    CVLiv2 = CLiv2 / PLiv2
   AUCLiv2 = INTEG(CLiv2, 0.0)

! Liver Metabolism (umoles)
  RMetLiv2 = (VMLiv2 * CVLiv2) / (KMLiv2 + CVLiv2)
   MetLiv2 = INTEG(RMetLiv2, 0.0)

! Amount remaining to be absorbed into liver from GI (umoles)
      RMR2 = kA * MR2
       MR2 = Dose2 - INTEG(RMR2, 0.0)

! Chamber concentration (umoles/mL)
     RACh2 = (Rats * QP * CEx2) + (kLoss1 * ACh1) - (FA2 * Rats * QP * CCh2) &
           - (kLoss2 * ACh2) - (kLoss2R * Rats * ACh2)
      ACh2 = INTEG(RACh2, ACh02)
      CCh2 = ((ACh2 / VCh) * ClOn) + (Conc2 * (1.0 - ClOn))
    CCPPM2 = CCh2 * 24450.0
    CLoss2 = INTEG(((kLoss2 * ACh2) + (kLoss2R * Rats * ACh2)), 0.0)

        IF (SCINPPM2 .GT. 0.) THEN
               CCPPM2PCT=100.*CCPPM2/SCINPPM2  !BuOH conc. as % of intitial <GD>
        ELSE
               CCPPM2PCT = 0.
        END IF

! Lungs
!  Amount in Lungs (umoles)
   RALung2 = (QP * ((FA2 * CCh2) - CEx2)) + (QC * (CVB2 - CVLung2))
    ALung2 = INTEG(RALung2, 0.0)
    CLung2 = ALung2 / VLung
   CVLung2 = CLung2 / PLung2

!  Amount Inhaled
   RInhal2 = FA2 * QP * CCh2
   AInhal2 = INTEG(RInhal2, 0.0)
  AInhal2C = AInhal2 * Rats

!  Amount Exhaled
      CEx2 = CvLung2 / PB2
     RAEx2 = QP * CEx2
      AEx2 = INTEG(RAEx2, 0.0)
            AEx2C = AEx2 * Rats

```

```
! Other Perfused Tissues (umoles)
    RAOth2 = ((QOth + QFat) * (CAB2 - CVOth2)) + RMetOth1 - RAMOth2
     AOth2 = INTEG(RAOth2, 0.0)
     COth2 = AOth2 / (VOth + VFat)
    CVOth2 = COth2 / POth2

! Metabolism in Other Perfused Tissues (umoles)
   RAMOth2 = kOth2 * AOth2
    AMOth2 = INTEG(RAMOth2, 0.0)

! Venous Blood
     RAVB2 = (QLiv*CVLiv2) + ((QOth+QFat)*CVOth2) + RMetVB1 + kIV2 &
           - (QC*CVB2) - RAFilt2
      AVB2 = INTEG(RAVB2, 0.0)
      CVB2 = AVB2 / VVB
     AUCB2 = INTEG(CVB2, 0.0)

!  Filtration
   RAFilt2 = kFilt2 * AVB2
    AFilt2 = INTEG(RAFilt2, 0.0)

! Average Blood (umoles)
      CTB2 = (AAB2 + AVB2) / (VAB + VVB)
    AUCTB2 = INTEG(CTB2, 0.0)


!************************   n-Butyraldehyde   ************************
! Arterial Blood (umoles)
     RAAB3 = QC * (CVLung3 - CAB3)
      AAB3 = INTEG(RAAB3, AAB30)
      CAB3 = AAB3 / VAB
                     AUCAB3 = INTEG(CAB3, 0.0)

! Liver (umoles)
    RALiv3 = (QLiv * (CAB3 - CVLiv3)) + RMetLiv2 + RMR3 - RMetLiv3
     ALiv3 = INTEG(RALiv3, 0.0)
     CLiv3 = ALiv3 / VLiv
    CVLiv3 = CLiv3 / PLiv3
   AUCLiv3 = INTEG(CLiv3, 0.0)

! Liver Metabolism (umoles)
  RMetLiv3 = (VMLiv3 * CVLiv3) / (KMLiv3 + CVLiv3)
   MetLiv3 = INTEG(RMetLiv3, 0.0)

! Amount remaining to be absorbed into liver from GI (umoles)
      RMR3 = kA * MR3
       MR3 = Dose3 - INTEG(RMR3, 0.0)

! Chamber concentration (umoles/mL)
     RACh3 = (Rats * QP * CEx3) - (FA3 * Rats * QP * CCh3) - (kLoss3 * ACh3) &
           - (kLoss3R * Rats * ACh3)
      ACh3 = INTEG(RACh3, ACh03)
      CCh3 = ((ACh3 / VCh) * ClOn) + (Conc3 * (1.0 - ClOn))
    CCPPM3 = CCh3 * 24450.0
         CLoss3 = INTEG(((kLoss3 * ACh3) + (kLoss3R * Rats * ACh3)), 0.0)

```

```
        IF (SCINPPM3 .GT. 0.) THEN
               CCPPM3PCT=100.*CCPPM3/SCINPPM3  !BuCOO conc. as % of intitial <GD>
        ELSE
               CCPPM3PCT = 0.
        END IF

! Lungs
!  Amount in Lungs (umoles)
   RALung3 = (QP * ((FA3 * CCh3) - CEx3)) + (QC * (CVB3 - CVLung3))
    ALung3 = INTEG(RALung3, 0.0)
    CLung3 = ALung3 / VLung
   CVLung3 = CLung3 / PLung3

!  Amount Inhaled (umoles)
   RInhal3 = FA3 * QP * CCh3
   AInhal3 = INTEG(RInhal3, 0.0)
  AInhal3C = AInhal3 * Rats

!  Amount Exhaled (umoles)
      CEx3 = CvLung3 / PB3
     RAEx3 = QP * CEx3
      AEx3 = INTEG(RAEx3, 0.0)
     AEx3C = AEx3 * Rats

! Other Perfused Tissues (umoles)
    RAOth3 = ((QOth + QFat) * (CAB3 - CVOth3)) + RAMOth2
     AOth3 = INTEG(RAOth3, 0.0)
     COth3 = AOth3 / (VOth + VFat)
    CVOth3 = COth3 / POth3

! Venous Blood (umoles)
     RAVB3 = (QLiv*CVLiv3) + ((QOth+QFat)*CVOth3) + kIV3 - (QC*CVB3)
      AVB3 = INTEG(RAVB3, 0.0)
      CVB3 = AVB3 / VVB
     AUCB3 = INTEG(CVB3, 0.0)

! Average Blood (umoles)
      CTB3 = (AAB3 + AVB3) / (VAB + VVB)
    AUCTB3 = INTEG(CTB3, 0.0)

!************************   Butyric Acid   *************************
! Arterial Blood (umoles)
     RAAB4 = QC * (CVLung4 - CAB4)
      AAB4 = INTEG(RAAB4, AAB40)
      CAB4 = AAB4 / VAB
                     AUCAB4 = INTEG(CAB4, 0.0)

! Liver (umoles)
    RALiv4 = (QLiv * (CAB4 - CVLiv4)) + RMetLiv3 + RMR4 - RMetLiv4
     ALiv4 = INTEG(RALiv4, 0.0)
     CLiv4 = ALiv4 / VLiv
    CVLiv4 = CLiv4 / PLiv4
   AUCLiv4 = INTEG(CLiv4, 0.0)

! Liver Metabolism (umoles)
  RMetLiv4 = (VMLiv4 * CVLiv4) / (KMLiv4 + CVLiv4)
   MetLiv4 = INTEG(RMetLiv4, 0.0)
```

```
! Amount remaining to be absorbed into liver from GI (umoles)
      RMR4 = kA * MR4
       MR4 = Dose4 - INTEG(RMR4, 0.0)
! Chamber concentration (umoles/mL)
     RACh4 = (Rats * QP * CEx4) - (FA4 * Rats * QP * CCh4) - (kLoss4 * ACh4) &
           - (kLoss4R * Rats * ACh4)
      ACh4 = INTEG(RACh4, ACh04)
      CCh4 = ((ACh4 / VCh) * ClOn) + (Conc4 * (1.0 - ClOn))
    CCPPM4 = CCh4 * 24450.0
    CLoss4 = INTEG(((kLoss4 * ACh4) + (kLoss4R * Rats * ACh4)), 0.0)

        IF (SCINPPM4 .GT. 0.) THEN
               CCPPM4PCT=100.*CCPPM4/SCINPPM4  !BuCOOH conc. as % of intitial <GD>
        ELSE
               CCPPM4PCT = 0.
        END IF
! Lungs
!  Amount in Lungs (umoles)
   RALung4 = (QP * ((FA4 * CCh4) - CEx4)) + (QC * (CVB4 - CVLung4))
    ALung4 = INTEG(RALung4, 0.0)
    CLung4 = ALung4 / VLung
   CVLung4 = CLung4 / PLung4

!  Amount Inhaled
   RInhal4 = FA4 * QP * CCh4
   AInhal4 = INTEG(RInhal4, 0.0)
  AInhal4C = AInhal4 * Rats

!  Amount Exhaled
      CEx4 = CvLung4 / PB4
     RAEx4 = QP * CEx4
      AEx4 = INTEG(RAEx4, 0.0)
     AEx4C = AEx4 * Rats

! Other Perfused Tissues (umoles)
    RAOth4 = (QOth + QFat) * (CAB4 - CVOth4) - RMetOth4
     AOth4 = INTEG(RAOth4, 0.0)
     COth4 = AOth4 / (VOth + VFat)
    CVOth4 = COth4 / POth4

! Other Perfused Tissue Metabolism (umoles)
  RMetOth4 = (VMOth4 * CVOth4) / (KMOth4 + CVOth4)
   MetOth4 = INTEG(RMetOth4, 0.0)

! Venous Blood (umoles)
     RAVB4 = (QLiv*CVLiv4) + ((QOth+QFat)*CVOth4) + kIV4 - (QC*CVB4) - RAFilt4
      AVB4 = INTEG(RAVB4, 0.0)
      CVB4 = AVB4 / VVB
     AUCB4 = INTEG(CVB4, 0.0)
!  Filtration
   RAFilt4 = kFilt4 * AVB4
    AFilt4 = INTEG(RAFilt4, 0.0)

! Average Blood (umoles)
      CTB4 = (AAB4 + AVB4) / (VAB + VVB)
    AUCTB4 = INTEG(CTB4, 0.0)
        TERMT(T.GE.TStop)

```

```
!************************   Mass Balance   *************************
! Total System Mass Balance
   MassBal = TMass1 + AEx1 + AFilt1 + TMass2 + AEx2 + AFilt2 + TMass3 + AEx3 &
           + TMass4 + AEx4 + AFilt4 + TMetab4
! Mass Balance for BuAc (umoles)
    TMass1 = AAB1 + AFat1 + ALiv1 + ALung1 + AOth1 + AVB1
  TMetab1a = MetAB1 + MetLiv1 +  MetVB1 + MetOth1
 TMassTot1 = TMass1 + TMetab1a  + AEx1 + AFilt1
    Delta1 = ((IVDose1 * 1000.0/MW1) * BW) + AInhal1 + Dose1 + AAB10 - TMassTot1
   Delta1C = ACh01 - ACh1 - AInhal1C - CLoss1 + AEx1C
! Mass Balance for BuOH (umoles)
    TMass2 = AAB2 + ALiv2 + ALung2 + AOth2 + AVB2
   TMetab2 = MetLiv2 + AmOth2
 TMassTot2 = TMass2 + TMetab2 + AEx2 + AFilt2
    Delta2 = ((IVDose2 * 1000.0/MW2) * BW) + AInhal2 + Dose2 + AAB20 &
           - TMassTot2 + TMetab1a
   Delta2C = ACh02 - ACh2 - AInhal2C - CLoss2 + AEx2C
! Mass Balance for BuCHO (umoles)
    TMass3 = AAB3 + ALiv3 + ALung3 + AOth3 + AVB3
   TMetab3 = MetLiv3
 TMassTot3 = TMass3 + TMetab3 + AEx3
    Delta3 = ((IVDose3 * 1000.0/MW3) * BW) + Dose3 + AAB30 + TMetab2 &
           + AInhal3 - TMassTot3
   Delta3C = ACh03 - ACh3 - AInhal3C - CLoss3 + AEx3C

! Mass Balance for Butyrate (umoles)
    TMass4 = AAB4 + ALiv4 + ALung4 + AOth4 + AVB4
   TMetab4 = MetLiv4 + MetOth4
 TMassTot4 = TMass4 + TMetab4 + AEx4 + AFilt4
    Delta4 = ((IVDose4 * 1000.0/MW4) * BW) + Dose4 + AAB40 &
           + AInhal4 - TMassTot4 + TMetab3
   Delta4C = ACh04 - ACh4 - AInhal4C - CLoss4 + AEx4C

    Total1 = MetLiv1 + MetAB1 + MetVB1
    Total2 = MetLiv2 + AMOth2
    Total3 = MetLiv3
    Total4 = MetLiv4

  IF (AInhal1.NE.0.0) THEN
     Recov = 100.0 * (TMassTot1 / AInhal1)
  ELSE
     Recov = 0.0
  ENDIF
! Check Blood Flows
      QTot = QFat + QLiv + QOth
    QRecov = 100.0 * (QTot / QC)

END               ! End of Derivative
END               ! End of Dynamic

TERMINAL

END               ! End of Terminal
END               ! End of Program
```

C.2. Summary of Model Evaluation

U.S. EPA performed an evaluation and quality control check of the PBPK model for n-butanol published by Teeguarden et al. (2005). The evaluation included confirmation of accurate implementation of the model described in Teeguarden et al. (2005) and evaluations of the performance of the rat and human PBPK models that were not reported in Teeguarden et al. (2005). These included evaluations of residuals for model predictions compared to observations made in rat i.v. and inhalation studies and in human inhalation studies; comparisons of observed and predicted AUC for blood n-butanol–time profiles in rats and humans; and alternative values for metabolism parameters were explored or estimated by statistical optimization. The conclusions from these analyses as follows:

1. The value adopted by Teeguarden et al. (2005) for the K_m of metabolism of n-butanol (model variable: KMLivC2) in rats did not have a strong empirical basis and, therefore, the value derived from in vitro studies in rat hepatic cytosol preparations (Carlson and Olson, 1995) was selected as the preferred value for use in dosimetry calculations. Use of this value was supported by statistical optimization analyses, which showed that model performance was not substantially improved or degraded by use of the empirically-based value. Since the value for KMLivC2 was modified from Teeguarden et al. (2005), values for the V_{max} for n-butanol metabolism in the liver (model variable: VMLivC2) and the rate constant for metabolism of n-butanol in other tissues (model variable: KOthC2) were re-optimized and evaluated with data from i.v. and closed chamber inhalation studies conducted in rats.

2. The same empirical value for KMLivC2for was also adopted for the human model and values for VMLivC2 and KOthC2 were re-optimized against data from human exposures to n-butanol (Astrand et al., 1976). The optimized values resulted in improved fit to the human data and were adopted for the human model.

3. The validity of the human model is not as strongly supported as the rat model; it has been tested against data from a single study. The metabolism parameter values for the human model are highly sensitive to observations made in a single individual, and the influence of these data on the parameter value estimates will affect the predicted n-butanol blood AUC. However, even with these uncertainties, the human model provides improved confidence for interspecies extrapolation of pharmacokinetics and, therefore, was used in making dosimetry extrapolations from rats to humans.

On the basis of this evaluation, U.S. EPA concluded that the PBPK model was suitable for use in deriving the RfC for n-butanol.

C.3. Uncertainties in PBPK Modeling of HECs Related to Selection of Values for Metabolism Parameters

Values for n-butanol metabolism parameters used for the dosimetry modeling are shown in Table C-1. Values for n-butanol metabolism parameters, V_{max} for metabolism in the liver (model variable: VMLivC2) and the rate constant for metabolism of n-butanol in other tissues (model variable: KOthC2) were optimized against data for blood n-butanol concentrations in humans during and following inhalation exposure to 100 or 200 ppm n-butanol (Astrand et al., 1976), while keeping the value for the K_m for liver metabolism (model variable: KMLivC2) set at the empirically-based value of 0.16 mM (Carlson and Olson, 1995). Optimizing these parameters resulted in improvement of model performance as judged by residuals for the Astrand et al. (1976) observations. Improved model performance was the basis for selecting the optimized values over allometric scaling of the rat values. The impact of this decision on the estimate of the HEC corresponding to the rat NOAEL is shown in Table C-2. The NOAEL HEC is 34% lower when the allometrically scaled rat values are used in the human model (39 vs. 59 mg/m^3).

With respect to the urinary elimination rate parameters, the PBPK model does not incorporate the interaction of butyric acid with colonocytes and it is well known that the basic form, butyrate, is a major substrate for colon cells (see Thibault et al., 2010 for a recent review). However, kinetic information on urinary clearance for butyric acid following n-butanol administration is not available and was therefore not incorporated into the model.

Table C-1. n-Butanol metabolism parameters

Parameter	Abbreviation	Values		Basis
		Rat	Human	
Maximum metabolic rate in liver	VMLivC2	2.17	0.62	Optimized (see model evaluation report)
Affinity constant in liver	KMLiv2	0.16	0.16	Estimate for rat (Carlson and Olson,1995)
First-order metabolic rate constant in other tissues	KOthC2	4.0	20.1	Optimized (see model evaluation report)

Units: V_{max} (mmol/hr/kg$^{0.75}$); K_m (mM); K_{other} (kg$^{0.25}$/hr).

Table C-2. Results of PBPK modeling of NOAEL concentration from subchronic rat study

Source of human parameter values	NOAEL rat mg/m^3	NOAEL rat ppm	AUCAB2_24 rat mM·24hr	AUCAB2_24 human mM·24hr	NOAEL HEC human ppm	NOAEL HEC human mg/m^3
Optimized[a]	154	50	0.01282	0.01282	19.21	59.2

Allometrically scaled from rat values[b]	154	50	0.01282	0.01282	12.76	39.3

[a]KMLivC2=0.16 mM, VMLivC2=0.62 mmol/hr/kg$^{0.75}$, KOthC2=20.1 kg$^{0.25}$/hr.
[b]KMLivC2=0.16 mM, VMLivC2=2.17 mmol/hr/kg$^{0.75}$, KOthC2=4.0 kg$^{0.25}$/hr.

Source: Korsak et al. (1994).

1

2