Voluntary Children's Chemical Evaluation Program (VCCEP)

Tiers 1, 2, and 3 Pilot Submission For 1,4-Dioxane

(CAS No. 123-91-1)

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List of Abbreviations and Acronyms

ACGIH	American Conference of Governmental Industrial Hygienists
ADD	average daily dose
ALAT	alanine aminotransferase
AP	alkaline phosphatase
ASAT	aspartate aminotransferase
atm	atmospheres
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
AUC-Liver	average area under liver dioxane concentration time curve per day
AUC-MET	average area under metabolite concentration time curve for whole body per day
AZ	Arizona
BCEE	bis (2-chloroethyl) ether
BCF	bioconcentration factor
BUN	blood urea nitrogen
bw	body weight
°C	degrees Celsius
$CaCl_2$	calcium chloride
cal	calories
CalEPA	California Environmental Protection Agency
CCL	contaminant candidate list
CERCLIS	Comprehensive Environmental Response, Compensation and Liability Information System
CHO	Chinese hamster ovary
cm	centimeter
cm^3	centimeter cubed
CNS	central nervous system
CO_2	carbon dioxide
CPFs	cancer potency factors
CYP	cytochrome P-450
d	day
DE	Delaware
DNA	deoxyribonucleic acid
DMBA	7,12-dimethylbenzanthracene
DW	drinking water
EC	European Commission
ECETOC	European Chemical Industry Ecology and Toxicology Centre
EPIWIN	Estimation Programs Interface for Windows
EROD	ethoxyresorufin O-deethylase

°F	degrees Fahrenheit
f	female
FDA	US Food and Drug Administration
g	gram
GGT	gamma-glutamyl transferase
GJIC	gap-junction intercellular communication
GSH	glutathione
H_2O_2	hydrogen peroxide
HEAA	β-hydroxyethoxyacetic acid
Hg	mercury
HGPRT	hypoxanthine-guanine phosphoribosyl transferase
HI	Hazard Index
hPa	hectopascals
hr	hour
HSDB	Hazardous Substances Data Bank
IARC	International Agency for Research on Cancer
inhal	inhalation
inj	injection
ip	intraperitoneal
IPCS	International Program on Chemical Safety
IRIS	Integrated Risk Information System
iv	intravenous
K_{aw}	log air water coefficient
kcal	kilocalorie
kg	kilograms
K_M	Michaelis constant
K_{oc}	log soil sorption coefficient
Kp	permeability constant
kPa	kilopascal
L	liters
LADD	lifetime average daily dose
lb	pounds
L_{cl}	plume length for chloride
LC_{50}	lethal concentration for 50% of the population
LD_{50}	lethal dose for 50% of the population
LOAEL	Lowest-Observed-Adverse-Effect-Level
L_{org}	plume length for contaminant of interest
m	male
M	mole
m^3	meters cubed
MA	mutagenic activity

max	maximum
m/z	mass to charge ratio
MFO	mixed function oxidases
mg	miligrams
min	minute
ml	mililiter
MLD	minimum lethal dose
MLE	maximum likelihood estimate
mm	milimeters
mM	milimoles
MPC	maximum permissible concentration
MPE	micronucleated polychromatic erythrocytes
mRNA	messenger ribonucleic acid
mol	moles
NaCl	sodium chloride
NADPH	nicotinamide adenine dinucleotide phosphate
NaOH	sodium hydroxide
NAS	National Academy of Sciences
NCI	National Cancer Institute
ND	nondetect
neg	negative
NICNAS	National Industrial Chemicals Notification and Assessment Schemes
NIOSH	National Institute for Occupational Safety and Health
nm	nanometers
NMR	nuclear magnetic resonance
NO	nitrogen oxide
NOAEL	No-Observed-Adverse-Effect-Level
NPL	National Priority List
NRC	National Research Council
NTP	National Toxicology Program
OCWD	Orange County Water Department
OECD	Organization for Economic Co-operation and Development
Pa	Pascals
PBPK	physiologically based pharmacokinetics
pos	positive
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
PROD	pentoxyresorufin O-depentylase
q_{animal}	animal cancer potency
q_{human}	human cancer potency

RDS	replicative DNA synthesis
REL	recommended exposure level
R_f	retardation factor
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
RIVM	National Institute for Public Health and the Environment
s	seconds
SAR	structure-activity relationship
sc	subcutaneous
SCEs	sister chromatid exchanges
SPC	Science Policy Council
SPIR	standardized proportionate incidence ratio
$t_{1/2}$	half-life
TARP	Tucson Airport Area Remediation Project
TCE	trichloroethylene
TEAM	Total Exposure Assessment Methodology
T_{max}	maximum accumulation time
TNO	The Netherlands Organization for Applied Scientific Research
TRI	Toxic Chemical Release Inventory
TSCA	Toxic Substances Control Act
UDS	unscheduled DNA synthesis
UF	uncertainty factors
UFa	uncertainty factor (animal to human)
UFh	uncertainty factor (human to human)
UFc	uncertainty factor (subchronic to chronic)
UFl	uncertainty factor (LOAEL to NOAEL)
Ufd	uncertainty factor (completeness of database)
µg	micrograms
µl	microliters
µmol	micromoles
US	United States
USEPA	United States Environmental Protection Agency
UV	ultraviolet
v/v	volume per volume
VCCEP	Voluntary Children's Chemical Exposure Program
V_{max}	maximum velocity
w/w	by weight
WHO	World Health Organization
wk	week
w/o	without

wt	weight
yr	year

Executive Summary

USEPA in selecting compounds for the pilot of the Voluntary Children's Chemical Exposure Program (VCCEP) elected to focus on those compounds that had been detected in various biological monitoring programs. 1,4-Dioxane was added to the VCCEP as the result of its being detected in the breath of individuals monitored during the Total Exposure Assessment Methodology (TEAM) studies of the mid-1980s. The source of this 1,4-dioxane in breath was never determined although low levels of the compound were detected in both ambient and indoor air in the same locale during the same period.

1,4-Dioxane possesses unique properties that made it valuable in a number of industrial applications (Sections 2.0 and 3.0). In the United States, in the 1970s and 1980s, 90% of the 1,4-dioxane was used as a stabilizer in chlorinated solvents (*i.e.*, 1,1,1-trichloroethane). As these compounds have been phased out due to environmental concerns, the demand for 1,4-dioxane has also declined markedly. Only one US producer remains and the amount of 1,4-dioxane produced annually has declined by over 80% of that produced in the mid-1980s.

1,4-Dioxane has been detected in drinking and surface water, indoor, outdoor, and workplace air, food (from both natural and artificial sources), and various consumer products; however, the data is dated (Section 6.0). The extent and amount of current exposure to children and adults is unknown although it is expected that the exposure potential in many situations has also declined in proportion to the product demand. In other cases (*i.e.*, ground water), the true extent of exposure to 1,4-dioxane may be greater than previously recognized. However, Toxic Release Inventory data suggest that amount of 1,4-dioxane being released into the environment is decreasing. Therefore, exposure to 1,4-dioxane via ingestion of surface or ground water is also declining with time. The occurrence of 1,4-dioxane as an impurity in surfactants used in various foods and consumer products suggests that, even today, the majority of the US population including children are exposed to 1,4-dioxane, albeit it at low levels.

Children can be exposed to 1,4-dioxane via the ingestion of foods containing 1,4-dioxane as an impurity, dermal contact with contaminated water during showering or bathing and through the use of consumer products containing 1,4-dioxane as an impurity, and through inhalation of 1,4-dioxane in ambient and indoor air (assumed to be the same air concentrations). Children and youths are also exposed to 1,4-dioxane through ingestion of contaminated water whereas infants are exposed through contaminated breast milk or formula. In the case of breast milk, the exposed mother was assumed to be a worker and the dose of 1,4-dioxane in breast milk under these circumstances would be greater than and subsume the dose from a solely environmentally exposed mother or through formula. The decision to employ the 1980s vintage data in estimating environmental exposure in combination with probabilistic modeling was viewed as a conservative step that allowed data

gaps and other uncertainties to be identified and addressed. These findings expressed as the average daily dose in **Table ES-1** support the conclusion that the current exposure of children to 1,4-dioxane is likely to be low with upper bound estimates of total average daily dose ranging from 0.14 mg/kg-d (infants) to 0.04 mg/kg-d (youths).

Table ES-1. Mean (and 95th Percentile) Average Daily Dose (ADD) Estimate For 1,4-Dioxane Exposure in Infants, Children, and Youths

Exposed Age Group	Ingestion Dose (mg/kg-d)	Inhalation Dose (mg/kg-d)	Dermal Dose (mg/kg-d)	Total Dose (mg/kg-d)
Pregnant Worker (Fetus)	1.7E-2 (4E-2)	4.1E-1 (1.1E+0)	6.9E-1 (2.0E+0)	1.1E+0 (3.2E+0)
Infants (0-1 years)	1.1E-2 (2.5E-2)	1.0E-3 (2.7E-3)	3.3E-2 (1.1E-1)	4.5E-2 (1.4E-1)
Children (1-2 years)	2.6E-2 (6.8E-2)	1.0E-3 (2.8E-3)	2.7E-3 (8.7E-3)	3E-3 (7.9E-2)
Children (2-3 years)	2.4E-2 (6.1E-2)	8.4E-4 (2.2E-3)	2.7E-3 (8.7E-3)	2.7E-2 (7.2E-2)
Children (3-6 years)	2.1E-2 (5.5E-2)	6.0E-4 (1.6E-3)	2.7E-3 (8.7E-3)	2.4E-2 (6.5E-2)
Children (6-11 years)	1.4E-2 (3.7E-2)	3.6E-4 (9.9E-4)	2.7E-3 (8.7E-3)	1.7E-2 (4.6E-2)
Youths (11-16 years)	1.1E-2 (2.8E-2)	2.3E-4 (6.4E-4)	2E-3 (6.4E-3)	1.3E-2 (3.5E-2)
Youths (16-21 years)	1.2E-2 (3.1E-2)	2E-4 (5.4E-4)	2.7E-3 (8.7E-3)	1.4E-2 (4E-2)

Much of the uncertainty is associated with environmental measurements that could be addressed (if warranted) by sampling the respective media for current 1,4-dioxane concentrations, extent of contamination, and frequency of occurrence.

Review of the toxicity of 1,4-dioxane finds a relatively robust database (Section 4.0). 1,4-Dioxane is a compound that displays low acute and chronic toxicity. Liver and kidney damage are the major endpoints most consistently associated with target organ toxicity in

sub-chronic and chronic toxicity animal studies, although other tissues (*e.g.*, nasal) have been associated with these tests as well. 1,4-Dioxane is clearly carcinogenic in animal studies with liver and nasal tumors predominating in various experiments, but it is also very likely a non-genotoxic compound requiring high, prolonged dosing to induce tumors. Numerous pharmacokinetic studies have been carried out that suggest that the metabolism of 1,4-dioxane is saturable and above these doses the kinetics become decidedly non-linear. It is postulated that the build-up of 1,4-dioxane in tissues above these levels results in cytotoxicity in the target organs with resultant hyperplasia and hypertrophy as a precursor to the development of tumors. Indeed, tumors are not reported to occur in the absence of cytotoxicity. Additionally pharmacokinetic models of 1,4-dioxane find that the levels of 1,4-dioxane predicted in humans at the animal no observed adverse effect levels (NOAELs) for cytotoxicity (and cancer) are many times lower than that predicted in experimental animals.

NOAELs for 1,4-dioxane that were protective of adverse reproductive outcomes and cytotoxicity were identified from animal studies and used to derive health-protective reference doses (RfDs) (Section 5.0). An RfD of 0.1 mg/kg-d was determined for ingested 1,4-dioxane for *ex utero* neonates and older children and an RfD of 5.2 mg/kg-d was derived to protect *in utero* exposures. A reference concentration (RfC) of 1.1 mg/kg-d was determined for inhalation exposures. Based on its lack of genotoxicity, it was assumed that if 1,4-dioxane cytotoxicity was prevented (*e.g.* exposures at or below the RfD and RfC above), no cancer risk would occur in exposed populations. This same rationale has been associated with regulatory decisions for other non-genotoxic carcinogens. Comparison of the appropriate RfDs to estimated exposures for fetal, infant, child, and youth life stages as well as routes of exposure found no excursions above unity using a Hazard Index Approach as illustrated in **Table ES-2**.

Reitz *et al.* (1990) also conducted a cancer risk assessment for 1,4-dioxane using physiologically based pharmacokinetic (PBPK) model derived internal doses (the most appropriate dose metric was determined to be area under the 1,4-dioxane concentration curve in the liver) and assuming a non-linear threshold for tumor response. The most conservative values derived using this safety factor approach gave virtually safe doses for humans of 1,900 ppb in air and 51,000 ppb in water, or 1.9 and 1.5 mg/kg-d, respectively. These values are comparable to the RfD and RfC described above and add further confidence that the exposures determined in this assessment do not pose a cancer risk to children or prospective parents.

Table ES-2. Comparison of Estimated Exposures of 1,4-Dioxane to Derived Reference Doses

Life Stage	Hazard Index[1]	>1.0
Pregnant Worker (Fetus)		
Best Estimate (mean)	**0.2**	**No**
Upper Bound (95th percentile)	**0.5**	**No**
Infant (0-1 yr)		
Best Estimate (mean)	**0.4**	**No**
Upper Bound (95th percentile)	**1**	**No**
Child (1-2 yrs)		
Best Estimate (mean)	**0.3**	**No**
Upper Bound (95th percentile)	**0.8**	**No**
Child (2-3 yrs)		
Best Estimate (mean)	**0.3**	**No**
Upper Bound (95th percentile)	**0.7**	**No**
Child (3-6 yrs)		
Best Estimate (mean)	**0.2**	**No**
Upper Bound (95th percentile)	**0.6**	**No**
Child (6-11 yrs)		
Best Estimate (mean)	**0.2**	**No**
Upper Bound (95th percentile)	**0.5**	**No**

Life Stage	Hazard Index[1]	>1.0
Youth (11-16 yrs)		
Best Estimate (mean)	**0.1**	**No**
Upper Bound (95th percentile)	**0.3**	**No**
Youth (16 -21 yrs)		
Best Estimate (mean)	**0.1**	**No**
Upper Bound (95th percentile)	**0.4**	**No**

[1] Hazard Indexes were derived using chronic RfDs/RfC while the exposure durations were subchronic. Therefore, the HIs are considered conservative.

Although cancer potency factors (CPFs) have been derived for 1,4-dioxane by the United States Environmental Protection Agency (USEPA) and California Environmental Protection Agency (CalEPA), their relevance is questionable if a cancer threshold based on cytotoxicity is assumed for this compound (or others with the same mode of action). Additionally, they are somewhat dated and were derived using methods that have since been superceded, rely on tumor endpoints or animals models that are of uncertain relevance to humans, and do not include the pharmacokinetic data that is critical for interpreting the significance of the animal study results. Alternate CPFs have been derived assuming a no-threshold response and incorporating the results of the physiologically based pharmacokinetic model for 1,4-dioxane of Reitz *et al.* (1990) to take the pharmacokinetics of animals and humans into account. This approach was described by Reitz *et al.* for comparison to the non-linear approach described above. If it is assumed that the cancer response is linear in the low-dose region (contrary to the weight-of-evidence), then multiplying the lifetime average daily doses for each life-stage by the CPFs from Reitz *et al.* (1990) give the added lifetime cancer risk (**Table ES-3**). Even with this conservative (and likely incorrect) assumption, none of the added cancer risks by life-stage or total exceed the 1×10^{-5} acceptable lifetime risk levels for children identified by the VCCEP. These results also suggest that the existent CPFs (USEPA and CalEPA) would overstate the added cancer risk to humans by two to four orders of magnitude and ought to be revised in accordance with the regulatory use of pharmacokinetics and PBPK modeling for cancer risk assessment.

Table ES-3. Estimated Added Lifetime Cancer Risk From Exposure to 1,4-Dioxane by Life-Stage (Based on Reitz *et al.*, 1990 PBPK Model)

Life Stage	Added Lifetime Cancer Risk
Infant (0-1 yr)	
Best Estimate (mean)	**1.2E-8**
Upper Bound (95th percentile)	**3.7E-8**
Child (1-2 yrs)	
Best Estimate (mean)	**7.75E-9**
Upper Bound (95th percentile)	**1.8E-7**
Child (2-3 yrs)	
Best Estimate (mean)	**7.15E-9**
Upper Bound (95th percentile)	**1.9E-8**
Child (3-6 yrs)	
Best Estimate (mean)	**1.95E-8**
Upper Bound (95th percentile)	**5.2E-8**
Child (6-11 yrs)	
Best Estimate (mean)	**2.2E-8**
Upper Bound (95th percentile)	**6.05E-8**
Youth (11-16 yrs)	
Best Estimate (mean)	**1.75E-8**
Upper Bound (95th percentile)	**4.6E-8**
Youth (16 -21 yrs)	
Best Estimate (mean)	**1.9E-8**
Upper Bound (95th percentile)	**5.3E-8**
Total[1]	
Best Estimate (mean)	**1.05E-7**
Upper Bound (95th percentile)	**2.6E-7**

[1] Total (0-21 years) exposure was determined by summing the LADD values across age groups within the Monte Carlo simulation

Based on the results of this evaluation and the conclusion that the actual current exposure to 1,4-dioxane is lower than the estimates used, it is concluded that exposures to 1,4-dioxane as determined in this assessment do not pose a significant health threat to children. While additional toxicity data could be helpful in strengthening this conclusion, it is not viewed as a pressing need given the existent data. Better exposure data should be collected prior to undertaking additional animal experimentation to determine if the current exposure patterns and concentrations warrant such an effort. Consideration can also be given to employing the existing PBPK models to fill data gaps and address age-specific issues without carrying out further experimentation as has been done in the Hazardous Air Pollutants testing program (*i.e.*, 1,1,2-trichloroethane and 1,2-dichloroethane) and by the Agency for Toxic Substances and Disease Registry (ATSDR) to fill age-specific data gaps for methylene chloride.

1.0 Introduction

The USEPA nominated 1,4-dioxane as one of the 20 compounds to be assessed under the VCCEP. It was selected based on its occurrence in breath samples collected during the California portion of the TEAM Studies carried out in the mid-1980s. In the United States, 1,4-dioxane is presently only produced by the Ferro Corporation at its Baton Rouge, Louisiana plant. The other producers outside the United States are BASF AG in Ludwigshafen, Germany, and Osaka Yuki and Toho Chemical in Japan (NICNAS, 1998; TNO and RIVM, 2002). In general, the world-wide production of 1,4-dioxane is decreasing because of changing use patterns such as the elimination of its use as a stabilizer in chlorinated solvents and increased recovery of the solvent in manufacturing processes. The world-wide production capacity in 1985 was estimated to be 11,000 to 14,000 tons/year. In 1995, the production capacity of known producers and the world wide production volume was estimated at 8,000 tons/year and 10,000 tons/year, respectively (TNO and RIVM, 2002), although the US production in the period between 1995 and 2000 averaged less than 1500 tons/year.

Ferro volunteered to carry out the VCCEP Tier I assessment for 1,4-dioxane in accordance with USEPA's request. Because of the availability of data, it was decided to provide the Tier II and III toxicity data along with an interpretation of the information in terms of the potential risk to humans, including children, for cancer and non-cancer endpoints. Exposure to 1,4-dioxane was estimated for relevant media including water, air, food, and consumer products using available data, conservative assumptions, probabilistic modeling, and children's exposure factors as developed by USEPA. Finally, a data needs assessment was performed based on sensitivity analysis of the exposure estimates developed and review of the toxicity database for data gaps and uncertainties with particular focus on children's health issues.

2.0 Identity and Physical-Chemical Properties

2.1 Chemical Name (IUPAC)

1,4-Dioxane

2.2 Registry Numbers

CAS number 123-91-1
EINECS number 204-661-8
EC number 603-024-00-5
RTECS number JG8225000
Shipping Name/ Number DOT/UN/NA/IMO: UN 1165; Dioxane

2.3 Synonyms

- Diethylene dioxide
- Diethylene-1,4-dioxide
- 1,4-Diethylene dioxide
- 1,4-Diethylene oxide
- Diethylene ether
- Di(ethylene oxide)
- 1,4-Dioxacyclohexane
- 1,4-Dioxan
- Dioxan
- *para*-Dioxan
- Dioxane
- *p*-Dioxane
- *para*-Dioxane
- Dioxyethylene ether
- Ethylene glycol ethylene ether
- Glycol ethylene ether
- Tetrahydro-1,4-dioxin
- Tetrahydro-1,4-dioxine
- 1,4-dioxan, Tetrahydro-
- Tetrahydro-*para*-dioxin
- Tetrahydro-*para*-dioxine
- NCI- C03689
- WLN: T50 DOJ
- WLN: T60 DOTJ

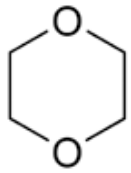
2.4 Trade Names

- 1,4-Dioxan
- NE 220

2.5 Molecular Formula

- $C_4H_8O_2$

2.6 Structural Formula



2.7 Molecular Weight

- 88.10 (Budavari, 1989)

2.8 Manufacture and Composition of 1,4-Dioxane

There are three main ways that 1,4-dioxane can be produced (NICNAS, 1998; TNO and RIVM, 2002):

- acid-catalyzed conversion of diethylene glycol via ring closure, dehydration, and distillation in a closed system. (TNO and RIVM, 2002). The use of mono-, tri-, and polyethylene glycol and their ethers as raw material has also been reported;

- catalyzed cyclodimerization of ethylene oxide (TNO and RIVM, 2002) on acid-ion exchanger resins via oligoethylene sulphonates;

- ring closure of 2-chloro-2'-hydroxyethyl ether (bis(2-chloroethyl)ether) through heating with 20% sodium hydroxide.

The latter processes are especially useful for the production of substituted dioxanes; however, there are no data indicating that these latter processes have any commercial importance.

Industrially, the first production process is the most important one. The manufacture of 1-4-dioxane is carried out at a temperature range of 130 and 200^{o}C and a pressure ranging from

250 to 1,100 hPa with dehydration and purification taking place by distillation (TNO and RIVM, 2002).

To facilitate this type of manufacture, sulphuric acid, phosphoric acid, p-toluenesulphonic acid, zeolites, and strongly acidic ion exchangers are used as catalysts. For continuous synthesis of 1,4-dioxane, a heated vessel is utilized whereby the raw product forms an azeotrope with water and the dioxane is separated by distillation. The main by-products of this process are acetaldehyde and 2-methyl-1,3-dioxalane, and 2-ethyl-1,3-dioxolane and, to a lesser extent, glycol, crotonaldehyde, and polyglycol. The crude 1,4-dioxane is further cleaned by distillation (to remove glycol and acetaldehyde), heating with acids, salting out with NaCl, $CaC1_2$ or NaOH, and fine subsequent distillation (TNO and RIVM, 2002).

1,4-Dioxane is available in the US in reagent, technical, spectrophotometric, and scintillation grades. The specifications for typical commercial products are (Santodonato *et al*, 1985; NICNAS, 1998; HSDB, 2007):

- 1,4-Dioxane — 99.9% minimum.
- 2-Ethyl-1,3-dioxolane — 0.1% max. (1000 ppm)
- 2-Methyl-1,3-dioxolane — not reported (certified), 0.05% max. (tech.)
- Water — 0.02% max. (certified), 0.1% max. (tech.)
- Acidity (as acetic acid) — 0.01% max. (certified), 0.1% max. (tech.)
- Peroxides (as H_20_2) — 0.003% max. (certified), 50 mg/kg max. (tech.)
- Non-volatile matter — 0.004% max. (certified), 0.0025% max. (tech.)
- Suspended matter — nil

Other impurities reported (HSDB, 2007; TNO and RIVM, 2000; NICNAS, 1998) for different grades/sources of product include:

- Bis (2-chloroethyl) ether (starting product)
- Hydroquinone (stabilizer)
- 2,6-Di-tert-butyl-*p*-cresol (stabilizer)
- Acetaldehyde
- Crotonaldehyde
- Paraldehyde
- Glycidol
- Ethylene diformate
- Methyl diformate
- Carbonyl (0.05% max.)
- Iron (0.25 ppm max.)
- Lead (0.25 ppm max.)

2.9 Physical and Chemical Properties

2.9.1 Physical State

1,4-Dioxane is a colorless liquid (or solid below 53ºF) with a mild pleasant, ether-like odor (NTP, 2005). The odor threshold has been reported to be between 6.5 mg/m^3 (NICNAS, 1998) and 9.8 mg/m^3 for detection and approximately 20 mg/m^3 for identification (NIOSH,1977). The most sensitive individual was able to detect 1,4-dioxane at 0.011 mg/m^3 and identify it at 20 mg/m^3 while the least sensitive individual detected it at 612 mg/m^3 and identified it at 972 mg/m^3. The irritating concentration was reported to be between 792 and 972 mg/m^3 (Ruth, 1986). An odor threshold in water was reported to be 230 ppm (Amoore and Hautala, 1983). The conversion factor for 1,4-dioxane (at 25ºC) is 1 mg/m^3 = 0.28 ppm or 1 ppm = 3.60 mg/m^3. The weight per gallon of 1,4-dioxane at 20ºC is 8.61 lb (HSDB, 2007).

2.9.2 Physical and Chemical Properties

The physical and chemical properties of 1,4-dioxane are listed in **Table 2-1**.

Table 2-1. Physical and Chemical Properties of 1,4-Dioxane

Parameter	Value	Source
Boiling point	101.1˚C @ 760 mm Hg	TNO and RIVM (2002), HSDB (2007)
Melting point	11.8˚C	Sax (1989), HSDB (2007)
Distillation range	95-103 ˚C @ 760 mm Hg	HSDB (2007)
Critical Temperature & Pressure	312˚C; 50.7 atm	Budavari (1989)
Density	1.034 kg/L @ 20˚C	NICNAS (1998)
Specific gravity	1.036 @ 20˚C	Santodonato *et al.* (1985)
Viscosity	0.0120 centipoises @ 25˚C	HSDB (2007)
Surface Tension	36.9 dynes/cm @ 25˚C	HSDB (2007)
Spectral Properties	Refraction index: 1.4175@ 20˚C IR: 6181 NMR: 1193 Mass: 155 (intense mass spectral peaks: 58 m/z, 88 m/z)	HSDB (2007)
Vapor density	3.03 (relative to air =1)	NICNAS (1998), HSDB (2007)
Vapor pressure	3.9 kPa @ 20˚C 4.9 kPa @ 25˚C 37 mmHg @ 25˚C	NIOSH (1994) NICNAS (1998) HSDB (2007)
Evaporation rate	7.3 (diethyl ether = 1) 2.7 (butyl acetate= 1)	NICNAS (1998) HSDB (2007)
Latent heat of vaporization	98.6 cal/g	Sax (1989) HSDB (2007)
Partition coefficient (Log K_{ow})	-0.27 to -0.49	Howard (1990) NICNAS, 1998
Henry's law constant	2.8 x 10^{-6} atm/m^3/mol	NICNAS, 1998 Sax (1989)
Adsorption coefficient (Log K_{oc})	1.07	NICNAS, 1998
Autoignition temperature	180˚C (356˚F)	Sax (1989), HSDB (2007)
Heat of combustion	581 Kcal/mol	HSDB (2007)
Flash point	Closed cup: 12˚C (54˚F) Open cup: 23˚C (73˚F)	ECETOC (1983)
Explosive limits	Lower limit: 2% v/v Upper limit: 22% v/v	Sax (1989) HSDB (2007)

2.9.3 Reactivity and Stability

2.9.3.1 Solubility

1,4-Dioxane is infinitely soluble in water and most organic solvents (*i.e.*, alcohol, ether, acetone, benzene, acetic acid). It is also miscible with aromatic hydrocarbons and oils. (HSDB, 2007).

2.9.3.2 Azeotropic Mixtures

Azeotropes can be formed by 1,4-Dioxane in the presence of water and a number of other organic compounds (NICNAS, 1998; HSDB, 2007). **Table 2-2** lists some binary azeotropes of 1,4-dioxane.

Table 2-2. Examples of Binary Azeotropes of 1,4-Dioxane

%1,4-Dioxane (w/w)	2nd Component	% 2nd Component (w/w)	Mixture Boiling Point (˚C)
82	Water	18	87.8
9.3	Ethanol	90.7	78.1
44	Heptane	56	91.8
45	n-Propyl alcohol	55	95.3

2.9.3.3 Hydrolysis

1,4-Dioxane does not form any readily hydrolyzable groups (NICNAS, 1998).

2.9.3.4 Flammability

Highly flammable (NICNAS, 1998).

2.9.3.4.1 Combustion Products

There is the potential for toxic gases and vapors to be released during combustion of 1,4-dioxane (NICNAS, 1998).

2.9.3.5 Reactivity

2.9.3.5.1 Polymerization

1,4-Dioxane does not polymerize (NICNAS, 1998).

2.9.3.5.2 Explosivity

1,4-Dioxane is hygroscopic and reacts with water in the presence of air to form explosive peroxides (NICNAS, 1998; HSDB, 2007). The tendency of 1,4-dioxane to form peroxides may be lessened by the addition of a reducing agent, such as stannous chloride or ferrous sulfate. The following substances form explosive mixtures with 1,4-dioxane:

- Hydrogen and hot Raney nickel
- Silver perchlorate
- Sulfur trioxide
- Nitromethane
- Boron trifluoride
- Decaborane (NICNAS, 1998).

3.0 Environmental Behavior of 1,4-Dioxane

At one time, 1,4-dioxane was produced and sold in the millions of pounds per year. Fifteen million pounds were produced in the US in 1982 alone. While 1,4-dioxane has been used in a great variety of applications because of its unique physical-chemical properties, it has been mainly used as a processing solvent (*i.e.*, waxes, fats, lacquers, paints, varnishes, paint and varnish removers, wetting and dispersing agents in textile processing, cleaning and detergent preparations, adhesives, cosmetics, deodorants, fumigants, emulsions and polishing compositions, dye baths, stain and printing compositions, cements, stabilizer for chlorinated solvents, scintillation fluids, and pulping of wood). It has also been used as an extraction medium for animal and vegetable oils; as a laboratory chemical (eluent in chromatography); as a carrier solvent in plastic, rubber, and insecticides and herbicides; and in surfactants or emulsifiers (HSDB, 2007; TNO and RIVM, 2002; NICNAS, 1998). Other uses include measuring optical activity, cryoscopic determination, as a chemical intermediate, in the manufacture of membrane filters, and as part of a catalyst (plastics polymerization) (NICNAS, 1998; TNO and RIVM, 2002). 1,4-Dioxane has been used as a wetting and dispersing agent for cellulose acetate, ethyl cellulose, benzyl cellulose, resins, oils, waxes, oil and spirit-sol dyes, and it may occur as an impurity in certain surfactants used in foods and cosmetics via ethoxylation reactions. The acid or base catalyzed addition of ethylene oxide as part of the ethoxylation process involved in the production of many anionic, cationic, amphoteric, and nonionic surfactants creates 1,4-dioxane. The impurity can be removed through a stripping process so these unintentional sources may also result in both direct and indirect 1,4-dioxane releases to the environment.

The U.S. production of 1,4-dioxane in 1982 was estimated at 15 million pounds. However, in recent years the production of 1,4-dioxane has dropped off markedly due to decreased demand resulting from phase-out of some of 1,4-dioxane's uses as well as improved recovery in certain industry using the solvent. For example, in 1985 about 90% of the 1,4-dioxane produced in the US was used as a stabilizer for chlorinated solvents, particularly 1,1,1-trichloroethane with the remaining 10% being used as a solvent in various applications. However, at the end of 1995 the use of 1,4-dioxane as a stabilizer (3 to 4%) in 1,1,1-trichloroethane stopped due to the ozone depletion potency of 1,1,1-trichloroethane (TNO and RIVM, 2002). Emissions of 1,4-dioxane arising from its use as a stabilizer for 1,1,1-trichloroethane are expected to be virtually non-existent due to this change in use patterns. Furthermore, increased 1,4-dioxane recovery has reduced overall 1,4-dioxane demand by industries (*e.g.*, pharmaceuticals) using it as a recovery solvent and reaction media for various organic synthesis reactions.

In the late 1970s to mid-1980s, between 5 to 10 firms were identified as producer of 1,4-dioxane (Hartung, 1989). That number has now dropped to one. Since 1995, the sole remaining US producer has manufactured less than 3 million pounds annually, largely to

service one customer. There has also been a sharp drop in the 1,4-dioxane content of cosmetics since 1986, emissions to the environment via this source should also have decreased; however, 1,4-dioxane remains as a minor impurity in several end-products. According to the most recent information from industry, 1,4-dioxane is primarily used in the production processes for flame retardant materials, followed by more limited uses in making specialty chemicals, pharmaceuticals, magnetic tape, adhesives, and others (TNO and RIVM, 2002).

When released into water, 1,4-dioxane is not expected to hydrolyze. Due to the very high water solubility, low partition coefficient, and vapor pressure of this product, a high level of partitioning to the water compartment would be expected (its infinite water solubility precludes estimating the volatilization half-life). Modeling suggests that less than 10% will volatilize from water. Based on its infinite water solubility and low estimated soil sorption partition coefficient, 1,4-dioxane released to soil is expected to leach to groundwater, but by the same token, 1,4-dioxane is not expected to bioaccumulate or bioconcentrate in fish. 1,4-Dioxane is not expected to significantly biodegrade in soil or water, but airborne 1,4-dioxane is expected to degrade fairly quickly. After 3.4 hr, 50% of the dioxane mixed with nitrogen monoxide and subjected to environmental UV radiation had degraded. The half-life for the reaction of 1,4-dioxane with atmospheric hydroxyl radicals was estimated to be 6.69 hours (NICNAS, 1998). The expected products of this reaction are aldehydes and ketones. The NICNAS (1998) and TNO and RIVM (2002) reports give a comprehensive description of the different environmental transformation and degradation routes of 1,4-dioxane. This information is summarized below.

3.1 Water

No volatilization data for 1,4-dioxane from water are available, and since 1,4-dioxane is infinitely soluble in water, a volatilization half-life cannot be estimated. 1,4-Dioxane has a moderate vapor pressure at 25° C (37 mm Hg), therefore, volatilization may be significant; however, the Level I MacKay fugacity model indicates that at equilibrium, 91% of 1,4-dioxane will partition to water, with 9% partitioning to air (NICNAS, 1998). These results compare favorably to those predicted in a Level II fugacity model used in section 6.3 as well as the results of a Level III fugacity model reported by Edwards *et al.* (1999). Additionally, NICNAS (1998) asserts that compounds with a Henry's Law constant in the range of 10^{-5} to 10^{-7} atm/m^3/mol will volatilize only slowly, and the rate is controlled by slow molecular diffusion through air. 1,4-Dioxane has an estimated Henry's Law constant of between 2.3 $\times 10^{-4}$ and 2.8 $\times 10^{-6}$ atm/m^3/mol or 4.34 Pa/m^3/mol (vapor pressure is 49.3 hPa at 25°C) at a temperature of 25°C (Hartung, 1989; NICNAS, 1998; Sax, 1989; TNO and RIVM, 2002). From a measured activity coefficient, a Henry-constant of 0.29 Pa/m^3/mol at 20°C was calculated (TNO and RIVM, 2002). In addition, an experimental value for the air/water partition coefficient at 25°C (log K_{aw}) was determined. This log K_{aw} of -3.70 corresponds to

a Henry's Law constant of 0.49 Pa. (TNO and RIVM, 2002). Howard (1990) also states that the estimated Henry's Law constant of 1,4-dioxane suggests its volatilization will be slow. Overall, these estimates support the conclusion that 1,4-dioxane volatilizes from water at a low rate.

The distribution of 1,4-dioxane in a sewage treatment plant is estimated as follows (log K_{ow}= -0.32 and H= 4.34 Pa-m^3-mol^{-1}):

- Fraction directed to air 0.07
- Fraction directed to water 0.93
- Fraction directed to sludge 0.001
- Fraction degraded 0

These results compare favorably to those estimated by the Level I MacKay fugacity model as well as the Level II and III models (Section 6.3).

Despite 1,4-dioxane's high water solubility and moderate vapor pressure, high removal rates have been found in stripping tests (30 to 70%). These stripping rates are much higher than the estimated fraction directed to air from a sewage treatment plant (7%) (TNO and RIVM, 2002).

In studying the movement of contaminants in groundwater, the retardation factor of a number of organic compounds, including 1,4-dioxane using chloride ions as a marker for comparison was identified. Retardation factors were defined as the ratio of the plume length for chloride to the plume length for the contaminant of interest ($R_f = L_{cl}/L_{org}$). The R_f for 1,4-dioxane was between 1.4 and 1.6 compare to 8.8 for benzene and 23.8 for carbon tetrachloride, suggesting at 1,4-dioxane moves rapidly once it reaches groundwater (Hartung, 1989).

1,4-Dioxane is not expected to biodegrade extensively in the aquatic environment and in several activated sludge experiments, the compound has been classified as relatively undegradable (ATSDR, 2006; Heukelekian and Rand, 1955, Ludzack and Ettinger, 1960; Sasaki, 1978; Kawasaki, 1980; Hartung, 1989; HSDB, 2007). Based upon estimated unacclimated aqueous aerobic biodegradation half-lives, Howard *et al.* (1991) provides half-lives for 1,4-dioxane in surface water ranging from 1 to 6 months, and in ground water ranging from 2 to 12 months. The photooxidation half-life of 1,4-dioxane in water is higher with a range from 67 days to over 9 years (NICNAS, 1998). This half-life is based upon measured rates for reaction with hydroxyl radicals in water. In TNO and RIVM (2002), a study is described the abiotic degradation of 1,4-dioxane with ozone. A half life of 60 hours for 1,4-dioxane in water with an ozone concentration of 10^{-5} mol/L is mentioned. There are no experimental data available on the hydrolysis of 1,4-dioxane (NICNAS, 1998; TNO and RIVM, 2002). Since there are no hydrolyzable groups on this compound and ethers have

been classified as generally resistant to hydrolysis, 1,4-dioxane is not expected to hydrolyse significantly (ATSDR, 2006). With an estimated K_{oc} of 1.07 to 1.23, 1,4-dioxane is also not expected to significantly adsorb on suspended sediments

3.2 Air

The Level I MacKay fugacity model indicates that at equilibrium, 9% of 1,4-dioxane will partition to air (NICNAS, 1998). This is approximately the percentage estimated from the Level II model described in section 6.3. There are two degradation pathways for organic substances in the atmosphere: direct photolysis with UV light and photooxidation through reaction with hydroxyl free radicals or ozone. Studies of direct photolysis of liquid 1,4-dioxane at 185 nm result in the generation of formaldehyde, glycol monovinyl ether, and ethylene. Gas-phase photolysis at 147 nm results in the generation of principally formaldehyde and ethylene. However, since the wavelength of light in the troposphere is greater than 290 nm, photolysis does not occur in the lower atmosphere (TNO and RIVM, 2002, HSDB, 2007).

Any 1,4-dioxane that enters the atmosphere is expected to degrade fairly quickly. 1,4-Dioxane vapor was mixed with NO at 27°C and subjected to UV radiation equal to about 2.6 times the intensity of natural sunlight on a summer day in Freeport, Texas. After 3.4 hr, 50% of the 1,4-dioxane had degraded. The half-life of the reaction of vapor phase 1,4-dioxane with hydroxyl radicals in the atmosphere was estimated to be 6.69 hr. A half-life of 9.6 hr was estimated for the reaction of 1,4-dioxane with hydroxyl radicals in the atmosphere. The products of the reaction of ethers with hydroxyl radicals are likely to be aldehydes and ketones (Howard, 1990). 1,4-Dioxane is photooxidized by aqueous hydroxyl radicals with a half-life of 336 days at pH 7.

A rate constant of $10.8 \pm 1.3 \times 10^{-12}$ cm^3-$molecule^{-1}$-s^{-1} has been determined experimentally for the photo-oxidation reaction of 1,4-dioxane with hydroxyl-radicals (TNO and RIVM, 2002). This rate constant corresponds with an atmospheric lifetime of 36 hours or 1.5 days (hydroxyl-concentration is 5×10^5 $molecule.cm^{-3}$). According to a quantitative structure activity relationship approach, a rate constant of 1.3×10^{-11} cm^3-$molecule^{-1}$-s^{-1} can be calculated, which results in an atmospheric lifetime of 1.2 days (29 hours) (hydroxyl-concentration of 5×10^5 $molecule.cm^{-3}$). These estimates confirm that 1,4-dioxane is expected to have a short persistence in the atmosphere.

3.3 Soil

Alexander (1973) pointed out that ether linkages such as exist in 1,4-dioxane impart resistance to degradation to organic compounds, and soil bacteria have been reportedly unable to use 1,4-dioxane as a sole source of carbon in experiments (Fincher and Payne,

1962). No adsorption data for 1,4-dioxane are available. Using a measured log octanol/water partition coefficient of -0.27, a log soil-sorption coefficient (K_{oc}) of 1.07 to 1.23 was estimated for 1,4-dioxane (NICNAS, 1998, HSDB, 2007). Compounds with a K_{oc} of this magnitude are considered mobile in soil, so 1,4-dioxane may be expected to leach to groundwater if released to soil, especially considering its infinite water solubility and low biodegradation potential. It can be concluded that 1,4-dioxane has a low adsorption potential and thus a high mobility/leaching potential. No data concerning the volatilization of 1,4-dioxane from soil are available. Although the infinite water solubility prevents the estimation of a volatilization half-life, 1,4-dioxane should volatilize at a moderate rate from dry soil based on its low vapor pressure (37 mmHg at 25°C). 1,4-Dioxane is not expected to bioconcentrate in fish or biodegrade in soil or water (Howard, 1990). Little 1,4-dioxane is predicted to occur in soil based on the results of a Level II fugacity model (section 6.3). 1,4-Dioxane may photooxidize at the soil surface. Experimental conditions demonstrated that 1,4-dioxane was readily photo-oxidized in the presence of 17 mM H_2O_2 and UV light, with approximately 96% removal within 2 hours (Kim *et al.*, 2006).

3.4 Biodegradation Potential

As discussed above, experimental evidence and theoretical estimates suggest that 1,4-dioxane is not biodegradable. 1,4-Dioxane has been found to be resistant to biodegradation and has been classified as relatively undegradable. Therefore, 1,4-dioxane is not expected to biodegrade rapidly in the environment. Results of the biochemical oxygen demand test for 1,4-dioxane indicate that negligible oxygen is consumed over a 20-day test period. Furthermore, degradation of 1,4-dioxane was not observed in cultures of sewage microorganisms exposed for one year to wastewater treatment plant effluent containing 1,4-dioxane at concentrations ranging from 100 to 900 ppm (NICNAS, 1998). Although an actinomycete has been identified in 1,4-dioxane adapted sludge as using the substance as a sole carbon and energy source, the occurrence of this organism in sewage sludge is unknown. Experimental studies with an actinomycete, *Amycolata* sp. CB1190, added to planted and unplanted soil demonstrated enhancement of 1,4-dioxane biodegradation, indicating that it would be useful for remediation. The actinomycete was most effective when it was grown and induced on a substrate other than 1,4-dioxane, such as tetrahydrofuran or 1-butanol (Kelley *et al.*, 2001). A strain of fungus (*Cordyceps sinensis*) has also been identified as being capable of using 1,4-dioxane as a sole carbon and energy source, but this fungus is only found in the mountainous regions of China. Using the fungus to degrade 1,4-dioxane, ethylene glycol was reported as a degradation product (Nakamiya *et al.*, 2005). NICNAS (1998) and TNO and RIVM (2002) reviewed several other biodegradation tests performed on 1,4-dioxane. From these standardized (OECD) and non-standardized tests it also concluded that 1,4-dioxane does not undergo biodegradation and is eliminated in part from open systems simply by stripping. 1,4-Dioxane, therefore, is not expected to biodegrade rapidly in the environment.

An *in vitro* study further examined the effects of 20 different bacteria strains on 1,4-dioxane. Two of the strains (*Pseudonocardia dioxanivorans* CB1190 and *P. Benzenivorans* B5) were reported to sustain growth by using 1,4-dioxane as a sole carbon and energy source. These two strains, plus an additional 11 other bacteria strains, were able to degrade 1,4-dioxane. However, all 13 of these strains were initially grown on primary substrate (*i.e.* methane, propane, tetrahydrofuran, and toluene) to activate their capability to express mono- or dioxygenase enzymes. Further experimentation with *Escherichia coli* containing recombinant plasmids coded for specific toluene monooxygenases confirmed the importance monooxygenase in degrading 1,4-dioxane (Mahendra and Alvarez-Cohen, 2006). While this information may be useful for remediation, it does not change the previous expectation about the lack of biodegradation of 1,4-dioxane in the natural environment.

3.5 Bioaccumulation and Bioconcentration Potential

No bioaccumulation or bioconcentration data for 1,4-dioxane are available. Due to its high hydrophilicity and low log K_{ow} (-0.27 to -0.49), the potential for bioaccumulation is considered to be extremely low, and it is assumed that 1,4-dioxane will not bioconcentrate significantly in aquatic organisms (HSDB, 2007). Hartung (1989) estimated the bioconcentration factor (BCF) for 1,4-dioxane as between 0.28 and 0.52 based on the relationship reported by Veith (1979) for BCFs in fish. In the 1986 MITI (Japan) list, 1,4-dioxane is classified having little or no accumulation potential (NICNAS, 1998). This conclusion is further supported by a bioaccumulation study in which very low BCF values (0.2 to 0.7) were found (TNO and RIVM, 2002) as well as the results of the Level II fugacity model described in Section 6.3.

4.0 Hazard Assessment

4.1 Tier 1 Toxicity Data for 1,4-Dioxane

4.1.1 Summary - Acute Toxicity of 1,4-Dioxane

In animals, 1,4-dioxane has low acute toxicity by all routes of exposure in different species. The animal studies are summarized in **Table 4-1**. It should be noted that many of these studies are older with little detail provided and that few of them were performed according to current guidelines or Good Laboratory Practices.

The reported oral LD_{50} for rats is >5,000 mg/kg (varies between 5,170 and 7,339 mg/kg) and the two-hour inhalation LC_{50} is >12,500 ppm (46,000 mg/m^3). The two-hour LC_{50} was 36,700 to 65,000 mg/m^3 for mice. No data were available for dermal LD_{50} in rats, although a level of 8,300 mg/kg reportedly produced no evidence of toxicity in Wistar rats (DeRosa *et al.*, 1996). The dermal LD_{50} in rabbits was >7,000 mg/kg. In animals, the main acute effects are central nervous system (CNS) depression, kidney and liver damage, convulsions, coma, and death. Four hours exposure of rats to 3,660 or 7,320 mg/m^3 1,4-dioxane caused elevated alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and ornithine carbamyl transferase activity. At oral doses of 1,000 and 2,000 mg/kg dose-dependent induction of drug metabolizing enzymes in mice was also seen. Only limited data on irreversible effects after single exposure exist. Clinical effects have been reported in rats at concentrations above 300 mg/kg 1,4-dioxane with subtle effects on CNS function above 1,000 mg/kg. (NICNAS, 1998). Depression of tonic extension after electroshock in rats was seen at concentrations at or above 6,800 mg/m^3 and an oral administration of 1,050 mg/kg caused a decrease in dopamine and serotonin levels in the hypothalamus and a decrease in serotonin in the medulla oblongata. Acute histopathological effects on the liver have been reported above 2,500 mg/kg, but the reversibility of these lesions was not investigated. (NICNAS, 1998).

4.1.1.1 Acute Toxicity of 1,4-Dioxane in Humans

Fairley *et al.* (1934) exposed four volunteers to 1,000 ppm (3,600 mg/m^3) of 1,4-dioxane for five minutes and six volunteers to 2,000 ppm (7200 mg/m^3) for three minutes. Although the odor was detectable, there was no irritation, no tearing, and no desire to cough. No adverse effects were noted in these volunteers. The intensity of the odor decreased over the course of the experiment. In a study of four male volunteers exposed to 50 ppm (180 mg/m^3) for six hours, the only effect reported was eye irritation (Young *et al.*, 1977). Twelve subjects were exposed to 1,4-dioxane for a maximum of 15 minutes and observed olfactory fatigue with a concentration of 720 mg/m^3 (200 ppm) as the highest concentration acceptable. At 1,080 mg/m^3 (300 ppm) for 15 minutes, irritation of eyes, nose, and throat was reported,

although the odor was not recognized (TNO and RIVM, 2002). Volunteers exposed to 5,760 mg/m^3 (1,600 ppm) for 10 minutes or 19,800 mg/m^3 (5,500 ppm) for 1 minute complained of eye, nose, and throat irritation whereas no such effects were reported in volunteers exposed to 3,600 mg/m^3 (1,000 ppm) for 5 minutes or 7,200 mg/m^3 (2,000 ppm) for 3 minutes (DeRosa *et al.*, 1996; Yant *et al.*, 1930).

The effect of 1,4-dioxane on human hemoglobin was investigated spectrophotometrically. At concentrations of 0.1-0.5% oxyhemoglobin was converted into methemoglobin. At concentrations of 10-20% a hemoglobin-1,4-dioxane complex formation occurred in addition to the methemoglobin conversion. Protein coagulation occurred as the 1,4-dioxane concentration was further increased (40%) (TNO and RIVM, 2002).

4.1.1.2 Acute Toxicity of 1, 4-Dioxane in Experimental Animals

A number of acute lethality studies have been conducted with 1,4-dioxane using different routes of administration. Based on the results of these tests, 1,4-dioxane would be considered to have low acute toxicity. The acute toxic effects reported in animals are primarily CNS depression, kidney, and liver damage. (NICNAS, 1998). 1,4-Dioxane was observed to produce anesthetic effects (*i.e.*, narcosis) at LD_{50} or higher doses, progressing from weakness, depression, incoordination, and coma to death. Autopsies usually revealed hemorrhagic areas in the stomach and enlarged kidneys (Gingell *et al.*, 1994). The acute lethality of 1,4-dioxane is summarized in **Table 4-1**.

Overt CNS effects including convulsions have been reported in rabbits administered 5 ml (2,060 mg/kg) of 1,4-dioxane via intravenous (iv) solution (Ware, 1988). Subtle effects on CNS function, as assessed by changes in various neurotransmitters in male Sprague-Dawley rats was reported following an oral dose of 1,050 mg/kg 1,4-dioxane (NICNAS, 1998). Using the electrically evoked seizure discharge (a sensitive indicator of neurotropic effects), a 30% depression in response was observed following inhalation of 1,860 ppm (four-hour) and 2,400 ppm (two-hour) of 1,4-dioxane in rats and mice, respectively (Frantik *et al.*, 1994).

Acute renal effects are generally reported as glomerular and tubular damage characterized by slight proteinuria clinically and by tubular cell vacuolation and necrosis histologically (Ware, 1988; NICNAS, 1998). A study by Fairley *et al.* (1934) reported degeneration of the renal cortex and medulla (plus hemorrhaging) in rabbits up to one month following iv administration of 400 to 2,000 mg/kg 1,4-dioxane. Significantly increased levels of aniline hydroxylase in kidney microsomes were reported in rats exposed via gavage to 2,000 mg/kg 1,4-dioxane. A western blot reported a 8-fold increase in CYP2E1 protein band in the kidney microsomes compared to control and northern blot analyses detected increased levels of CYP2E1 mRNA (Nannelli *et al.*, 2005).

Table 4-1. Summary of Acute Toxicity Studies of 1,4-Dioxane

Route	Species	Results	Source
	Rat	2,000 mg/kg (gavage)	Nannelli *et al.*, 2005
Oral	Rat	5400-7300 mg/kg (LD_{50})	DeRosa *et al*., 1996
	Rat	5345 mg/kg (LD_{50})	Laug *et al*., 1939
	Rat	approx. 6200 mg/kg (LD_{50})	Nelson, 1951
	Rat	approx. 5170 mg/kg (LD_{50})	TNO and RIVM, 2002
	Rat	6370 mg/kg (LD_{50})	Pozzani *et al*.,1959
	Rat	6500 mg/kg (LD_{50})	TNO and RIVM, 2002
	Rat	7339 mg/kg (LD_{50})	Smyth *et al*.,1941
	Mouse	5700 mg/kg (LD_{50})	ECETOC, 1983
	Mouse	5850 mg/kg (LD_{50})	Laug *et al*., 1939
	Mouse (m)	4500 mg/kg (MLD_4)	Mirkova, 1994
	Mouse (f)	>5000 mg/kg (MLD_4)	Mirkova, 1994
	Guinea Pig	3256 mg/kg (LD_{50})	Smyth *et al*.,1941
	Guinea Pig	4000 mg/kg (LD_{50})	Laug *et al*., 1939
	Guinea Pig	1270-3900 mg/kg (LD_{50})	NICNAS, 1998
	Rabbit	2000 mg/kg (LD_{50})	DeRosa *et al*., 1996
	Rabbit	6500 mg/kg (LD_{50})	Knoefel, 1934
	Rabbit	2100 mg/kg (LD_{50})	Nelson, 1951
	Cat	2000 mg/kg (LD_{50})	Gingell *et al.*, 1994
Inhalation	Rat	46000 mg/m^3 (2hr LC_{50})	ECETOC, 1983; TNO and RIVM, 2002
	Rat (f)	51300 mg/m3 (4hr LC_{50})	Pozzani *et al*., 1959: DeRosa *et al*., 1996
	Mouse	37000 mg/m^3 (2hr LC_{50})	TNO and RIVM, 2002
	Mouse	65000 mg/m^3 (2hr LC_{50})	ECETOC, 1983
	Cat	44000 mg/m^3 (7hr lethal dose)	TNO and RIVM, 2002
Dermal	Rabbit	7855 mg/kg (LD_{50})	TNO and RIVM, 2002
	Rabbit	7600 mg/kg (LD_{50})	DeRosa *et al.*, 1996
	Rat	>8300 mg/kg (lethal dose)	DeRosa *et al*., 1996

Route	Species	Results	Source
IV	Rabbit	1500 mg/kg (LD_{50})	TNO and RIVM, 2002
IP	Rat	2,000 mg/kg	Nannelli *et al.*, 2005
	Rat	5300 mg/kg	Appel, 1988
	Mouse	790 mg/kg	Karel *et al.*, 1947
	Mouse	4100 mg/kg	NICNAS, 1998
	Mouse	5790 mg/kg	TNO and RIVM, 2002
	Rat (f)	3976-5910 mg/kg (LD_{50})	Lundberg *et al.*, 1986

m = male
f = Female
MLD = 4 day minimum lethal dose

Acute hepatic effects include increased serum enzymes, glutamic oxalacetic transaminase, glutamic pyruvic transaminase, ornithine carbamyl transferase and sorbitol dehydrogenase, at an estimated 600 mg/kg in an inhalation test while increased cytochrome P-450 activity and vacuolar degeneration were noted at an oral dose in excess of 2,500 mg/kg (Kitchin and Brown, 1990; NICNAS, 1998). Additionally, increased aniline hydroxylation, p-nitrophenol hydroxylation, erythromycin N-demethylase, pentoxyresorufin O-dependentylase (PROD), and lauric acid hydroxylase was observed in rat liver microsomes after exposure via oral gavage at a dose level of 2,000 mg/kg. While these particular enzymes are associated with CYP2E1, no effect was observed with ethoxyresorufin O-deethylase (EROD) or cytochrome P-450 (CYP) levels. 1,4-Dioxane was also able to increase induction of liver microsome 2B1/2-dependent 16 β-testosterone hydroxylase (by approximately 20 fold), 17OT-testosterone hydroxylase, 16α-testosterone hydroxylase, and 2α-testosterone hydroxylase linked to CYP2C11. CYP3A-linked 6 β-testosterone hydroxylase was only weakly induced. Western blot analysis reported significantly increased levels of CYP2E1 protein band. In rats pre-induced with CYP2E1 or 2B1/2, 1,4-dioxane (2,000 mg/kg via intraperitoneal) did not cause any effects on hepatic glutathione (GSH) depletion or ALAT activity under normal exposure conditions. However, when the control and exposed rats were fasting, a decrease in hepatic GSH levels was reported (Nannelli *et al.*, 2005). However, no overt symptoms or histopathology were observed in rat liver following administration of 1,000 mg/kg 1,4-dioxane by gavage or 8,300 mg/kg applied dermally (DeRosa *et al.*, 1996; NICNAS, 1998).

Other organs affected following acute exposures include spleen, thymus, lungs (pulmonary congestion and atelectasis), brain (edema), and blood dyscrasias (leucocytosis and anisocytosis) (NICNAS, 1998).

4.1.1.2.1 Acute Oral Toxicity of 1,4-Dioxane in Experimental Animals

Signs of toxicity after oral administration to rats, mice and guinea-pigs included narcotic effects, coma, irritation of the gastrointestinal mucous membranes, and damage to liver and kidneys (Laug *et al.*, 1939; Nelson, 1951; Smyth *et al.*, 1941), while in rabbits, dose-related narcotic effects were also seen (Nelson, 1951). After a single oral dose of 5.66, 5.17 or 3.9 g/kg 1,4-dioxane to mice, rats and guinea pigs, symptoms progressed from weakness, depression, incoordination, and coma to death. Autopsy revealed hemorrhagic areas in pyloric region of the stomach, bladders distended with urine, enlarged kidneys, and slight proteinuria, but no hematuria (Gingell *et al.*, 1994). 1,4-Dioxane (2 g/kg) administered orally increased liver microsomal protein content significantly in male and female mice (Mungikar and Pawar,1979). The acute lethal dose of 1,4-dioxane following oral dosing has been determined in a number of species. In the rat, DeRosa *et al.* (1996) reported the oral LD_{50} to range between 5,400 and 7,300 mg/kg. This is similar to the oral LD_{50} reported by TNO and RIVM (2002) for rats of 5,170 mg/kg. Mice exhibited a similarly high oral LD_{50} of 5,700 mg/kg (ECETOC, 1983). Mirkova (1994) reported that a 4-day minimum lethal dose (MLD_4) for male and female mice was 4,500 mg/kg and >5,000 mg/kg, respectively. In the guinea pig, the oral LD_{50} was reported to range from1,270 to3,900 mg/kg (NICNAS, 1998) while the oral LD_{50} in both the rabbit and cat was reported to be 2,000 mg/kg (Gingell *et al.*, 1994; DeRosa *et al.*, 1996).

After implantation with [$^{6\text{-}3}$H]thymidine, groups of four male Sprague-Dawley rats received single gavage doses of 0, 10, 100, or 1,000 mg/kg 1,4-dioxane in saline, were sacrificed after seven days, and their livers were examined. No hepatic cytotoxicity was observed as judged by the lack of significant changes in organ to body weight ratios, the amount of DNA/g tissue, the rate of DNA synthesis as measured by [$^{6\text{-}3}$H]thymidine incorporation, or the presence of histopathological changes in the liver (Stott *et al.*, 1981). Groups of five male mice received 0, 500, 1000, or 2,000 mg 1,4-dioxane/kg administered orally once daily for two days. One day after the last dose, the animals were sacrificed and the livers were examined. At 1,000 and 2,000 mg/kg, the relative liver weights were increased and microsomal protein content in the liver was also increased. The same dose levels enhanced the rate of *in vitro* metabolism of aminopyrine, ethylmorphine, and acetanilide substrates and increased levels of microsomal NADPH cytochrome c reductase and cytochrome P450 content (TNO and RIVM, 2002).

4.1.1.2.2 Acute Inhalation Toxicity of 1, 4-Dioxane in Experimental Animals

Irritation of the eyes, nose and lung has been reported following inhalation of 1,4-dioxane (>2000 ppm) in guinea pigs, mice, and cats (Wirth and Klimmer, 1937; ACGIH, 1991). Groups of six Sprague-Dawley rats (three/sex) were exposed to a nominal concentration of 155,000 mg/m^3 for one, three, and seven hours and then were observed for 14 days. After one

hour of exposure, no deaths had occurred, after three hours 6/12 animals died, and after seven hours 4/18 animals (the discrepancy in number of animals exposed is unexplained). Effects observed after inhalation exposure included dyspnea, apathy, narcosis, irritation of mucous membranes of the eyes and respiratory tract, eyelid reflex-loss, unkempt coat, and staggering as well as acute heart dilatation, hemorrhagic erosion of the mucous membranes of the stomach, and bloody contents in stomach and intestines (TNO and RIVM, 2002)..

Fairley *et al.* (1934) exposed rats, mice, rabbits, and guinea pigs to concentrations of 1,4-dioxane ranging from 3,600 to 36,000 mg/m^3 for 3 to 202.5 hours (8.5 days). The primary organs affected were the kidney and liver. The lungs were affected only at very high concentrations.

Grasso *et al.* (1984) reviewed a study that exposed rats via inhalation to dioxane at concentrations of 1,500, 3,000, and 6,000 ppm (5,400, 10,800 and 21,600 mg/m^3, respectively) for 4 hours/day, 5 days per week for 2 weeks. After two days, all rats exposed to the two higher concentrations reported an inhibited avoidance response to the buzzer, but there was no effect observed on the escape response. However, the effect dissipated and some animals reported a complete recovery by the end of the two weeks of treatment.

Guinea pigs exposed to 3,660, 7,320, 10,980, 36,600, and 109,800 mg/m^3 for a maximum of eight hours showed irritation of the mucous membranes of the nose and eye. The highest concentration caused mortality within two days. Male rats exposed two times for four hours within one day to 3,660 or 7,320 mg/m^3 1,4-dioxane exhibited elevated serum enzymes ALAT, ASAT, and ornithine carbamyl transferase activities (TNO and RIVM, 2002). In terms of acute lethality, the two hour and four hour LC_{50} in rats and female rats was reported as 12,780 ppm (46,000 mg/m^3) and 14,250 ppm (51,300 mg/m^3), respectively (ECETOC, 1983; DeRosa *et al.*, 1996). In the mouse, the two hour LC_{50} was reported to be 18,000 ppm (65,000 mg/m^3) (ECETOC, 1983) while a seven hour lethal dose in the cat was observed at 10,900 ppm (44,000 g/m^3) (TNO and RIVM, 2002).

4.1.1.2.3 Acute Dermal Toxicity of 1,4-Dioxane in Experimental Animals

Clark *et al.* (1984) tested three doses of 1,4-dioxane (2.1, 4.2, and 8.3 g/kg) using male COBS/Wistar Rats (two/dose). Using non-occluded methods, 2.0 ml of the compound was applied to shaved areas on the backs and flanks of the animals. "Toby" collars were used to prevent grooming. After 24 hours, the skin was washed and the animals observed for 14 days. No mortality resulted from this treatment and no systemic toxicity or irritation was observed. A dermal LD_{50} has been reported for 1,4-dioxane in the rabbit of 7,600 mg/kg (Hartung, 1989, DeRosa *et al.*, 1996) while the lethal dose of 1,4-dioxane in the rat was in excess of 8,000 mg/kg. No clinical signs or symptoms were reported in these tests.

4.1.1.2.3.1 Dermal Irritation and Sensitization of 1,4-Dioxane

1,4-Dioxane is not generally irritating to the skin. However, being a fat solvent, 1,4-dioxane can cause eczema upon prolonged or repeated contact. Isolated cases of 1,4-dioxane-induced skin irritation have been seen in workers. Two skin tests carried out in rabbits indicate that 1,4-dioxane is a mild skin irritant; however, one test was carried out on unoccluded skin and the other did not provide sufficient study details. Insufficient details were available to assess the reported lack of irritation from repeated dermal application of 1,4-dioxane in rabbits, guinea pigs and mice. In particular, these studies did not report whether doses were applied under occlusion. A few cases of eczema and dermatitis (including a positive patch test response to 1,4-dioxane) have been reported in humans following repeated exposure to 1,4-dioxane. However, these cases would appear to be circumstantial or idiosyncratic in nature. 1,4-dioxane was not a sensitizer in a well conducted guinea pig maximization test (TNO and RIVM, 2002).

4.1.1.2.3.1.1 Dermal Irritation and Sensitization of 1,4-Dioxane in Humans

According to Gingell *et al.* (1994) 1,4-dioxane is a fat solvent and prolonged and repeated contact can cause skin irritation and eczema in humans. A 52-year old man who developed dermatitis on his left hand after daily dipping in a 1,4-dioxane based containing solvent for three years scored positive in a patch test (0.5% in water). No irritation was reported of neat 1,4-dioxane on the skin although a slight burning sensation was noted on mucous membranes of the mouth however, no details were presented (TNO and RIVM, 2002).

No controlled studies have been conducted to evaluate the sensitization potential of 1,4-dioxane in humans. Several weeks of dermal exposure to 1,4-dioxane resulted in inflammatory skin changes in the upper extremities and to a lesser extent in the face of a 47 year old female laboratory technician (NICNAS, 1998). Histological examinations of the skin irritation showed signs of eczema and renewed exposure, some four weeks later, led to a relapse with clinical symptoms of eczema. However, it was concluded from negative results on two other volunteers that this reaction was idiosyncratic and may have been related to a previously sustained chemical burn, which is a confounder in assessing the skin changes. A single positive patch test response to 1,4-dioxane was reported in a worker presenting with dermatitis apparently caused by skin contact with 1,4-dioxane used as a degreasing solvent (NICNAS, 1998).

4.1.1.2.3.1.2 Dermal Irritation and Sensitization of 1,4-Dioxane in Experimental Animals

In an epicutaneous study in rabbits (one of each sex), a 2.5 × 2.5 cm cotton patch was soaked with undiluted 1,4-dioxane (approximately 0.5 ml) and applied to the shaven back (for 1, 5,

and 15 minutes as well as for 20 hours) and on the ear (for 20 hours) under occlusive conditions. Application to the skin for 1 to15 minutes caused very slight erythema after 24 hours and slight scale formation after 8 days. This scale formation is most likely caused by the defattening properties of 1,4-dioxane. One day after the 20 hours application, slight erythema and slight edema were observed on the back of one animal. Seven days later moderate scale formation was seen. On the ear slight erythema was observed 24 hours as well as 8 days after the 20 hours application although scores were not provided (TNO and RIVM, 2002).

A special irritation test using six Wister rats and six ddY mice (three/sex) indicated that the lowest irritating concentration was 80% 1,4-dioxane in physiological saline. However, more data about concentrations, as well as scores, were not presented (TNO and RIVM, 2002). Mild irritation was also observed in rabbit skin following an application of 515 mg 1,4-dioxane in an open Draize Test. However, skin irritation was not seen in rats exposed (unoccluded) to 8,300 mg/kg 1,4-dioxane (NICNAS, 1998).

Evidence of skin irritation was also not seen in guinea pigs, rabbits or mice following repeated dermal exposure to 1,4-dioxane at concentrations above 50 mg applied two or three times per day in studies ranging from 50-100 days (NICNAS, 1998).

In a maximization test (performed to OECD guidelines), 1,4-dioxane did not show skin-sensitizing properties. After a pre-test, in which undiluted 1,4-dioxane caused no skin irritation, B6 female Pirbright White guinea pigs were induced with 5% (injection) and 100% (epidermal) 1,4-dioxane for the main test. Upon intradermal induction, well-defined signs of erythema and edema were observed while percutaneous induction resulted in incrustation, well-defined erythema and slight edema. However, the percutaneous results were caused by the intradermal induction. After the challenge with the undiluted 1,4-dioxane, no sensitization reactions were observed. 1,4-Dioxane, therefore, is unlikely to be a sensitizing agent (TNO and RIVM, 2002).

4.1.1.2.3.2 Irritancy of 1,4-Dioxane

1,4-Dioxane is an irritant of the eye and the respiratory tract. Eye irritation has been reported in humans exposed to 50 ppm (180 mg/m^3) 1,4-dioxane for six hours. Acute 1,4-dioxane exposure causes slight irritation of eyes, nose, and throat in humans above 280 ppm (1,000 mg/m^3), a concentration not recognizable by odor, with more severe irritation occurring above 1,400 ppm (5,000 to 10,000 mg/m^3). Acute eye irritation (transient corneal damage) has been reported in animals (rabbits and guinea pigs) from liquid and vapor 1,4-dioxane. Respiratory irritation (nose and lung) has been reported in guinea pigs (above 2,000 ppm (7,000 mg/m^3) 1,4-dioxane), mice, and cats (NICNAS, 1998).

4.1.1.2.3.2.1 Eye and Respiratory Irritancy of 1,4-Dioxane in Humans

Immediate slight burning of the eyes accompanied by lacrimation and slight irritation of the nose and throat was reported for an exposure of 5,760 mg/m^3 for 10 minutes. After exposure to 19,800 mg/m^3 for 1 minute, eye irritation and burning sensation in the nose and throat were noted. At 36,000 mg/m^3, pulmonary irritation occurred (TNO and RIVM, 2002).

Eye irritation has been reported after inhalation exposure. In a study of four male volunteers exposed to 50 ppm (180 mg/m^3) for six hours, the only effect reported was eye irritation (Young *et al.*, 1977). In subjects exposed to concentrations ranging from 2.5 to 10,000 mg/m^3 (0.7 to 2,800 ppm) for unspecified durations, slight mucous membrane and throat irritation was reported at 1,000 mg/m^3 (280 ppm), becoming more severe (*i.e*., strong throat irritation) at 5,000 and 10,000 mg/m^3 (1,400 and 2,800 ppm) (Wirth and Klimmer, 1937; DeRosa *et al.*, 1996). Twelve subjects were exposed to 1,4-dioxane for a maximum of 15 minutes to observe olfactory fatigue with a concentration of 720 mg/m^3 (200 ppm) was the highest concentration acceptable. At 1,080 mg/m^3 (300 ppm) for 15 minutes, irritation of eyes, nose, and throat was reported, although the odor was not recognized (TNO and RIVM, 2002). Volunteers exposed to 5,760 mg/m^3 (1,600 ppm) for 10 minutes or 19,800 mg/m^3 (5,500 ppm) for 1 minute complained of eye, nose, and throat irritation whereas no such effects were reported in volunteers exposed to 3,600 mg/m^3 (1,000 ppm) for 5 minutes or 7,200 mg/m^3 (2,000 ppm) for 3 minutes (DeRosa *et al.*, 1996; Yant *et* al., 1930).

4.1.1.2.3.2.2 Eye Irritancy of 1,4-Dioxane in Experimental Animals

1,4-Dioxane has been reported to have a miotic effect in rabbits at concentrations (not provided) below that causing alterations in the conjunctiva or cornea, with pupils returning to normal 10 to 15 minutes after administration. Liquid 1,4-dioxane has been reported to cause eye irritation in rabbits (NICNAS, 1998). Two male White Vienna rabbits received an installation of 0.05 ml undiluted 1,4-dioxane for an undetermined exposure. One day after instillation, slight corneal opacity and conjunctival redness as well as slight to severe chemosis (swollen eyes and eyelids) were observed in both rabbits. Additionally, smeary deposition was noted. Eight days after application, at study termination, slight conjunctival redness was observed in one animal. The authors suggested that this finding was expected to reverse if the observation period had been longer. In addition, this animal showed small retraction of the eyelid. Because the dose level is very low in comparison to the current guidelines and only two animals were used, 1,4-dioxane is considered as an eye irritant (TNO and RIVM, 2002).

In guinea pigs, both liquid 1,4-dioxane (10 ml) and exposure to 2,000 ppm 1,4-dioxane vapor produced eye irritation. In addition, damage to rabbit cornea induced by 1,4-dioxane that correlated with *in vitro* studies on bovine cornea opacity (NICNAS, 1998). In an *in vitro* test

with isolated bovine cornea, irritation, including changes in opacity and thickness of the isolated cornea, were observed at 1,4-dioxane concentrations of 5-100% (TNO and RIVM, 2002).

4.1.1.2.3.2.3 Respiratory Irritancy of 1,4-Dioxane in Experimental Animals

Groups of six Sprague-Dawley rats (three/sex) were exposed to a nominal concentration of 155,000 mg/m^3 for one, three, and seven hours and then were observed for 14 days. After one hour of exposure, no deaths had occurred, after three hours 6/12 animals died, and after seven hours 4/18 animals (the discrepancy in number of animals exposed is unexplained). Effects observed after exposure included irritation of the respiratory tract in rats. In this study, histopathology was performed which indicated that the animals that died showed swollen lungs. No other details were presented (TNO and RIVM, 2002).

Gingell *et al.* (1994) cite two studies, one in which guinea pigs were exposed for three hours to concentrations of 1,000 to 30,000 ppm 1,4-dioxane, and another in which rats, mice, guinea pigs, and rabbits were exposed for eight hours to 1,4-dioxane concentrations of 4,000 to 11,000 ppm. At the higher concentrations, marked irritation of the mucous membranes was apparent and deaths occurring during exposure or shortly afterward were usually due to respiratory failure because of lung edema, but the animals also exhibited congestion of the brain. Delayed deaths were due to pneumonia. Histological evidence of liver and kidney toxicity was observed in animals that died after exposure as well as in surviving animals evaluated several days after exposure (TNO and RIVM, 2002).

4.1.1.2.4 Acute Toxicity of 1,4-Dioxane in Experimental Animals *by Other Routes*

Administration via other routes resulted in LD_{50} values for rats of 799 to 5,600 mg/kg (intraperitoneal or ip) (Woo *et al.*, 1978; Argus *et al.*, 1973) and for the mouse of 4,350 mg/kg (subcutaneous or sc). After ip administration of 1,4-dioxane to mice, a LD_{50} of approximately 5,790 mg/kg was derived and observed effects before death included dyspnea, narcosis, convulsions, and ventral body position. Microscopic examination revealed a discolored liver (TNO and RIVM, 2002). 1,4-Dioxane (2 g/kg) administered ip increased liver microsomal protein content significantly in male and female mice. Male mice injected ip showed significant increase in cytochrome B5 and P450 contents as compared to female (Mungikar and Pawar,1979). A single iv injection of 1,4-dioxane in guinea pigs, rabbits, and cats caused a selective action on convoluted tubules of kidney characterized by acute hydropic degeneration. Deaths were due to uremia caused by intrarenal obstruction and anuria (Gingell *et al.*, 1994). The iv LD_{50} of 1,4-dioxane in rabbits was reported to be 1,500 mg/kg (TNO and RIVM, 2002) while the ip LD_{50} of 1,4-dioxane in rats was determined to 5,300 mg/kg (Appel,1988) and between 3,976 to 5,910 mg/kg in female rats (Lundberg *et al.*, 1986). In mice, the LD_{50} of 1,4-dioxane following ip administration ranged from a low

of 790 mg/kg or 8.97 mM/kg in early testing (Karel *et al.*, 1947) to 4,100 and 5,790 mg/kg in more recent studies (TNO and RIVM, 2002; NICNAS, 1998).

4.1.1.3 Summary - Mutagenicity of 1,4-Dioxane

The genotoxicity studies conducted with 1,4-dioxane are summarized in **Tables 4-2, 4-3, and 4-4**. *In vitro,* clastogenic and mutagenic effects were not reported. Negative results were seen in all Ames Salmonella reverse mutation assays; *in vitro* 'germ cell' cytogenetic assays; all *in vitro* unscheduled DNA synthesis (UDS) assays as well as other miscellaneous *in vitro* genotoxicity assays. *In vivo,* a dominant lethal assay was negative as was a test for sex linked recessive lethals in *Drosophila melanogaster* (except at high doses). Meiotic non-disjunctions were also reported in the progeny of *Drosophila melanogaster* after oral exposure to 1,4-dioxane. From the seven micronucleus tests performed, only two tests orally performed with C57BL6 or CD-1 mice showed a positive result. Three other oral tests using C57BL6, BALB/c, and CBA mice and two ip tests with B6C3F 1 and CD-1 mice showed negative results. In four of these negative tests, the target organ was reached. Results from *in vitro* as well as *in vivo* alkaline elution tests points to DNA strand breaks at high dose levels. 1,4-Dioxane can also induce sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells and cell transformation in BALB/3T3 cells (TNO and RIVM, 2002).

This series of tests would be expected to identify most genotoxic chemicals (NICNAS, 1998). A feature of the assays for DNA effects (*in vitro* and *in vivo*) in which 1,4-dioxane displayed positive results was that the effects were mainly seen at cytotoxic concentrations. 1,4-Dioxane produced positive results in replicative DNA synthesis (RDS) at high doses in one study (NICNAS, 1998), while another study reported only equivocal results at the highest dose tested after 24 hours (Uno *et al.*, 1994). Additionally, positive results were observed with 1,4-dioxane for cell transformation, DNA synthesis-inhibition (Heil and Reifferscheid, 1992), and gap-junction intercellular communication (GJIC) assays (Chen *et al.*, 1984), all of which have been used to screen for non-genotoxic carcinogens and, in particular, tumor-promoting agents (Swierenga and Yamasaki, 1992). 1,4-Dioxane has also been shown to inhibit transcription regulation (RNA-polymerase activity) *in vivo* (Kurl *et al*, 1981), an effect that has been linked with non-mutagenic carcinogenesis (NICNAS, 1998).

Table 4-2. Summary of Gene Mutation Assays of 1,4-Dioxane

Assay	*In Vitro*/ *In Vivo*	Test System	Dose	Result	Source
Ames Test (reverse mutation)	*In Vitro*	*S. typhimurium.* (TA1535, TA1537, TA98, TA100)	100, 333, 1000, 3333, 10000 µg/plate	Neg. (+ or - MA); consistent results between 2 labs	Haworth *et al*., 1983
Ames Test (reverse mutation)	*In Vitro*	*S. typhimurium.* (TA1535, TA100)	10-103 mg/vessel	Neg. (+ or - MA); dioxane added to open vessels	Nestmann *et al.*, 1984
Ames Test (reverse mutation)	*In Vitro*	*S. typhimurium.* (TA1535, TA1537, TA1538, TA98, TA100)	0-103 mg/plate	Neg. (+ or - MA); cytotoxicity at 62 mg/plate w/o MA	Stott *et al*., 1981
Point Mutation	*In Vitro*	*S. cerevisiae*	1.4% - 4.31%	Neg. (severe effects on cell morph at 3%)	Zimmermann *et al*., 1985
Mammalian cell gene forward mutation assay	*In Vitro*	Mouse (L5178Y) lymphoma cells	312-5000 µg/ml	Neg. (+ or - MA); Duplicate trials , no toxic effects	McGregor *et al*., 1991

Table 4-3. Summary of Miscellaneous Genotoxicity Assays of 1,4-Dioxane

Assay	*In Vitro/ In Vivo*	Test System	Dose	Result	Source
DNA damage (alkaline elution)	*In Vitro*	Rat hepatocytes	0.03 -30.0 mM	Pos. (max response at 3 mM w/ >30% cytotoxicity)	Sina *et al.*, 1983
DNA repair	*In Vitro*	Rat primary hepatocytes	0.001 - 1.0 mM (animals pretreated with 1-2% dioxane in water fro 1 wk)	Neg.	Goldsworthy *et al.*, 1991
Differential DNA Repair	*In Vitro*	*E. coli* (K12- uvrB/recA) measures differential lethality in DNA repair deficient and proficient bacteria	1150 mM/L	Neg (+ or - MA)	Hellmer and Bolcsfoldi, 1992
Unscheduled DNA synthesis	*In Vitro*	Rat primary hepatocytes	0.001 - 1.0 mM (animals pretreated with 1-2% dioxane in water for 1 wk)	Neg.	Goldsworthy *et al.*, 1991
Unscheduled DNA synthesis	*In Vitro*	Rat primary hepatocytes	10-8 - 1 M (88 mg/ml)	Neg. (+ or - MA)	Stott *et al.*, 1981; NICNAS, 1998
Miotic recombination	*In Vitro*	*S. cerevisiae* (D61.M)	1.48%-4.31%	Neg. (severe effects on cell morphology at 3%)	Zimmermann *et al*., 1985
DNA synthesis inhibition	*In Vitro*	HeLa S3; many nongenotoxic carcinogens pos. in this test	DI50 = 400 mM/L	Pos.	Heil and Reifferscheid, 1992

Assay	*In Vitro/ In Vivo*	Test System	Dose	Result	Source
Sex-linked Recessive Lethal	*In Vivo*	*D. melanogaster*	35000 ppm (feed) & 50000 ppm (inj.) for 3 days	Neg. (pos = >0.2% lethals)	Yoon *et al*., 1985
Aneuploidy induction	*In Vitro*	*S. cerevisiae* (D61.M)	1.48%-4.31%	Neg. (severe effects on cell morphology at 3%)	Zimmermann *et al*., 1985
Cell transformation assay	*In Vitro*	BALB/3T3 mouse cells	0.25-4.0 mg/ml; treated at 48 hrs & 13 days	Pos. (- MA); type II foci induced at 0.5 & 2 mg/ml (indicates transformation)	Sheu *et al*., 1988
Cell transformation assay	*In Vitro*	SA7/SHE test system	62-100 µl/ml	Neg. (no increase in frequency of viral transformed foci)	Heidelberger *et al*., 1983
Gap Junction Intracellular Communication	*In Vitro*	Chinese Hamster V79 cells	5-80 µl/5 ml	Pos. (above 10 µl/5ml incr recovery of HGPRT cells	Chen *et al*., 1984
DNA alkylation	*In Vivo*	Male rat hepatic DNA	1000 mg/kg (4 hrs prior to sacrifice)	Neg.	Stott *et al*., 1981; NICNAS, 1998
DNA damage (alkaline elution)	*In Vivo*	Female rat hepatocytes	0, 168, 840, 2550, or 4200 mg/kg (gavage 21 & 4 hrs before sacrifice)	Weakly Pos. (43-50% hepatic DNA damage at 2 highest doses w/o cytotoxicity)	Kitchin and Brown, 1990
DNA damage	*In Vivo*	Male rat kidney cells	Dose = approx. 1/2 LD_{50}	Neg.	NICNAS, 1998

Assay	*In Vitro/ In Vivo*	Test System	Dose	Result	Source
Unscheduled DNA synthesis	*In Vivo*	Male rat hepatocytes	1000 mg/kg (2 -12 hrs prior to sacrifice) or up to 2% in water for 1 wk	Neg.	Goldsworthy *et al*., 1991
Unscheduled DNA synthesis	*In Vivo*	Rat hepatocytes	10,100, 1000 mg/kg (gavage 7 days prior to sacrifice)	Neg.	Stott *et al*., 1981
Unscheduled DNA synthesis	*In Vivo*	Rat hepatocytes	10 & 1000 mg/kg-d in water for 11 wks	Neg. at 10 mg/kg; Pos. at 1000 mg/kg	Stott *et al*., 1981
Unscheduled DNA synthesis	*In Vivo*	Male rat nasal epithelium (from nasoturbinate or maxilloturbinate)	1% in water for 8 days and 10-1000 mg/kg by gavage 12 hr prior to sacrifice	Neg.	Goldsworthy *et al*., 1991
Replicative DNA synthesis	*In Vivo*	Male rat nasal epithelium (from nasoturbinate or maxilloturbinate)	1% in water for 2 wks	Neg.	Goldsworthy *et al*., 1991
Replicative DNA synthesis	*In Vivo*	Male rat hepatocytes	1 gavage dose of 1000 mg/kg or 1% in water for 2 wks	Neg. (one dose); Pos. (repeat dose - 2x incr. in labeling index)	Goldsworthy *et al*., 1991
Replicative DNA synthesis	*In Vivo*	Male rat	1000 & 2000 mg/kg (single oral dose at 24, 39 & 48 hrs)	Equivocal (1.1% RDS incidence in hepatocytes after 24 hrs w/o decrease in cell viability; decrease cell viability after 39 & 48 hrs in both groups w/o RDS increase)	Uno *et al*., 1994

Assay	*In Vitro/ In Vivo*	Test System	Dose	Result	Source
Replicative DNA synthesis	*In Vivo*	Male rat	2000 mg/kg (single oral dose at 24, 39 & 48 hrs)	Pos. (4% RDS incidence in hepatocytes after 24 hrs. No histopathology seen)	NICNAS, 1998
Replicative DNA synthesis	*In Vivo*	Male CBA/J mouse	Daily inj of 0.1- 20% dioxane for 7 days	Neg. (incorporation rates recorded for isolated lymphocytes, no histopathology. noted)	Thurman *et al*., 1978
RNA-polymerase transcription inhibition (hepatic nuclei)	*In Vivo*	Male rat	10 & 100 mg iv 24 hrs prior to sacrifice	Pos. (decrease. levels of RNA polymerase A & B peaked 4 hr post-injection)	Kurl *et al*., 1981
Meiotic non-disjuction induction	*in Vivo*	Female *Drosophila melanogaster*	1, 1.5, 2, 3, and 3.5% (single oral dose)	Pos	Munoz and Barnett, 2002

Table 4-4. Summary of Chromosomal Aberration Assays of 1,4-Dioxane

Assay	*In Vitro*/*In Vivo*	Test System	Dose	Result	Source
Chromosome Aberration	*In Vitro*	*C. capillaris* (wheat)	1% in phosphate buffer	Neg. (-MA) at 2 optimal pH values	NICNAS, 1998
Chromosome Aberration	*In Vitro*	CHO cells	1,050 - 10,500 µg/ml	Neg. (+ or - MA)	Galloway *et al*., 1987
Sister Chromatid Exchange	*In Vitro*	CHO cells	1,050-10,500 µg/ml	Weakly Pos. (pos. at highest dose tested)	Galloway *et al*., 1987
Micronucleus (bone marrow)	*In Vivo*	CBA & C57BL6 Mouse (male)	1800 & 3600 mg/kg (single oral dose)	Neg. (3 ind. assays done; P/N ratio 0.6:1.0)	Tinwell and Ashby, 1994
Micronucleus (bone marrow)	*In Vivo*	C57BL6 & BALB/c Mouse (male/female)	450-5000 mg/kg in C57BL6 by gavage; 5000 mg/kg in BALB/c; 24 & 48 hrs before sacrifice	Pos. in C57 BL6 (dose-related increase in MPE from 900 mg/kg in males and females at 5000 mg/kg (only dose tested)	Mirkova, 1994
Micronucleus (bone marrow)	*In Vivo*	B6C3F1 Mouse	500-4000 mg/kg (ip) 24 & 48 hrs before sacrifice	Neg.	McFee *et al*., 1994
Micronucleus (peripheral erthrocyte)	*In Vivo*	CD-1 Mouse (male)	500-3200 mg/kg (ip); 2 inj. 24 hrs apart	Neg. (blood analyzed 24, 48 & 72 hrs after inj.)	NICNAS, 1998

Assay	*In Vitro*/*In Vivo*	Test System	Dose	Result	Source
Dominant Lethal (germ cells)	*In Vivo*	Mouse (male)	2.5 ml/kg (ip); approx. 2500 mg/kg, 1 inj.	Neg.	TNO and RIVM, 2002; Appel, 1988
Clastogenicity (lymphocytes)	*In Vivo*	Human (male)	up to 48 mg/m^3 of 1,4-dioxane for 25 yrs in 24 workers; no data on exposure to 11 workers with other exposures	Neg. in 24 workers exposed for 25 yrs to 1,4-dioxane; Pos. in 11 workers also exposed to ethylene and propylene oxide	Thiess *et al.*, 1976 NICNAS, 1998

MA = mutagenic activity
MPE = micronucleated polychromatic erythrocytes

Although there are some indications that 1,4-dioxane may be weakly genotoxic, overall the total weight of evidence from *in vitro* and *in vivo* tests indicates that 1,4-dioxane is unlikely to be a mutagen. This is further supported by the absence of DNA-adducts at hepatotoxic doses. No alkylation of hepatocellular DNA was seen in rats at 1,4-dioxane doses associated with carcinogenicity. In addition, this conclusion is further supported by evidence from structure-activity relationship (SAR) modeling using the Computer Automated Structure Evaluation, which indicates a lack of intrinsic electrophilicity for 1,4-dioxane and metabolites (NICNAS, 1998).

4.1.1.3.1 In Vitro Gene Mutation Assays of 1,4-Dioxane

These mutational assays are summarized in **Table 4-2**. In a gene mutation assay in CHO cells (HGPRT test), negative results were found both with and without metabolic activation. Although the test concentrations ranged from 0.05 to10.0 mg/ml, the necessary cytotoxicity was not observed in this assay (TNO and RIVM, 2002). In yeast, there was no increase in aneuploidy following exposure to 1,4-dioxane (Zimmermann *et al.*, 1985). 1,4-Dioxane also tested negative in an UDS-test using primary isolated rat hepatocytes (Goldsworthy *et al.*, 1991). In the mouse lymphoma forward mutation assay (L5178Y), both with and without S9 metabolic activation, 1,4-dioxane produced negative results (McGregor *et al.*, 1991). A cell transformation assay with BALB/3T3 mouse cells tested without metabolic activation was positive (Sheu *et al.*, 1988) while another test (both with and without metabolic activation) showed negative results (TNO and RIVM, 2002). 1,4-Dioxane also produced negative results in the cell transformation assay with SA7/SHE cells (Heidelberg *et al.*, 1983).

In vitro incubation of 1,4-dioxane and DNA in the presence of microsomes showed no signs of covalent DNA binding when benzo[a]pyrene was used as a positive control (Woo *et al.*, 1977a).

The 1,4-dioxane metabolite, 1,4-dioxan-2-one, also gave negative results in an *in vitro* UDS assay (Goldsworthy et al, 1991) and an HGPRT-test with CHO cells. A cell transformation test with BALB/3T3 mouse cells was negative with metabolic activation and positive without metabolic activation (TNO and RIVM, 2002).

4.1.1.3.2 Bacterial Reverse Mutation Assays of 1,4-Dioxane

These mutational assays are summarized in **Table 4-2**. Bacterial assays in *Salmonella typhimurium* have been carried out in 2 to 8 strains at several dose levels (including bacteriostatic concentrations) according to the protocol of Ames *et al.* (1975). All tests were negative with and without metabolic activation (Stott *et al.*, 1981; TNO and RIVM, 2002). The metabolite 1,4-dioxan-2-one also gave negative results in the Ames and HGPRT tests

despite the fact that a number of lactones with a similar structure to this metabolite have been demonstrated as carcinogenic (NICNAS, 1998).

4.1.1.3.3 Other Genotoxicity Assays

These miscellaneous genotoxicity assays are summarized in **Table 4-3**. A dominant lethal assay in male mouse was negative after a single ip injection. The rate of conception, mean number of implantations, percentage of living fetuses, and mutagenicity index were unchanged (Appel, 1988). At high dosages of 1,4-dioxane, positive results were obtained in a sex-linked recessive lethal test in *Drosophila melanogaster* (Yoon *et al.*, 1985; TNO and RIVM, 2002). An additional *Drosophila melanogaster* study reported significantly increased meiotic non-disjunctions observed in all broods born to female *Drosophilia* exposed orally to 1, 1.5, 2, 3, and 3.5% 1,4-dioxane concentration (Munoz and Barnett, 2002).

Neither a single application of 1,000 mg/kg, nor treatment with 1% 1,4-dioxane in drinking water for two weeks, or with 2% 1,4-dioxane for one week induced UDS in primary rat hepatocytes. In addition, negative results for UDS were also found in rat nasal respiratory epithelial cells (from the nasoturbinate or the maxilloturbinate) after treatment of rats with 1% 1,4-dioxane in drinking water for 8 days, or after treatment with 1% in the drinking water for 8 days with an additional single gavage dose of up to 1,000 mg/kg 1,4-dioxane (Goldsworthy *et al.*, 1991). Rats treated with a single oral dose of 1,000 mg 1,4-dioxane/kg after pre-treatment of 0.01%, 0.1%, 1.0%, or 2.0% 1,4-dioxane for 1 day up to 9 weeks in drinking water showed increased incorporation of [$^{6\text{-}3}$H]thymidine into liver DNA after pretreatment at concentrations at or above 0.1% in drinking water. These effects remained the same after several weeks of administration suggesting cytotoxicity is probably involved (Goldsworthy *et al.*, 1991).

A single gavage administration of 1,000 mg/kg1,4-dioxane to rats did not result in hepatocyte cell proliferation since no increases in the liver to body weight ratio and the labeling index (with ^{3}H-methyl thymidine) were found. In contrast, continuous administration of 1% 1,4-dioxane in the drinking water for 1 to 2 weeks produced a two-fold increase in the hepatic labeling index, suggesting cell proliferation was occurring (Goldsworthy *et al.*, 1991). After mapping the nasal tumors as found in the 1978 National Cancer Institute (NCI) chronic rat bioassay, Goldsworthy *et al.* (1991) investigated cell proliferation in the nasal epithelium where the majority of the tumors originated. No histopathological lesions were present in rats given 1% 1,4-dioxane in the drinking water for up to two weeks, and no increases in labeling index (with ^{3}H-methyl thymidine) were observed at any site (TNO and RIVM, 2002).

Despite the observed hepatotoxicity at 1,000 mg/kg-d, no *in vivo* DNA alkylation or an increase in hepatic DNA repair were observed in rats dosed by gavage at this dose level

(Stott *et al.*, 1981). 1,4-Dioxane was also negative in a differential DNA repair *in vitro* assay using derivatives of *Escherichia coli* K-12 343/113 without and without metabolic S9 activation (Hellmer and Bolscfoldi, 1992). CBA/J mice (number unknown) were injected ip seven times over seven days with 0.5 ml of a 0.1%, 1.0%, 5%, 10%, or 20% 1,4-dioxane solution. The 20% concentration caused mortality even before all seven injections were given and no biologically significant changes in ^{3}H-thymidine incorporation rates were recorded for isolated lymphocytes. In another study, lymphocytes from untreated mice were incubated with 1,4 dioxane in concentrations of 0.25% and 0.5%. The rate of ^{3}H-thymidine incorporation into the lymphocytes fell and the ability of the T-lymphocytes to be stimulated by mitogens was reduced, while that of the B-lymphocytes was greatly increased. Levels of 1.0 % 1,4-dioxane and above were cytotoxic. Human lymphocyte cultures treated for two hours with 1,4-dioxane in concentrations of 0.25% to 1.0% showed no significant effects. However, a 1,4-dioxane level of 2.5% resulted in a marked increase in phytohemagglutinin-stimulated DNA synthesis (TNO and RIVM, 2002).

4.1.1.4 Summary - Repeated Dose Testing of 1,4-Dioxane

1,4-Dioxane has been administered in several repeated oral dose studies over short and long periods of exposure. Although most of these studies can be considered as chronic toxicity and carcinogenicity studies (section 4.3.1; **Table 4-7**), there were some sub-acute and sub-chronic studies also available (section 4.2.1; **Table 4-5**). In a limited study with rats, effects on the kidneys were seen after administration of 5% 1,4-dioxane in the drinking water for 1 to 10 days. A rise in [$^{6\text{-}3}$H]-thymidine incorporation into liver DNA accompanied by a minimal degree of hepatocellular swelling was observed after exposure to oral doses higher than 10 mg/kg for 11 weeks. In 2- and 13-week oral studies and in the longer term oral studies (drinking water doses ranging from 0.05 to 9% for mice and from 0.01 to 9% for rats), toxicological effects observed included severe effects on the nasal cavity, lungs, liver, and kidneys. A NOAEL of 0.01% (equivalent to 10 mg/kg-d) for liver effects was identified from sub-chronic and chronic rat studies (TNO and RIVM, 2002; NICNAS, 1998).

For inhalation exposure, a 2-year chronic toxicity and carcinogenicity study with rats identified a NOAEL for toxic effects of 400 mg/m^3 1,4-dioxane (equivalent to 108 mg/kg-d), the highest (and only) dose tested (TNO and RIVM, 2002).

In very limited dermal experiment in rabbits and guinea-pigs, effects on liver and kidneys were observed, indicating dermal absorption may be significant. CNS Effects (avoidance response) were dose dependently increased in rats at concentrations at or above 5,400 g/m^3 (TNO and RIVM, 2002).

Under extreme conditions, occupational exposure resulted in adverse effects in humans. For example, a woman with a skin burn developed inflammatory skin changes and clinical

symptoms of eczema after occupational dermal exposure. Furthermore, a male alcoholic who received an occupational inhalation exposure to concentrations of 720 to 2340 mg/m^3 demonstrated hypertonia and neurological symptoms followed by death due to kidney failure. Necropsy showed renal cortex and centrilobular liver necrosis and brain damage (TNO and RIVM, 2002).

4.1.1.4.1 Repeated Dose Toxicity of 1,4-Dioxane in Humans

Fatalities in humans from repeated short-term exposure (*i.e.*, 6 days to 2 months) to 1,4-dioxane have been reported. Exposure concentrations and durations associated with these adverse effects are largely unknown or uncertain (in one case, the air concentration was estimated to be 470 ppm or1,700 mg/m^3), but have been classified as "acute" exposure to high levels. Exposure via skin absorption was also likely in these cases.

Six occupational fatalities associated with exposure (primarily inhalation but potentially dermal as well) to 1,4-dioxane have been reported in the literature including five fatalities among a group of workers exposed to 1,4-dioxane vapors during textile (artificial silk) manufacture (NICNAS, 1998). Symptoms included irritation of upper respiratory passages, coughing, irritation of eyes, drowsiness, vertigo, headache, anorexia, severe stomach pains, nausea, vomiting, uremia, coma, and ultimately death 5 to 8 days after the symptoms appeared. Blood counts showed no abnormalities other than considerable leucocytosis. Autopsy revealed congestion and edema of lungs and brain, and marked injury of liver and kidney (*i.e.*, centrilobular liver necrosis and symmetrical necrosis (outer cortex) of the kidney). Hemorrhagic nephritis was reported as the ultimate cause of death. All deaths occurred within a two week period, between four and eight weeks after an alteration in the process that led to an increase in potential inhalation exposure to 1,4-dioxane. Dermal contact may also have contributed to body burden. While Johnstone (1959) reported the exposure period was up to 16 months for this study, no estimates of 1,4-dioxane exposure levels or duration were reported, even though one death occurred following only five days of exposure. The author concluded that the deaths resulted from 'intensive acute exposure' to 1,4-dioxane, rather than cumulative exposure, based on the fact that three out of five of the cases worked extended shifts (up to 12 hours) prior to the onset of illness, but it is debatable whether exposure was chronic or acute. A further four workers were reported as similarly exposed in the above process, of which two exhibited symptoms of liver toxicity. These symptoms are similar to those described by another study which reported the case of a 21-year old worker who had been exposed to 1,4-dioxane (as a solvent to remove glue) for one week (presumably 5 days) in a closed, non-ventilated room without respiratory equipment. The estimated 1,4-dioxane concentrations ranged from 720 mg/m^3 to 2,340 mg/m^3 (208-650 ppm) with an average concentration of 1,692 mg/m^3 (470 ppm). Additionally, he had repeatedly dipped his hands into a tub containing liquid 1,4-dioxane so dermal absorption in addition to inhalation of the vapors was likely in this case. He was

admitted to hospital with severe pain in the upper abdomen, emanating into the sides, followed by hypertonia, and neurological symptoms, and died six days later of kidney failure. Necropsy included renal cortex necrosis with severe interstitial hemorrhages. Severe centrilobular necrosis was found in the liver. The brain showed signs of demyelination and partial loss of nerve fiber tissue (TNO and RIVM, 2002). The man had been an alcoholic and, since other workers with similar exposures were unaffected, the author concluded that alcohol consumption may have increased the susceptibility of the worker to 1,4-dioxane intoxication, but made no conclusions as to the nature of the exposure (*i.e.*, acute or cumulative) associated with the elicited effects (NICNAS, 1998).

Finally, a worker died as a result of exposure to a concrete sealant containing 1,1,1-trichloroethane (80%) and 1,4-dioxane (2.5%) as a stabilizer . The autopsy report listed the cause of death as trichloroethane intoxication and the sealant product was subsequently recalled by the manufacturer (NICNAS, 1998).

4.1.1.4.2 Repeated Dose Testing of 1,4-Dioxane in Experimental Animals

The results of sub-acute and sub-chronic toxicity testing of 1,4-dioxane are summarized in **Table 4-5**.

4.1.1.4.2.1 Oral Repeat Dose Studies in Experimental Animals

1,4-Dioxane has been administered in several repeated oral studies; however, most of these studies are not sub-acute or sub-chronic toxicity studies, but rather are chronic toxicity or carcinogenicity studies, sometimes with shortened application or exposure periods. These studies, including the toxicological effects observed, are described in the Sub-Chronic Toxicity and Chronic Toxicity and Carcinogenicity Sections (sections 4.2.1 and 4.3.1). CNS, kidney, and liver damage are the most frequently reported effects seen in the sub-acute and sub-chronic animal studies. While few of the older sub-acute or sub-chronic studies are of sufficient quality to derive lowest observed adverse effect levels (LOAELs), recent Japanese studies of 1,4-dioxane in drinking water over 2- and 13-week exposure periods do appear to be of high quality (TNO and RIVM, 2002).

Dogs given 1,4-dioxane orally over a period of 9 days died after a total consumption of about 3 g/kg, with severe liver and kidney damage (ACGIH, 1986).

Table 4-5. Summary of Sub-Acute and Sub-Chronic Toxicity Studies of 1,4-Dioxane

Species	Route	Dose	Duration	Results	Source
Oral					
Rabbit	Oral (DW)	500-1000 mg/kg	3x wk	214% increase in blood urea; kidney damage (cortical necrosis)	Appel, 1988
Dog	Oral	3000 mg/kg (total)	9 days	Death due to liver/kidney damage	ACGIH, 1986
Rat	Oral (DW)	400 mg/kg-day	10 days	Significant increase in aniline hydroxylation, p-nitrophenol hydroxylation, PROD, and lauric acid hydroxylase in liver microsomes; no effect on EROD or CYP levels; significant increase in levels of aniline hydroxylase in kidney and nasal mucosa microsomes; significant increase of CYP2E1 protein brand concentration in kidney microsomes.	Nannelli *et al.*, 2005
Rat	Oral (DW)	5% (4150 mg/kg-d)	up to 10 days	35/50 deaths; after 3 days, kidney damage/necrosis observed	David, 1964
Mouse (m&f)	Oral (DW)	0- 90,000 ppm	2 weeks	95% mortality in high dose males & females; body wt.& food consumption reduced at 30000 & 90000 ppm; water consumption reduced $\geq$10000 ppm; single cell necrosis & swelling of liver central area at 30000 & 90000 ppm	TNO and RIVM, 2002
Rat	Oral (DW)	0- 90,000 ppm	2 weeks	100% mortality at high dose; 10% at	TNO and RIVM, 2002

Species	Route	Dose	Duration	Results	Source
(m&f)				30000 ppm; body wts reduced at 30000 & 90000 ppm; food consumption reduced ≥10000 ppm (m) & ≥30000 ppm (f); water consumption reduced ≥1110 ppm (m) & ≥3330 ppm (f); nuclear enlargement of olfactory epithelium at 10000 & 30000 ppm; swelling & vacuolic change of liver central area, hydropic change of proximal renal tubule & vacuolic change in brain at 30000 ppm.	
Rat	Oral (DW)	1% (1000 mg/kg-d)	up to 2 wks	No nasal lesions	Goldsworthy *et al*., 1991
Rat & Mouse	Oral (DW)	4-5% or 50000 mg/l (7200 mg/kg-d - rats; 9800 mg/kg-d - mouse)	up to 67 days	Some deaths; congestion & degeneration of renal cortex & hepatocellular degeneration	DeRosa *et al*., 1996; Appel, 1988
Rat	Oral (DW)	10-1000 mg/kg-d	up to 11 wks	No effects - low dose; minimal hepatic effects - high dose	Stott *et al*., 1981

Species	Route	Dose	Duration	Results	Source
Mouse (m&f)	Oral (DW)	0-25,000 ppm	13 weeks	Body wt & food consumption slightly reduced $\geq$10000 ppm (m) & in high dose females; water consumption decrease $\geq$4000 ppm. In males, hematology, biochemistry or urinary affected at 10000, $\geq$4000 & 10000 ppm, respect. In females, simple effects at 10000 ppm; absolute & relative lung wts increased at 25000 ppm (m) & $\geq$10000 ppm (f); kidney wt also increased at these doses (f);non-neoplastic lesions in nasal cavity, trachea, lung, and liver $\geq$4000 ppm (M) & $\geq$1600 ppm (F). No effects on the reproductive organs.	TNO and RIVM, 2002

Species	Route	Dose	Duration	Results	Source
Rat (m&f)	Oral (DW)	0-25,000 ppm	13 weeks	Body wts reduced at 10000 and 25000 ppm; food consumption. decreased. at 25000 ppm (m) & ≥10000 ppm (f); water consumption decreased ≥1600 ppm. In males, hematology, biochemistry, or urinary parameters affected at 25000, ≥4000 and ≥4000 ppm, respect. In females, simple effects ≥10000, ≥4000 & ≥10000 ppm, respect.; absolute.& relative kidney wts increased ≥1,600 ppm (f);non-neoplastic lesions in the nasal cavity, trachea, liver, kidney & brain ≥1600 ppm. No effects on the reproductive organs.	TNO and RIVM, 2002
Gavage					
Mouse	Gavage/IP	1000 mg/kg	3x wk for 8 wks	No macroscopic hepatic effects	Stoner *et al*., 1986
Inhalation					
Cat	Inhalation	5000-350000 mg/m^3	NR	Hematology effects at low dose; cardiac effects above 10000ppm with cardiac arrest within 5 min of highest dose exposure	Wirth and Klimmer, 1937
Rat (f)	Inhalation	100 mg/m^3	4 hr/d, 5 d/wk, 4 wks	significantly increased glutathione peroxidase activation in both the brain and ovaries	Burmistrov *et al.*, 2001

Species	Route	Dose	Duration	Results	Source
Rat	Inhalation	5400, 10800 & 21600 mg/m^3	4 hr/d, 5d/wk, 2 wks	NOAEL = 5400 mg/m^3; behavioral effects at 10800 mg/m^3 and above, most pronounced after 2 days, recovery (sometimes complete) during study course	Grasso *et al*., 1984
Rabbit	Inhalation	2900 mg/m^3	up to 30 days	Fatalities due to severe kidney damage	ACGIH, 1991
Cat, Rabbit & Guinea Pig	Inhalation	9700 mg/m^3	up to 34 days	Fatal to majority; emaciation, narcosis, renal & hepatic toxicity	ACGIH, 2001
Guinea Pig	Inhalation	180 mg/m^3	5d/wk for 12 wks	No effects on growth, organ wts, mortality, hematology, clinical chemistry or pathology	Torkelson *et al*., 1974; TNO and RIVM, 2002
Dog, Rat & Rabbit	Inhalation	180-360 mg/m^3 in rats & rabbits; 180 mg/m^3 in dogs	5d/wk for 18 wks	No effects on growth, organ wts, mortality, hematology, clinical chemistry or pathology	Torkelson *et al*., 1974; TNO and RIVM, 2002
Dermal					
Rabbit & Guinea Pig	Dermal (80% aq sol.)	10 drops/rabbit; 5 drops/guinea pig	11x/wk for 14 wks; non-occlusive	Renal tubular cell & glomeruli damage; renal medulla hemorrhages & liver degeneration observed	Fairley *et al*., 1934; ACGIH, 2001; NICNAS, 1998: Appel, 1988

In a limited repeated dose study, 50 white rats of an unspecified inbred strain were given drinking water containing 5% 1,4-dioxane for 1 to 10 days (corresponding to a dose of approximately 4,150 mg/kg). Thirty-five rats died and were not examined while the remaining 15 surviving animals were sacrificed for macroscopic and electron microscopic examination of the kidneys on days 1, 3, 5, 7, 8, and 10 during treatment. No macroscopic changes were seen in rats sacrificed during the first seven days of exposure. However, later sacrificed rats showed frequent enlargements of the kidneys with superficial aberrations. Microscopic examination of the kidneys from rats sacrificed after three days of exposure showed swollen epithelial cells in the proximal section of the nephron. Vesicular degeneration of tubular epithelium was first observed at five days of exposure and became more severe on the seventh day of exposure and continued through the treatment. An accumulation of intracellular hyaline droplets was observed by electron microscopy, followed by enlargement of the basal labyrinth. Subsequent changes were noted in the tubular epithelium followed by degeneration and ultimately resulting in necrosis (David, 1964).

In a two week study, groups of 10 male and 10 female Crj:BDF1 mice received drinking water containing 0, 1,110 ppm, 3,330 ppm, 10,000 ppm, 30,000 ppm, or 90,000 ppm 1,4-dioxane (equivalent to 0, 0.21, 0.66, 1.38, 2.55, or 3.63 g/kg-d for males of the 0 to 90,000 ppm groups, and 0, 0.24, 0.75, 1.78, or 3.23 g/kg-d for females of the 0 to 30,000 ppm groups, respectively). Observations included clinical signs, body weight, food and water consumption, necropsy, and histopathological examination (on 2 to 4 animals per sex per group). Mortality occurred in the 90,000 ppm male (9/10) and female (10/10) groups. Body weights and food consumption were decreased in males and females at 30,000 and 90,000 ppm. Water consumption was decreased in males at or above10,000 ppm and in females at or above 3,330 ppm. In the liver, single cell necrosis and swelling of the central area were observed in both males and females from the 30,000 and 90,000 ppm groups, respectively (TNO and RIVM, 2002).

In a related two week study, groups of 10 male and 10 female F344/DuCrj rats received drinking water containing 0, 1,110 ppm, 3,330 ppm, 10,000 ppm, 30,000 ppm, or 90,000 ppm 1,4-dioxane (equivalent to 0, 0. 13, 0.37, 1.01, or 2.96 g/kg-d and 0, 0. 16, 0.40, 1.04 or 2.75 g/kg-d for males and females of the 0 to 30,000 ppm groups, respectively). Observations again included clinical signs, body weight, food and water consumption, necropsy, and histopathological examination (on 2-4 animals per sex per group). In the 90,000 ppm group, all males and females died. In the 30,000 ppm group, two females died. Body weights were reduced in the 30,000 and 90,000 ppm male and female groups. Food and water consumption were decreased in a dose-related manner in males (at or above 10,000 and at or above1,110 ppm, respectively) and in females (at or above 30,000 and at or above 3,330 ppm, respectively). Histopathological examination revealed nuclear enlargement of the olfactory epithelium, swelling and vacuolic change of the central area in the liver, hydropic

change of the proximal renal tubule, and vacuolic change in the brain in 30,000 ppm male and female groups. Nuclear enlargement of the olfactory epithelium was also seen in the 10,000 ppm male and female groups (TNO and RIVM, 2002).

A recent study exposed rats to 1,4-dioxane via drinking water (1.5% v/v; corresponding to 400 mg/kg-day) for 10 days which resulted in significantly increased aniline hydroxylation, p-nitrophenol hydroxylation, PROD, and lauric acid hydroxylase in liver microsomes, but did not effect either EROD or CYP levels. A NOAEL was not identified as only one dose was administered. Additionally, chronic 1,4-dioxane exposure increased induction of liver microsome 2B1/2-dependent 16 β-testosterone hydroxylase by approximately 18 fold. 17OT-Testosterone hydroxylase, 16α-testosterone hydroxylase, and 2α-testosterone hydroxylase linked to CYP2C11 were also induced at increased concentrations. Using a western blott analysis, significantly increased levels of CYP2E1 protein band (670% of control) were reported (Nannelli *et al.*, 2005).

Significantly increased levels of aniline hydroxylase in kidney and nasal mucosa microsomes were observed in rats exposed to 400 mg/kg-d (1.5 v/v) 1,4-dioxane via drinking water and a significant increase (9-fold) of CYP2E1 protein brand concentration compared to control was also reported in kidney microsomes (Nannelli *et al.*, 2005).

4.1.1.4.2.2 Inhalation Repeat Dose Studies in Experimental Animals

Rats, rabbits, mice, and guinea pigs were exposed by inhalation for 1.5 hr/d to concentrations of 1,000, 2,000, 5,000, and 10,000 ppm of 1,4-dioxane vapor (duration unknown). At the higher levels, mortality was high and deaths were usually due to lung injury. Animals that survived repeated exposures at all levels suffered marked liver and kidney injury (Gingell *et al.*, 1994). Repeated inhalation of 800 ppm of 1,4-dioxane vapor for 30 days by rabbits resulted in fatal kidney injury to some (ACGIH, 1986).

An additional short term inhalation study focused on glutathione peroxidase and catalase activation and protein peroxidation within the rat brain and ovaries. Female rats were exposed via inhalation to 10 or 100 mg/m^3 1,4-dioxane for 4 hours per day, 5 days per week for one month. These doses are the maximum permissible concentration (MPC) for Russian industrial plant working zones and 10-fold MPC, respectively. Neither dose level had any effect on glutathione catalase activation or protein peroxidation, while the highest dose level resulted in significantly increased glutathione peroxidase activation in both the brain and ovaries (Burmistrov *et al.*, 2001).

4.1.1.4.2.3 Dermal Repeat Dose Studies in Experimental Animals

No sub-acute dermal toxicity studies with 1,4-dioxane were located.

4.1.1.5 Chromosomal Aberration Assays of 1,4-Dioxane

These chromosomal aberration assays are summarized in **Table 4-4**. Studies on the chromosomal aberration frequency in lymphocytes from 1,4-dioxane exposed humans have provided conflicting results, although it would appear that positive results have only been seen in workers with a history of exposure to other known mutagens, such as ethylene oxide and propylene oxide. In a study of 74 workers exposed to 1,4-dioxane during manufacture and handling for an average duration of 25 years, and with an estimated exposure to 0.02 to 48 mg/m^3 (0.006 to 13 ppm), chromosomal aberrations were not increased in lymphocytes of exposed subjects (Thiess *et al.*, 1976). In a further study, a significant increase in mean lymphocyte chromosomal aberration frequency was found in 11 workers exposed (>20 yr) to alkylene oxides (including 1,4-dioxane). Exposures to known mutagens such as ethylene oxide and propylene oxide confound any conclusions with regard to causation (NICNAS, 1998).

A total of seven micronucleus tests have been performed on 1,4-dioxane. In the most recent study, CD-1 mice were exposed to 1,4-dioxane via oral gavage and resulted in the significantly increased induction of micronucleated erythrocytes at all doses (1,500, 2,500, and 3,500 mg/kg). Chromosome breakage resulted in 90% of the micronucleus induction, while a portion of the remaining percentage resulted from chromosome loss. The results of this study do suggest that 1,4-dioxane may interfere with cell proliferation at the two highest doses (Roy *et al.*, 2005). However, this data has yet to be corroborated. The only other positive chromosome aberration (*in vitro* or *in vivo*) assay was a mouse micronucleus test (bone marrow) which, when repeated (using the same strain of animals), gave a negative result, in agreement with similar tests in other mouse strains. In C75BL6 mice, oral dosing with 1,4-dioxane resulted both in micronucleus induction (Mirkova, 1994) as well as in negative results (Tinwell and Ashby, 1994). Negative results were also observed after oral dosing with 1,4-dioxane in BALB/c mice (Mirkova, 1994) and CBA mice (Tinwell and Ashby, 1994) as well as after ip application in B6C3F1 mice (McFee *et al.*, 1994) and CD-1 mice (NICNAS, 1998). Except for the oral study with BALB/c mice and the ip study with CD-1 mice, the polychromatic erythrocytes/normochromatic erythrocytes ratio was decreased, indicating that the bone marrow was reached.

An alkaline elution test for DNA breaks was positive in rat hepatocytes at cytotoxic concentrations (Sina *et al.*, 1983) while in another alkaline elution test, 1,4-dioxane induced DNA breaks in liver cells especially at dose levels higher than 2,500 mg/kg (TNO and RIVM, 2002). In a species of wheat (*Crepis capillaris*) without metabolic activation, 1,4-dioxane did not produce any chromosome aberrations. An in vivo study in male rats reported no chromosome aberrations in kidney cells (NICNAS, 1998). Negative results were also obtained in a test for chromosomal aberrations both with and without S9 metabolic activation. A test for SCEs in CHO cells was positive without metabolic activation at the

highest dose tested but negative with metabolic activation (Galloway *et al.*, 1987, TNO and RIVM, 2002).

4.2 Tier 2 Toxicity Data for 1, 4-Dioxane

4.2.1 Sub-Chronic Toxicity Testing of 1,4-Dioxane

The majority of sub-chronic studies conducted with 1,4-dioxane in rats, mice, guinea pigs, rabbits, dogs and cats were carried out between 1930 and 1960. CNS, (*i.e.*, narcosis, behavioral changes, brain lesions), hematological effects, cardiac effects, and kidney and liver damage have been reported in these sub-chronic and chronic animal studies. In general, the doses used in these studies are very high and as such provide little useful information on critical effects (*i.e*., most sensitive effects) and NOAELs. There is concern over the adequacy of testing protocols and many of these studies lack details (ECETOC, 1983). The sub-chronic studies are summarized in **Table 4-5**.

4.2.1.1 Sub-Chronic Oral Studies in Experimental Animals

Rats given 1.0% or 0.1% 1,4-dioxane in drinking water for 4 to 24 months showed renal tubular and hepatocellular degeneration, necrosis, and regeneration (Kociba *et al.*, 1974).

Groups of 10 male and 10 female Crj:BDF1 mice received drinking water containing 0, 640 ppm, 1,600 ppm, 4,000 ppm, 10,000 ppm, or 25,000 ppm 1,4-dioxane (equivalent to 0, 0.10, 0.26, 0.58, 0.92 or 1.83 g/kg-d and 0, 0.17, 0.41, 0.92, 1.71 or 2.70 g/kg-d for males and females of the 0 to 25,000 ppm groups, respectively) for 13 weeks. Observations included clinical signs, body weight, food and water consumption, hematology, biochemistry, urinalysis, necropsy, organ weights and histopathological examination. One male in the 25,000 ppm group died and body weights and food consumption were slightly reduced in the 10,000 and 25,000 ppm male groups and in the 25,000 ppm female group. Water consumption was decreased in all treated males and in females at or above 4,000 ppm. In males, effects on hematology, biochemistry, or urinalysis parameters were observed at 10,000 ppm, at or above 4,000 ppm, and 10,000 ppm, respectively. In females, these effects occurred at 10,000 ppm. Absolute and relative lung weights were increased in males at 25,000 ppm and in females at or above10,000 ppm. In females, kidney weight was also increased at these dose levels. Histopathological examination revealed non-neoplastic lesions in the nasal cavity (*i.e*., nuclear enlargement and eosinophilic change of the olfactory and respiratory epithelium, and vacuolic change of the olfactory nerve), trachea (*i.e.*, nuclear enlargement of the epithelium), lung (*i.e*., accumulation of foamy cells, and degeneration and nuclear enlargement of the bronchial epithelium), and liver (*i.e*., necrosis of single cell and swelling of the central area) in males at 4,000 ppm or greater groups and in females at 1,600 ppm or greater. No effects were found on the reproductive organs. Based on the

histopathology findings in females at 1,600 ppm, the NOAEL in this study can be established at 640 ppm (equivalent to 0.17 g/kg-d) (TNO and RIVM, 2002).

Groups of 10 male and 10 female F344/DuCrj rats also received drinking water containing 0, 640 ppm, 1,600 ppm, 4,000 ppm, 10,000 ppm, or 25,000 ppm 1,4-dioxane (equivalent to 0, 0.06, 0.15, 0.33, 0.76, or 1.90 g/kg-d and 0, 0.10, 0.20, 0.43, 0.87 or 2.01 g/kg-d for males and females of the 0 to 25,000 ppm groups, respectively) for 13 weeks. Observations again included clinical signs, body weight, food and water consumption, hematology, biochemistry, urinalysis, necropsy, organ weights, and histopathological examination. One female in the 25,000 ppm group died. Body weights were reduced in the 10,000 and 25,000 ppm male and female groups. Food consumption was decreased in males at 25,000 ppm and in females at or above 10,000 ppm. Water consumption was decreased in a dose-related manner in all treated males and in females at or above 1,600 ppm. In males, effects on hematology, biochemistry, or urinalysis parameters were observed at 25,000 ppm, at or above 4,000 ppm, and at or above 4,000 ppm, respectively. In females, this occurred at or above10,000 ppm, at or above 4,000 ppm, and at or above10,000 ppm, respectively. Absolute and relative kidney weights were increased in females at or above1,600 ppm. Upon histopathology, non-neoplastic lesions were observed in the nasal cavity (*i.e.*, nuclear enlargement of the olfactory and respiratory epithelium), trachea (*i.e.*, nuclear enlargement of the epithelium), liver (*i.e.*, vacuolic change and swelling of the central area, and granulation), kidney (*i.e.*, hydropic change and nuclear enlargement of the proximal tubule) and brain (*i.e.*, vacuolic change) in both males and females in the 1,600 ppm or greater groups. No effects were found on the reproductive organs. Based on the findings at 1,600 ppm (histopathology in males and females, and kidney weight changes in females), the NOAEL in this study can be established at 640 ppm (equivalent to 0.06 g/kg-d for males and 0.10 g/kg-d for females) (TNO and RIVM, 2002).

4.2.1.2 Inhalation Studies

Torkelson *et al*. (1974) described some sub-chronic inhalation studies with rats, rabbits, guinea pigs, and dogs at concentrations ranging from 180 to 360 mg/m^3 over 82 to 136 seven-hour exposures (12 to 18 weeks). It is stated that, in all of these studies, no adverse effects were noted with respect to appearance, demeanor, growth, mortality, hematological and clinical chemical studies, organ weights, or gross and microscopic pathological examination, but no details of these studies are available. On this basis, NOAELs of approximately 100 mg/kg-d, 65 mg/kg-d, 30 mg/kg-d, and 20 mg/kg-d for 1,4-dioxane in rats, rabbits, guinea pigs, and dogs respectively are suggested and consistent with that observed in the two-year rat study by the same group, but cannot be independently verified.

Continuous (24 hour/day) exposure of white rats to 4 and 20 mg/m^3 1,4-dioxane for 90 days decreased weight gain, increased activity of glutamate-aspartate and glutamate-alanine

transaminases, prolonged duration of sleep, increased urine protein, decreased diuresis, and altered content of chlorides, and decreased motor activity (HSDB, 2007).

4.2.1.3 Dermal Studies

Fairley *et al.* (1934) Repeated application of an 80% aqueous 1,4-dioxane solution to the skin of four rabbits and guinea-pigs under non-occlusive conditions led to damage of the renal tubular cells and glomeruli as well as hemorrhages in the renal medulla, and liver degeneration within 50 to 100 days (7 to 14 weeks). From this study the only conclusion that can be drawn is that dermal absorption occurs and that the same effects were obtained as after oral administration (ACGIH, 2001; Appel, 1988).

4.2.2 Summary - Reproductive and Developmental Toxicity Testing of 1,4-Dioxane

Limited evidence exists in humans regarding gonadotoxic effects from occupational exposure to 1,4-dioxane. An increased incidence of effects on 'reproductive outcome,' including miscarriages, premature births, and decreased birth weights, was reported in women exposed to chemicals (including 1,4-dioxane) in the electronics industry. Concurrent exposures to other chemicals preclude any conclusions with respect to 1,4-dioxane causation. Testicular tumors were seen in rats in a carcinogenicity study carried out by NCI; however, other chronic studies failed to corroborate this finding. In the oral 13-week studies and in the oral and inhalation chronic toxicity/carcinogenicity studies, no histopathological effects were observed in the reproductive organs of mice and rats (NICNAS, 1998).

No effects on fertility in a two-generation study were reported in mice administered 1,1,1-trichloroethane containing 1,4-dioxane (up to 30 mg/kg-d). However, doses used in this study were an order of magnitude lower than those required to elicit toxic effects in chronic mouse studies. Limited evidence exists in rats that 1,4-dioxane has effects on certain sex hormones. No effects on implantation numbers, live fetuses, post-implantation loss, or major malformations were seen following administration (oral) of up to 1.0 ml/kg-d (1,033 mg/kg-d) 1,4-dioxane to pregnant rats. This dose caused slight maternal toxicity and embryotoxicity as evidenced by reduced maternal and fetal weight gain (NOAEL = 517 mg/kg-d) (NICNAS, 1998).

4.2.2.1 Reproductive Toxicity of 1,4-Dioxane in Humans

Studies on 'reproductive outcome' were conducted in pregnant women (314 workers) exposed to chemicals (including 1,4-dioxane) in the electronics industry. Effects included an increased incidence of miscarriages, premature births, maternal toxicosis, fetal ossifications, and decreased birth weights. Gonadotoxic effects, associated with 1,4-dioxane exposure, also in the electronics industry, were also reported. Insufficient data were

available in these studies to draw any conclusions with respect to 1,4-dioxane exposure and the effects observed. A PBPK model, developed for lactating women, indicated that exposure to 25 ppm 1,4-dioxane in air may give rise to a significant lactational transfer (Fisher *et al.*, 1997).

4.2.2.2 Reproductive Toxicity of 1,4-Dioxane in Experimental Animals

Testicular tumors were seen in rats in a chronic study (NCI, 1978); however, other sub-chronic and chronic studies have failed to corroborate this finding. In addition, 1,4-dioxane inhibits GJIC *in vitro*, a mechanism which has been associated with reproductive dysfunction in adult germ tissue. 1,4-Dioxane did not induce chromosomal aberrations *in vitro* (in CHO cells) or *in vivo* (male mouse germ cells), indicating a low potential for reduced fertility or inherited genetic effects (Appel 1988; NICNAS, 1998). In the oral 13-week studies and in the oral and inhalation chronic toxicity/carcinogenicity studies, no histopathological effects were observed in the reproductive organs of mice and rats (sections 4.2.1 and 4.3.1; **Tables 4-5 and 4-7**).

In a multi-generation study, modified to include screening for dominant lethal and teratogenic effects, with ICR Swiss mice, 1, 1, 1-trichloroethane (containing 3% 1,4-dioxane as stabilizer) and 1,2-dichloroethane were tested via the drinking water. A control group was treated with 0.17 mg 1,4-dioxane/ml in 1% Emulphor in deionized water. A naive control group which received only deionized water was also included in this study. In the 1,4-dioxane/Emulphor control group, no adverse effects were found in adults, reproductive performance, litter survival and growth, teratogenesis, or general pathology. With respect to dominant lethal screening, the frequency of dominant lethal factors was somewhat increased (TNO and RIVM, 2002). While this study was not performed with 1,4-dioxane as the test substance, it has some relevant supporting information for evaluation of the reproductive toxicity of 1,4-dioxane (Lane *et al.*, 1982).

4.2.3 Fertility Testing of 1,4-Dioxane in Experimental Animals

No effects on fertility were reported in OCR Swiss mice given 1,1,1-trichloroethane containing 3% 1,4-dioxane stabilizer (doses of 1,4-dioxane were estimated as 3, 10, and 30 mg/kg-d) during a two-generation drinking water study (DeRosa *et al.*, 1996). The validity of this study has been questioned due to the nature of the test material and the fact that the upper dose level was not shown to be approaching maternally toxic doses (NICNAS, 1998). The study of Lane *et al.* (1982) cited above found no effects on reproductive performance or outcome following treatment with 0.17 mg 1,4-dioxane/ml in 1% Emulphor in deionized water in a multi-generation study with ICR Swiss mice.

4.2.4 Developmental Toxicity of 1,4-Dioxane in Experimental Animals

The potential for 1,4-dioxane to induce developmental effects in the offspring of groups of 17 to 20 pregnant Sprague-Dawley rats given 0, 0.25, 0.5, and 1.0 ml/kg-d (0, 258, 517, and 1,033 mg/kg-d) by gavage, on gestational days 6 to15, has been reported (NICNAS, 1998). Food consumption and weight gain of the dam were followed. Rats were sacrificed on gestational day 21. During laparotomy, the numbers of corpora lutea, implantations, resorptions and live fetuses were recorded. Fetuses were weighed and inspected for external malformations. Fifty percent were examined for visceral and 50 percent for skeletal malformations. The females treated with 1.0 ml/kg-d showed a slightly smaller weight gain during treatment, which continued during the second stage of gestation suggesting slight maternal toxicity. Food consumption in these females decreased during treatment, this was especially evident in the first two days of treatment. There were no significant differences between control and treated groups in implantation numbers, live fetuses, or post-implantation loss. The frequency of major malformations remained within normal limits for all groups, and no deviations were found regarding minor anomalies and variants when compared with controls. Slight embryotoxicity, manifested by reduced average fetal weight (*i.e.*, 3.6 g compared to 3.8 g for controls, a significant difference) and reduced sternebral ossifications, occurred only at the highest dose level (*i.e.*, dams treated with 1.0 ml/kg-d), but there was no indication of teratogenicity. The NOAEL in this study for maternal and embryotoxicity can be established at 0.5 ml/kg-d, which is equivalent to 517 mg/kg-d.

In a developmental study, 1, 1, 1-trichloroethane (containing 3% 1,4-dioxane as a stabilizer) was given to CD rats. The 'vehicle control' group was given 0.05% Tween 80 with 0.9 ppm 1,4-dioxane as a stabilizer. When compared to a deionized/filtered water control group, no significant changes were seen in the vehicle controls however some very minor differences for maternal body weight and water consumption were observed (TNO and RIVM, 2002). While this study was not performed with 1,4-dioxane as the test substance, it also provides some relevant supporting information for evaluation (George *et al.* 1989).

In another study, no treatment-related developmental effects were seen in the offspring of Sprague-Dawley rats or Swiss Webster mice exposed (seven hr/d) by inhalation to 1,1,1-trichloroethane containing 3.5% 1,4-dioxane on gestational days 6 to 15 (Schwetz *et al.*, 1975). The exposure concentration for 1,4-dioxane was estimated to be 32 ppm (0.12 mg/L) (NICNAS, 1998).

4.2.5 Immuntoxicity of 1,4-Dioxane in Experimental Animals

Immunological effects for 1,4-dioxane have been evaluated in mice both *in vivo* and *in vitro* (NICNAS, 1998). Thurman *et al.* (1978) exposed murine and human lymphocytes *in vitro* to graded concentrations of 1,4-dioxane in culture medium, while exposing them to various

lymphocyte stimulating agents to assess potential immunologic responses. At 1% (10.3 mg/ml), 1,4-dioxane was toxic to murine lymphocytes while at 0.25% and 0.5%, it enhanced mouse B-cell responses while inhibiting T-cell responses. Human lymphocytes showed a slight enhancement of responses to phytohemagglutinin P when the 1,4-dioxane concentration reached 2.5%, but there was no noticeable reaction at lower concentrations. While mice were injected ip with 0.5 ml of 0.1% to 20% 1,4-dioxane every day for a week, the highest concentration was lethal to all animals. Lymphocytes were harvested from the spleen, lymph nodes, and thymus glands, and then tested with a series of lymphocyte stimulating agents. The lymphocytes from mice treated with 10% 1,4-dioxane for a week showed only slight differences from controls in response to mitogens.

Although induction of B-cell responses and inhibition of T-cell responses were seen at 0.5% 1,4-dioxane *in vitro*, little immunosuppression was seen *in vivo* even at near lethal doses. The meaning of such observations in terms of possible effects on immune function is unclear (NICNAS, 1998).

In sub-chronic and chronic bioassays carried out with 1,4-dioxane, no increased mortality associated with disease was noted, clinical chemistry parameters were normal, and tissues associated with the immune system reported were not affected by 1,4-dioxane treatment when examined histopathologically. In the NCI (1978) chronic bioassay both treated rats and mice were reported to have a higher incidence of respiratory infections (i.e., pneumonia) than controls (Hartung, 1989), but this finding has not been reported in other sub-chronic or chronic studies. As previously discussed, 1,4-dioxane was also negative in a well-conducted guinea pig maximization test and, therefore, is unlikely to be a sensitizing agent (TNO and RIVM, 2002).

4.2.6 Metabolism and Pharmacokinetics of 1,4-Dioxane

1,4-Dioxane vapor is rapidly absorbed from lungs in humans (~80%) and animals (~100%). Radiolabeled 1,4-dioxane was rapidly and almost completely absorbed after oral and inhalation exposure by rats. Therefore, it can be concluded that significant skin absorption of 1,4-dioxane may occur. In an *in vitro* study, it was demonstrated that 1,4-dioxane can penetrate human skin when occluded, but rapidly evaporates from human skin when not occluded (TNO and RIVM, 2002). Around 3% of an applied dermal dose was absorbed from unoccluded skin in monkeys (over 24 hrs); however, evidence indicates that higher levels are likely to be absorbed if evaporation is prevented (NICNAS, 1998). For the risk assessment purposes, 100% absorption has been chosen for the oral route and for inhalation. Dermal absorption can be calculated based on measured and estimated permeability constants.

In animals, 1,4-dioxane is widely distributed to the various organs, including target organs (liver and kidney), where selective accumulation has been reported. Covalent binding was

only demonstrated in liver, spleen, and colon. Administration of inhibitors and inducers of cytochrome P-450 results in an increase and decrease in the LD_{50} for 1,4-dioxane respectively, indicating a role for the microsomal mixed function oxidase system in metabolism and potentiation of acute toxicity. 1,4-Dioxane is rapidly metabolized in both humans and animals to β-hydroxyethoxyacetic acid (HEAA), which is predominantly excreted in urine with a small amount of unchanged 1,4-dioxane. Both HEAA and 1,4-dioxan-2-one have been identified as major metabolites of 1,4-dioxane in rat urine. Identification of these metabolites is, however, pH dependent. At a low pH, HEAA will be detected and at a high pH, HEAA will be converted to 1,4-dioxan-2-one. In humans, clearance of HEAA (from kidneys) is much faster (approximately 400 fold) than for 1,4-dioxane. Metabolism and plasma half-lives for 1,4-dioxane are similar in animals and humans (NICNAS, 1998).

In rats and humans, the pharmacokinetic and metabolic fate of 1,4-dioxane is comparable suggesting that the rat is a good surrogate for humans. In rats, metabolism has been shown to be dose-dependent due to a limited capacity to metabolize 1,4-dioxane to HEAA mediated by cytochrome P450. A metabolic threshold for biotransformation of 1,4-dioxane has been demonstrated in rats, above which a larger proportion (increasing with dose) of unchanged 1,4-dioxane is eliminated (in urine and expired air). Although the dose of 1,4-dioxane at which metabolic saturation occurs has not been fully elucidated in either animals or humans, it has been estimated that saturation occurs at a plasma level (steady state) of 100 µg/ml 1,4-dioxane in rats (NICNAS, 1998). A single oral dose of 10 mg/kg to rats was rapidly metabolized and excreted via the urine. A single oral dose of 1,000 mg 1,4-dioxane/kg saturated the metabolism of 1,4-dioxane to HEAA, resulting in decreased urinary excretion of HEAA and increased 1,4-dioxane in the expired air. In rats, 1,4-dioxane was eliminated from the plasma by linear kinetics with a $t_{1/2}$ of 1 hour after iv doses up to 10 mg/kg and after inhalation exposure to 180 mg/m^3. At higher iv doses (at or above100 mg/kg) elimination occurred progressively more slowly until plasma peak levels of 100 µg/ml were reached, thereafter elimination occurred with the same $t_{1/2}$ of lower doses. Hence, saturation of metabolism is likely to occur at 1,4-dioxane doses resulting in plasma levels above 100 µg/ml in rats. After inhalation exposure of humans to 50 ppm (180 mg/m^3) 1,4-dioxane, 1,4-dioxane was rapidly eliminated from plasma ($t_{1/2}$ of 1 hour) and excreted via urine (TNO and RIVM, 2002). No evidence of metabolic saturation was seen in humans exposed to this level of 1,4-dioxane for 8 hours, which lead to plasma levels (steady state) of 10 µg/ml 1,4-dioxane and 8 µg/ml HEAA (NICNAS, 1998).

Repeated oral administration of 1,4-dioxane to rats at high doses causes further alterations in the pharmacokinetics of 1,4-dioxane including changes in oxidizing enzyme capacity and a reduction in 1,4-dioxane accumulation in plasma. This correlates with the observed reduction in the 1,4-dioxane exhaled with respiratory air and the increase in the amount of CO_2 and possibly also with the shift in the ratio of oxidation products (HEAA, 1,4-dioxane-

2-one) to the possible intermediate products (*i.e.*, 1,4-dioxane-2-ol; β-hydroxyethoxy acetaldehyde) (TNO and RIVM, 2002). Toxicological data indicate that the metabolic saturation dose may be associated with chronic tissue damage which may be a precursor of neoplastic effects. Although increased retention of unmetabolized 1,4-dioxane has been proposed as a primary cause of liver/kidney damage, a number of metabolites, including 1,4-dioxan-2-one, β-hydroxyethoxy acetaldehyde, diethylene glycol, and oxalic acids, have also been implicated in the toxic/carcinogenic effects of 1,4-dioxane. However, available data are inconclusive as there is no evidence that any of the above metabolites are increased during metabolic saturation and evidence indicates that induction of metabolic enzymes occurs during repeated dosing (above metabolic saturation levels) with a concomitant reduction in 1,4-dioxane body burden (Young *et al.*, 1978a). Route of administration would also appear to have a bearing on toxicity and carcinogenicity (Kociba *et al.*, 1975), which may be due to differences in distribution to target organs (Reitz *et al.*, 1990). Thus, extrapolations between ingested and inhaled doses may be appropriate if differences in toxicokinetics are appreciated.

4.2.6.1 Absorption

No studies on absorption of 1,4-dioxane following ingestion by humans were located; however, it is assumed that it would be well absorbed from the gastrointestinal tract based on results in animals and from behavior of similar compounds. Male rats (three) were given single oral doses of 10, 100, or 1,000 mg ^{14}C-1,4-dioxane/kg. Radioactivity in urine, feces and expired air was determined after 24 hours for rats treated once with 10 mg/kg and after 72 hours for rats given a single dose of 100 or 1000 mg/kg. In another experiment, two male rats were treated with 10 or 1,000 mg ^{14}C-1 4-dioxane/kg for 17 days. Excreta were collected up to 20 days. At the experiment's end, the rats were sacrificed and analyzed for radioactivity (**Table 4-6**). After both single and repeated administration, high absorption of ^{14}C-1,4-dioxane occurs in rats, as demonstrated by urinary excretion of 75.74 to 98.74 % of the administered dose and fecal excretion of only 0.46 to 2.05 % of the applied dose. The concentration of expired 1,4-dioxane increases in a dose related manner from 0.43 % at 10 mg/kg to 25.25 % at 1,000 mg/kg indicating saturation of urinary excretion and metabolism. After multiple dosing, saturation also occurs and, in addition, the amount of expired CO_2 increases. When 1,000 mg/kg was given repeatedly, expired 1,4-dioxane decreased and expired $^{14}CO_2$ increased compared with single dosing. This effect was not observed when 10 mg/kg was given repeatedly (TNO and RIVM, 2002). These experiments showed that greater than 95% 1,4-dioxane was absorbed from the gastrointestinal tract (Young *et al.* 1978a-b; DeRosa *et al.*, 1996).

Table 4-6. Cumulative Excretion of Radioactivity in Rats after Oral Dosing with 1,4-Dioxane

	10 mg/kg (single dose)	100 mg/kg (single dose)	1000 mg/kg (single dose)	10 mg/kg (repeat dose)	1000 mg/kg (repeat dose)
Time (hrs)	24	72	72	480	480
% of the dose					
urine	98.74	85.52	75.74	98.87	82.32
feces	0.95	1.95	1.06	0.46	2.05
expired 1,4-dioxane	0.43	4.69	25.25	1.33	8.86
expired $^{14}CO_2$	3.07	3.13	2.39	4.17	6.95
body	3.11	1.47	1.02	0.63	0.53

Young *et al.*, 1978a

An inhalation study was carried out in four healthy male volunteers exposed to 50 ppm (180 mg/m^3) 1,4-dioxane for six hours in a chamber under dynamic airflow conditions. Blood was sampled at regular intervals up to 12 hours after the start of the experiment. Urine was collected during and after exposure for a total of 48 hours. The maximum uptake (10.9 mg/kg) was around 50% of that measured in rats following similar exposure (Young *et al.*, 1977). Because of its rapid biotransformation to HEAA, the body burden of 1,4-dioxane was estimated to be no more than 1.2 mg/kg at steady state (NICNAS, 1998). A simulation of repeated daily exposures to 180 mg/m^3 1,4-dioxane for eight hr/d indicated that 1,4-dioxane would never accumulate to concentrations above those attained after a single eight hour exposure as long as the exposure concentration of 1,4-dioxane was 180 mg/m^3 or less (Young *et al.*, 1977).

Four male Sprague-Dawley rats with jugular vein cannulas were placed in a one liter "head-only" chamber under dynamic air flow conditions. The flow rate of 1,4-dioxane vapor was adjusted to give a chamber concentration of 180 mg/m^3 (50 ppm). During and after the six hour exposure period, urine was collected and analyzed. The radioactivity expressed as 1,4-dioxane in plasma at the end of exposure was 7.3 µg/ml. Thereafter, the plasma concentration of 1,4-dioxane decreased in a log-linear manner until it was not detectable (<0.3 µg/ml) 11 hours after the start of experiment. A $t_{1/2}$ of 1.01 hour was calculated. The amounts of 1,4-dioxane and HEAA in urine during exposure (six hours) were 5.1 and 7,613 µg, respectively, and 48 hours afterwards 1.7 and 13,659 µg, respectively (*i.e.*, a total of 7

μg of 1,4-dioxane and 21 mg of HEAA were excreted after 48 hours). Hence, more than 99.9% of the total urinary excretion of the inhaled 1,4-dioxane was HEAA. When estimated from the total 1,4-dioxane (6.8 μg) and 1,4-dioxane equivalents of HEAA (21,271 μg * 0.73 [= ratio of molecular weights] excreted in urine), the rats absorbed at least 72 mg 1,4-dioxane/kg during the six hour exposure period. Assuming a respiratory minute volume of 240 ml/minute for rats, these data indicate almost complete absorption (DeRosa et al., 1996; Young *et al.* 1978a).

No data exist to assess the *in vivo* dermal uptake for 1,4-dioxane in humans, although skin absorption has been considered a potential route of exposure in case reports of human fatalities from short term exposures.

The ability of ^{14}C-1,4-dioxane to penetrate excised human skin has been examined. The substance was applied to the epidermis in three different vehicles representative for cosmetic products. In *in vitro* diffusion cell studies on human skin, significant differences exist for the ability of 1,4-dioxane to cross the skin under occluded and non-occluded conditions. Since ^{14}C-1,4-dioxane is a volatile compound, the evaporation after application to the skin was also determined. When evaporation was prevented, the absorption rate values for 1,4-dioxane in each vehicle were: water, 4.3×10^{-4} cm/hr; isopropopyl myristate, 11.2×10^{-4} cm/hr; and 'popular lotion', 2.7×10^{-4} cm/hr. When 1,4-dioxane was applied in the 'popular lotion' and evaporation was allowed to occur, skin permeation was reduced approximately 10 to 20-fold. Up to 3.2% of applied 1,4- dioxane (dissolved in lotion) was absorbed under occlusion for 3.5 hour, whereas only 0.3% absorption occurred under non-occluded conditions (TNO and RIVM, 2002). These differences in the amount of absorption were solely associated with the high volatility of 1,4-dioxane. From the solubility characteristics alone, Grandjean (1990) predicted that "considerable uptake" by the skin could be expected for 1,4-dioxane, but that oxidation and evaporation from the skin surface would limit the total amount absorbed. Almost 90% (as a percentage of applied dose) evaporation of ^{14}C-1,4-dioxane from a thin layer of the lotion was demonstrated within 15 minutes of application (to a non-absorbent test material), with the remainder evaporating slowly over the next 24 hour (Bronaugh, 1982). A permeability constant (Kp) of 2.7×10^{-4} cm/hr was determined for the occluded test system. This value is similar to that calculated for undiluted 1,4-dioxane using the formula of Potts and Guy (1992). The absorption rate for 1,4-dioxane (under occlusion) was calculated to be approximately 0.3 mg/cm^2/hr (NICNAS, 1998). According to the authors, these results rank 1,4-dioxane as a rapidly penetrating compound and compares with other solvents reported as being readily absorbed in *in vitro* skin (human) tests.

In a skin-penetration study male and female Pitman-Moore Rhesus monkeys (n= 3 to 6) received applications of ^{14}C-1,4-dioxane in methanol or a skin lotion on the forearm for 24 hours (dose: 4 μg/cm^2; skin area: 3 to 15 cm^2). After the 24 hour treatment period, the treated area was washed with water and soap. One and five minutes after treatment with 1,4-dioxane

in skin lotion, 36% and 15% of the applied dose, respectively, was still detectable on the skin. Urine was collected over a 5-day period and was analyzed for the radiolabel. Within 24 hours after treatment, 2.3% and 3.4% of the applied radioactivity was absorbed from unoccluded skin and excreted in the urine (Marzulli *et al.*, 1981). The peak rate of absorption was within four hours after treatment when estimated on urinary excretion. The extent of evaporation from the application site during the course of the experiment was not determined, but according to Appel (1988), the results could be affected by evaporation because only 15% of the applied dose was detectable after 5 minutes. Despite this, the fact that between 30 to 50% of the dose was absorbed within the first four hours indicates that dermal absorption can be high.

In a very limited study by Fairley *et al.* (1934), repeated application of an 80% aqueous 1,4-dioxane solution to the skin of four rabbits and guinea pigs under non-occlusive conditions led to damage of the renal tubulus cells and glomeruli as well as hemorrhages in the renal medulla, and liver degeneration within 50 to 100 days. The only conclusion possible from this study is that significant dermal absorption of 1,4-dioxane can occur.

4.2.6.2 Distribution

At various times (up to 16 hours) after ip injection of 6.97 mg ^{3}H-1,4-dioxane/kg to male Sprague-Dawley rats, the distribution of radioactivity was studied in whole blood, liver, kidney, spleen, lung, colon, and skeletal muscle. One to two hours after treatment, the kidneys had 1.5 to 2 times higher levels of radioactivity than the other tissues which is consistent with preferential excretion in the urine. Distribution among other tissues was more or less uniform. Radioactivity in all examined tissues decreased in time. In the blood, radioactivity was higher than in examined tissues at all sampling points, except for kidneys after 1 hour. Studies of the nature of 1,4-dioxane binding revealed that the extent of 'covalent' binding (as measured by incorporation of radioactivity into lipid free, acid-insoluble tissue residues) increased up to six hours post-injection and was clearly higher in the liver, spleen, and colon than in other tissues. Much lower amounts of 'covalent' binding occurred in the skeletal muscle and blood. Investigations of the subcellular distribution in liver indicated that most of the radioactivity was in the cytosol, followed by the microsomal, mitochondrial, and nuclear fractions. The specific activity of all three particulate fractions reached a maximum at 6 hours after 1,4-dioxane administration. The percent 'covalent binding' (as measured by incorporation into lipid free, acid insoluble tissue residues) was highest in the nuclear fraction, followed by microsomal and mitochondrial fractions and the whole homogenates. Pre-treatment of rats with inducers of microsomal mixed function oxidases [3-methylcholanthrene (dissolved in corn oil, given as a single ip dose of 40 mg/kg 24 hours prior to 1,4-dioxane administration), polychlorinated biphenyls (dissolved in corn oil and administered ip at 500 mg/kg four days prior to 1,4-dioxane administration) and phenobarbital (dissolved in 0.9% saline and administered ip at a dose of 80 mg/kg daily for

four consecutive days prior to 1,4-dioxane treatment)] had no significant effect on the 'covalent binding' of 1,4-dioxane to the various subcellular fractions of the liver. There was no microsome-catalyzed *in vitro* binding of ^{3}H- or ^{14}C-1,4-dioxane to DNA under conditions that brought about substantial binding of ^{3}H-benzo[a]pyrene (Woo *et al.*, 1977a; DeRosa *et al.*, 1996). Binding to macromolecules was non-specific and not associated with DNA (DeRosa *et al.*, 1996). This finding was consistent with investigations into hepatic DNA alkylation in rats (Sprague-Dawley) carried out by Stott *et al.* (1981).

In a similar study, Sprague-Dawley rats received a single ip dose corresponding to 1/10 of the LD_{50} (799 to 5600 mg/kg) of ^{14}C-1,4-dioxane/kg (no details provided). Six rats/time point were sacrificed after 5, 15, and 30 minutes and after 1, 3, and 6 hours. After 5 minutes, a maximal 1,4-dioxane level was found in liver and kidney, and after 15 minutes in blood, brain, and testes. The tissue/blood partition coefficient in the liver was 0.8, remaining constant throughout the experiment. In kidneys, the tissue/blood partition coefficient was also 0.8, but it increased to 1.0 by the end of the experiment. In the testes, the tissue/blood partition coefficient was 0.6 after five minutes and 1.3 by the end of the experiment. In the brain, the tissue/blood partition coefficient was 0.7 remaining constant throughout the experiment. The subcellular tissue/blood partition coefficient for the nuclear fraction of liver cells was 0.06 and for mitochondrial liver fractions 0.01 after six hours (Mikheev *et al.*, 1990). The authors concluded that selective uptake of 1,4-dioxane takes place in liver and kidney, due to the fact that T_{max} (maximum accumulation time) values for these organs were less than that for blood.

PBPK modeling of rat data by Reitz *et al.* (1990) indicated that liver concentrations of 1,4-dioxane were approximately 2.5 times greater from ingestion than from inhalation of similar doses (*i.e.*, 0.1% in water or 111 ppm in air) of 1,4-dioxane.

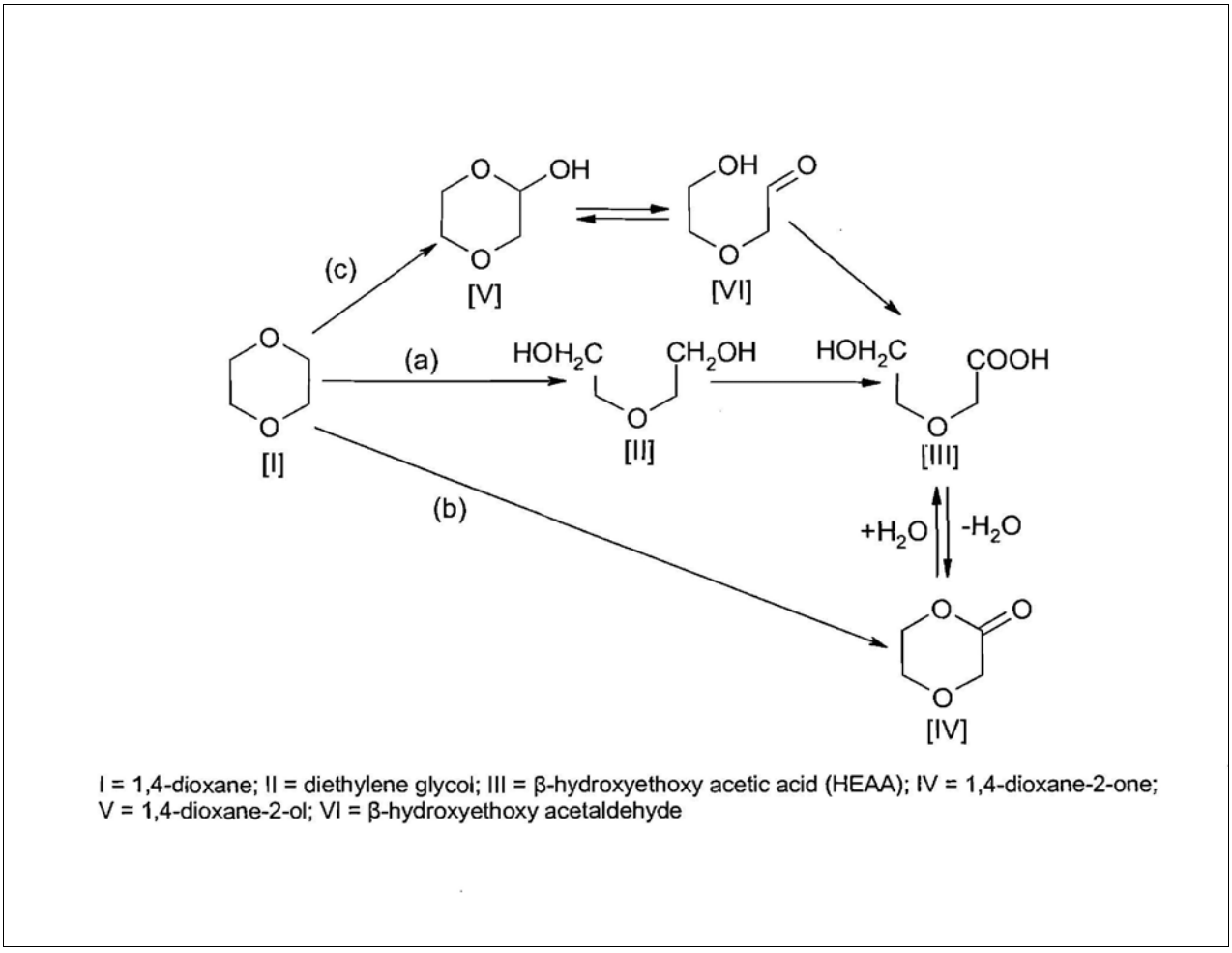
4.2.6.3 Metabolism

NIOSH proposed the following pathway for 1,4-dioxane biotransformation: initial formation of an oxonium ion; nucleophilic attack by water to open the ring, with the formation of the corresponding alcohol; rapid reduction of the alcohol to β-hydroxyethoxy acetaldehyde; and rapid oxidation of the aldehyde to HEAA (DeRosa *et al.*, 1996). The possible metabolic pathways of 1,4-dioxane are depicted in **Figure 4.1** (I=1,4-dioxane; II=diethylene glycol; III = HEAA; IV=1,4-dioxane-2-one; V=1,4-dioxane-2-ol, VI= β-hydroxyethoxy acetaldehyde). This pathway includes: a) hydrolysis to diethylene glycol, followed by oxidation of one of the hydroxyl groups, b) direct conversion via a possible ketoperoxyl radical intermediate, and c) through α-hydroxylation, followed by the oxidation of the hemiacetal or hydroxyaldehyde intermediate (Woo *et al.*, 1977b).

Pharmacokinetic and toxicological data indicate that 1,4-dioxane toxicity occurs only after exposure to doses large enough to saturate processes for detoxification and elimination (Kociba *et al.*,1974).

In humans, the major metabolite of 1,4-dioxane is HEAA. Four volunteers were exposed to 50 ppm 1,4-dioxane for six hours (Young *et al.*, 1977). A steady state plasma level of 10 µg/ml 1,4-dioxane was reached after three hours inhalation exposure, with a steady state plasma concentration of 8 µg/ml HEAA reached one hour after cessation of exposure (*i.e.*, after seven hours). The plasma half-lives for 1,4-dioxane and HEAA were around 1 and 2.5 hours, respectively. HEAA accounted for around 99% of recovered 1,4-dioxane in urine. Clearance of 1,4-dioxane from kidneys was approximately 400 times slower than HEAA (DeRosa *et al.*, 1996). The authors concluded that the pharmacokinetics of 1,4-dioxane in humans can be described by a one compartment model with zero order uptake and first order elimination, and that repeated exposure to 50 ppm 1,4-dioxane would not lead to accumulation in plasma. In workers exposed to a time-weighted average concentration of 1.6 ppm (5.8 mg/m^3) 1,4-dioxane for 7.5 hours (Young *et al.*, 1976), the average concentrations of 1,4-dioxane and HEAA in samples of urine collected at the end of each workday were 3.5 and 414 µmol/l, respectively. The high ratio of HEAA to 1,4-dioxane suggests that, at low-exposures, 1,4-dioxane is rapidly metabolized to HEAA, with no evidence of non-linear pharmacokinetics, that is, no evidence of saturation of biotransformation of 1,4-dioxane to HEAA (Dietz *et al.*, 1982). The metabolite HEAA accumulates in tissues with a high oxidative capacity (Hecht & Young, 1981; Hecht *et al.*, 1983). Metabolic rate constants developed for 1,4-dioxane in humans in a PBPK model were K_m = 3.0 mg/L and V_{max} = 6.35 mg/kg-hr (Reitz *et al.*, 1990). 1,4-Dioxane may also inhibit the oxidative metabolism of other substances as it has been shown to inhibit human CYP2A6 activity in liver microsomes *in vitro* (Draper *et al.*, 1997).

It is unclear whether 1,4-dioxane is metabolized directly to HEAA or whether 1,4-dioxane-2-one is the principle metabolite that undergoes hydrolysis to HEAA (NICNAS, 1998). Male Sprague-Dawley rats received ip 1,000 (in Woo *et al.*, 1977c, the lowest dose is stated as 500) to 4,000 mg 1,4-dioxane/kg. Urine samples were collected in 8 to12 hour intervals for two days and analyzed for volatile compounds. Two major peaks were identified, one for



I = 1,4-dioxane; II = diethylene glycol; III = β-hydroxyethoxy acetic acid (HEAA); IV = 1,4-dioxane-2-one; V = 1,4-dioxane-2-ol; VI = β-hydroxyethoxy acetaldehyde

Figure 4-1. Possible Metabolic Pathways of 1,4-Dioxane (Woo *et al.*, 1977c)

1,4-dioxane and one for a metabolite which was identified as 1,4-dioxane-2-one. The identification of HEAA verus 1,4-dioxane-2-one may depend on the methods of analysis and appears to be pH dependent (DeRosa *et al.*, 1996). It has been suggested that 1,4-dioxane-2-one is an artifact resulting from the cyclization of HEAA to 1,4-dioxane with the loss of water at might occur at the elevated temperatures associated with gas chromatography (Hartung, 1989). 1,4-Dioxane-2-one was undetectable at a pH greater than12, but reappeared upon re-acidification of the urine. At low pH, HEAA was detected as the major metabolite. At high pH, HEAA is converted to 1,4-dioxane-2-one, which was then identified as the major metabolite by Woo *et al.* (1977b). These two substances are in chemical equilibrium. The excretion of this metabolite was also dose- and time-dependent, reaching a maximum between 20 to 28 hours after dosing. The amount of unchanged 1,4-dioxane in urine accounted for 2.9, 6.8, 10.8, and 10.8% of the 1,000 (or 500), 2,000, 3,000, and 4,000 mg/kg doses, respectively. At a dose of 3,000 mg/kg, 33% was excreted as 1,4-dioxane-2-one (Woo *et al.*, 1977b-c).

Apart from the above mentioned oxidation products, 1,4-dioxane-2-one and HEAA, Hecht and Young (1981) postulated the formation of 1,4-dioxane-2-ol as a result of hydroxylation. The substance is in equilibrium with the reactive and presumably cytotoxic β-hydroxyethoxy acetaldehyde. Toxicologically significant amounts presumably can be formed in cells in which the oxidative capacity has been saturated by high doses of 1,4-dioxane prohibiting the complex oxidation to HEAA. Studies of Braun and Young (1977) show that 1,4-dioxane forms an artifact during gas chromatographic separation as a cyclization product of HEAA (TNO and RIVM, 2002).

In experimental animals, increased and decreased acute toxicity following administration of inducers and inhibitors of cytochrome P-450 indicate a role for microsomal mixed function oxidases in 1,4-dioxane metabolism (DeRosa *et al.*, 1996; NICNAS, 1998). The effect of typical inducers and inhibitors of hepatic mixed function oxidases (MFO) on the excretion of 1,4-dioxane-2-one, the main 1,4-dioxane metabolite in urine, was studied in rats treated ip with 3 g/kg 1,4-dioxane. Pre-treatment with the inducers phenobarbital (dissolved in 0.9% NaCl solution and administered ip at a dose of 80 mg/kg daily for four consecutive days prior to 1,4-dioxane treatment), the polychlorinated biphenyl Aroclor 1254 (dissolved in corn oil and administered as a single oral ip of 500 mg/kg four days prior to 1,4-dioxane administration) and, to a much lesser extent, 3-methylcholanthrene (dissolved in corn oil, given as a single ip dose of 40 mg/kg 24 h prior to 1,4-dioxane administration) increased the metabolite excretion and shortened the time of onset of peak excretion of the metabolite. In contrast, inhibitors of MFO such as 2,4-dichloro-6-phenylphenoxyethylamine (dissolved in 0.9% NaCl solution and given ip at doses of 15.9 mg/kg at 0.5 hours before and 8, 16, 24 hours after 1,4-dioxane administration) and cobaltous chloride (injected sc at 60 mg/kg 24 hours prior to 1,4-dioxane administration) decreased the metabolite excretion. The effects observed were independent of renal excretory function. Of the inducers, phenobarbital had the greatest effect, followed by Aroclor 1254. 3-Methylcholanthrene was the weakest (Woo *et al.*, 1977d; 1978).

Two male Sprague-Dawley rats were gavaged with a single oral dose of 1,000 mg ^{14}C-1,4-dioxane/kg in distilled water. Urine was collected for 24 hours and analyzed for radioactivity. Metabolites were identified by thin layer chromatography, gas chromatography, nuclear magnetic resonance (NMR), and gas chromatography/mass spectrometry. HEAA was identified as the major metabolite in urine and amounted to about 85% of the excreted material. The remaining 15% in urine were attributed to unchanged 1,4-dioxane and diethylene glycol (TNO and RIVM, 2002).

The fate of 1,4-dioxane in rats is markedly dose-dependent because of limited capacity of rats to metabolize it to HEAA, and there is an apparent threshold for toxic effects of 1,4-dioxane that coincides with saturation of metabolic pathway for its detoxification. The pharmacokinetics of 1,4-dioxane appeared to be non-linear. Young *et al.* (1978a-b)

demonstrated that the pharmacokinetics of 1,4-dioxane in rats differ markedly depending on the dose and suggested that the metabolism of 1,4-dioxane in rats is saturated at plasma levels above 100 µg/ml. Six rats received iv doses of 3, 10, 30, 100, 300 or 1,000 mg ^{14}C-1,4-dioxane/kg and samples of blood were obtained via the right jugular vein every five minutes for estimating radioactivity in plasma. Two additional rats were used to estimate radioactivity in expired air (1,4-dioxane and $^{14}CO_2$) as well as in urine and feces. These rats were equipped with jugular and ureter cannulas. At the lowest iv doses, 3 and 10 mg/kg, radioactivity was eliminated from the plasma by apparently linear kinetics with a $t_{1/2}$ of 1.1 hour. At higher doses, the metabolism of 1,4-dioxane to HEAA appears to be saturated at high doses as a larger fraction of 1,4-dioxane is retained in the body and eliminated in the breath. The radioactivity from high doses was eliminated from plasma progressively more slowly. At dose levels at or above 100 mg/kg, elimination was retarded until a peak level of approximately 100 µg/ml plasma was reached, thereafter, elimination occurred with the same $t_{1/2}$ as that at lower doses.

As the dose increased from 3 to 1,000 mg/kg, plasma clearance decreased from 3.33 to 0.25 ml/min. The renal clearance rates of 1,4-dioxane were low: 0.032 ml/min at 10 mg/kg and 0.029 ml/min at 1,000 mg/kg, indicating that 1,4-dioxane is extensively reabsorbed by the kidney. The pulmonary clearance of 1,4-dioxane was also low: 0.032 at 10 mg/kg and 0.055 ml/min at 1,000 mg/kg. The metabolic clearance (*i.e.*, the difference between plasma clearance and the total of renal and pulmonary clearance) decreased from 2.82 ml/min at 10 mg/kg to 0.17 ml/min at 1,000 mg/kg (TNO and RIVM, 2002), indicating saturation of the metabolic capacity of rats at high dose levels. Repeated daily administration of 1,000 mg/kg also results in a marked decrease in the body burden of 1,4-dioxane after a few days, indicating that, at high daily doses, 1,4-dioxane induced its own metabolism. Similar body burdens of 1,4-dioxane were observed following repeated exposure to 10 mg/kg and 1,000 mg/kg following this induction period. Pharmacokinetic parameters determined for 1,4-dioxane in rats (3 to1,000 mg/kg by iv) were K_m = 20.93 mg/L and V_{max} = 13.3 mg/kg-hr. At saturation, the maximum velocity of metabolism to HEAA was about 18 mg/kg. The saturation curve for 1,4-dioxane in this experiment suggested that doses of 1,4-dioxane below the plateau of 100 µg/ml plasma are metabolized and eliminated rapidly, while doses of 1,4-dioxane resulting in higher plasma levels are removed progressively more slowly from the body due to saturation of metabolism (Young *et al.*, 1978a). Dietz et al. (1982) suggested that liver damage (and hence carcinogenicity) in rats only occurs when metabolizing enzymes are saturated and pharmacokinetics becomes non-linear.

In a similar study, rats received a single gavage dose of 10, 100, or 1,000 mg ^{14}C-1,4-dioxane/kg. At the low dose level of 10 mg/kg, almost all of the administered dose was rapidly excreted in the urine as HEAA and only a small amount of parent compound was excreted in the exhaled air. At higher doses (100 and 1,000 mg/kg) more unchanged ^{14}C-1,4-dioxane was excreted in the expired air. In rats given a daily oral dose of 1,000 mg ^{14}C-1,4-

dioxane/kg for 17 days, increased biotransformation of 1,4-dioxane was seen as demonstrated by a higher excretion of metabolites in urine and exhalation of CO_2. At this dose level, induction of the metabolizing enzymes also took place. At repeated administration of 10 mg/kg, no increase in biotransformation was seen (Dietz *et al.*, 1982; Reitz *et al.*, 1990; Young *et al.*, 1978a).

Doses of 1,4-dioxane in excess of that required for metabolic saturation have been associated with toxicity (including carcinogenicity) in rats (Dietz *et al.*, 1982; Kociba *et al.*, 1975). 1,4-Dioxan-2-one and β-hydroxyethoxy acetaldehyde, diglycolic, and oxalic acids have been proposed as possible metabolites associated with toxic/carcinogenic effects resulting from 1,4-dioxane exposure. However, there is no evidence that these or other metabolites are increased during metabolic saturation. There is evidence to suggest that metabolic induction occurs during repeated dosing (above metabolic saturation levels) with a concomitant reduction in 1,4-dioxane body burden over time (Young *et al.*, 1978a).

4.2.6.4 Excretion

During the chamber exposure of four healthy male exposed to 50 ppm (180 mg/m^3) 1,4-dioxane for six hours, a plateau concentration was reached in plasma after an initial rapid rise (Young *et al.*, 1977). After exposure, the plasma 1,4-dioxane concentration decreased linearly, indicating first-order elimination, which was not saturated at 50 ppm. The plasma $t_{1/2}$ for elimination of 1,4-dioxane was 59 minutes (0.98 $\pm$ 0.12 hr) . The plasma HEAA concentration was maximal after 7 hours, thereafter, it fell log-linearly. After the exposure period, the plasma HEAA concentration was higher than the plasma 1,4-dioxane concentration.

From the total administered 1,4-dioxane 99.3% was eliminated via the urine as HEAA. In the 0 to 6 hour interval, 47% of the total HEAA was excreted, and none was detectable after 24 hours. The $t_{1/2}$ for elimination of HEAA in urine was 2.7 hours. Only 0.7% of the total administered dose was eliminated by excretion of 1,4-dioxane in the urine, 90% of which was already recovered in the 0 to 6 hour period. None was detectable after 12 hours. The $t_{1/2}$ for elimination of 1,4-dioxane in urine was 48 hours. When estimated from the total 1,4-dioxane and 1,4-dioxane equivalents of HEAA excreted in urine, at least 5.4 mg 1,4-dioxane/kg was absorbed during the six hour exposure period (*i.e.* at least 50% of the administered dose, assuming a respiratory volume of 20 m^3/day). Since a large fraction of both 1,4-dioxane and HEAA was eliminated during the exposure period, the calculated dose of 5.4 mg/kg was not in the body at one time. The maximum amount in the body occurred at six hours. To calculate this amount, the total 1,4-dioxane and 1,4-dioxane equivalents of HEAA excreted from 0 to 6 hours were subtracted from the total dose to obtain 2.86 mg equivalents of 1,4-dioxane per kilogram in the body at six hours (TNO and RIVM, 2002).

1,4-Dioxane and HEAA were found in the urine of 1,4-dioxane plant personnel exposed to a time weighed average concentration of 1.6 ppm (5.8 mg/m^3) for 7.5 hours. The average concentration of 1,4-dioxane and HEAA in samples of urine collected at the end of the working day amounted 3.5 and 414 µmol/L, respectively (Young *et al.*,1976). Hence, 1,4-dioxane composed only 0.8% of the total concentration of 1,4-dioxane and HEAA in urine, suggesting that metabolism of 1,4-dioxane to HEAA in humans is very rapid and not saturated at a vapor concentration of 1.6 ppm (Young *et al.*, 1976). A PBPK model, developed for lactating women, indicated that exposure to 25 ppm 1,4-dioxane in air may give rise to a significant lactational transfer (Fisher *et al.*, 1997).

Following inhalation exposure of rats for 6 hours to 50 ppm 1,4-dioxane, the excretion of 1,4-dioxane in urine and expired air followed the same pattern of 1,4-dioxane in plasma, indicating first order processes. The metabolite HEAA accounted for approximately 99% of urinary metabolites (Young *et al.*, 1978b). In this study, 75% of 1,4-dioxane and 36% of total HEAA was eliminated during the exposure period. The concentration of 1,4-dioxane in plasma decreased in a first order manner from 7.3 µg/ml at the end of exposure to non-detectable levels at 11 hours (*i.e.*, 5 hours post-treatment).

Oral dosing (single) of male rats to 10, 100, and 1,000 mg/kg of labeled ^{14}C 1,4-dioxane resulted in approximately 99%, 85%, and 75% of dose in urine and approximately 0.5%, 5%, and 25% of dose as expired 1,4-dioxane, respectively. Metabolites in feces were 1% to 2% irrespective of dose. The excretion of 1,4-dioxane *per se* was measured in rats administered iv doses of labeled 1,4-dioxane. Total 1,4-dioxane in urine was 4 $\pm$ 1% and 11 $\pm$ 27% of dose at 10 mg/kg and 1,000 mg/kg, respectively (NICNAS, 1998). Following 10 mg/kg, 92% was excreted as HEAA in urine, while following 1,000 mg/kg bw this was only 60% (Young *et al.*, 1978a).

Young *et al.* (1978a) also measured plasma levels of 1,4-dioxane following single iv administration of 3, 10, 30, 100, 300, and 1,000 mg/kg 1,4-dioxane. Doses up to 10 mg/kg were eliminated from plasma by linear kinetics, whereas above 30 mg/kg plasma clearance was markedly nonlinear and could be described by Michaelis-Menten kinetics. Plasma half-lives increased from 1.1 hours to 14.2 hours after injection of 10 mg/kg and 1,000 mg/kg, respectively. The area under the curve (AUC) for plasma concentrations of 1,4-dioxane also increased disproportionately with dose, indicating that elimination of 1,4-dioxane from blood is a saturable process (Dietz *et al.*, 1982). Neither pulmonary or renal clearance rates were significantly different, at low (10 mg/kg) or high (1,000 mg/kg) 1,4-dioxane doses, to account for the dose-dependent decrease in plasma clearance rates. This was interpreted by the study authors as evidence for saturation of 1,4-dioxane biotransformation rather than elimination (Young *et al.*, 1978a).

4.3 Tier 3 Toxicity Data for 1,4-Dioxane

4.3.1 Summary - Chronic Toxicity and Carcinogenicity of 1,4-Dioxane

Toxicological effects observed in these longer term studies in rats and mice after oral administration in the drinking water included severe effects on the liver, kidneys, and nose. LOAELs for1,4-dioxane of 0.02% (equal to 0.016 g/kg-d) and 0.1% (90 to 150 mg/kg-d for male and female rats) for chronic oral exposure has been established based on these effects (TNO and RIVM, 2002; NICNAS, 1998). A dose of 0.01% (equivalent to 10 mg/kg-d) showed no effects and can be considered a NOAEL. LOAELs were not determined in either chronic inhalation or dermal studies, although no effects were seen in rats or mice exposed to 111 ppm (108 mg/kg-d) and 1,500 mg/kg-d, respectively so these may be considered as NOAELs (NICNAS, 1998).

4.3.1.1 Chronic Toxicity

4.3.1.1.1 Epidemiological Studies

In a matched-pair study of 151 textile factory workers exposed from one to six years to atmospheric concentrations up to 1,350 mg/m^3 (250 ppm) of 1,1,1-trichloroethane blended with 4% 1,4-dioxane (exposure levels not reported) stabilizer, no significant differences in health, particularly on heart (*i.e.*, electrocardiogram changes) or liver were reported when compared to a control group (TNO and RIVM, 2002).

In a study of 74 workers exposed to 1,4-dioxane during manufacture and handling, for an average duration of 25 years, with an estimated exposure to 0.02 to 48 mg/m^3 (0.006 to13 ppm), an increased serum transaminase levels was seen in 6 of 24 workers currently exposed. However, since all six workers were known to consume at least 80 grams of alcohol per day for several years, the authors concluded these effects may have been related to alcohol consumption. Additionally, other exposures were concurrent in the workplace (*i.e.*, dichloroethane and ethylene chlorohydrin). Chromosome analyses of lymphocyte cultures in these six showed 5.65% gaps and 1.74% fragments, dicentric chromosomes, and other aberrations compared to 5.24% gaps and 2.62% other aberrations in unexposed controls (Thiess *et al.*, 1976).

In a study of 165 production workers exposed to 1,4 -dioxane (less than 25 ppm for 1 to 4 years), 12 deaths were reported, including a death from chronic hepatitis/cirrhosis. Results are inconclusive given the small cohort size and relatively short exposure duration (Buffler *et al.*, 1978).

4.3.1.1.2 Chronic Toxicity in Experimental Animals

Several chronic (*i.e.*, greater than one year in duration) studies have been carried out in rats, mice and guinea pigs. In general, the non-neoplastic lesions observed and organs affected (liver/kidney) are consistent with observations from acute and sub-chronic studies. The chronic toxicity bioassays are summarized in **Table 4-7**.

4.3.1.1.2.1 Chronic Oral Toxicity Studies in Experimental Animals

Several oral drinking water studies have been carried out in rats, mice and guinea pigs. Argus et al. (1965) exposed rats to 1% 1,4-dioxane in drinking water for 63 weeks. Changes found in the kidney resembled glomerulonephritis. Liver changes were only poorly described, but no cirrhosis was noted. Lungs of both exposed and control rats showed severe inflammation suggestive of a respiratory infection. In rats, gross effects (decreased body weight) were observed at 0.5% with increased relative and absolute liver weights at 1% 1,4-dioxane. Hepatic and renal histopathological effects (*i.e.*, hepatic and renal tubular degeneration/necrosis and regeneration) were seen at 0.1% (Kociba *et al.*, 1974).

The NOAEL for non-neoplastic effects was 0.01 to 0.02% 1,4-dioxane (equivalent to 10 to 40 mg/kg-d) derived from Kociba *et al.* (1974) and Yamazaki *et al.* (1994) and based on the increased incidence (dose-related) of spongiosis hepatis seen in males at and above 0.02% (statistically significant at 0.1%). Rats exposed via drinking water to 0.5% or 1.0% 1,4-dioxane for 110 weeks (NCI, 1978) showed tubular degeneration of the kidney, hepatic cytomegaly, and stomach ulceration (in males). In mice, gross effects (decreased body weight) were observed at 0.2% (Yamazaki *et al.*, 1994), with pulmonary, hepatic, and nasal effects at and above 0.5% (equivalent to 400 to700 mg/kg-d) 1,4-dioxane (NCI, 1978). A NOAEL was not identified in this study.

In guinea pigs, pulmonary effects were reported at 0.5 to 2.0% (equivalent to >2000 mg/kg-d, calculated from total intake data) 1,4-dioxane (Hoch-Ligeti and Argus, 1970). A NOAEL was not identified in this study.

Table 4-7. Summary of Chronic Toxicity and Carcinogenicity Bioassays for 1,4-Dioxane

Route	Species	Number	Dose	Exposure	Clinical & pathology (non-neoplastic)	Tumor Incidence	Source
Oral (DW)	Male Wistar Rats	26/test group 6 controls	1.0%	*ad. libitum* - 63 wks.	Severe kidney damage, enlarged hyperchromic hepatic nuclei. Study considered inadequate	6/26 animals with liver tumors, of which 1 also had a renal pelvic carcinoma & another with myeloid leukemia; 1/6 controls with lymphosacroma.	Argus *et al*., 1965
Oral (DW)	Male CD Rats	30/group	0.75%, 1.0%, 1.4%, 1.8%	*ad. libitum* - 13 mo.	Precancerous nasal lesions, no other pathology	Hepatic carcinoma at 2 highest doses (2 each); nasal tumors: adenocarcinoma (1 at 0.75% & 1 at 1.4%), epidermoid carcinoma (1 at 1.0% & 1 at 1.8%), squamous cell carcinoma (1 at 1.0%), epithelial papilloma (1 at 0.75%). Nasal tumor latency > 330 days. 1 control with nasal fibroma	Hoch-Ligeti *et al*., 1970
Oral (DW)	Male CD Rats	30/group	0.75%, 1.0%, 1.4%, 1.8%	*ad. libitum*- 13 mo.	Kidney damage in all groups	Liver hepatomas at 2 highest dose (3 and 12, respectively); liver nodules at all doses (4, 9, 13, 11, respectively). Some liver tumors malignant	Argus *et al*., 1973

Route	Species	Number	Dose	Exposure	Clinical & pathology (non-neoplastic)	Tumor Incidence	Source
Oral (DW)	Sherman Rats	60/sex/group	0, 0.01%, 0.1%, 1.0%	*ad. libitum* - 2 yr.	Body wt decrease. at high dose; relative liver wt increase at high dose; hepatic degeneration & necrosis & kidney damage at 2 highest doses	Hepatic carcinomas: 1 in controls; 1 at 0.1% & 10 at 1.0%; 2 cholangiomas and 3 nasal squamous cell carcinomas at highest dose. Nasal tumors and high liver tumors statistically significant	Kociba *et al*., 1974
Oral (DW)	Osborne-Mendel Rats	35/sex/group	0, 0.5%, 1.0%	*ad. libitum* - 110 wks.	Body wt decrease at 2 high doses in males and at highest dose in females; fatty/ hyperplastic liver at 2 high doses; hemosiderosis & splenic atrophy in high dose males. Survival low in test groups	Hepatic adenomas: low dose (0 males, 10 females), high dose (0 males, 11 females); nasal carcinoma: low dose (12 males, 10 females), high dose (16 males, 8 females); testicular mesothelioma: low dose (3 males), high dose (5 males). Nasal tumors statistically significant, and appeared after 1 yr.	NCI, 1978

Route	Species	Number	Dose	Exposure	Clinical & pathology (non-neoplastic)	Tumor Incidence	Source
Oral (DW)	F344/DuCrj Rat	35/sex/group	0, 0.02%, 0.1%, 0.5%	*ad. libitum* - 2 yr.	Body wt decrease in high dose animals; dose-related liver hyperplasia at 2 highest dose & hepatic spongiosis in all treated males & high dose females; nasal squamous cell metaplasia & proliferation of nasal gland at highest dose. Decreased survival at highest dose related to nasal tumor incidence.	Hepatic carcinoma: high dose (14 males, 10 females); hepatic adenoma: controls (1 female), low dose (2 males), mid dose (4 males, 5 females), high dose (24 males, 38 females); nasal squamous cell carcinomas: high dose (3 males, 7 females); 5 other nasal tumors in high dose males & females; 52% of high dose males with peritoneal mesothelioma; 20% of high dose females with mammary adenomas. All high dose tumors except other nasal tumors statistically significant.	Yamazaki *et al.*, 1994: TNO and RIVM, 2002

Route	Species	Number	Dose	Exposure	Clinical & pathology (non-neoplastic)	Tumor Incidence	Source
Oral (DW)	Crj:BDF1 Mouse	50/sex/group	0, 0.05%, 0.2%, 0.8%	*ad. libitum* - 2 yr.	Body wt decreased at 2 highest doses; nasal and lung epithelial atrophy & nuclear enlargement at highest dose; fatty liver change & kidney nuclear enlargement in high dose males. Decreased survival at 2 highest doses related to liver tumor incidence in females.	Hepatic carcinoma: control (15 males), low dose (20 males, 6 females), mid-dose (23 males, 30 females), high dose (36 males, 45 females); hepatic adenomas: controls (7 male, 4 females), low dose (16 males, 30 females), mid dose (22 males, 20 females), high dose (8 males, 2 females); nasal tumors: 1 adenocarcinoma in a high dose female and 1 esthesioneuroepithelioma in a high dose male.	Yamazaki *et al.*, 1994: TNO and RIVM, 2002
Oral (DW)	B6C3F1 Mouse	50/sex/group	0, 0.5%, 1.0%	*ad. libitum.*- 90 wks.	Body wt incr in high dose males & mid-dose females; body wt decreased in high dose females; lung inflammation in all test groups; nasal turbinate inflammation in treated females; hepatic hyperplasia at mid-dose only & necrosis at mid and high dose; decreased survival at high dose	Hepatic adenomas: mid-dose (1 male, 9 females), high dose (4 males, 6 females); hepatic carcinoma: controls (2 males), mid-dose (18 males, 12 females), high dose (24 males, 29 females); nasal adenoma: mid-dose (1 female), high dose (1 male). Tumors appeared after 81 wks in females & 58 wks in males	NCI, 1978

Route	Species	Number	Dose	Exposure	Clinical & pathology (non-neoplastic)	Tumor Incidence	Source
Oral (DW)	Male Guinea Pig	22/test group 10 controls	0, 0.5-2.0%	*ad. libitum* - 23 mo.	9/22 animals developed bronchial epithelial hyperplasia (1/10 controls) & infiltration of mononuclear cells (4/10 controls)	3 Liver hepatomas; 1 kidney adenoma; 2 gallbladder carcinomas in treated animals	Hoch-Ligeti and Argus, 1970
Inhalation	Wistar Rat	288/sex/ test group; 192/sex/ controls	400 mg/m3 (111 ppm); whole body study, therefore, est. body burden (105 mg/kg-d) may be low	7h/d, 5d/wk, 2 yr	No clinical effects	Tumor incidence. similar in test & control animals. No liver or nasal tumors. Slight increased in mammary adenomas & recticulum cell sacromas in both sexes.	Torkelson *et al*., 1974
Dermal	Male C3H/HeJ Agouti Mouse	30/test group	0.05 ml (50 mg) 100% dioxane (4 grades); ethanol controls; uncertain if applied under occlusion	3x wk/78 wks	No clinical effects. Early deaths due to respiratory infect. 40/120 survived to end of experiment.	5 Hepatic and 1 pulmonary tumor reported; all within normal limits	NICNAS, 1998

Route	Species	Number	Dose	Exposure	Clinical & pathology (non-neoplastic)	Tumor Incidence	Source
Dermal	Swiss Webster Mouse	30/sex/test group	0.2 ml of test substance solution in acetone; no dose info given; in promotion portion of study, 50 µg of DMBA applied as initiator; control data not reported; uncertain if applied under occlusion	3x wk/60 wks	Cancer study: no pre-neoplastic lesions reported. Promotion study: mild liver lesions & hypertrophic/ hyperplastic skin lesions.	Cancer study: 1/25 females with skin carcinoma; 1/22 males with subcutaneous tumor. Promotion study: 80% of males had skin tumors after 20 wks; at study end, 15/30 animals had skin tumors (incl nasal septum squamous cell carcinoma), lung, liver & kidney tumors.	King *et al*., 1973

4.3.1.1.2.2 Chronic Inhalation Toxicity Studies in Experimental Animals

A chronic rat inhalation (whole body) study with 1,4-dioxane was available for assessment (Torkelson *et al.*, 1974). Groups of 288 male and 288 female Wistar rats were exposed to air containing 400 mg 1,4-dioxane vapor/m^3, 7 hrs/d, 5 d/wk for a total of 2 years. Based on 100% absorption, 240 ml/min breathed air, a body weight of 400 g, and 7 hrs/d, 5d/wk exposure, a dosage of 108 mg/kg-d was calculated. A control group of 192 rats/sex was used. No treatment-related effects were seen on clinical signs (including activity, demeanor, eye and nasal irritation, skin condition, and respiratory distress), body weights, or mortality. Some slight changes were observed in hematological values, but these were within the normal physiological limits and not considered of toxicological importance. Blood urea nitrogen (BUN) and alkaline phosphatase (AP) values in treated male rats were slightly decreased. Changes in liver, kidney, or spleen weights were not observed. Upon gross and microscopic examination carried out on over 20 organs and tissues including liver, kidney, nose, testes, lung, and spleen, no treatment-related non-neoplastic effects were found in tissues or organs, including the reproductive organs. The NOAEL for toxic effects can be considered to be 400 mg 1,4-dioxane/m^3 (0.4 mg/L or 111 ppm) or 108 mg/kg-d (Torkelson *et al.*, 1974).

4.3.1.1.2.3 Chronic Dermal Toxicity Studies in Experimental Animals

Two chronic dermal toxicity studies have been conducted in mice. Only one study reported non-neoplastic lesions; however, these were only reported following 7,12-dimethylbenzanthracene (DMBA) pre-treatment (*i.e.*, a promotion study). Insufficient data were available in this study to estimate the applied doses of 1,4-dioxane. In another study, no gross or compound-related histological lesions were seen in animals treated with approximately 50 mg 1,4-dioxane (100%) applied three days/wk (estimated applied dose of 1,500 mg/kg-d, assuming a mean animal body weight of 20 grams and averaging the three day dose over a five day week) over 78 weeks. It was not stated in either study whether doses were applied under occlusion (NICNAS, 1998).

4.3.1.2 Carcinogenicity

The USEPA has classified 1,4-dioxane as a 'B2' carcinogen ('probable human carcinogen') (DeRosa *et al.*, 1996; USEPA, 2000) based on the induction of nasal cavity and liver carcinomas in multiple strains of rats, liver carcinomas in mice, and gall bladder carcinomas in guinea pigs. The ACGIH (2001) has classified 1,4-dioxane as 'A3' ('confirmed animal carcinogen with unknown relevance to humans'). IARC (1999) has concluded that there is inadequate evidence in humans, but sufficient evidence in experimental animals for the carcinogenicity of 1,4-dioxane. IARC classifies 1,4-dioxane as probably carcinogenic to humans (Group 2B). 1,4-Dioxane has been classified by the European Commission (EC,

1993) as a category 3 carcinogen (Risk phrase R40). Germany has classified 1,4-dioxane as a group IIIB carcinogen ('suspected of possessing significant carcinogenic potential'). Denmark and Norway have classified 1,4-dioxane as a carcinogen category 'K3' with risk phrase R215 ('risk of cancer cannot be excluded with prolonged exposure') and Sweden with risk phrase R340 ('some risk of cancer cannot be excluded after frequently repeated exposure') (NICNAS, 1998).

Overall, the weight of evidence from *in vitro* and *in vivo* tests indicates that 1,4-dioxane is unlikely to be a mutagen. Although the mechanism for carcinogenicity for 1,4-dioxane has not been established, the apparent lack of genotoxic effects of 1,4-dioxane metabolites, together with the fact that 1,4-dioxane exhibits tumor promoter properties support a non-genotoxic mechanism of carcinogenicity (NICNAS, 1998).

Limited retrospective studies on workers who inhaled 1,4-dioxane concentrations up to 184 mg/m^3 for some years showed no evidence of occupational disease or an increased tumor incidence when compared to the general population. The chromosome aberration rate in lymphocytes in six 1,4-dioxane exposed workers was also comparable to the controls.

In a two-year inhalation study, rats exposed to 400 mg/m^3 showed no 1,4-dioxane characteristic tumors. However, in chronic oral bioassays (via drinking water) with rats and mice, 1,4-dioxane clearly causes liver and kidney damage and liver adenomas and carcinomas. In rats, nasal adenomas and carcinomas were also seen, accompanied by non-neoplastic lesions in the nasal cavity. These lesions were also observed in mice, but in mice 1,4-dioxane induced no increased incidence of nasal tumors. The liver, kidney, and nasal damage were still seen at concentrations of 0.02%, 0.1% and 0.1%, respectively, in drinking water, while at 0.01% (equivalent to 10 mg/kg-d) no effects were seen. The liver tumors were seen at 1,4-dioxane drinking water concentrations at or above 0.05% for mice and at or above 0.l% for rats. The nasal tumors in rats were observed at 1,4-dioxane drinking water concentrations at or above 0.5%. Some indication of liver tumors were also obtained in guinea pigs, but no information on non-neoplastic lesions was provided.

Based on these results, 1,4-dioxane can be considered as a carcinogen in laboratory animals. Since 1,4-dioxane is considered a non-genotoxic compound, a threshold approach seems justified. The liver tumors are considered to be associated with cytotoxicity and organ damage, which seem to occur in particular at dose levels at which 1,4-dioxane metabolism becomes saturated. The nasal tumors cannot be explained from a drinking water study; however, it also appears that nasal toxicity plays a role in the nasal carcinogenicity. Reitz *et al.* (1990) offer an interesting perspective on the nasal tumor issue based on a personal communication from Stott who examined the turbinates of rats drinking solutions of a water soluble dye from a bottle. He noted that significant amounts of dye were present in the turbinates. Reitz *et al.* (1990) speculate that, although impossible to quantify, this

observation suggests that the nasal tissues of rats in the drinking water studies may have been repeatedly splashed with water containing 5,000 to 10,000 mg/L of 1,4-dioxane. Since the configuration of the human nose differs from that of the rat and water does not normally contact the nasal tissues of humans during drinking, Reitz *et al.* (1990) suggest that these nasal lesions and tumors are probably irrelevant to man.

A NOAEL, based on liver damage, can be considered to be 0.01% (equivalent to 10 mg/kg-d). 1,4-Dioxane is a low potency carcinogen and the available data indicate it most likely functions via a non-genotoxic mechanism. For both liver (and nasal) tumors, cytotoxic effects and organ damage are involved, which are subject to non-linear kinetics, indicating a threshold. A NOAEL for all liver tumors in rats was established to be 0.02% 1,4-dioxane (equivalent to 40 mg/kg-d) in studies by Kociba *et al.* (1974) and Yamazaki *et al.* (1994), based on the dose-related increased incidence of adenomas in male animals at and above 0.02% (statistically significant at 0.5%). Hepatocellular adenomas and carcinomas were also significantly increased in mice at the lowest dose level, 0.05% (equivalent to 40 to 70 mg/kg-d) 1,4-dioxane. However, a clear dose-response relationship for adenomas was not evident in mice (Yamazaki *et al.*, 1994). A NOAEL was also not identified in this mouse study. Statistically significant increases in nasal carcinomas were also seen in rats at 0.5% 1,4-dioxane (NCI, 1978; Yamazaki *et al.*, 1994). The NOAEL for nasal tumors in rats was 0.1% (equivalent to 90 to150 mg/kg-d) (Kociba *et al.*, 1974; Yamazaki *et al.*, 1994). The NOAEL for nasal tumors in mice (Yamazaki *et al.*, 1994) was 0.2% (equivalent to 160 to 280 mg/kg-d). Other tumors reported in drinking water studies included mammary adenomas and mesotheliomas of the testes and peritoneum seen in rats and renal pelvis carcinoma, myeloid leukemia, kidney adenoma, and gallbladder carcinomas seen in guinea pigs at and above 0.5% 1,4-dioxane (Argus *et al.*,1965; Hoch-Ligeti and Argus, 1970; NCI, 1978; Yamazaki *et al.*, 1994).

4.3.1.2.1 Epidemiological Studies

Available cancer mortality studies of workers exposed to 1,4-dioxane indicate that observed cancer rates are not significantly higher than the number expected, although the populations studied were considered of insufficient size to detect a 'weak' carcinogenic effect (Goodman and Wilson, 1991). Four epidemiologic studies on workers exposed to 1,4-dioxane are available. A cross sectional study of 74 current (n= 24) and former (n= 23 working else where; 15 retired, and 12 deceased) workers (aged between 32 and 62 years) employed in the 1,4-dioxane manufacture and handling and exposed for between 3 and 41 years (average duration of 25 years = 1,840 man-years) to 1,4-dioxane concentrations estimated at 0.02 to 48 mg/m^3 (0.006 to13 ppm) was carried out by Thiess *et al.* (1976). Six of the then current workers also had a history of exposure to dichloroethane and ethylene chlorohydrin. Twelve deaths were reported among this group (expected = 14.5). The group showed no evidence of liver or kidney damage, nor had a higher incidence of cancer deaths than did the

population at large. Two pensioned employees contracted cancer. One, 66-year old, died of liver and kidney insufficiency with diabetes mellitus, and was diagnosed having metastasis of a lamellar squamous epithelial carcinoma with unknown primary turnout. The other, 69 years old, died of circulatory failure with fluid from the pericardial space and uraemia. Myelofibrotic leukemia was also noted. No statistically significant increase was noted based on these few cases of cancer. In six active workers, no increased rate of chromosome aberrations in lymphocytes compared to controls was noted (Thiess *et al.*, 1976).

In a retrospective mortality study conducted on 165 employees engaged for one month to ten years or more in 1,4-dioxane production and processing (as well as vinyl chloride, perchloroethylene, methylene chloride, trichloroethylene and carbon tetrachloride) were studied (Buffler *et al.*, 1978). These workers were discontinuously exposed to 1,4-dioxane at an average concentration of <0.1 to 17 ppm or <0.36 to 61.2 mg/m^3 (maximum concentrations ranges between 1.5 and 32 ppm [5.4 to 115.2 mg/m^3] with a high of 800 ppm [2880 mg/m^3] around storage tanks) for an unstated duration (although 41% of the workers were exposed for at least 10 years). Twelve deaths were also reported in this cohort (9.8 expected). Three of these deaths were due to cancer: one stomach cancer, one alveolar carcinoma, and one mediastinal malignancy (1.7 expected). At least two of these cases were smokers. No significant difference in observed deaths for total deaths or overall cancer was found when compared to the expected numbers. Exposure periods for tumor onset were between one and four years. The study had sufficient power to detect a doubling of cancer mortality with a probability of 0.21, a threefold increase with a probability of 0.58, and a five fold increase with a probability of 0.9.

In an unpublished report to NIOSH (Santodonato *et al.*, 1985), four cancers were reported among 80 dioxane workers with potential exposure of 0.18 to 184 mg/m^3 of 1,4-dioxane. The cancers included a colonic cancer, a pulmonary cancer, a lymphosarcoma, and a glioblastoma. Again, the observed number of cancer cases was not different from the expected cancer deaths. The investigation also showed no signs of 1,4-dioxane related non-cancer health effects.

In a comparative mortality study of over 19,000 cases in the Danish cancer register, a standardized proportionate incidence ratio (SPIR) of 1.64 was determined for liver cancer in male workers employed in companies between 1970 to 1984 using 1,4-dioxane. It was concluded that this increase (64%) was significant ($p = 0.04$) and that confounding factors, particularly alcohol consumption, could not account for this increase. However, when a latency period (minimum 10 years) was incorporated in the analysis, the SPIR was reduced to 1.15. Statistically, the confidence intervals (1.03 to 2.48) indicate the possibility of a real effect; however, uncontrolled factors, such as the potential for exposure to other carcinogenic chemicals, particularly 1,1,1- trichloroethane, and the lack of quantitative exposure data for 1,4-dioxane prevent any firm conclusions regarding a causal association with liver cancer in

this study. An increase in liver cancer of 50% was identified in one workplace where only 1,4-dioxane was used. Again, alcohol consumption alone could not account for this increase. A workplace exposure survey (1983 to 1991) reported that the majority of 1,4-dioxane levels measured were less than 10 mg/m^3 (3 ppm). However, these data were insufficient to speculate on workplace exposure levels in the comparative mortality study (NICNAS, 1998).

4.3.1.2.2 Carcinogenicity Studies in Experimental Animals

1,4-Dioxane has been shown to cause malignant tumors (multiple sites) in more than one animal species. Tumor sites associated with 1,4-dioxane exposure in animal studies were: liver (rat, mouse and guinea pig), nose (rat and mouse) and gall bladder (guinea pig). Liver tumors were the only tumors seen in all species tested and were elicited at lower doses than nasal tumors in rats and mice (only one dose was tested for guinea pigs). The carcinogenicity bioassays are summarized in **Table 4-7**.

4.3.1.2.2.1 Oral Carcinogenicity Studies in Experimental Animals

1,4-Dioxane administered in drinking water has produced tumors in rats, mice, and guinea pigs.

Liver neoplasms were induced after chronic ingestion of cytotoxic doses of dioxane in rats. Treatment of rats with 1 g/kg-d of 1,4-dioxane, a tumorigenic dose, in drinking water for 11 weeks resulted in a 1.5 times increase in hepatic DNA synthesis. Cytotoxicity was not detected in rats dosed orally with 10 mg/kg-d of 1,4-dioxane, a non-tumorigenic dose. Alkylation of hepatic DNA and DNA repair was also not detected in rats dosed with 1 g/kg ^{14}C-1,4-dioxane orally. The lack of genotoxic activity of 1,4-dioxane and its cytotoxicity at tumorigenic dose levels suggest a non-genetic mechanism of liver tumor induction in rats (Stott *et al.*, 1981).

In a drinking water study, 26 Wistar rats received 300 mg 1,4-dioxane/animal (equivalent to 1 g/kg-d) for 63 weeks. A control group of six animals was used. In two rats that died 21.5 weeks after the beginning of the experiment, histological changes appeared in the entire liver. Groups of cells with strongly enlarged hyperchromic nuclei were found, generally located periportally. There were similar changes in rats that died or were sacrificed after 63 weeks on study. In addition, groups of large cells with reduced cytoplasmic basophilia were evident. At the end of treatment period, six of the treated animals had hepatomas. One of these six also had renal pelvis carcinoma and another myeloid leukemia. Severe kidney damage was also reported. These changes often resembled glomerulonephritis. There are no data provided about the control group. This study is somewhat dated and not performed according to current guidelines; however, the results show a potential for kidney damage and liver tumors seen in other studies (Argus *et al.*, 1965).

In a later study, groups of 30 male Charles River CD rats were given daily via the drinking water (freshly prepared) 0%, 0.75%, 1.0%, 1.4%, and 1.8% 1,4-dioxane (equivalent to 750, 1,000, 1,400, and 1,800 mg/kg-d) for 13 months, followed by a 3-month observation period. Tumors of the nasal cavity occurred in 0/30, 1/30, 1/30, 2/30, and 2/30 rats in the controls, 0.75%, 1.0%, 1.4%, and 1.8% groups, respectively. The earliest effects observed (time and dose levels were unclear) was an increase in the nuclear size of hepatocytes, mostly in the periportal areas. Groups of large cells with reduced cytoplasmic basophilia gave the liver a slightly nodular appearance. Two of the animals in the highest dose group also developed hepatocellular carcinomas. Histological examination at termination showed epidermoidal carcinoma with adenocarcinoma-like areas in the nose. Epithelial papillomas were also observed (Hoch-Ligeti *et al.*, 1970). Argus *et al.* (1973) reported in a later publication of the same study, a dose dependent increase in liver tumors (nodules and hepatomas). In the control group, no nodules were seen, 4 were seen in the 0.75% group, 9 in the 1.0% group, 13 in the 1.4% group, and 11 in the 1, 3% group. Hepatomas (3 and 12) were seen at the 1.4% and 1.8% level, respectively. Furthermore, marked kidney damage, including glomerulonephritis and pyelonephritis with epithelial thickening of Bowman's capsules, periglomerular fibrosis, localized extended distal tubulus lumina, nuclear atypia and numerous multinuclear giant cells were seen at all dose levels. No mortality data was provided. This study is somewhat dated and not performed according to current guidelines; however, the results show a potential for liver tumors seen in other studies.

Groups of 60 male and 60 female Sherman rats received 1,4-dioxane via the drinking water at doses of 0%, 0.01%, 0.1%, and 1.0% (equivalent to 0, 9.6, 94, and 1,015 mg/kg-d for males and 0, 19, 148, and 1,599 mg/kg-d for females) for 716 days. Within two days after initiating the study, the body weights of both sexes at 1.0% 1,4-dioxane were significantly lower than that of the controls. The body weights remained depressed throughout the study. The 1.0% 1,4-dioxane concentration led to a severe reduction of survival rates in both sexes within two to four months, nearly half of the group succumbing after four months. The survival rate after four months was essentially the same for all groups. No effects on hematology were observed and the only significant alteration in terminal organ weights was a significantly increased liver weight in rats receiving 1.0% 1,4-dioxane. In rats at the 0.1% and 1.0% doses, gross and histopathological examination revealed variable degrees of renal tubular epithelial and hepatocellular degeneration and necrosis, accompanied by regenerative activities in liver (*i.e.*, hepatocellular hyperplastic nodule formation) and renal tubuli. No effects were seen on male and female reproductive organs. Only in the highest dose group were treatment-related tumors found in both sexes: in the liver, carcinomas were found in 10/66 animals surviving at 12 months and cholangiomas in 2/66 animals, while squamous cell carcinomas of the nasal cavities were found in 3/66 animals. No statistically significant increase in incidence of tumors was seen in rats given the two lower dose levels. The NOAEL in this study was 0.01% 1,4-dioxane, equal to 9.6 or 19 mg/kg-d in males and females, respectively (Kociba *et al.*, 1974).

In a NCI drinking water lifetime study, groups of 50 male and 50 female B6C3F1 mice were exposed to 0%, 0.5% and 1% 1,4-dioxane (≥99.9% pure) for 90 weeks. The mean doses were 720 and 830 mg/kg-d for males and 380 and 860 mg/kg-d for females. Observations included clinical signs, body weight, food and water consumption, necropsy, and histopathology. Body weights were not consistently affected, although the weight of the high dose females was lower than that of the controls during the second year of the study. The high dose animals exhibited some aversion to water and consumed less than the controls and low dose animals. Survival rates of the dosed mice (46/50 in low and 45/50 in high dose males, 39/50 in low and 28/50 in high dose females) were lower than those of the controls (48/50 in males and 45/50 in females), but sufficient number of animals were at risk for development of late-appearing tumors. Given the fact that the exposed female mice had lower survival rates than controls, the dose administered to these animals probably exceeded the MTD. Treatment-related non-neoplastic lesions in males and females included hepatic cytomegaly, pneumonia, and rhinitis. In both sexes, an increased incidence of hepatocellular carcinomas was seen. The incidences were 2/49, 18/50, and 24/47 for males and 0/50, 12/48, and 29/37 for females at 0%, 0.5%, and 1.0%, respectively. Also an increase in the incidence of hepatocellular adenomas plus carcinomas was seen at 0%, 0.5%, and 1%: 8/49, 19/50, and 28/47 for males and 0/50, 21/48, and 35/37 for females, respectively. One nasal adenomacarcinoma was seen in a low dose female and one in a high dose male. No effects were seen on male or female reproductive organs (NCI, 1978).

The NCI (1978) study also tested groups of 35 male and 35 female Osborne-Mendel rats exposed via drinking water to 0%, 0.5% and 1.0% 1,4-dioxane (≥99.9% pure) for 110 weeks. The mean dose levels were 240 and 530 mg/kg-d for male rats, and 350 and 640 mg/kg-d for female rats. High-dose and matched control male rats were placed in the study one year after the study began to replace two original groups of male rats that had died during an air-conditioning failure. Observations included clinical signs, body weight, food and water consumption, necropsy, and histopathology. Body weights were not consistently affected, although the weight of the high dose animals was lower than that of the controls during the second year of the study. The high dose animals exhibited some aversion to water and consumed less than the controls and low dose animals. The survival rates of the rats of both dose groups were significantly lower than that of controls, but sufficient animals of each sex were alive at 52 weeks (33/35, 26/35, and 33/35 for males and 135/35, 30/35, and 29/35 for females at 0%, 0.5% and 1.0%, respectively) to be at risk for the detection of late-appearing tumors. Given the fact that all exposed rats had lower survival rates than controls, the dose administered to these animals probably exceeded the MTD. Non-neoplastic lesions associated with 1,4-dioxane treatment were observed in the kidney (tubular degeneration), liver (cytomegaly), and stomach (ulceration). A higher incidence of pneumonia and rhinitis occurred in males and females of both dose groups. Rats of both sexes developed squamous cell carcinomas in the nasal cavities (0/33, 12/33 and 16/34 for control, low, and high dosed males and 0/34, 10/35, and 8/35 for control, low, and high dosed females, respectively). In

one high dose male, these carcinomas extended to the retrobulbar tissues of the eye and in one low dose male into the brain. In addition, adenocarcinomas arose from the nasal mucosal epithelium in three high dose males, one high dose female, and one low dose female. The first nasal carcinomas developed after one year. A follow-up examination localized nasal tumors in the front third of the posterior meatus of the nasal cavities (Goldsworthy *et al.*, 1991). An increase in hepatocellular adenomas was also seen in females. The incidence was 0/31, 10/33, and 11/32 for control, low, and high dosed females, respectively. No effects were seen on male or female reproductive organs (NCI, 1978).

In a long-term drinking water experiment groups of 50 male and 50 female mice (Crj:BDF1) were administered 1,4-dioxane in drinking water for 104 weeks. The dose rates were 0%, 0.05%, 0.2%, and 0.8% (equivalent to 0, 0.066, 0.25, and 0.77 g/kg-d in males and 0, 0.077, 0.32, and 1.07 g/kg-d for females, respectively). All animals were examined for clinical signs, body weight, food and water consumption, and clinical chemistry (*i.e.*, hematology, biochemistry, and urinalysis). After 105 weeks, the animals were sacrificed. Necropsy and histopathology were performed on all animals, including dead and moribund animals. The survivals of females at the 0.2% and 0.8% dose levels were significantly lower than those of the controls (17/50 and 5/50 vs 29/50, respectively) due to liver tumors. Mean body weights of females at 0.2% and 0.8% and males at 0.8% were lower than those of controls. Food and water consumption were decreased in high dose males and females. In males, effects on hematology, biochemistry, and urinalysis parameters were observed at 0.8%, at or above 0.2%, and 0.8%, respectively. In females, these effects occurred at or above 0.2%. Absolute and relative lung weights were increased in males at 0.8% and in females at or above 0.2%. Upon histopathological examination, non-neoplastic lesions were observed in the nasal cavity (*i.e.*, nuclear enlargement and atrophy of the olfactory and respiratory epithelium, and inflammation), trachea (*i.e.*, atrophy or nuclear enlargement of the epithelium), lung (*i.e.*, accumulation of foamy cells, and nuclear enlargement and atrophy of the bronchial epithelium), kidney (*i.e.*, nuclear enlargement of the proximal tubule) in males and females at or above 0.2%. In males, lesions were also observed in liver (angiectasis) at 0.8% and in testis (decreased mineralization) at or above 0.2%. Hepatocellular carcinomas occurred with significantly increased incidences in males at 0.8% and in all treated female groups (incidence in males was 15/50, 20/50, 23/50 and 36/50, and in females 0/50, 6/50, 30/50 and 45/50 for controls, 0.05%, 0.2%, and 0.8%, respectively). An increased incidence of hepatocellular adenoma was seen in males and females at 0.05% and 0 2% (incidence in males was 7/50, 16/50, 22/50, and 8/50, and in females 4/50, 30/50, 20/50, and 2/50 for controls, 0.05%, 0.2%, and 0.8%, respectively). One nasal esthesioneuroepithelioma was seen in one male at 0.8% and one nasal adenocarcinoma was seen in one female at 0.8%. A LOAEL was established at 0.05%, equivalent to 0.066 g/kg-d for males and 0.077 g/kg-d for females (Yamazaki *et al*, 1994).

In the companion study to the mouse study described above, groups of 50 male and 50 female rats (F344/DuCrj) were administered 1,4-dioxane in drinking water for 104 weeks. The dose levels were 0%, 0.02%, 0.1%, and 0.5% in drinking water (equivalent to 0, 0.016, 0.081, and 0.398 g/kg-d for males and 0, 0.021, 0.103, and 0.514 g/kg-d for females, respectively). All animals were examined for clinical signs, body weight, food and water consumption, and clinical chemistry (*i.e.*, hematology, biochemistry, and urinalysis). After 105 weeks, the animals were sacrificed. Necropsy and histopathology were performed on all animals, including dead and moribund animals. The survivals of males and females were significantly lower than those of the control group (22/50 vs 40/50 and 24/50 vs 38/50, respectively) due to nasal and liver tumors. Mean body weights at 0.5% males and females were lower than those of controls. Food and water consumption were not affected. In males, effects on hematology, biochemistry, and urinalysis parameters were observed at or above 0. 1%, 0.5%, and 0.5%, respectively. In females, this occurred at 0.5%, 0.5%, and at or above 0.1%, respectively. Absolute and relative liver weights were increased in males at or above 0.1% and in females at 0.5%, while lung and kidney weights were also increased in females at this dose level (TNO and RIVM, 2002). Histopathological examination revealed non-neoplastic lesions in the nasal cavity (*i.e.*, respiratory metaplasia, nuclear enlargement and atrophy of the olfactory epithelium, nuclear enlargement, squamous cell metaplasia and squamous cell hyperplasia of the respiratory epithelium, hydropic change and sclerosis in the lamina propria, adhesion, and inflammation and proliferation of the nasal gland), liver (*i.e.*, spongiosis and hyperplasia) and kidney (*i.e.*, nuclear enlargement of the proximal tubule) in males at or above 0.02%, and in females at or above 0.1%. Malignant neoplasms of the nasal cavity occurred only in 0.5% males and females, not in controls or the 0.02 or 0.1% animals.

These tumors included squamous cell carcinoma (3/50 and 7/50 for males and females, respectively), sarcoma (not otherwise specified; 2/50 in males), esthesioneuroepithelioma (1/50 and 1/50 for males and females, respectively), and rhabdomyosarcoma (1/50 in males). Higher incidences of non-neoplastic lesions in the nasal cavity (see above) were also observed in 0.5% males and females. The lesions in the olfactory epithelium also tended to occur at a somewhat higher incidence in the 0.1% groups. Hepatocellular adenoma and carcinoma incidence was significantly increased in high dose males (24/50 and 14/50, respectively) and high dose females (38/50 and 10/50, respectively). Hepatocellular adenomas were also seen at low incidences in males at 0.02% (2/50) and 0.1% (4/49) and in females at 0.1% (5/50) incidence in control males and females was 0/50 and 1/50, respectively. The incidence of non-neoplastic lesions in the liver (see above) was increased 0.1% and 0.5% in both males and females. The incidence of hyperplasia in males was 3/50, 2/50, 10/50, and 24/50 and in females 3/50, 2/50, 11/50, and 47/50 for controls, 0.02%, 0.1%, and 0.5%, respectively. The increased incidence of spongiosis was dose-related in males at all dose levels and in females at 0.5% (the incidence in males was 12/50, 20/50, 25/50 and 40/50, and in females 0/50, 0/50, 11/50, and 20/50 for controls, 0.02%, 0.1%, and 0.5%, respectively). In males, the incidences of mesothelioma of the peritonium, fibroma of the

subcutis, and fibroadenoma of the mammary gland at 0.5% were greater than in the control group. In females at 0.5%, the incidence of adenoma of the mammary gland was also increased. In this study, an effect on the target organ liver (spongiosis) was seen in males even at the lowest dose tested 0.02% (although not statistically significant, there was a dose-related trend). Therefore, 0.02% (equivalent to 0.016 g/kg-d) was established as the LOAEL (Yamazaki *et al.*, 1994).

In a small study, a group of 22 guinea pigs was exposed for 23 months to drinking water containing 1,4-dioxane in concentrations that ranged from 0.5 to 2.0% (equivalent to >2000 mg/kg-d). An untreated control group was used. Nine treated animals developed peri- or bronchial and nodular mononuclear infiltration in the lung. In addition, two guinea pigs developed gallbladder carcinomas, three had early hepatomas, and one had an adenoma of the kidney. In the control animals, 4/10 guinea pigs developed peripheral mononuclear cell accumulation and hyperplasia of the bronchial epithelium was observed in one. A NOAEL for guinea pigs was not identified in this study. This study is also somewhat dated and not performed according to current guidelines; however, the results show some indication for liver and gall bladder tumors seen in other studies (Hoch-Ligeti and Argus, 1970).

4.3.1.2.2.2 Inhalation Carcinogenicity Studies in Experimental Animals

Groups of 288 male and 288 female Wistar rats were exposed to air containing 400 mg/m^3 99.9% pure 1,4-dioxane 7 hrs/d, 5 d/wk for a total of two years. Based on 100% absorption, 240 ml/min breathed air, a body weight of 400 g and seven hours exposure/day, a dosage of 108 mg/kg-d was calculated. A control group of 192 rats/sex exposed to air was used. Half of the animals survived 20 to 24 months of exposure. No effects were seen on clinical signs (including activity, demeanor, eye and nasal irritation, skin condition and respiratory distress), body weights, or mortality. Some slight changes were observed in hematological values, but these were within the normal physiological limits and not considered of toxicological importance. BUN and AP values in treated male rats were slightly decreased. Changes in liver, kidney or spleen weights were not observed. Upon comprehensive gross and microscopic examination, no characteristic nasal and liver tumors, as observed after oral administration with 1,4-dioxane, were seen. It is, however, unclear whether the nasal cavity was adequately examined. No statistically significant increase in incidence of other tumors was observed in the 525 treated rats examined compared with 347 controls, and the incidence of tumors observed in other organs and tissues appeared to be unrelated to exposure. The only difference from the control groups was an increase in lymphoreticular cell sarcomas in males (18% [37/206] versus 12% [18/150]) and in mammary gland adenoma in females (13% [2 9/217] versus 8% [11/139]), neither of which were statistically significant. For neoplastic effects, the NOAEL appears to be 400 mg/m^3 (0.4 mg/L or111 ppm), since there was no increase in tumor incidence and no gross pathological or histopathological evidence of organ injury (Torkelson *et* al., 1974).

In addition to the Torkelson *et al.* (1974) study, the results (no study details provided) of a rat carcinogenicity study on 1,1,1-trichloroethane containing approximately 4% 1,4-dioxane have been described (NICNAS, 1998). Exposure to 1,4-dioxane was estimated as 103 ppm (0.4 mg/L), assuming that its airborne concentration was directly proportional to its concentration in 1,1,1-trichloroethane. No increase in tumors was reported in this study.

4.3.1.2.2.3 Dermal Carcinogenicity Studies in Experimental Animals

1,4-Dioxane was found to be a promoter in a two-stage skin carcinogenesis study in Swiss-Webster mice (King *et al.*, 1973). A single dermal application of 50 µg of the initiator DMBA was followed one week later by thrice-weekly paintings of 1,4-dioxane (unspecified concentration in 0.2 ml of acetone) for 60 weeks. Tumors of skin, nose, lung, kidney and liver were reported in excess of that observed in controls (32 tumors in 15 experimental animals compared to 9 in 55 control animals), but apparently these findings could not be reproduced in a repeat study (NICNAS, 1998). In both the King *et al.* (1973) and NICNAS (1998) studies, however, increases in tumors (lymph node, skin, hepatic, and pulmonary neoplasms) were reported to be within normal limits following similar applications of 1,4-dioxane without DMBA initiation (NICNAS, 1998).

4.3.1.2.3 Supporting Carcinogenicity Studies in Experimental Animals

1,4-Dioxane was administered both by gavage (1000 mg/kg, three times a week for eight weeks) and ip injection (200, 500, 1000 mg/kg, three times a week for eight weeks) to male and female A/J mice to test for the induction of lung tumors (Stoner *et al.*, 1986). A significant increase (38%) in lung tumors (compared with controls) was seen only in males dosed at 500 mg/kg by ip injection. The authors concluded that this finding was the result of a low incidence of tumors in control animals. Since many known carcinogens have demonstrated false negative effects in this assay, a negative result is not considered meaningful in the absence of other bioassays (NICNAS, 1998).

In groups of 20 to 40 female Sencar mice administered a single dose of up to 1,000 mg/kg 1,4-dioxane (oral, dermal, or subcutaneous) as an initiator, followed by dermal treatment with the promoter 12-O-tetradecanoylphorbol-13-acetate, three times a week for 20 weeks, no significant increases in the formation of papillomas were observed after 24 weeks. Therefore, 1,4-dioxane was not functioning as an initiator. The promotional activity of 1,4-dioxane was not tested (Bull *et al.*, 1986). 1,4-Dioxane (881 mg/kg) administered by ip injection to male Sprague Dawley rats (one day after partial hepatectomy), followed by administration of 500 ppm sodium phenobarbitone (in drinking water) for 49 days, also showed a lack of initiation activity as demonstrated by measurement of gamma-glutamyl transferase (GGT) positive foci in the liver (NICNAS, 1998).

In an initiation/promotion assay, 1,4-dioxane was used as a potential initiator and sodium phenobarbital as a promoter. The 1,4-dioxane was administered ip to male Sprague-Dawley rats at 881 mg/kg 24 hours after a 2/3 partial hepatectomy. Seven days after the hepatectomy, the rats were administered 500 ppm sodium phenobarbital in their drinking water for a total of 49 days. One week later, the rats were sacrificed and their livers examined for increased GGT positive foci. There were no increases in the number of foci and, therefore, 1,4-dioxane was not considered an initiator in this assay. It was concluded it may cause a tumor response via an epigenetic mechanism (Hartung, 1989).

In a similar initiation/promotion assay, 1,4-dioxane was used as a potential initiator protocol in male Sprague-Dawley rats (nine/group) by Lundberg *et al.* (1987). In this experiment, however, a statistically significant increase in the number and total volume of GGT positive foci in the liver was observed following ip administration of 30 mg/kg of the initiator diethylnitrosamine (one day after 2/3 partial hepatectomy), with subsequent gavage dosing of 1,4-dioxane at 100 or 1,000 mg/kg-d, 5 d/wk for seven weeks. The rats were administered 500 ppm sodium phenobarbital in their drinking water as a positive control. Ten days after the last administration, the animals were sacrificed. Liver sections were stained for GGT and the number and total volume of GGT-positive foci was studied. 1,4-Dioxane at 1,000 mg/kg showed a clear positive result (63% of the number of foci as seen with sodium phenobarbital). The control and 100 mg/kg group gave negative results (Lundberg *et al.*, 1987).

Significant dose-related increases in hepatic ornithine decarboxylase activity in the liver of Sprague Dawley rats were observed following a single dose of 840, 2,550, and 4,200 mg/kg 1,4-dioxane, suggestive of strong promoter activity. In addition, cytochrome P-450 was also induced at these doses. Other studies supporting the role of 1,4-dioxane as a tumor promoting agent, include its ability to inhibit GJIC *in vitro*, a property shared by a number of non-genotoxic carcinogens and promoters (NICNAS, 1998). In addition, 1,4-dioxane has been shown to induce cell proliferation (in nasal turbinates and hepatocytes) *in vivo* at cytotoxic doses (Goldsworthy *et al.*, 1991). Regenerative cell proliferation has been linked to 'preferential' growth of pre-cancerous cells (Butterworth *et al.*, 1992).

4.3.1.2.4 Mechanism of Carcinogenic Action

The mechanism behind the carcinogenic effects of 1,4-dioxane has not yet been fully elucidated and insufficient data (from animal or human studies) exist to discount the relevance to humans of tumors, particularly liver tumors, seen in animal studies. It is, however, considered most likely that 1,4-dioxane carcinogenicity occurs through a non-genotoxic mechanism as discussed above. The weight of evidence indicates that neither 1,4-dioxane nor its major metabolite, 1,4-dioxan-2-one, are genotoxic. Both 1,4-dioxane and its metabolite, 1,4-dioxan-2-one, have been generally negative when tested in a battery of *in*

vitro and *in vivo* genotoxicity assays (with and without metabolic activation) including the Ames assay, HGPRT tests with CHO cells, UDS tests on rat hepatocytes, and so forth (sections 4.1.1.3 and 4.1.1.5; **Tables 4-2, 4-3, and 4-4**). A cell transformation assay with Balb/3T3 mouse cells was negative with metabolic activation and positive without metabolic activation.

The specific mechanism by which 1,4-dioxane elicits its carcinogenic effect is likely associated with one or more of the following: **cytotoxicity** (tissue damage) with increased cell proliferation and RDS, **promotion** of endogenous carcinogens, cytochrome P-450 **induction** (the majority of hepatocarcinogenic promoters are P-450 inducers), or **saturation pharmacokinetics**.

A likely mechanism of action occurs as a function of high dose testing. Tissue damage (cytotoxicity or cell killing) is followed by increased cell proliferation and RDS resulting in increased errors of DNA with increased potential for tumorigenicity. There is no good evidence to determine whether 1,4-dioxane or a metabolite (*e.g.*, diethylene glycol, 1,4-dioxan-2-ol, or HEAA) is better associated with carcinogenicity. Treatment of mice with 1,4-dioxane for three days increased lipid peroxidation (Mungikar and Pawar, 1978), a potential mechanism of cell damage and precursor of carcinogenicity. However, the same authors suggested that metabolites of 1,4-dioxane acted as antioxidants given the significant reduction in the 'diene conjugation band' in the ultraviolet spectrum of liver microsomal lipids (NICNAS, 1998). In the study by Stott *et al.* (1981), male Sprague-Dawley rats (4 to 6/group) received 1,4-dioxane at 0, 10, or 1000 mg/kg-d via drinking water for 11 weeks (7d/wk). Seven days prior to sacrifice, the rats received [$^{6\text{-}3}$H]thymidine. After sacrifice the livers were examined. 1,4-Dioxane was cytotoxic to hepatic tissue at the highest dose level, as evidenced by an increase in liver to body weight ratio and a significant rise in hepatic DNA synthesis as measured by [$^{6\text{-}3}$H]-thymidine incorporation, accompanied by a minimal degree of hepatocellular swelling. Since this effect remained the same after several weeks of application, replacement of damaged cells and cytotoxicity are probably involved. No changes relative to controls were observed in rats dosed with 10 mg/kg-d.

1,4-Dioxane has also been demonstrated to possess protein denaturing activity *in vivo* (Argus *et al.*, 1965). Irrespective of whether such effects are related, perturbations in hormonal regulation and protein damage have also been linked to carcinogenicity in humans and animals. Whether these changes are biologically significant remains to be demonstrated.

The possibility of 1,4-dioxane inducing liver tumors via proliferation of hepatic peroxisomes is also a possibility; however, this mechanism of tumor formation is not thought to be relevant to humans. In studies for peroxisomal proliferation, no effect of 1,4-dioxane on peroxisome proliferation was observed. Oral treatment of five male Fischer 344 rats with 1.0% 1,4-dioxane in the drinking water for five days showed neither a dose related increase

in liver/body weight ratios, nor an increase in the peroxisomal enzyme palmitoyl-CoA-oxidase (Goldsworthy *et al.*, 1991). In another study on peroxisomal proliferation, male Fischer 344 rats (seven/group) received 2,000 mg/kg-d via saline gavage in nine doses over 11 days. The animals were sacrificed 16 hours after the last exposure. Body weights were significantly decreased in comparison to the controls from the fifth day onward. No induction of palmitoyl-CoA-P activity was observed, despite increases in absolute and relative liver weights (the protein concentration in the liver remained constant). These results indicate that 1,4-dioxane does not elicit tumors via peroxisome proliferation (NICNAS, 1998).

Non-linear toxicokinetics of 1,4-dioxane have been demonstrated in the rat and pharmacokinetic data indicate similarities between rat and human metabolism of 1,4-dioxane. Saturation of oxidation of 1,4-dioxane to HEAA and 1,4-dioxane-2-one at doses greater than 10 mg/kg results in accumulation of 1,4-dioxane. The metabolites 1,4-dioxane-2-ol and HEAA may also accumulate in tissues with oxidative capacity. These effects may be related in view of the correlation between increased rates *in vitro* of DNA strand breaks, sister chromatid exchange at cytotoxic concentrations, and the *in vivo* damage observed in the dose ranges associated with organ (*e.g.*, liver and kidney) damage. In combination with the cytotoxicity observed at high doses, this suggests accumulation of toxic metabolites not removed via oxidative metabolic pathways may play a role in the expression of a cancer response. Evidence from animal studies indicates the existence of a threshold dose for toxicity and carcinogenicity at doses where 1,4-dioxane metabolism becomes saturated.

The possibility of carcinogenicity elicited by impurities in 1,4-dioxane is also considered unlikely. Argus *et al.* (1973) analyzed 1,4-dioxane used in a chronic bioassay for the presence of carcinogenic hydroperoxides. None were detected (detection limit = 15 µM) either in original 1,4-dioxane or following mixing with tap water used in drinking water studies. A number of other impurities have been reported in different grades of 1,4-dioxane, including the recognized human carcinogen, bis (2-chloroethyl) ether (BCEE). BCEE is reported as a starting product in one method of 1,4-dioxane manufacture; however, it was not reported as a potential impurity by researchers.

Overall, indications are strong that the primary mechanism of tumorigenicity for 1,4-dioxane in animals is non-genotoxic. A number of possible epigenetic mechanisms (*i.e.*, cytotoxicity, mitogenicity, cell proliferation, tumor promotion, endocrine-modification, immunosuppression) have been suggested, but based on the above mentioned findings, the mode of action is most likely cytotoxic in nature; the cytotoxic effects and organ damage via increased cell turnover may pave the way for liver carcinogenesis. This conclusion has also been reached by independent researchers (Goldsworthy *et al.*, 1991; Butterworth *et al.* 1992; Reitz *et al.*, 1990; Leung and Paustenbach, 1990; Neumann *et al.*, 1997; Stickney *et al.*, 2003) as well as authorative agencies (NICNAS 1998; TNO 2002).

The underlying mechanism for the nasal tumors observed in drinking water studies is unclear, however, it seems that nasal toxicity (as evidenced by the non-neoplastic lesions in the nasal cavity) and differences in nasal anatomy plays a role in nasal carcinogenesis (Reitz *et al.*, 1990). This toxicity is also more likely associated with cytotoxicity and organ damage triggered by reactive metabolites than by a local effect due to volatilization of the 1,4-dioxane from the water since no nasal tumors were observed after inhalation exposure. It is noted that no cell proliferation was observed in the nasal epithelium of rats given 1.0% 1,4-dioxane in their drinking water for two weeks, but did induce cell proliferation (in nasal turbinates and hepatocytes) *in vivo* at cytotoxic doses (Goldsworthy *et al.*, 1991). However, it is possible that another novel mechanism may be involved (DeRosa *et al.*, 1996).

4.3.2 Neurotoxicity

Neurotoxicity studies of 1,4-dioxane are limited in number and generally confined to acute exposure studies. Human fatalities have reported brain edema and demylination, but these are the results of extremely high doses. Although CNS depression is reported consistently in acute animal toxicity studies, this appears to be a dose-related, non-specific effect observed with most compounds with solvent (*i.e.*, defattening) characteristics and likely associated with the disruption of the membrane potentials of excitable tissues similar to that observed with anesthetics.

The inhibition of the propagation and maintenance of an electrically evoked seizure discharge was investigated as a criterion of the acute neurotropic effect of 1,4-dioxane. The testing was performed in parallel on male albino Wistar rats (four per group) and female H strain mice (eight per group) under two conditions: the shortening of the duration of maximal tonic extension after electroshock in male rats exposed to 1,4-dioxane vapor for four hours, and the slowing of the development of tonic extension after electroshock in female mice exposed for two hours. Three concentrations between 25% and 75% of the maximum effect level were tested (exact concentrations not mentioned). The concentration at which a 30% depression of the maximum attainable effect was obtained was 6808 mg/m^3 for rats and 8784 mg/m^3 for mice. No information was provided as to behavioral changes (*e.g.* narcosis or depressed activity) (Frantik *et al.*, 1994).

Kanada *et al.* (1994) investigated the effect of oral administration of 1,4-dioxane on monoamine neurotransmitters and metabolites in the rat brain. Male Sprague-Dawley rats received a single oral administration of 1050 mg 1,4-dioxane/kg. Two hours after administration the rats were sacrificed and acetylcholine, 3,4-dihydroxyphenylalanine, dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, norepinephrine, 3-methoxy-4-hydroxyphenylglycol, serotonin, and 5-hydroxyindoleacetic acid contents in various brain regions were measured. Significant effects of 1,4-dioxane administration included a decrease in dopamine and serotonin concentrations in the hypothalamus and a decrease of serotonin

concentrations in the medulla oblongata. Concentrations of the other neurotransmitters and metabolites were not significantly influenced after the administration of 1,4-dioxane. No information was provided as to behavioral changes (*e.g*. narcosis or depressed activity).

In a study for effects on behavior, female CFE rats (8 to10/group) were exposed to 1,4-dioxane concentrations of 5,490, 10,980, and 21,960 mg/m^3 4 h/d, 5 d/wk for two weeks. The avoidance response was decreased in a dose related manner. At 21,960 mg/m^3, a few animals also showed a decreased escape response. Maximal decrease for both parameters were seen after two days exposure. Thereafter, the effects became less severe, and all effects were ultimately reversible. Other severe behavioral effects (*e.g*. motor imbalance, frank depression or ataxia) were not seen (TNO and RIVM, 2002).

No chronic neurotoxicity studies have been performed for 1,4-dioxane; however, some histopathological changes in nervous system tissues (*i.e*., brain vacuoles) have been reported from some sub-chronic and chronic oral bioassays with 1,4-dioxane (TNO and RIVM, 2002) but not all. The biological significance of these inconsistent effects is unclear, but the weight of evidence does not support the nervous system as a specific target organ at relevant doses.

4.3.3 Developmental Neurotoxicity

No specific study of the developmental neurotoxic potential of 1,4-dioxane has been performed to date. However, as pointed out above, the nervous system does not appear to be a target organ for the effects of 1,4-dioxane except at high, lethal or near lethal doses. These effects are most likely due to disruption of the membrane potential as the result of the common defattening property possessed by many solvents as opposed to a unique effect of 1,4-dioxane. Most sub-chronic and chronic toxicity studies have not found the nervous system to be a specific target for the effects of 1,4-dioxane, albeit in adult animals.

In reproductive and developmental tests of 1,4-dioxane, no unusual effects on offspring that might be associated with neurotoxicity have been reported although specific neurotoxicity test batteries were not conducted. In a multi-generation study of ICR Swiss mice, one group was treated with 0.17 mg 1,4-dioxane/ml in 1% Emulphor in deionized water. In this group, no adverse effects were found in litter survival and growth, teratogenesis, or general pathology compared to a water control (Lane *et al.*, 1982). In a developmental study in CD rats, exposure to the 'vehicle control' (0.05% Tween 80 with 0.9 ppm 1,4-dioxane as a stabilizer) also resulted in no significant effects when compared to a deionized/filtered water control group. Only some very minor differences for maternal body weight and water consumption were observed (George *et al.* 1989). No treatment-related developmental effects were seen in the offspring of Sprague-Dawley rats or Swiss Webster mice exposed by inhalation on gestational days 6 to 15 (seven hr/d) to 1,1,1- trichloroethane containing

3.5% 1,4-dioxane (estimated 1,4-dioxane concentration= 32 ppm or 0.12 mg/L) (Schwetz *et al.*, 1975).

5.0 Dose-Response Evaluation for 1,4-Dioxane

Assessment of dose-response for a chemical's toxicity (sometimes referred to as toxic potency assessment, is an integral part of both (1) estimating the degree of injury that a chemical may impart (*i.e.*, poisoning) to an individual or a population and (2) estimating dose ranges in which no toxic injuries are likely to occur. This analytical process is predicated on the universally accepted principal that as the dose of a chemical increases so does the incidence and severity of the injuries it produces. The shape of any dose-response curve will be predicated on many factors, including the existence and location of biological thresholds, the susceptibility of those exposed, and the circumstances of exposure.

In practice, dose-response assessment is largely a process that translates the toxic potency of a substance as defined by high-dose, laboratory animals studies to the toxic potency for humans exposed to lower (often considerably lower) doses experienced by humans. Over the past 60 years, this process has evolved considerably in detail; however, it still retains two steps as its mainstays:

1) extrapolation from a test species to humans to take into account quantitatively the degree of variability in susceptibility that may exist between humans and other species; and

2) extrapolation from the high doses administered to laboratory animals and the lower doses experienced by humans in assorted situations (*e.g.*, workplace vs. home).

5.1 Non-Cancer Endpoints

The primary non-cancer endpoints for the effects of 1,4-dioxane are liver and kidney damage, typically associated with high dose exposure and cytotoxicity. Toxicological and pharmacokinetic data indicate that chronic tissue damage occurs at doses above metabolic saturation, which in turn may be a precursor of the neoplastic effects. Although increased retention of unmetabolized 1,4-dioxane has been proposed as a primary cause of liver/kidney damage, a number of metabolites, including 1,4-dioxan-2-one, β-hydroxyethoxy acetaldehyde, diethylene glycol, and oxalic acids, have also been implicated in the toxic and carcinogenic effects of 1,4-dioxane. Hecht and Young (1981) postulated the formation of 1,4-dioxane-2-ol as a result of hydroxylation. The substance is in equilibrium with the reactive and presumably cytotoxic β-hydroxyethoxy acetaldehyde. Toxicologically significant amounts could be formed in cells in which the oxidative capacity has been saturated by high doses of 1,4-dioxane prohibiting the complex oxidation to HEAA.

5.1.1 Oral Toxicity Studies

Two sub-chronic toxicity studies provide data of sufficient quality to identify NOAELs. In a 13-week study, groups of Crj:BDF1 mice (10/sex/dose) received 1,4-dioxane in drinking water at doses of 0, 0.10, 0.26, 0.58, 0.92 or 1.83 g/kg-d and 0, 0.17, 0.41, 0.92, 1.71 or 2.70 g/kg-d for males and females, respectively (TNO and RIVM, 2002). Histopathological examination revealed in males at 0.58 g/kg-d or greater groups and in females at 0.41 g/kg-d or greater. No effects were found on the reproductive organs. Based on the histopathology findings in females at 0.41 g/kg-d (*i.e.*, non-neoplastic lesions in the nasal cavity, trachea, lung, and liver), the NOAEL in this study was established at 0.17 g/kg-d.

In the equivalent rat 13-week study, F344/DuCrj rats (10/sex/dose) also received 1,4-dioxane in drinking water at doses of 0, 0.06, 0.15, 0.33, 0.76, or 1.90 g/kg-d and 0, 0.10, 0.20, 0.43, 0.87 or 2.01 g/kg-d for males and females, respectively (TNO and RIVM, 2002). Non-neoplastic lesions were observed in the nasal cavity, trachea, liver, kidney, and brain in both males at 0.15 mg/kg-d and females at 0.20 mg/kg-d. Based on these histopathology results along with increased kidney weights in females, the NOAEL in this study was established at 0.06 g/kg-d for males and 0.10 g/kg-d for females.

In a developmental toxicity test (Giavini *et al.*, 1985), no effects on implantation numbers, live fetuses, post-implantation loss, or major malformations were seen following administration (oral) of up to 1.0 ml/kg-d (1,033 mg/kg-d) 1,4-dioxane to pregnant rats. This dose caused slight maternal toxicity and embryotoxicity as evidenced by reduced maternal and fetal weight gain. The NOAEL was identified as 0.52 g/kg-d.

In chronic toxicity studies carried out in rats, mice and guinea pigs, NOAELS and LOAELs can also be identified. 1,4-Dioxane in drinking water caused severe effects on the liver, kidneys, and nose at high doses. In rats, gross effects (decreased body weight) were observed at 0.5% with increased relative and absolute liver weights at 1% 1,4-dioxane. Hepatic and renal histopathological effects were seen at 0.1% (Kociba *et al.*, 1974). LOAELs for1,4-dioxane of 0.02% (equal to 0.016 g/kg-d) and 0.1% (90 to 150 mg/kg-d for male and female rats, respectively) for chronic oral exposure have been identified based on these effects (TNO and RIVM, 2002; NICNAS, 1998). A NOAEL for non-neoplastic effects was 0.01 to 0.02% 1,4-dioxane in drinking water (equivalent to 10 to 40 mg/kg-d) derived from Kociba *et al.* (1974) and Yamazaki *et al.* (1994) based on the increased incidence (dose-related) of spongiosis hepatis seen in males at and above 0.02% (statistically significant at 0.1%). A dose of 0.01% (equivalent to 10 mg/kg-d) showed no effects and was considered a NOAEL in evaluations by the Dutch and Australian governments (TNO and RIVM, 2002; NICNAS, 1998).

Both the Dutch and Australian evaluation of 1,4-dioxane identified NOAELs for the cancer studies based largely on established protocol but also on the assumption that 1,4-dioxane is a non-genotoxic compound and is expected to exhibit a threshold response (TNO and RIVM, 2002; NICNAS, 1998). Although this approach is not often taken for assessing carcinogens in the US, it is another measure of chronic toxicity and can furnish additional insight into the hazard potential of the compound for purposes of arriving at health-protective criteria. In particular, the liver tumors are closely associated with cytotoxicity and organ damage that occur at dose levels at which 1,4-dioxane metabolism becomes saturated. The nasal tumors also appear to be associated with nasal cytotoxicity. In Sherman rats (60/sex/dose) administered 1,4-dioxane via the drinking water at doses of 0, 9.6, 94, and 1,015 mg/kg-d for males and 0, 19, 148, and 1,599 mg/kg-d for females, respectively, for two years (Kociba *et al.*, 1974). In rats at the two highest doses, gross and histopathological examination revealed renal and hepatocellular damage, accompanied by regenerative activities in liver and kidney. Only in the highest dose group were treatment-related liver and nasal tumors found in both sexes. The NOAEL in this study was identified as 9.6 or 19 mg/kg-d in males and females, respectively. Leung and Paustenbach (1990) identified a NOAEL of 14.3 mg/kg-d for liver tumors from the same study. A NOAEL for all liver tumors in rats of 0.02% 1,4-dioxane (equivalent to 10 to 40 mg/kg-d) was identified from the studies by Kociba *et al.* (1974) and Yamazaki *et al.* (1994), based on the dose-related increased incidence of adenomas in male animals at and above 0.02% (statistically significant at 0.5%). Hepatocellular adenomas and carcinomas were also significantly increased in mice at the lowest dose level, 0.05% (equivalent to 40 to 70 mg/kg-d) 1,4-dioxane. Overall, a NOAEL from the various studies can be identified as 0.01% (equivalent to 10 mg/kg-d) based on liver damage and cancer.

Statistically significant increases in nasal carcinomas were also seen in rats at 0.5% 1,4-dioxane (NCI, 1978; Yamazaki *et al.*, 1994). The NOAEL for nasal tumors in rats was identified at 0.1% (equivalent to 90 to150 mg/kg-d) (Kociba *et al.*, 1974; Yamazaki *et al.*, 1994). A NOAEL for nasal tumors in mice (Yamazaki *et al.*, 1994) was identified at 0.2% (equivalent to 160 to 280 mg/kg-d). Other tumors reported in drinking water studies included mammary adenomas and mesotheliomas of the testes and peritoneum seen in rats and renal pelvis carcinoma, myeloid leukemia, kidney adenoma, and gallbladder carcinomas seen in guinea pigs at and above 0.5% 1,4-dioxane suggesting this level as a LOAEL for chronic toxicity and tumorigenic effects (Argus *et al.*,1965; Hoch-Ligeti and Argus, 1970; NCI, 1978; Yamazaki *et al.*, 1994).

5.1.1.1 Oral Reference Dose

No oral reference dose has been established for 1,4-dioxane by USEPA. A one and ten day drinking water health advisory was developed in 1987 based on the iv rabbit data of Fairley *et al.* (1934). Using assumptions of a 10 kg child drinking 1 liter of water per day and a 1000 uncertainty factor resulted in a one-day health advisory of 4.12 mg/L. The ten-day health

advisory was derived by employing an additional 10-fold uncertainty factor to arrive at a value of 0.412 mg/L.

Review of the toxicity information in Section 4.0 supports the overall NOAEL of 10 mg/kg-d for 1,4-dioxane identified in the Kociba *et al.* (1974) and Yamazaki *et al.* (1994) by the Dutch and Australian governments (TNO and RIVM, 2002; NICNAS, 1998) as protective of chronic (primarily liver and renal) health effects as well as any potential carcinogenicity (based on an assumption of a high dose cytotoxicty and a non-gentoxic threshold effect). Using an uncertainty factor approach (*i.e*., values of 1, 3, or 10), an oral RfD for 1,4-dioxane can be developed. **Table 5-1** provides the uncertainty factors (UF) used in deriving the oral RfD.

Table 5-1. Uncertainty Factors used in Deriving the Oral RfD for 1,4-Dioxane

Uncertainty Parameter	UF Value
Intraspecies (human to human) - UFh	10
Interspecies (animal to human) - UFa	3
Duration (sub-chronic to chronic) - UFc	1
Endpoint (LOAEL to NOAEL) - UFl	1
Completeness of Database - UFd	3
Total UF	**100**

The careful selection of the uncertainty factors is the key point in deriving a RfD that is protective without being unnecessarily restrictive. The UFs employed typically fall within a range of one to ten and are applied to various aspects of an experimental study that might critically bear on its extrapolation to human health. It is recognized by numerous authoritative bodies (*i.e*., National Academy of Sciences/National Research Council (NAS/NRC), the World Health Organization (WHO), and USEPA) that UFs can and should accommodate a wide continuum of numerical expressions other than a single default value (most notably, 10). The NAS/NRC states, "*There is no strong scientific basis for using the same constant uncertainty factor for all situations.*." (NRC, 1994). USEPA in the Agency's draft report entitled "*A Review of the Reference Dose and Reference Concentration Process*" (USEPA, 2002), also stated "*.. that rigid application of log or ½ log units for UFs could lead to an illogical set of reference values.*"

USEPA has attempted to systematically structure the use of UFs, and increasingly has moved away from their rigid application of default values. There is growing support for chemical-specific of data-driven uncertainty factors in non-cancer risk assessment, which incorporate

toxicokinetic and toxicodynamic data, and as a consequence, the application of uncertainty factors other than 3 or 10 may become more frequent in human health risk assessment in the future (WHO, 1999; IPCS, 2001; USEPA, 2002). As a demonstration that each UF is indeed a continuum whereby a value is selected based on factual understanding of toxicity, when deriving an oral RfD for boron, USEPA recently adopted a set of chemical-specific uncertainty/variability factors of 4.08, 1.6, 2.5, 1.2, and 3.16 to yield a net uncertainty factor of 61.9 (USEPA, 2004). A similar approach was adopted by International Program on Chemical Safety (IPCS) in their assessment of boron as well (IPCS,1998).

In an earlier review, Cicmanec and Poirier, (1995), a number of Integrated Risk Information System (IRIS) entries that used other than the default UFs were examined. The five basic circumstances in which a UF less than 10 was used were:

1) The UF for sensitive subpopulations was reduced because the database did not support the use of this UF;

2) Gaps remained in the database but not so great as to require the default UF;

3) The compound was an essential nutrient and application of UFs would result in a RfD below the minimum daily dietary requirement;

4) The doses selected failed to define a NOAEL but the LOAEL was of minimal severity and application of the default UF would result in an unrealistically high value; and

5) The animal study used possesses definable traits that preclude the use of one or more default UF.

Decisions pertaining to the selection of specific UFs for deriving an oral RfD for 1,4-dioxane in terms of the individual uncertainty factors for intraspecies extrapolation (UFh), interspecies extrapolation (UFa), subchronic to chronic extrapolation (UFc), LOAEL to NOAEL extrapolation (UFl), and database uncertainty (UFd) are summarized below.

- *UFh* - The primary difference in exposure between adults and children in this case may simply be the increased dose due to differences in body size and relative intake of 1,4-dioxane. NRC (1993) has pointed out that, for most chemicals, the majority of people (including children) respond in a sufficiently similar manner that a tenfold safety factor is adequate to address the variability in the human population; therefore, the overall intraspecies uncertainty factor selected is somewhat more conservative. Derivation of chemical-specific factors for the intraspecies uncertainty factor often indicate that a factor less than 10 is usually sufficient (Renwick and Lazarus, 1998;

Renwick, 1998; Abdel-Megeed *et al.*, 2001; USEPA, 2002). Typically in such cases, 3 is chosen as a ½ log unit of 10. Calabrese and Gilbert (1993) suggested that UFs for inter-individual differences be reduced to 4 (normal animal lifetime experiment) or 5 (less than lifetime animal study or lifetime human study) because of the interdependence of uncertainty factors for inter- and intraspecies extrapolation, and intraspecies and sub-chronic to chronic extrapolations. Only extrapolations based on occupational epidemiology studies would use a 10×UF for inter-individual differences in this scheme. The critical endpoint of 1,4-dioxane is associated with chronic exposure to cytotoxic levels of the parent compound; unfortunately individual variabilities associated with age, gender, ethnicity and so forth are unknown in this case. Accordingly a UFh of **10** is adopted in this situation.

- *UFa* - USEPA (2002) has indicated that when the data suggests that humans are less (or more) sensitive than animals, the traditional default interspecies uncertainty factor can be lowered (or raised) to accommodate this fact. Based upon numerous analyses in which effective dose levels (*i.e.*, LOAEL, NOAEL, MTD) were found to correspond across species as a function of body weight or surface area (USEPA, 1992a), the default interspecies uncertainty factor of 10 could also be replaced using species-specific values derived using allometric scaling (Clewell *et al.*, 2002). Using rat and human body weights of 0.3 and 70 kg, respectively, with a default scaling factor of 0.75, the rat-to-human extrapolation uncertainty factor is approximately a factor of 4. In the case of 1,4-dioxane, however, the adverse effects are likely associated with cytotoxic levels of the parent compound. An evaluation of the pharmacokinetics and toxicity of 1,4-dioxane by Reitz *et al.* (1990) found that the levels of the best dose surrogate were 70 to 250 times higher at the doses associated with cytotoxicity and tumor production than at the NOAEL, and that the equivalent human level associated with 10 ppm (in water) was nearly 1300 times lower than the level at the NOAEL (*i.e.*, 90,000 to 320,000 times lower than the dose surrogate level associated with cytotoxicity). This would suggest that humans are less likely to be effected by 1,4-dioxane even under extreme exposure conditions that are not likely to exist in reality. The indications that humans are likely to be less sensitive to 1,4-dioxane since for a given concentration of 1,4-dioxane humans will experience an internal dose (AUC- Liver) 5 to 10 lower than the rat at levels below metabolic saturation and even lower if levels are above metabolic saturation, and that the default UFa can be reduced based on pharmacokinetic issues alone without considering the scaling issues discussed by Clewell *et al.* (2002). The UFa for this assessment is set equal to **3**.

- *UFl* - In the case of 1,4-dioxane, NOAELs are available and consistent. The *UFl* is accordingly set at **1**

- *UFc* - Chronic studies with NOAELS are available for 1,4-dioxane and thus a UFc of **1** is selected.

- *UFd* - USEPA's Technical Panel recommended discontinuing the application of an additional modifying factor, since it overlaps considerably with the database uncertainty factor (USEPA, 2002). With respect to the database uncertainty factor, the panel states, "*the size of the factor to be applied will depend on other information in the database and on how much impact the missing data may have on determining the toxicity of a chemical*" (USEPA, 2002). In this case, while the toxicological database for 1,4-dioxane is relatively robust, deficiencies in the reproductive and developmental toxicity studies database suggest a UFd of **3** is appropriate.

Two NOAELS were selected for deriving both an overall RfD as well as a reproductive RfD to protect against adverse reproductive outcome. The studies of Kociba *et al.* (1974) and Yamazaki *et al.* (1994) identify a NOAEL of 10 mg/kg-d that would be protective for all endpoints in rats (NICNAS, 1998; TNO and RIVM, 2002) while the reproductive study of Giavini *et al.*, (1985) identified a NOAEL of 517 mg/kg-d based on slight maternal and embyro- toxicity. Accordingly, using an oral NOAEL of 10 mg/kg-day and an overall UF of 100, an oral RfD of **0.1 mg/kg-d** is derived for ingested 1,4-dioxane for sensitive endpoints in *ex utero* neonates and older children while the NOAEL of 517 mg/kg-d is used with overall UF of 100, to derive an oral RfD of **5.2 mg/kg-d** to protect against *in utero* exposure to 1,4-dioxane.

5.1.2 Inhalation Toxicity Studies

Torkelson *et al.* (1974) described some sub-chronic inhalation studies conducted with 1,4-dioxane in rats, rabbits, guinea pigs, and dogs at concentrations ranging from 180 to 360 mg/m^3 during 82 to 136 (12 to 18 weeks) seven-hour exposures. No adverse effects were noted with respect to appearance, demeanor, growth, mortality, hematological and clinical chemical studies, organ weights, or gross and microscopic pathological examination, but no details of these studies are available. On this basis, a NOAEL of 360 mg/m^3 (100 mg/kg-d) for 1,4-dioxane in rats, is suggested but cannot be independently verified. This result is similar, however, to that observed in the chronic inhalation study described below.

The sole chronic inhalation study with 1,4-dioxane is that of Torkelson *et al.* (1974). Groups of 288 male and 288 female Wistar rats were exposed to air containing 400 mg 1,4-dioxane vapor/m^3 7 hrs/d, 5 d/wk for a total of 2 years. Based on 100% absorption, 240 mL/min breathed air, a body weight of 400 g, and 7 hrs/d, 5d/wk exposure, a dosage of 108 mg/kg-d was calculated. No treatment-related effects were seen on clinical signs (including activity, demeanor, eye and nasal irritation, skin condition, and respiratory distress), body or organs weights, clinical chemistry, histopathology, or mortality. The NOAEL for toxic effects can

be considered to be 400 mg 1,4-dioxane/m^3 (0.4 mg/L or 111 ppm) or 108 mg/kg-d (Torkelson *et al.,* 1974).

5.1.2.1 Inhalation Reference Concentration

No inhalation reference concentration (RfC) has been developed by the USEPA although California developed a chronic Recommended Exposure Level (REL) for 1,4-dioxane using the Torkelson *et al.* (1974) study, an uncertainty factor of 30, and corrected for the discontinuous dosing (7 hrs/d, 5 d/wk) (CalEPA, 2000). This chronic REL is 3 mg/m^3.

Aside from the fact that the study by Torkelson *et al.* (1974) used only a single dose, it appears to be a well-conducted study. No effects were seen in rats or mice exposed to 111 ppm (108 mg/kg-d) and is considered a NOAEL (TNO and RIVM, 2002; NICNAS, 1998). The actual NOAEL is higher than the NOAEL identified from the study so the use of this value can be considered an additional conservatism in deriving an inhalation RfC. Since 1,4-dioxane appears to be completely absorbed in animals by either inhalation or oral exposure, the toxicological database is reasonably complete. Using an uncertainty factor approach (*i.e.*, values of 1, 3, or 10), an inhalation RfC for 1,4-dioxane can be developed (a reference concentration could also be developed using the NOAEL of 400 mg/m^3). **Table 5-2** provides the uncertainty factors (UFs) used in deriving an inhalation RfC.

Table 5-2. Uncertainty Factors used in Deriving the Inhalation RfC for 1,4-Dioxane

Uncertainty Parameter	UF Value
Intraspecies (human to human)- UFh	10
Interspecies (animal to human) - UFa	3
Duration (sub-chronic to chronic) - UFc	1
Endpoint (LOAEL to NOAEL) - UFl	1
Completeness of Database - UFd	3
Total UF	**30**

Decisions pertaining to the selection of specific UFs for deriving an inhalation RfC for 1,4-dioxane in terms of the individual uncertainty factors for intraspecies extrapolation (UFh), interspecies extrapolation (UFa), subchronic to chronic extrapolation (UFc), LOAEL to NOAEL extrapolation (UFl), and database uncertainty (UFd) are summarized below.

- *UFh* - The primary difference in exposure between adults and children in this case may simply be the increased dose due to differences in body size and relative intake of 1,4-dioxane. NRC (1993) has pointed out that, for most chemicals, the majority of people (including children) respond in a sufficiently similar manner that a tenfold safety factor is adequate to address the variability in the human population; therefore, the overall intraspecies uncertainty factor selected is somewhat more conservative. Derivation of chemical-specific factors for the intraspecies uncertainty factor often indicate that a factor less than 10 is usually sufficient (Renwick and Lazarus, 1998; Renwick, 1998; Abdel-Megeed *et al.*, 2001; USEPA, 2002). Typically in such cases, 3 is chosen as a ½ log unit of 10. Calabrese and Gilbert (1993) suggested that UFs for inter-individual differences be reduced to 4 (normal animal lifetime experiment) or 5 (less than lifetime animal study or lifetime human study) because of the interdependence of uncertainty factors for inter- and intraspecies extrapolation, and intraspecies and sub-chronic to chronic extrapolations. Only extrapolations based on occupational epidemiology studies would use a 10×UF for inter-individual differences in this scheme. The critical endpoint of 1,4-dioxane is associated with chronic exposure to cytotoxic levels of the parent compound; unfortunately individual variabilities associated with age, gender, ethnicity and so forth are unknown in this case. Accordingly a UFh of **10** is adopted in this situation.

- *UFa* - USEPA (2002) has indicated that when the data suggests that humans are less (or more) sensitive than animals, the traditional default interspecies uncertainty factor can be lowered (or raised) to accommodate this fact. Based upon numerous analyses

in which effective dose levels (*i.e.*, LOAEL, NOAEL, MTD) were found to correspond across species as a function of body weight or surface area (USEPA, 1992a), the default interspecies uncertainty factor of 10 could also be replaced using species-specific values derived using allometric scaling (Clewell *et al.*, 2002). Using rat and human body weights of 0.3 and 70 kg, respectively, with a default scaling factor of 0.75, the rat-to-human extrapolation uncertainty factor is approximately a factor of 4. In the case of 1,4-dioxane, however, the adverse effects are likely associated with cytotoxic levels of the parent compound. An evaluation of the pharmacokinetics and toxicity of 1,4-dioxane by Reitz *et al.* (1990) found that the levels of the best dose surrogate were 70 to 250 times higher at the doses associated with cytotoxicity and tumor production than at the NOAEL, and that the equivalent human level associated with 10 ppm (in water) was nearly 1300 times lower than the level at the NOAEL (*i.e.*, 90,000 to 320,000 times lower than the dose surrogate level associated with cytotoxicity). This would suggest that humans are less likely to be effected by 1,4-dioxane even under extreme exposure conditions that are not likely to exist in reality. The indications that humans are likely to be less sensitive to 1,4-dioxane since for an given concentration of 1,4-dioxane humans will experience an internal dose (AUC- Liver) 5 to 10 lower than the rat at levels below metabolic saturation and even lower if levels are above metabolic saturation, and that the default UFa can be reduced based on pharmacokinetic issues alone without considering the scaling issues discussed by Clewell *et al.* (2002). The UFa for this assessment is set equal to **3**.

- *UFl* - In the case of 1,4-dioxane, NOAELs are available and consistent. The UFl is accordingly set at **1**

- *UFc* - Chronic studies with NOAELS are available for 1,4-dioxane and thus a UFs of **1** is selected.

- *UFd* - USEPA's Technical Panel recommended discontinuing the application of an additional modifying factor, since it overlaps considerably with the database uncertainty factor (USEPA, 2002). With respect to the database uncertainty factor, the panel states, "*the size of the factor to be applied will depend on other information in the database and on how much impact the missing data may have on determining the toxicity of a chemical*" (USEPA, 2002). In this case, while the toxicological database for 1,4-dioxane is relatively robust and consistent for oral, inhalation, and dermal routes as a whole, deficiencies in the reproductive and developmental toxicity studies database suggest a UFd of **3** is appropriate.

Using the inhalation NOAEL of 108 mg/kg-day and an overall UF of 100, an inhalation RfC of **1.1 mg/kg-d** is derived for 1,4-dioxane in air. A RfC of 13.3 mg/m^3 can also be utilized.

5.1.3 Dermal

Only one chronic dermal toxicity study of 1,4-dioxane conducted in mice is potentially suitable for risk assessment (NICNAS, 1998). In this study, no gross or compound-related histological lesions were seen in animals treated with approximately 50 mg 1,4-dioxane (100%) applied three days/wk over 78 weeks (estimated applied dose of 1,500 mg/kg-d, assuming a mean animal body weight of 20 grams and averaging the three day dose over a five day week). It was not stated whether doses were applied under occlusion (NICNAS, 1988). Since no effects were seen in mice exposed to 1,500 mg/kg-d, so this dose may be considered as NOAEL (NICNAS, 1998). However, this is an unpublished study and the necessary details are not available for review. Dermal reference doses are not standard and the need for deriving them is uncertain. Accordingly, this data is not used to derive any toxicological criteria; however, the information is useful in forming an impression of the hazard potential of 1,4-dioxane by the most likely route of exposure for many people.

5.1.3.1 Dermal Reference Dose

The dermal toxicity studies are not of sufficient quality to develop a Dermal RfD for 1,4-dioxane. No RfDs are typically available for the dermal route of exposure. Most RfDs are expressed as the amount of substance administered per unit time and unit body weight, whereas exposure estimates for the dermal route of exposure are eventually expressed as absorbed doses. In some cases, the non-carcinogenic risks associated with dermal exposure can be evaluated using an oral RfD (USEPA, 1992b). In brief, exposures via the dermal route generally are calculated and expressed as absorbed doses. These absorbed doses are compared to an oral toxicity value that has been adjusted (if necessary) so that it is also expressed as expected from human contact with the absorbed dose. For example, if an oral RfD, unadjusted for absorption, equals 10 mg/kg-d, and other information (or an assumption) indicates a 20% oral absorption efficiency in the species on which the RfD is based. The adjusted RfD that would correspond to the absorbed dose would be: 10 mg/kg-d × 0.20 = 2 mg/kg-d. The adjusted RfD of 2 mg/kg-d would then be compared with the amount estimated to be absorbed dermally each day.

In the case of 1,4-dioxane, oral absorption is assumed to be 100% based on experimental results so the adjustment for absorbed dose to assess dermal exposure would be:

$$RfD_a = 0.1 \text{ mg/kg-d} \times 1.0 = 0.1 \text{ mg/kg-d}$$

An adjusted RfD of **0.1 mg/kg-d** can be used to assess the significance of 1,4-dioxane absorbed across the skin.

5.2 Cancer Endpoints

5.2.1 Mode of Action

The mode of action by which 1,4-dioxane exerts its carcinogenic effects has not yet been completely established, but it seems likely that 1,4-dioxane carcinogenicity occurs through a non-genotoxic mechanism. The weight of evidence indicates that neither 1,4-dioxane nor its major metabolite, 1,4-dioxan-2-one, are genotoxic since both have been generally negative when tested in a battery of *in vitro* and *in vivo* genotoxicity assays (with and without metabolic activation).

The specific mechanism by which 1,4-dioxane elicits its carcinogenic effect is most likely associated with saturation pharmacokinetics followed by resultant cytotoxicity (tissue damage) with associated increased cell proliferation and RDS. Promotion of initiated and cytochrome P-450 induction (the majority of hepatocarcinogenic promoters are P-450 inducers) may also play a role in the final outcome.

These likely modes of action occur as a primarily function of high dose testing. Non-linear toxicokinetics of 1,4-dioxane have demonstrated in the rat and pharmacokinetic data indicate similarities between rat and human metabolism of 1,4-dioxane. Saturation of oxidation of 1,4-dioxane to HEAA and 1,4-dioxane-2-one at doses greater than 10 mg/kg results in accumulation of 1,4-dioxane (the metabolites 1,4-dioxane-2-ol and HEAA may also accumulate in tissues with oxidative capacity). The saturation of 1,4-dioxane metabolism leads to a build-up of the slowly excreted parent compound resulting in increased tissue damage (*i.e.*, cytotoxicity or cell killing). This, in turn, is followed by increased cell proliferation and RDS resulting in increased probability of errors in DNA with accompanying increased potential for tumorigenicity. These effects may be related in view of the correlation between increased rates *in vitro* of DNA strand breaks and sister chromatid exchange at cytotoxic concentrations, and the *in vivo* damage observed in the dose ranges associated with organ (*e.g.*, liver and kidney) damage. In combination with the cytotoxicity observed at high doses, this suggests accumulation of the parent compound or metabolites not removed via oxidative metabolic pathways may reach cytotoxic levels and play a role in the expression of a cancer response. Evidence from animal studies indicates the existence of a threshold dose for toxicity and carcinogenicity at doses where 1,4-dioxane metabolism becomes saturated. For instance, in the study by Stott *et al.* (1981), 1,4-dioxane was cytotoxic to hepatic tissue at the highest dose level tested (1,000 mg/kg-d), as evidenced by an increase in liver to body weight ratio and a significant rise in hepatic DNA synthesis as measured by [$^{6\text{-}3}$H]-thymidine incorporation, accompanied by a minimal degree of hepatocellular swelling. Since this effect remained the same after several weeks of application, substitution of toxically damaged cells and cytotoxicity are likely involved.

The question of 1,4-dioxane genotoxicity is particularly relevant to the observed induction of nasal cavity tumors. Goldsworthy *et al.* (1991) suggested that the nasal tumors observed in these studies are due to inspiration of water into the nasal cavity during drinking from sipper bottles (high 1,4-dioxane doses applied directly to nasal tissue) (Reitz et al., 1990; Stickney *et al.*, 2003) and subsequent cytotoxicity. This hypothesis is further supported bythe fact that rats have a more convoluted nasal turbinate system than humans, resulting in greater deposition in the upper respiratory tract. Although the mechanism for the nasal carcinogenicity of 1,4-dioxane is unclear, "DNA repair induction, peroxisome proliferation, and cell proliferation do not appear to be involved in nasal tumor formation" (Goldsworthy *et al.*, 1991; Stickney *et al.*, 2003).

Overall, indications are strong that the primary mechanism of tumorigenicity for 1,4-dioxane in animals is non-genotoxic and most likely cytotoxic in nature. The cytotoxic effects and organ damage via increased cell turnover may pave the way for the observed liver carcinogenesis seen at high doses. The underlying mechanism for the nasal tumors observed in drinking water studies is unclear, however, it seems that nasal toxicity (as evidenced by the non-neoplastic lesions in the nasal cavity) plays a role in resulting nasal carcinogenesis. This toxicity is also more likely associated with cytotoxicity and organ damage triggered by reactive metabolites than by a local effect due to volatilization of the 1,4-dioxane from the water since no nasal tumors were observed after inhalation exposure (Torkelson *et al.*, 1974). It is also noted that no cell proliferation was observed in the nasal epithelium of rats given 1% 1,4-dioxane in their drinking water for two weeks, but did induce cell proliferation (in nasal turbinates and hepatocytes) *in vivo* at cytotoxic doses (Goldsworthy *et al.*, 1991). The conclusion that 1,4-dioxane is acting through an epigenetic mode of action (*i.e.*, cytotoxicity accompanied with cell proliferation) has also been concurred by the Dutch, Australian, and German environmental regulatory authorities (Stickney *et al*., 2003).

5.2.2 Cancer Potency Factors

The USEPA has classified 1,4-dioxane as a B2 carcinogen (*i.e*., probable human carcinogen) based on the induction of nasal cavity and liver carcinomas in multiple strains of rats, liver carcinomas in mice, and gall bladder carcinomas in guinea pigs (IRIS, 2002). The cancer assessment dates from 1988 with minor revisions done in 1990.

The available human epidemiologic record was judged inadequate to assess the carcinogenicity of 1,4-dioxane (Section 4.3.1.2.1). In the IRIS documentation, three epidemiologic studies on workers exposed to 1,4-dioxane were cited. The study of Thiess *et al.* (1976) which reported 12 deaths, including two cancer deaths, among 74 workers exposed to 1,4-dioxane was discussed. No statistically significant increase was noted primarily due to the few cases of cancer. In the study by Buffler *et al.* (1978), 12 deaths were also reported among 165 production and processing workers exposed to 1,4-dioxane (as well

as vinyl chloride, perchloroethylene, methylene chloride, trichloroethylene, and carbon tetrachloride). Only three of these deaths were due to cancer (*i.e.*, stomach, alveolar carcinoma, and a mediastinal malignancy) and the rate was not different from the expected. In the unpublished NIOSH report (Santodonato *et al.*, 1985), four cancers (*i.e.*, colon, lung, lymphosarcoma, and glioblastoma) were reported among 80 1,4-dioxane workers. As before, the observed number of cancer cases was not different from the expected cancer deaths. The small number of cases and exposed, uncertain case ascertainment, exposure assessment, follow-up, and confounder control introduce potential flaws and biases into these studies and limits the utility of this data in drawing conclusion about the human risk from 1,4-dioxane exposure.

On the other hand, the USEPA judged the animal data to be sufficient to judge the cancer risk from ingestion of 1,4-dioxane (Section 4.3.1.2.2.1). The most consistent tumor response observed in these studies has been in the liver. Although nasal tumors have been reported as well, they are not seen as consistently as liver neoplasms (additionally, inhalation exposure to 1,4-dioxane failed to induce nasal tumors at all). The NCI (1978) administered 1,4-dioxane (greater than or equal to 99.9% pure) in the drinking water to Osborne-Mendel rats (35 rats/sex/dose) and mice (50 mice/sex/dose) for a significant portion of their life (110 weeks, rats; 90 weeks, mice). Male and female rats were given 530, 240, or 0 mg/kg-d and 640, 350, or 0 mg/kg-d, respectively. High-dose and matched control male rats were placed in the study one year after the study began to replace two original groups of male rats that had died during an air-conditioning failure. Male and female treated rats also had a statistically significant elevated incidence of nasal cavity squamous cell carcinomas and treated female rats had a statistically significant elevated incidence of liver adenomas, both dose-related. Male and female mice treated with 830, 720 or 0 mg/kg-d and 860, 380, or 0 mg/kg-d, respectively, developed a statistically significant elevated incidence of liver carcinomas and liver carcinomas or adenomas, both dose-related. Although the survival rate of treated rats and female mice was decreased compared with controls, the NCI concluded that sufficient numbers of treated animals survived to make this a valid study.

Kociba *et al.* (1974) administered 1%, 0.1%, 0.01% or 0% 1,4-dioxane in the drinking water to male and female Sherman rats for up to 716 days (60 rats/sex/treatment group). The incidences of hepatocellular carcinomas, liver cholangiomas, and nasal cavity squamous cell carcinomas showed a significant increase in the high-dose rats of both sexes. Similar administration of 0.5% to 2% 1,4-dioxane to male guinea pigs for 23 months induced gall bladder carcinomas (2/22) and liver hepatomas (3/22) (Hoch-Ligeti and Argus, 1970). Hoch-Ligeti *et al.* (1970) and Argus *et al.* (1973) treated male Sprague-Dawley rats with 1.8, 1.4, 1.0, 0.75, or 0% 1,4-dioxane in the drinking water for 13 months, followed by a 3-month observation period. Treatment-related hepatocellular carcinomas and nasal cavity carcinomas were observed at 1.8% and 1.4% 1,4-dioxane, and treatment-related nasal cavity carcinomas were observed at 1.0% and 0.75% 1,4-dioxane. Liver tumors (7/26) were

induced in male Wistar rats after oral administration of 1% 1,4-dioxane in the drinking water for 63 weeks (Argus *et al.*, 1965). One kidney transitional cell carcinoma and one myeloid leukemia were also observed in the treated animals. A lymphoid tissue lymphosarcoma was observed in 1 of 9 control rats.

1,4-Dioxane was also found to be a promoter in a two-stage skin carcinogenesis study in mice (King et al., 1973). A single dermal application of 50 µg of DMBA was followed 1 week later by thrice-weekly paintings of 1,4-dioxane (unspecified concentration in acetone) for 60 weeks. Similar applications of 1,4-dioxane without DMBA initiation did not result in a significantly increased incidence of subcutaneous carcinomas.

5.2.2.1 Oral Potency Factor

EPA chose the NCI (1978) drinking water study on which to base their dose-response assessment. In this study, 1,4-dioxane had been administered at multiple dose levels by a relevant route of exposure. NCI (1978) exposed male and female Osborne-Mendel rats to 0, 0.1, or 1.0 % dioxane in their drinking water for 110 or 90 weeks, respectively, and comprehensive histologic examinations were performed. The incidence of nasal tumors were 0/33, 12/33, and 16/33 in the males, and 0/34, 10/35, and 8/35 in the females. Although survival was affected by treatment and the doses used probably exceeded the MTD, adequate numbers of rats were at risk for development of late-appearing tumors. Transformed doses in mg/kg-d were provided by NCI (1978). The average daily doses from the mean consumption of dioxane solution per week at intervals during the second year of treatment was determined (**Table 5-3**). The weight of the animals in the study was assumed to be 0.55 kg. USEPA assumed human weight was 70 kg in order to calculate the human equivalent dose. From measured water consumption and body weight data, the human cancer potency from a multistage polynomial fit of these data was 9.5×10^{-3} $(mg/kg\text{-}d)^{-1}$ from male rat data, and 4.9×10^{-3} $(mg/kg\text{-}d)^{-1}$ from female rat data. An adjustment for early mortality following the procedure of USEPA yielded cancer potencies of 1.1×10^{-2} $(mg/kg\text{-}d)^{-1}$ and 6.0×10^{-3} $(mg/kg\text{-}d)^{-1}$ from male and female rat data, respectively. Based on the age of the assessment, the scaling factor used was 2/3 as opposed to 3/4 adopted by the USEPA under the Revised Guidelines for Carcinogen Risk Assessment (USEPA, 2005). On this basis alone, the estimate of cancer potency for 1,4-dioxane is probably over-stated by a factor of 2 to 3.

Table 5-3. Human Equivalent Doses Based on NCI (1978) 1,4-Dioxane Bioassay

Administered Dose (%)	Administered Dose (mg/kg-d)	Human Equivalent Dose (mg/kg-d)	Tumor Incidence
0	0	0	0/33
0.5	240	48	12/25
1.0	530	106	16/33

USEPA's oral cancer slope factor was based on a linearized multistage procedure for estimating extra risk was determined to be 1.1E-2 $(mg/kg\text{-}d)^{-1}$ based on the occurrence of squamous cell carcinoma of the nasal turbinates in male Osborne-Mendel rats administered 1,4-dioxane over their lifetimes (NCI, 1978). This extrapolation translates into a oral unit risk of 3.1×10^{-7} $(\mu g/L)^{-1}$. The added lifetime cancer risks associated with various 1,4-dioxane concentrations in drinking water concentrations are 1×10^{-4} (300 µg/L or ppb), 1×10^{-5} (30 µg/L or ppb), and 1×10^{-6} (3 µg/L or ppb). USEPA has stated that the potency factor should not be used if the water concentration exceeds 30,000 µg/L, since above this concentration the slope factor may differ from that predicted by the model.

The use of the nasal tumor data to derive the oral potency is questionable since the chronic inhalation study failed to detect the same lesion and the appearance of nasal lesions and tumors is inconsistent between species exposed via drinking water. The observation by Stott (cited in Reitz *et al.*, 1990) that the nasal tissue of rats appeared to be directly exposed to water from drinking (supported by the observations of Goldsworthy *et al.*, 1991) and the differences in nasal anatomy between rats and humans makes these lesions of uncertain relevance to humans and arguably inappropriate on which to base a toxicological criteria. The liver tumor data is both more consistent between studies and species and allows the incorporation of kinetic and mechanistic data (section 5.2.3).

The California EPA has derived an oral cancer potency factor from a drinking water study that differs from USEPA. Of the available studies (Argus *et al*., 1965; Hoch-Ligeti *et al*., 1969; Argus *et al*., 1973; Kociba *et al*., 1974; NCI, 1978), only the Kociba *et al*. (1974) and NCI (1978) studies were considered for the determination of the cancer potency factor for dioxane. The NCI (1978) study using B6C3F1 mice was used as the basis for California's oral cancer potency factor for 1,4-dioxane. They considered this study to contain the best data on the most sensitive species and sex, and the most sensitive target tissue. In this study, 50 male or female mice were exposed to 0, 0.5, or 1.0% dioxane for 90 weeks. Average doses were determined from weekly measurements of water consumption. The estimated doses were 0, 720, and 830 mg/kg-d for the males and 0, 380, and 860 mg/kg-d for the

females. The incidence of hepatocarcinomas were 2/49, 18/50, and 24/47 for males, and 0/50, 12/48, and 29/37 for the females. The incidence of hepatocarcinomas or adenomas were 8/49, 19/50, and 28/47 in males, and 0/50, 21/48, and 35/37 in females.

A linearized multistage procedure was applied to the female mouse combined hepatocellular carcinoma and adenoma incidence from the NCI (1978) study by CalEPA (CalEPA, 2002). The animal cancer potencies were 8.3×10^{-4} and 1.4×10^{-3} $(mg/kg\text{-}d)^{-1}$, for the males and females, respectively. The animal cancer potency, q_{animal}, was calculated from the linear slope using the lifetime scaling factor $q_{animal} = q_1^* \times (T/T_e)^3$, where T/T_e is the ratio of the experimental duration to the lifetime of the animal. The animal cancer potencies were therefore adjusted for the short duration of the experiment, using the factor $(104/90)^3$. A value for the human cancer potency was determined using the relationship $q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$, where bw is the default body weight of human or animal (mouse). Body weights for interspecies scaling were assumed to be 0.04 and 0.035 kg for males and females, respectively. The combined incidence of hepatocarcinomas and adenomas in males and females gave human cancer potencies of 1.5×10^{-2}, and 2.7×10^{-2} $(mg/kg\text{-}d)^{-1}$, respectively. The combined incidence of hepatocarcinomas and adenomas in females was used to derive the human cancer potency for dioxane of 2.7×10^{-2} $(mg/kg\text{-}d)^{-1}$.

The relevance of the USEPA and CalEPA oral potency factors is uncertain for some of the reasons stated above. In terms of CalEpA's database selection, the mouse may be the sensitive species, however, the rat seems more similarly pharmacokinetically to humans and is likely a better choice as an animal surrogate for that reason (especially given the importance that pharmacokinetics plays in assessing the hazard potential of 1,4-dioxane). Additionally, the extrapolation techniques (*i.e.*, scaling factors) employed are not the most current or recommended, and the assumption of a linear no-threshold response does not make full use of the available data.

5.2.2.2 Inhalation Potency Factor

USEPA has not generated an inhalation cancer potency factor for 1,4-dioxane. Although a 2-year inhalation study (Torkelson *et al.*, 1974) using male and female Wistar rats exposed to 111 ppm or 0 ppm 1,4-dioxane vapor was performed, a quantitative estimate of the carcinogenic risk from inhalation was not done. Three replicate groups of 288 rats/sex served as the treated and control groups, and comprehensive gross and microscopic examination of the major organs and tissues was done. Although analysis revealed no treatment-related lesions, the study is limited by having only one dose making an extrapolation of an inhalation potency factor impractical.

The California EPA, assuming that the route of administration is not critical to assessing the cancer potency factor of 1,4-dioxane via inhalation, has developed an inhalation cancer

potency factor from a drinking water study. Of the available studies (Argus *et al*., 1965; Hoch-Ligeti *et al*., 1969; Argus *et al*., 1973; Kociba *et al*., 1974; NCI, 1978), only the Kociba *et al*. (1974) and NCI (1978) studies were considered for the determination of the cancer potency factor for dioxane. The NCI (1978) study using B6C3F1 mice was used as the basis for California's cancer potency for dioxane. They considered this study to contain the best data on the most sensitive species and sex, and the most sensitive target tissue. In this study, 50 male or female mice were exposed to 0, 0.5, or 1.0% 1,4-dioxane for 90 weeks. Average doses were determined from weekly measurements of water consumption. The estimated doses were 0, 720, and 830 mg/kg-d for the males and 0, 380, and 860 mg/kg-d for the females. The incidence of hepatocarcinomas were 2/49, 18/50, and 24/47 for males, and 0/50, 12/48, and 29/37 for the females. The incidence of hepatocarcinomas or adenomas were 8/49, 19/50, and 28/47 in males, and 0/50, 21/48, and 35/37 in females.

A linearized multistage procedure was applied to the female mouse combined hepatocellular carcinoma and adenoma incidence from the NCI (1978) study by CalEPA (CalEPA, 2002). The animal cancer potencies were 8.3×10^{-4} and 1.4×10^{-3} $(mg/kg\text{-}d)^{-1}$, for the males and females, respectively. The animal cancer potency, q_{animal}, was calculated from the linear slope using the lifetime scaling factor $q_{animal} = q_1^* \times (T/T_e)^3$, where T/T_e is the ratio of the experimental duration to the lifetime of the animal. The animal cancer potencies were therefore adjusted for the short duration of the experiment, using the factor $(104/90)^3$. A value for the human cancer potency was determined using the relationship $q_{human} = q_{animal} \times (bw_h/bw_a)^{1/3}$, where bw is the default body weight of human or animal (mouse). Body weights for interspecies scaling were assumed to be 0.04 and 0.035 kg for males and females, respectively. The combined incidence of hepatocarcinomas and adenomas in males and females gave human cancer potencies of 1.5×10^{-2}, and 2.7×10^{-2} $(mg/kg\text{-}d)^{-1}$, respectively. The combined incidence of hepatocarcinomas and adenomas in females was used to derive the human cancer potency for dioxane of 2.7×10^{-2} $(mg/kg\text{-}d)^{-1}$. The airborne unit risk factor for dioxane of $7.7\ E^{-6}$ $(\mu g/m^3)^{-1}$ was calculated by California assuming a human body weight of 70 kg and an inhalation rate of 20 m^3/day.

The relevance of the CalEPA inhalation potency factor is uncertain for many of the same reasons that the USEPA potency factor is called into question. While the mouse may be the sensitive species, the rat seems more similarly pharmacokinetically to humans and is likely a better choice as an animal surrogate for that reason (especially given the importance that pharmacokinetics plays in assessing the hazard potential of 1,4-dioxane). Additionally, the extrapolation techniques (*i.e*., scaling factors) employed are not the most current or recommended, and the assumption of a linear no-threshold response does not make full use of the available data.

5.2.3 PBPK Assessments for 1,4-Dioxane

The available pharmacokinetic and toxicologic information on 1,4-dioxane indicates that its metabolism is saturable (section 4.2.6) and its metabolites are non-genotoxic. These factors suggest that the carcinogenic response observed at high doses may be due to cytotoxicity with the increased chance of error associated with hyperplasia. Pharmacokinetic variables were ignored in the USEPA and CalEPA evaluations of the cancer risk associated with 1,4-dioxane. Three groups have undertaken to incorporate this source information into PBPK models and use the results from exercising the model to better estimate human cancer risk. It should be noted, however, that use of a PBPK model does not imply acceptance or rejection of a particular hypothesis regarding whether a compound is a (human) carcinogen. It is simply a better tool for more accuratelye estimating the internal dose at the target organ(s). In this case, there is strong reason to believe that 1,4-dioxane is a threshold carcinogen and unlikely to pose a cancer risk at environmentally relevant, non-cytotoxic doses.

5.2.3.1 Reitz *et al.*, 1990

Reitz *et al.* (1990) pointed out that, although high doses of 1,4-dioxane resulted in liver and nasal tumors in rats, 1,4-dioxane is generally negative in short-term tests designed to detect genotoxic agents and, therefore, the neoplastic effect is likely due to an indirect or non-genotoxic mechanism. Since non-gentoxic carcinogens are often characterized by a threshold, Reitz *et al.* (1990) developed a PBPK model to assess whether humans exposed to the much lower doses that characterized environmental and occupational exposures are at risk of cancer. This model was used to provide quantitative estimates of delivered dose, time course, and response in experimental animals with extrapolation to humans. The PBPK model was based on that developed for styrene by Ramsey and Andersen (1984). Adjusted for differences in 1,4-dioxane solubility and metabolic rates. This is a six compartment model (*i.e.*, lungs, fat, liver, venous blood, slowly perfused tissues such as muscle, and rapidly perfused tissues such as brain) formulated to simulate exposure via inhalation, iv administration, bolus gavage, and ingestion via drinking water. Tissue/air partition coefficients for 1,4-dioxane in human and rat blood, rat fat, muscle and liver were determined in F344 rats and from human blood drawn from volunteers. The metabolic process was described as saturable as suggested by the data and organ volumes, blood flows and air flows were generally the same as employed by Andersen *et al.* (1987) except for increasing ventilation and cardiac outputs to better coincide with the blood dat a of Young *et al.* (1977). The model was formulated using a commercially available software program (SimuSolv) and metabolic rate constants from the data of Young *et al.* (1977; 1978a) for 1,4-dioxane in rats and in humans (Young *et al.*, 1976; 1977). Given the fact that 1,4-dioxane is a non-genotoxic carcinogen and likely to exhibit a threshold for its neoplastic activity, the output from the model (target doses) was used to determine NOAELs in animals and the dose in

humans that correspond to this dose divided by an uncertainty factor of 100. Additionally, Reitz *et al.* (1990) used the linearized multistage model to fit target dose and tumor response data in a manner similar to that done by USEPA, for comparison.

The model output was compared to that developed by Young *et al.* (1978b) and a reasonable agreement found in prediction of venous blood levels. When compared to the results of an oral dosing study (Young *et al.*, 1978a) using 10, 100 and 1000 mg/kg of 1,4-dioxane. While most (>95%) of the 1,4-dioxane was rapidly eliminated as metabolites at low doses, as the doses increased, 25% or more of the administered dose was eliminated unchanged suggesting a saturable metabolism as suggested by Young *et al.* Similar results were obtained with inhalation data for 1,4-dioxane in rats. The PBPK model was scaled up to simulate human responses by changing the physiological parameters for rats in the validated model to those of humans. Once this was done, the model output was compared to the results of human volunteers exposed to 1,4-dioxane (Young *et al.*, 1977). The PBPK model for humans gave reasonable agreement to the venous blood results obtained in the human volunteers.

Once the PBPK model was developed, dose surrogates (*i.e.*, target organ concentrations) for animals and humans were calculated based on reasonable hypotheses regarding the mechanism of action for 1,4-dioxane. Three different types of dose surrogates were considered based on the protocol developed by Andersen *et al.* (1987): 1) average parent chemical concentration in the target organ; 2) average concentration of stable metabolite(s) of the parent chemical in the target organ; and 3) production of short-lived reaction metabolites in the target organ. Since 1,4-dioxane causes both nasal and liver tumors, this suggests that six dose surrogates must be considered. The nasal tumors were eliminated for development of the dose surrogate given the lack of response in the inhalation study of Torkelson *et al.* (1974) despite significant exposure to 1,4-dioxane vapors for two years. Reitz *et al.* (1990) suggest that the discrepancy between the results of the drinking water studies and the inhalation study in terms of nasal tumor may lie in the observation that the nasal tissues of rats were repeatedly splashed with water containing high amounts of 1,4-dioxane during drinking. Since the nasal configuration differs between rodents and humans and human nasal tissue generally does not contact fluids during drinking, the nasal tumor data was not considered in dose surrogate selection. The reactive metabolite dose surrogate was also eliminated since 1,4-dioxane is inactive in tests of genotoxicity and fails to induce cytotoxicity for extended periods (up to 11 weeks). Therefore, Reitz *et al.* (1990) assumed that the parent compound or a stable metabolite affecting the liver were the most appropriate dose surrogates for 1,4-dioxane.

The two dose surrogates considered relevant to evaluate the biological response to 1,4-dioxane were AUC- Liver (*i.e.*, the average area under the liver dioxane concentration time curve per day) and AUC-Met (*i.e*, the average area under the metabolite concentration time curve for the whole body per day). In the case of the parent compound, it was possible to

quantitatively describe the liver concentration using the partition coefficients for the tissue and the chemical. In terms of AUC-Met, a different approach was taken. HEAA is known to be the principal metabolite of 1,4-dioxane in rats and humans; however, partition coefficients are lacking for this compound so liver concentrations cannot be predicted. Since the elimination of HEAA was described by Young et al. (1976; 1977), it was possible to estimate the AUC-Met for the whole body using these elimination rate constants. This was calculated using the PBPK model and used as a first approximation of the HEAA liver concentration. Disproportionate increases in 1,4-dioxane liver concentration (AUC-Liver) were seen in each species where the PBPK model predicts the saturation of metabolic enzymes would occur. Linear extrapolations of toxicity based on AUC-Liver from conditions where rats developed liver tumors (0.5-1.0% 1,4-dioxane in water) to environmentally relevant exposure would be inappropriate for this dose surrogate. Additionally, at low levels, the predicted human AUC-Liver values are always lower than that predicted for rodents suggesting that interspecies conversion factors based on body surface area (which predict that humans are more sensitive than experimental animals) would also be inappropriate for interspecies extrapolation based on this dose surrogate. Similarly, AUC-Met (the dose surrogate related to stable metabolites in the body) also predicted lower levels in humans than animals exposed to equivalent concentrations of 1,4-dioxane in water or air due to lower rates of metabolism in humans, again suggesting that interspecies extrapolation using body surface area would be inappropriate.

Use of the PBPK predictions for quantitative risk assessment required a decision as to which dose surrogate best predicted the cancer response. While the cancer response seems to be associated with metabolic saturation and build up of the parent chemical, HEAA has not been tested in a chronic bioassay and so could be the carcinogenic agent. In the absence of such data, either dose surrogate could be the appropriate choice. AUC-Liver values for rats and mice exposed to doses resulting in tumor formation (*i.e.*, 0.5 to 1.0% 1,4-dioxane in drinking water) were high (*i.e.*, 17,900 - 64,200 mg*hr/L for rats and 15,200 - 43,400 mg*hr/L for mice) and increased disproportionately as the water concentration increased from 0.5% to 1.0%. AUC-Liver levels increased three to fourfold for a twofold increased in 1,4-dioxane concentrations consistent with the pattern of tumor development observed in mice. The response in rats was less marked (18.5% to 19.8%) and less consistent with the AUC-Liver results. At the tumor NOAELs for rats exposed to 1,4-dioxane in water (*i.e.*, 0.1%) and air (*i.e.*, 400 mg/m^3) (Kociba *et al.*, 1974; Torkelson *et al.*, 1974), the AUC-Liver values were much lower, 257 and 109 mg*hr/L, respectively. The large (70-fold) difference between the predicted levels of AUC-Liver at 0.5% 1,4-dioxane and 0.1% 1,4-dioxane (the NOAEL dose) correlates well with the dose response and observed tumor frequencies than AUC -Met (see below). The model also predicted that humans exposed to air (24 hr/day) or water (2 L/day) containing up to 10,000 ppb of 1,4-dioxane had AUC-Liver values several orders of magnitude below that of rats at the NOAEL. Humans exposed to 10,000 ppb 1,4-dioxane in water were predicted to have an AUC-Liver value of 0.2 mg*hr/L, a value nearly 1,300

times lower than the drinking water NOAEL for rats and nearly 550 times lower than the air NOAEL for rats. The AUC-Met at tumorigenic doses gave values of 1,500 mg*hr/L (and were virtually identical for both the 0.5% and 1.0% dose levels even though the tumor response was dose-related) while the levels at water and air NOAELs were 470 and 197 mg*hr/L, a much smaller ratio than that observed with the AUC-Liver dose surrogate. These levels were only threefold higher than that predicted for rats consuming 0.1% (the NOAEL dose). Such a relatively small difference in dose surrogate values does not correlate well with the increasing tumor frequencies observed in the rats, suggesting that AUC-Met may not be the best dose surrogate for 1,4-dioxane for this endpoint. Accordingly, Reitz *et al.* (1990) selected AUC-Liver as the best dose surrogate for 1,4-dioxane.

Reitz *et al.* (1990) evaluated the PBPK model prediction in terms of predicted human risk in two ways: an uncertainty factor approach and extrapolation using the linearized multi-stage model. In the uncertainty factor approach, the NOAELs from the chronic ingestion and inhalation studies (Kociba *et al.*, 1974; Torkelson *et al.*, 1974) were identified and the equivalent human AUC-Liver estimated using the PBPK model with an uncertainty factor (100 in this case) used to estimate a "virtually safe human dose" in water or air. The AUC-Liver for the water NOAEL of 0.1% 1,4-dioxane was 257 mg*hr/L and for the inhalation NOAEL of 400 mg/m^3 1,4-dioxane was 109 mg*liter. These values were divided by a 100-fold uncertainty factor and the corresponding dose in water (51,000 to 118,000 ppb) and air (1900 to 3700 ppb) was identified. Use of a larger uncertainty factor (*i.e.*, 1,000, 3,000, 10,000, etc.) would decrease the "virtually safe dose" by the same amount that the uncertainty factor was increased.

Reitz *et al.* (1990) also employed the linearized multistage model to identify the "risk specific dose" (95th percentile upper confidence limit) associated with exposure to 1,4-dioxane in water and air. Relying again on the drinking water study of Kociba *et al.* (1974) and the NCI chronic bioassay (1978). The inhalation study of Torkelson *et al.* (1974) could not be used since only a single dose was used and no tumor response was observed. At a risk level of 1×10^{-5}, the PBPK risk specific dose using the AUC-Liver dose surrogate was 2,800 ppb for continuous exposure to air (via route to route extrapolation) and 83,000 ppb when consuming 2 L of water daily. By comparison, the USEPA unit risk for ingestion of 1,4-dioxane developed without considering pharmacokinetic parameters is 30 ppb at the 1×10^{-5} risk level. Risk specific doses developed using mouse data or the AUC-Met dose surrogate were lower; however, the B6C3F1 mouse strain used in the bioassay is prone to developing tumors and the AUC-Met dose surrogate was considered less reliable for predicting human hazard than the AUC-Liver since the parent compound is thought to be responsible for the tumor response as previously discussed. When the mouse and AUC-Met data were added into a weighted evaluation (*i.e.*, rat and AUC-Liver results were given twice the weight of the mouse and AUC-Met data), the weighted risk specific dose (1×10^{-5}) for 1,4-dioxane in air and water was 740 ppb and 20,000 ppb, respectively.

Even though these quantitative risk estimates for human populations exposed to 1,4-dioxane are still regarded as plausible upper bounds on risk rather that actual estimates of risk, the incorporation of pharmacokinetic data and PBPK modeling adds another dimension and improved realism in the dose and risk estimates. The PBPK model allows pertinent information on species, dose routes, target organs, physiology and metabolism to be used in evaluating risk and make better judgements about potential human hazards accordingly. In this case, incorporating mechanistic and physiologic data in the evaluation of the cancer risk associated with 1,4-dioxane results in an assessment 650 to 2,750 times less conservative (in drinking water) without sacrificing health protection.

5.2.3.2 Leung and Paustenbach, 1990

Leung and Paustenbach (1990) also reviewed the data available at the time and noted that, although high doses of 1,4-dioxane caused liver and nasal tumors in rats and increased liver tumors in mice when administered in drinking water, and similarly concluded that the evidence supports cancer induction through a non-genotoxic mechanism. 1,4-Dioxane is inactive in a variety of genotoxicity assays, does not alkylate DNA even at high doses, and is inactive as an initiator. The fact that tumors are observed only at high doses and in the presence of extensive cellular necrosis suggests an epigenetic mechanism associated with cytotoxicity is responsible for the tumorigenic response. While the earlier pharmacokinetic work conducted by Young *et al.* and others provides insight into the relationship between metabolism and toxicity (section 4.2.6), it was unable to determine the target organ concentrations. The PBPK modeling was undertaken to better understand the target organ concentrations associated with various administered dose levels and extrapolate the results observed in rats to that expected in humans.

The model developed and validated by Leung and Paustenbach (1990) describes the disposition, metabolism, and excretion of 1,4-dioxane in the rat and human along with a revised assessment of the excess cancer risk associated with delivered dose. Similar to the approach of Reitz *et al.* (1990), the validated Ramsey and Andersen (1984) PBPK model for styrene was used as the basis for the 1,4-dioxane PBPK model. The physiological and biochemical parameters (*e.g.*, weights, organ volumes, blood flow, partition coefficients, and metabolic rate constants) for rats and humans used in the model are detailed in the published paper and will not be repeated here.

The liver tumor response in the rat was selected as the most consistent tumor type seen in chronic drinking water bioassays. Unlike USEPA, nasal tumors were not used due to their inconsistent occurrence in various studies (and the failure of a chronic inhalation study to induce them), and mouse data was rejected both due to inconsistent responses and lack of pharmacokinetic data needed for the PBPK model. The time weighted average concentration of 1,4-dioxane in the liver over the entire lifetime exposure was chosen to reflect the putative

carcinogenic mechanism since the parent chemical rather than the metabolite was assumed to be the toxic species and the response was associated with attainment of a level associated with chronic insult and resultant cytotoxicity. The rodent and human data of Young *et al.* (1977; 1978a-b) was used to validate the model predictions and the results of the modeling were extrapolated using the linearized multistage model to reflect the expected response at low levels.

The model supports the finding that the metabolism of 1,4-dioxane is saturable. At low administered doses, the metabolism follows first order kinetics and the relationship between administered dose and liver concentration is linear. As the administered dose increases, the metabolic enzymes become saturated and the concentration of unmetabolized 1,4-dioxane becomes proportionally greater than the administered dose. Once saturated, the levels of 1,4-dioxane persist longer than the metabolite and results in the aforementioned damage. The results of Leung and Paustenbach (1990) suggest that the dose of 1,4-dioxane associated with 1×10^{-5} risk in humans using the PBPK approach is as much as 60 to 60,000 times greater than that derived using the conventional USEPA risk assessment methodology. The maximum likelihood estimate (MLE) for the 1×10^{-5} risk level is 50 mg/kg-d, which translates to a drinking water level of approximately 1,750 ppm, while the lower confidence limit is 0.06 mg/kg-d, which translates to a drinking water level of approximately 2 ppm.

The use of rodent tumor incidence data and administered dose provides only a crude estimate of cancer risk that is markedly improved by incorporating pertinent biological and mechanistic data. In the case of 1,4-dioxane, Leung and Paustenbach (1990) use of PBPK modeling provides a more realistic and relevant prediction of human response than a simple mathematical model and qualitatively supports the findings and conclusions of Reitz *et al.* (1990).

5.2.3.3 Balter, 1989

An unpublished PBPK model of 1,4-dioxane is cited by Hartung (1989). This evaluation was conducted by Nancy J. Balter, Ph.D. at the Georgetown University School of Medicine to simulate drinking water exposure to 1,4-dioxane and determine if saturation kinetics would be observed was delivered as a continuous as opposed to a bolus dose. Using the Young *et al.* (1978a) data, the model was exercised to simulate exposure in drinking water over a 7-day period. Total doses of 10 to 1000 mg/kg-d as a continuous infusion to the gastrointestinal tract over a 12 hour period each day were used. The results of the simulation are presented in terms of various dose surrogates: peak liver concentrations, 24 hr area under the curve for the liver, and the amount of metabolite formed in 24 hours (**Table 5-4**). Although only the AUC-Liver is reported, the model would predict the same relationship overall between drinking water exposure and other organ dose or blood level. The data are normalized for the total daily dose to demonstrate the influence of non-linear pharmacokinetics.

5.2.3.4 Stickney *et al.*, 2003

Stickney *et al.* (2003) evaluated the Leung and Paustenbach (1990) and Reitz *et al.* (1990) PBPK models in conjunction with a larger effort to update the carcinogenic potential of 1,4-dioxane. The authors noted that each of these models account for the nonlinear pharmacokinetics of 1,4-dioxane as evidenced in experimental studies as well as physiological differences between the rodent models and humans. In addition, these PBPK models also provide a methodology whereby accurate human cancer risks can be estimated through the derivation of target organ doses. The authors evaluated the metabolism and pharmacokinetic studies in rodents and humans (Braun and Young, 1977; Kociba et al., 1975; Young et al., 1977; Young et al., 1978a-b; Woo *et al.*, 1977 a-c) utilized in both PBPK models and discussed earlier in this report (see Section 4, above). These data indicate that 1,4-dioxane is readily absorbed by rodents and humans after oral and inhalation exposure and is metabolized into HEAA, which is then excreted via urine. These studies also demonstrate that the threshold for toxic effects corresponds with the saturation of metabolism and detoxification mechanisms. Based on these findings, the authors concluded that nonlinear pharmacokinetics play a critical role in the liver toxicity and subsequent carcinogenicity of 1,4-dioxane. This conclusion is particularly true at high dose levels typically used in the cancer bioassays conducted on 1,4-dioxane. The authors also noted that PBPK modeling is accurate in estimating the saturation of metabolism and subsequent cytotoxicity at high doses and, therefore, appropriate in evaluating human cancer risk.

Metabolic saturation, seen as an increase in the values for unmetabolized 1,4-dioxane in tissues or excreted, and as a decrease in normalized values of 1,4-dioxane as the metabolite, is seen beginning at a total daily dose between 30 and 100 mg/kg. The influence of non-linear pharmacokinetics on tissue accumulation associated with chronic dosing is seen by comparing the values for the liver concentrations and the amounts exhaled or metabolized for the first and seventh 24 hour period in the simulation. At daily doses where these values are the same (10 to 300 mg/kg), there is no accumulation of 1,4-dioxane from one day to the next; however, when the total daily dose of 1,4-dioxane is 1,000 mg/kg, such accumulation is evident. These findings again support the results reported in the other PBPK models and the supposition that cancer and non-cancer endpoints observed in animal study are associated with the non-linear pharmacokinetics displayed by 1.4-dioxane and the cytotoxicity and resultant tissue damage and tumor production can be avoided if target doses are below those associated with metabolic saturation.

Table 5-4. PBPK Model Output for Simulated Seven Day Exposure to 1,4-Dioxane in Drinking Water

Parameter	Daily Dose (mg/kg)	1st 24 hours	7th 24 hours
Peak [Liver] (mg/L)			
	10	1 (0.1)*	1 (0.1)
	30	4 (0.1)	4 (0.1)
	100	20 (0.2)	20 (0.2)
	300	101 (0.3)	152 (0.5)
	1000	827 (0.8)	1498 (1.5)
*24hr Liver AUC (mg*hr/L)*			
	10	13 (1.3)	13 (1.3)
	30	44 (1.5)	44 (1.5)
	100	213 (2.1)	213 (2.1)
	300	1686 (5.6)	1723 (5.8)
	1000	12471 (12.5)	28793 (28.8)
24hr Metabolite (mg as dioxane)			
	10	2.4 (0.2)	2.4 (0.2)
	30	7.3 (0.2)	7.3 (0.2)
	100	23.9 (0.2)	23.9 (0.2)
	300	65.2 (0.2)	65.9 (0.2)
	1000	91.1 (0.1)	94.4 (0.1)

* normalized for the daily dose

5.3 Summary and Conclusion

A number of authors (Dietz *et al.*, 1982; Stott *et al.*, 1988; Stott 1988; Stott and Watanabe, 1982; Hartung, 1989, Reitz *et al.*, 1990; Leung and Paustenbach, 1990; Stickney *et al.*, 2003) have interpreted the pharmacokinetic, genotoxicity, and dose-response data for 1,4-dioxane as evidence of an epigenetic carcinogen, which is characterized by the existence of a

threshold of effect. 1,4-Dioxane typically causes liver, kidney, and nasal lesions that can progress to tumor if exposure is sufficiently high and prolonged. Accordingly, if the oral or inhalation RfD is protective against the relevant target organ histopathologies, they are likely protective against tumorigenicity. While the relevance of the nasal tumor data to man has been called into question, the liver and renal toxicity and liver tumor data is consistent across species and tests, and is a stronger candidate for use in risk assessment. The weight -of-evidence indicates that 1,4-dioxane is a non-genotoxic carcinogen and therefore likely has a threshold both for the non-carcinogenic as well as the carcinogenic effects observed. Based on the pharmacokinetic and toxicological data available, it is appears that, at high doses (*i.e.*, 5,000 to 10,000 ppb in water), the metabolism of 1,4-dioxane becomes saturated and the parent compound builds up to cytotoxic levels. If the exposure continues at the same level for a prolonged period of time, the damage appears first as lesions in the aforementioned target organs and ultimately progresses to tumors as a consequence of cell proliferation, hyperplasia, and hypertrophy in the target organs, and the increased probability of repair and replication error being conserved in an altered cell line (Stott and Watanabe, 1982, Ames, 1989). Presumably if cytotoxic levels are avoided, neither the non-cancerous lesions or tumors should express themselves in individuals exposed to 1,4-dioxane.

Humans appear unlikely to be at risk from environmentally relevant levels of 1,4-dioxane. When evaluated using a validated PBPK model for 1,4-dioxane, the dose surrogate levels predicted in humans hypothetically exposed to the same dose that resulted in liver lesions and tumors in rodents was two to three orders of magnitude lower than the dose surrogate levels associated with the NOAEL doses in the same studies. Since 1,4-dioxane is likely to be an irritant at cytotoxic doses (assuming such concentrations would realistically ever be encountered), exposure to critical concentrations would be self-limiting. Most current relevant exposures to 1,4-dioxane are expected to be intermittent and to relatively low concentrations as opposed to the prolonged, high dose exposures necessary to result in harm.

Although no reference doses for 1,4-dioxane have been developed by USEPA, the toxicological database is sufficiently robust to identify both LOAELs and NOAELs for relevant routes of exposure. An oral NOAEL of 10 mg/kg-d, an inhalation NOAEL of 108 mg/kg-d, and a dermal NOAEL of 1500 mg/kg-d have been identified in 1,4-dioxane evaluations conducted by the Dutch and Australian governments (TNO and RIVM, 2002; NICNAS, 1998). Employing an uncertainty factor approach and including an additional uncertainty factor to address the potential sensitivity of children furnished reference doses of 0.33 mg/kg-d (oral) and 3.6 mg/kg-d (inhalation) that are well below the doses associated with saturated metabolism or cytotoxicity and therefore protective of human and specifically children's health.

USEPA has drinking water health advisories and an oral cancer potency factor for 1,4-dioxane; however, these evaluations are somewhat dated. The health advisories are based

on a 1934 iv study in rabbits. The potency factor is based on nasal tumors which are of uncertain relevance to humans for reasons previously mentioned, the wealth of pharmacokinetic data for 1,4-dioxane is not utilized at all, and the extrapolation assumes no threshold for the carcinogenic effects of 1,4-dioxane which is unlikely to be the case for non-genotoxic compounds. Evaluating the toxicologic and cancer data with a PBPK model while still applying the linear multistage extrapolation model suggests that the USEPA potency factor overestimates the hypothetical human cancer risk by two to three orders of magnitude (Reitz *et al.*, 1990; Leung and Paustenbach, 1990; Stickney *et al.*, 2003) assuming that 1,4-dioxane is, in fact, a human carcinogen at environmentally relevant doses. In this case, use of the derived reference doses and estimated children's exposure to 1,4-dioxane using a RfD approach is viewed as an appropriate assessment of potential hazard.

From this evaluation, RfDs for 1,4-dioxane were derived to protect children from sensitive endpoints (*i.e.*, liver, kidney, and other target organ damage) as well as avoid adverse reproductive outcomes. A reproductive RfD of **5.2 mg/kg-d** will be used to assess the hazards to pregnancy and *in utero* exposure while RfD/RfCs of **0.1 mg/kg-d**, **1.1 mg/kg-d**, and **0.1 mg/kg-d** will be used to assess the potential hazards posed to children from ingestion, inhalation, and dermal exposure to 1,4-dioxane, respectively.

Should a cancer risk assessment be desired, cancer potency factors based on the PBPK model of Reitz *et al.* (1990) can be derived that take into account the issues of metabolic saturation and kinetic issues since the currently available CPFs from USEPA and CalEPA do not reflect the current state of dose extrapolation and do not make full use of the data.

The various toxicological criteria for 1,4-dioxane are listed in **Table 5-5**.

Table 5-5. Toxicity Criteria for 1,4-Dioxane

Toxicity Criteria	Oral	Inhalation	Dermal
RfD/RfC (Derived Herein)	0.1 mg/kg-d	1.1 mg/kg-d	0.1 mg/kg-d
RfD/RfC (CalEPA, 2000)	NA	0.82 mg/kg-d	NA
CPF (Reitz *et al.*, 1990)	1.8E-5 $(mg/kg\text{-}d)^{-1}$	1.3E-5 $(mg/kg\text{-}d)^{-1}$	NA
CPF (USEPA, 2002)	1.1E-2 $(mg/kg\text{-}d)^{-1}$	NA	NA
CPF (CalEPA, 2002)	2.7E-2 $(mg/kg\text{-}d)^{-1}$	2.7E-2 $(mg/kg\text{-}d)^{-1}$	NA

6.0 Exposure Assessment

Children can be exposed to 1,4-dioxane in a variety of ways. Children of workers manufacturing or using 1,4-dioxane can be exposed *in utero* or through ingestion of breast milk containing 1,4-dioxane. Additionally, children can be exposed via inhalation to 1,4-dioxane present in ambient and indoor air originating from its industrial use and occurrence in consumer products, ingestion via water and in food where it is present both as a natural constituent and as an unintentional additive, and dermally through the use of consumer products (*i.e*, shampoos and lotions) again containing it as an unintentional additive and contact with contaminated water.

Assessing the exposure of children to 1,4-dioxane is challenging for a number of reasons. 1,4-Dioxane has never been the subject of extensive occupational or environmental monitoring, so data is often lacking altogether, limited in scope, and of uneven (or uncertain) quality. In addition, while production and use of 1,4-dioxane has dramatically dropped in the past 20+ years, 1,4-dioxane is now only produced at one site in the US. The majority of this 1,4-dioxane is used in the industrial processes that would not result in children's exposure. Some of the data gaps can be addressed using probabilistic techniques combined with conservative assumptions to meet the goals of a Tier I exposure assessment. Physiologic and behavioral issues that are specific to and influence the exposure of children were drawn from USEPA's recent Children's Exposure Factors Handbook (USEPA, 2006). Where exposure factors were missing for children, the Exposure Factors Handbook was employed to estimate children's exposure (USEPA, 1997). Probabilistic modeling decisions also followed recent USEPA guidance on the subject (SPC, 1997)

The environmental and occupational data that does exist is somewhat dated. Most of it is from the 1980s or earlier when the use of 1,4-dioxane was much more extensive than it is now. The change in use patterns and consumption, particularly associated with the curtailing of 1,4-dioxane's use as a stabilizer, has resulted in a dramatic decrease in the production of 1,4-dioxane. Additionally, the makers of ethoxylated surfactants that contain 1,4-dioxane as an impurity have taken steps to reduce the levels of 1,4-dioxane in their products both in response to consumer pressure and FDA regulations. Use of this data for estimating exposures should also be considered conservative since the actual exposure to 1,4-dioxane is likely much reduced at this point in time.

6.1 1,4-Dioxane Uses and Occurrences

Commercial production of 1,4-dioxane in the United States was first reported in 1951, but commercial quantities were produced before that time. The 1979 TSCA Inventory identified seven U.S. companies producing approximately 11.6 million lb, while in 1997, the *Directory of Chemical Producers* listed two producers with undisclosed amounts. In 1998, the

Chemical Buyers Directory identified seven domestic suppliers or distributors of 1,4-dioxane, while *Chemcyclopedia 98* named five. Three companies were reported as importing 1.1 million pounds of 1,4-dioxane in 1977. In 1985, four companies produced approximately 25 million pounds of 1,4-dioxane, and none was reportedly imported into the United States. Approximately 15 million pounds of 1,4-dioxane were produced by three companies in the United States during 1982 and sales of 1,4-dioxane in the United States were reported to be approximately 7.4 million pounds in 1981 (NTP, 2005). USEPA's 1997 and 2001 TSCA inventory reports show only one US producer of 1,4-dioxane with a range code both years as 1 to 10 million pounds. No imports were reported in either the 1997 or 2001 inventory update reports. Currently, there is only one US producer of 1,4-dioxane, Ferro Corporation, which produces the chemical at its Fine Chemical manufacturing plant in Louisiana, which sells a significant portion of its output to one client that uses it in the production of fire retardant chemicals. In 2000, Ferro produced less than 3 million pounds of 1,4-dioxane, primarily for one customer, and 2003 production dropped to 1 million pounds. The amount of 1,4-dioxane imported is reported as less than 50,000 lbs in 2001 (Ferro, 2002; 2006). Overall this represents a reduction of over 80% from the peak production in 1982 when much of the environmental data was also collected.

For many years, the major use for 1,4-dioxane was as a stabilizer for chlorinated solvents, particularly, 1,1,1-trichloroethane. Approximately 90% of the 1,4-dioxane produced annually was used as a stabilizer for chlorinated solvents, particularly 1,1,1-trichloroethane. The remainder of the 1,4-dioxane production is used in other solvent applications such as an extraction solvent for animal and vegetable fats and oils, waxes and natural and synthetic resins, in the production of fire retardant chemicals and cassette tapes, as a plastics and rubber solvent, as a wetting and dispersing agent in textile processing, as a carrier solvent for pesticides, as a fumigant, in the pulping of wood, in dye-baths, lacquers, paints, varnishes, paint and varnish removers, and in stain and printing compositions. With 1,4-dioxane's relatively high cost, these applications have become few and wide. In many cases, 1,4-dioxane will not be present in the end product, but is only used as a solvent in the production process.

1,4-Dioxane has also been found in cleaning and detergent preparations, as a degreasing agent, in adhesives, cosmetics, deodorants, and emulsions and polishing compositions. It is used as a solvent in spectroscopic and photometric measurements, an eluent in chromatography, a working fluid for scintillation counting, and in the purification of drugs. It was formerly used as well in the preparation of tissue sections for histology. 1,4-Dioxane may be formed as a by-product of reactions based on condensing ethylene oxide or ethylene glycol during the production of certain consumer products (*i.e*, detergents, shampoos, surfactants, and certain pharmaceuticals). Although steps are taken to reduce this impurity, 1,4-dioxane has been found in end-use cosmetic and personal care products, such as shampoos and bath preparations. 1,4-Dioxane residues may also be present in food packaged

in 1,4-dioxane-containing materials, since it is an indirect food additive allowed for use as a component of adhesives. 1,4-Dioxane also occurs as a natural component of food and is found in foods that contain stabilizer, solubilizers, surfactants, and emulsifiers similar or identical to those used in cosmetics. Most exposure to 1,4-dioxane from these niche uses or inadvertent occurrences is likely to be low or infrequent or both.

6.2 Occupational Exposure

Occupational exposure to 1,4-dioxane is of interest in this evaluation only in respect to potential *in utero* exposure to the children of workers and the resultant impact this exposure might have on reproductive outcome and development.

Exposure to 1,4-dioxane at the workplace occurs most likely via the skin and the respiratory tract. The number of women currently exposed to 1,4-dioxane is unknown (no male-mediated reproductive toxicity is assumed for this compound). The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that 334,000 workers were potentially exposed to 1,4-dioxane, including 100,000 workers possibly exposed due to 1,4-dioxane contamination of 1,1,1-trichloroethane (NICNAS, 1998). In 1977, NIOSH estimated that 2,500 workers were potentially occupationally exposed to 1,4-dioxane, not including the 100,000 workers who may have been exposed to both 1,1,1- trichloroethane and 1,4-dioxane (NICNAS, 1998). Another survey, the National Occupational Exposure Survey (1981-1983) estimated that 429,330 workers, including 149,697 women, were possibly exposed to 1,4-dioxane (NIOSH, 1984). Occupational and Safety Health Administration reported that as many as 466,000 workers may have been occupationally exposed to 1,4-dioxane. This estimation was derived from actual use observations of the compound (25% of total observations) and the further use of trade name products known to contain the compound (75% of total observations) (NTP, 2005). Since the use of 1,4-dioxane as a stabilizer in chlorinated solvents has dropped dramatically as use of these chlorinated solvents has decreased, this source of exposure has largely been eliminated. Additional reductions in 1,4-dioxane use and potential exposure are associated with improved recovery and reuse within the industry. Given that only 65 companies report using or handling 1,4-dioxane in the 2000 Toxic Release Inventory (TRI), and 51 companies in the 2004 TRI, it can be assumed that there is something less than 10,000 workers currently exposed to this compound in the workplace.

1,4-Dioxane was used as a stabilizer in concentrations of 3-4% in 1,1,1-trichloroethane, which was itself used as a cleaning agent for metals. Its use has been prohibited since 1995, as a result of the agreement in Montreal, which dealt about the handling of substances depleting the ozone layer (EC, 1996). The University of Louvain, Belgium has made 179 measurements on 1,4-dioxane over the period 1985-1996 in industries in which cleaning/degreasing, painting and the use of adhesives was done. The range of measured

concentrations was 0.9 to 302.4 mg/m^3 with a 90th percentile of 40 mg/m^3. In 97.8% of the samples, 1,1,1-trichloroethane was also present and the correlation between the concentration of the two substances in the samples was high (r = 0.8). It is, therefore, concluded that these data relate to the use of 1,4-dioxane as a stabilizer for 1,1,1-trichloroethane and that these data are not relevant for the present it occupational exposure situation (TNO and RIVM, 2002).

Workers involved in the manufacture of ethoxylated chemicals may be exposed to 1,4-dioxane from its occurrence as an impurity, and in particular, during the 'stripping' process used to remove 1,4-dioxane from ethoxylated surfactants and emulsifiers. The principal ethoxylated chemicals manufactured are alkyl and alkyl phenol ether sulphates, polyethylene glycols, and ethoxylates of alcohols, alkylphenols, sorbitan esters, amides and amines. The highest levels produced during manufacture (before any stripping occurs) were reported in a specialist industrial ethoxylate/thionyl chloride surfactant (2,000 ppm 1,4-dioxane w/w), ethoxylated amines (typically 300 ppm) and alkyl ether sulphates (typically 100-200 ppm) (NICNAS, 1998). Manufacture of ethoxylated chemicals is generally a closed process involving automated feedstock addition to a reactor and automatic feed of reactor product(s) to ancillary 1,4-dioxane stripping (if present) plant. Product(s) are transferred to blenders which are covered, except for feed ports (which one manufacturer reported may be left open) and stripper condensate transferred to the site effluent pond. At one site, possible sources of 1,4-dioxane emissions in this process were identified as the sulphonation plant, blender feed ports, stripper vacuum exhaust, and the site effluent pond. Limited air monitoring data were available for assessment. Personal monitoring carried out at one Australian surfactant manufacturing plant indicated that levels of 1,4-dioxane were below 1 ppm in the drumming area (NICNAS, 1998). Another surfactant manufacturer estimated (based on equilibrium vapor concentrations) that levels of 1,4- dioxane in product handling are unlikely to exceed 9 ppm (32.4 mg/m^3) and in well-ventilated areas would be less than 1 ppm (3.6 mg/m^3). Levels of 1,4-dioxane measured in stripper vacuum exhaust and air above the effluent pond were below the level of detection (NICNAS, 1998).

Occupational exposure data for 1,4-dioxane is sparse, but enough is available to develop exposure estimate for use in this assessment of potential hazards to the embryo and fetus. **Table 6-1** represents the available personal sampling data (in mg/m^3) for potential manufacturing and processing, and end use exposure to 1,4-dioxane.

Table 6-1. Occupational Exposure to 1,4-Dioxane

Manufacture and Processing	# Samples	Mean (or median) mg/m^3	Range	90th Percentile	Source
Processing	Not Reported	1.08	ND - 57.6	Not Reported	Buffler *et al.*, 1978
Loading	Not Reported	1.8	ND - 79.2	Not Reported	Buffler *et al.*, 1978
Storage	Not Reported	0.72	ND- 39.6	Not Reported	Buffler *et al.*, 1978
Processing	9	1.26	0.11 - 2.3	Not Reported	Thiess *et al.*, 1976
Measurement Station	2	3.85	2.3 - 5.4	Not Reported	Thiess *et al.*, 1976
Drum Filling	1	24.0	Not Reported	Not Reported	Thiess *et al.*, 1976
Processing	30	40.9	0.18 - 183.6	Not Reported	NIOSH, 1977
Processing	44	15.4	0.18 - 132.1	Not Reported	NIOSH, 1977
Processing	46	32.4	0.18 - 183.6	Not Reported	NIOSH, 1977
Closed Synthesis	59	0.18 (median)	<0.004 - 1.3	0.9	TNO and RIVM, 2002
Closed Synthesis	18	0.08 (median)	<0.007 - 1.14	1.1	TNO and RIVM, 2002
Drum Filling	37	0.07 (median)	<0.07 - 574	40	TNO and RIVM, 2002
Drum Filling	8	0.1 (median)	0.07 - 12	10	TNO and RIVM, 2002
Pilot Plant	264	2.6 (median)	ND - 173	47	TNO and RIVM, 2002
Pilot Plant	52	0.18 (median)	0.07 - 30	4.8	TNO and RIVM, 2002

Manufacture and Processing	# Samples	Mean (or median) mg/m^3	Range	90th Percentile	Source
Processing	15	0.63	ND - 43	Not Reported	Ferro, 2002
Processing	1	3.6	Not Reported	Not Reported	Young *et al.*, 1976
Processing	5	5.8 ± 1.8	Not Reported	Not Reported	Young *et al.*, 1976
Processing	4	7.2 ± 3.6	Not Reported	Not Reported	Young *et al.*, 1976
Processing	5	6.6 ± 1.4	Not Reported	Not Reported	Young *et al.*, 1976
Processing	5	4.1 ± 2.2	Not Reported	Not Reported	Young *et al.*, 1976
Manufacturing	6	1.22	0.25-3.66	Not Reported	Ferro, 2006
End Use					
Laboratory	305	0.11 (median)	ND - 166	0.6	TNO and RIVM, 2002
Laboratory	29	<0.07 (median)	<0.07 - 018	0.15	TNO and RIVM, 2002
Laboratory	1	165	Not Reported	Not Reported	TNO and RIVM, 2002
Laboratory	8	ND	Not Reported	Not Reported	NICNAS, 1998
Repair	10	0.72 (median)	<0.036 - 4.7	Not Reported	TNO and RIVM, 2002
Cleaning	1	12.6	Not Reported	Not Reported	Thiess *et al.*, 1976
Metal Cleaning	4	31	15 -55	Not Reported	TNO and RIVM, 2002
Pharmaceutical	20	6.5	1.8 -18	Not Reported	TNO and RIVM, 2002

Manufacture and Processing	# Samples	Mean (or median) mg/m^3	Range	90th Percentile	Source
Pharmaceutical	<30	<3.6	Not Reported	Not Reported	TNO and RIVM, 2002
Textiles	2	1.1	0.7 - 1.8	Not Reported	TNO and RIVM, 2002
Textiles	3	ND	Not Reported	Not Reported	TNO and RIVM, 2002
Magnetic Tape	>100	Not Reported	37 - 75	Not Reported	TNO and RIVM, 2002
Solvent Use	194	0.11 (median)	0.01 - 184	1.8	TNO and RIVM, 2002
Solvent Use	49	0.07 (median)	<0.04 - 72	0.62	TNO and RIVM, 2002
Solvent Mixing	estimated	Not Reported	37 -180	Not Reported	TNO and RIVM, 2002
Plastics (closed system)	estimated	Not Reported	ND - 0.4	Not Reported	TNO and RIVM, 2002
Paint mixing (40% dioxane)	estimated	Not Reported	37 - 180	Not Reported	TNO and RIVM, 2002
Rubber Solvents	11	ND	Not Reported	Not Reported	NICNAS, 1998
Waste Disposal	2	Not Reported	0.15 - 4.7	Not Reported	TNO and RIVM, 2002

Area samples near storage tanks and production or drumming areas typically give higher values (*i.e.*, up to 2880 mg/m^3, but are probably not appropriate for use in estimating personal exposure). For purposes of estimating occupational (and pregnancy) exposure, the median values were used to develop the probable personal exposures since these values are more likely representative of such exposures when averages are driven by a few outlier values. Accordingly, an average daily exposure of 0.54 mg/m^3 is assumed and a range of 0

to 47 mg/m^3 following a triangular distribution. This is intended to address both exposure during production, processing, and use. Exposure duration and frequency for a normal (pregnant) worker, breathing rates, and a woman's body weight along with 50% absorption from the lung (Section 4.2.6; Young *et al.*, 1977) is used in a probabilistic model as appropriate to assess workplace inhalation dose from 1,4-dioxane (assuming no personal protective equipment is in use). Although production is often a closed system, some dermal exposure may occur during manufacture or use. For purposes of assessing dermal exposure, the area of a woman's hands are included in the assessment along with an average daily duration of dermal exposure of 30 minutes (range of 0 to 2 hours) to a 1,4-dioxane product containing an average of 40% 1,4-dioxane with a range of 5% to 100%, and dermal absorption rates identified from tests of unoccluded and occluded skin. All parameters used follow a triangular distribution for purposes of this screening exposure evaluation.

6.3 Environmental Exposure

The Toxic Release Inventory estimated that 437,349 pounds of 1,4- dioxane were released to the environment from 43 facilities that produced, processed, used, or otherwise handled the chemical in the United States in 1995. Of that total, 49.5% was released to water, 50.4% to air, and <1% to land. Seven facilities releasing more than 10,000 lb of 1,4-dioxane accounted for 58% of the total air emissions, and one facility located in Kingsport, Tennessee, reporting for the industrial classifications for manufacture of cellulosic man-made fibers (SIC Code 2823), plastics materials and resins (2821), industrial organic chemicals not elsewhere listed (2869), cyclic crudes and intermediates (2865), and printing ink (2893), represented 30% of total water releases (USEPA, 1997). The 2000 TRI reported a total of 286,864 pounds of 1,4-dioxane released to the environment (57.1% to water, 36.5% to air, and 6.4% to land). A significant amount of the 1,4-dioxane used industrially is recovered on-site and recycled or combusted in energy production (USEPA, 2002).

The 2004 Toxic Release Inventory shows 51 sites reported Form R's for 1,4-dioxane for a total of 821,067 lb. for on- and off-site disposal and other releases. Of this 233,349 lb. was released directly to the environment (38.4% to water, 49.3% to air and 12.4% to land). Off-site disposal or other releases was the largest portion at 587,718 lb.

6.3.1 Inhalation

6.3.1.1 Ambient Air Levels

The 2000 TRI reports that approximately 105,000 pounds of 1,4-dioxane was released to the air from over 60 facilities in the United States (primarily in southern states). This is down over 50% from the amount reported released in 1995 (USEPA, 2002). The completeness and accuracy of the information contained in the TRI is uncertain, given that 360,000 to 500,000

pounds of 1,4-dioxane was reportedly released to the air in California alone in the late 1980s and more recent estimates of annual statewide emissions from facilities reporting under the Air Toxics Hot Spots Act in California were 155,549 pounds of 1,4-dioxane (Grosjean, 1990; CalEPA, 2000). In spite of the discrepancies, these estimates qualitatively reflect the changes in production and use of 1,4-dioxane that has occurred over the past ten years. The potential for inhalation exposure to 1,4-dioxane likely mirrors the reductions in production and consumption patterns. Based on available data, it is assumed that virtually everyone has some exposure to airborne 1,4-dioxane, albeit at often low levels inside and out.

Air samples at three urban sites in New Jersey were collected for 40 days in the summer of 1981. The geometric mean 1,4-dioxane concentrations ranged from 0.01 to 0.02 ppb (0.036 to 0.072 $\mu g/m^3$). Fifty-one percent of the samples were positive for 1,4-dioxane. The same three sites were also sampled for a similar period in the winter of 1982. The geometric means of these samples ranged from 0 to 0.01 ppb (0 to 0.036 $\mu g/m^3$), but only 20% of samples were positive (Harkov *et al.*,1984). A report from Bellingham, Washington of air sampling results for a period of 1995 to 1999 reported relatively high concentrations of 1,4-dioxane among other volatile organic compounds sampled. The geometric mean (and range) from three sampling locations were 4.9 $\mu g/m^3$ (nondetect [ND] to 5.8 $\mu g/m^3$), 5.7 $\mu g/m^3$ (ND to 27.8 $\mu g/m^3$), and 9.9 $\mu g/m^3$ (ND to 20.9 $\mu g/m^3$). One ground level grab sample collected on a "stagnant" air day during the evening commute reported a 1,4-dioxane level of 19.5 $\mu g/m^3$. These results are suspect, however, both because they appear so out of line with other sampling results from the period of heaviest 1,4-dioxane use and because the study reported 1,4-dioxane contamination of sample blanks and spike and duplicate Quality Assurance/Quality Control samples (Keel, 2000).

Shah and Singh (1988) examined nearly 125,000 outdoor air samples from 300 cities in 42 states. The data are neither spatially or temporally uniform. California, New Jersey, and Texas records predominate although 20 of the states have more than 1000 analyses each. More than 50% of the records are from 1981, 1984, or 1985 and 90% of the data cover the period 1975 to 1985. Sampling periods ranged from 1 hour to 24 hours in length. A total of 617 outdoor samples were analyzed for 1,4-dioxane with a reported daily ambient air concentration of 0.107 ppb (0.39 $\mu g/m^3$). The median and lower quartile (25%) value were both 0, suggesting that most values were below the detection limit. A value of 0.04 ppb (0.144 $\mu g/m^3$) was reported for the upper quartile (75%). Since this value was lower than the average, it implies that the average was influenced by a few high values, possibly associated with source dominated areas. A study in Japan measured atmospheric concentrations of 1,4-dioxane around residential zones near an industrial plant at 5.1- 275 $\mu g/m^3$ at Yanai. Using this data, it was estimated that individuals living near this industrial plant were exposed to 1.5-82.5 $\mu g/kg\text{-}d$. The Margin of Exposure for the residents living within the higher range of concentrations was 300-750, less than the uncertainty factor of 1,000, indicating that these residents may feel adverse effects from exposure. A second industrial plant was evaluated

and atmospheric 1,4-dioxane concentrations in residential zones were 2.6-5.2 μg/m^3 and daily exposure for individuals was calculated to be 0.8-1.6 μg/kg-d. The Margin of Exposure for these residents was well above the uncertainty factor indicating that there was no significant health risks (Makino *et al.*, 2006).

USEPA compiled ambient air quality data for 1,4-dioxane from 45 locations in 12 cities between 1979 and 1984 and reported a mean ambient air concentration for 1,4-dioxane of 0.44 μg/m^3 with air levels ranging from ND to 30 μg/m^3 (ATSDR, 2006). The TEAM study (Wallace, 1987) that identified 1,4-dioxane in human breath and resulted in its being nominated for the VCCEP also analyzed 1,4-dioxane in the outdoor air in California (Los Angeles and Contra Costa County). This study reported median and 90th percentile 1,4-dioxane levels in overnight ambient air as 0.26 and 1.4 μg/m^3 (February, 1984 - LA), 0.02 and 0.76 μg/m^3 (May, 1984 - LA), and 0.03 and 0.53 μg/m^3 (June, 1984 - CC). The maximum outdoor air levels for 1,4-dioxane in this study were 5.0, 2.0 and 1.0 μg/m^3 in Los Angeles (February, 1984), Los Angeles (May, 1984) and Contra Costa County (June, 1984), respectively.

The information from these studies is dated, but represents the most recent reliable data available for this compound. It is used while recognizing that the actual current exposure to 1,4-dioxane is probably lower (perhaps as much as an order of magnitude or more) due to the significant reductions in production and use that have occurred in the interim. Shah and Singh (1988) suggest that the median value may best represent the data due to the influence of a few high results, therefore, the median value from the February 1984 Los Angeles TEAM study for 1,4-dioxane was adopted for use as a value in estimating children's exposure. Accordingly, 0.26 μg/m^3 was used as the most likely outdoor air concentration for 1,4-dioxane with a range of 0 to 5.0 μg/m^3 and a triangular distribution. A probabilistic analysis was used as appropriate to estimate the likely ambient air concentrations of 1,4-dioxane to which children might be exposed using standard exposure factors relevant for children of different ages along with a pulmonary absorption rate of 50% for 1,4-dioxane (Section 4.2.6; Young *et al.*, 1977).

6.3.1.2 Indoor Air Levels

Sack *et al.* (1992) analyzed 1159 common household products distributed among 65 product categories for 31 volatile organic compounds, including 1,4-dioxane, that might contribute to indoor air exposure. The product categories included automotive products, electronic equipment cleaners, oils, greases, and lubricants, adhesive products, household cleaners and polishes, fabric and leather treatments, paint-related products, and miscellaneous products (*i.e.*, rust removers, caulking, correction fluid, etc.). 1,4-Dioxane was analyzed for in 1043 products and found in 3 (0.3%). It was found in 2.7% of adhesive-related products at an average concentration of 2.7 (%w/w) and reported in 0.9% of household cleaners and

polishes at an average concentration of 150 (%w/w). This latter value is assumed to be an error given the fact that it exceeds unity and that the authors neither discuss it or included it as one of the compounds with the highest average concentration in this category (*i.e.*, 1,1,2-trichlorotrifluoroethane at 49.7, 1,1,1-trichloroethane at 30.2, toluene at 22.6, and tetrachloroethylene at 21.6). It is presumed that the actual concentration is more likely 15.0 or 1.5 as these values would also be more consistent with its use as a stabilizer in solvents at 3-5% of the mixture.

In terms of indoor air exposure, Shah and Singh (1988) examined 52,180 records from analyses carried out in 30 cities in 16 states. The majority of these records (98%) date from 1981 to 1984 and so represent a period when the use of 1,4-dioxane was more extensive and much higher than it is today. A total of 585 indoor samples were analyzed for 1,4-dioxane with a reported daily indoor air concentration of 1.03 ppb (3.71 $\mu g/m^3$). The median and lower quartile (25%) value were both 0, suggesting that most values were below the detection limit. A value of 0.9 ppb was reported for the upper quartile (75%). Since this value was lower than the average, it implies that the average was influenced by a few high values, possibly associated with source dominated areas.

The TEAM study (Wallace, 1987) that identified 1,4-dioxane in human breath and resulted in its being nominated for the VCCEP also analyzed 1,4-dioxane in indoor air of California homes (Los Angeles and Contra Costa County). 1,4-Dioxane was detected in 12 to 55% of the samples collected. This study reported median and 90^{th} percentile 1,4-dioxane levels in overnight air as 0.24 and 1.4 $\mu g/m^3$ (February, 1984 - LA), 0.03 and 3.0 $\mu g/m^3$ (May, 1984 - LA), and 0.03 and 0.36 $\mu g/m^3$ (June, 1984 - CC). The maximum indoor air levels for 1,4-dioxane in this study were 4.0, 4.0 and 1.0 $\mu g/m^3$ in Los Angeles (February, 1984), Los Angeles (May, 1984) and Contra Costa County (June, 1984), respectively. In June of 1990, 125 homes in Woodland, California were monitored for a variety of indoor air pollutants, including 1,4-dioxane. Approximately 21% of the indoor samples contained measurable amounts of 1,4-dioxane. The average concentration of 1,4-dioxane was below the quantifiable limit of 0.11 $\mu g/m^3$ with a range of ND to 140 $\mu g/m^3$ (Sheldon *et al.*, 1992).

The information from these studies is dated, but are the most recent available for this compound. It is used while recognizing that the actual current exposure to 1,4-dioxane is likely lower (perhaps as much as an order of magnitude or more) due to the significant reductions in production and use of 1,4-dioxane that have occurred in the interim. Shah and Singh (1988) suggest that the median value may best represent the data due to the influence of a few high results, therefore, the median value from the February 1984 Los Angeles TEAM study for 1,4-dioxane was adopted for use as a value in estimating children's exposure. Accordingly, 0.24 $\mu g/m^3$ was used as the average indoor air concentration for 1,4-dioxane with a range of 0 to 4.0 $\mu g/m^3$ and a triangular distribution. A probabilistic analysis was used as appropriate to estimate the likely indoor air concentrations of 1,4-dioxane to

which children might be exposed which incorporates standard exposure assumptions relevant for children along with a pulmonary absorption rate of 50% for 1,4-dioxane (Section 4.2.6; Young *et al.*, 1997).

The ratio between indoor and ambient air levels of 1,4-dioxane was examined by Wallace (1987) who found no difference in Los Angeles and indoor air levels were only a factor of 2.9 higher than outdoor air in Contra Costa county. Since the distributions identified for ambient and indoor air levels are virtually identical, a decision was made to employ the most conservative of the two data sets (*i.e.*, ambient air) and use it to address both indoor and outdoor inhalation exposure to 1,4-dioxane. Therefore as a simplifying step, the outdoor air concentrations of 1,4-dioxane (*i.e.*, 0.26 $\mu g/m^3$ was used as the most likely outdoor air concentration for 1,4-dioxane with a range of 0 to 5.0 $\mu g/m^3$ and a triangular distribution) will be used to estimate indoor air exposure probabilistically as well. The fact that 1,4-dioxane was only detected in 12% to 55% of samples in California and 20% to 55% in New Jersey serves as the basis for exposure frequency distributions since this would suggest that exposure to 1,4-dioxane is not continuous. Age-specific behavior and physiologic parameters will be used to estimate inhalation dose of 1,4-dioxane to children along with a pulmonary absorption rate of 50% for 1,4-dioxane.

6.3.2 Ingestion

6.3.2.1 Breast Milk

Mothers working in an industry using 1,4-dioxane or exposed to 1,4-dioxane via air, water, or consumer products may expose nursing infants to the compound via the breast milk. Fisher *et al.* (1997) examined the infant exposure via breast milk for a variety of industrial chemicals including 1,4-dioxane using a PBPK model. For mothers exposed to 25 ppm 1,4-dioxane, a dose of 0.56 mg/day of 1,4-dioxane was predicted for a breast-feeding infant. Since the model results reflect an exposure below the level of metabolic saturation where linear kinetics are observed, a linear relationship between airborne exposure to the mother in the workplace or the environment and 1,4-dioxane content of the milk is assumed for purposes of carrying out an exposure assessment. One hundred percent (100%) of ingested 1,4-dioxane is assumed to be absorbed and the standard exposure assumptions relevant to infants and probabilistic modeling are used as appropriate to estimate the dose. Since the dose received under these conditions is probably the highest likely to occur, it can also address the dose received from non-occupationally exposed mothers.

6.3.2.2 Water

The number of individuals potentially exposed to 1,4-dioxane in drinking water cannot be estimated since no statistical survey for its occurrence in public or private water systems has

ever been performed. A review of the USEPA National Contaminants Occurrence Database for both public water supply and ambient water contaminants as well as the occurrence data for preliminary contaminant candidate list (CCL) found no reported instances of 1,4-dioxane contamination of drinking water. This may be more reflective of the fact that it is not a Safe Drinking Water Act regulated contaminant or found on the CCL. Hartung (1989) reported that 2.5% of hazardous waste sites reported the presence of 1,4-dioxane in 1988. A review of current hazardous waste site data sets (including the CERCLIS, National Priority List (NPL) Fact Sheets, Record of Decision System, and 1999 Superfund NPL Assessment Program Database) found 1,4-dioxane listed as a contaminant at just six sites. Plumb (1991) reported only four detections of 1,4-dioxane in groundwater from three waste sites after a review of monitoring data from 379 site investigations; however, 1,4-dioxane at concentrations ranging from 0.1 to 2.5 ppb was detected in 37% of the samples of groundwater collected near a solid waste landfill located 60 miles southwest of Wilmington, DE (ATSDR, 2006). Wells located near low level radioactive waste disposal sites in Kentucky and New York have also been reported to contain 1,4-dioxane from leachate, but again no quantitative data were presented and it is unclear if these were drinking water or monitoring wells (Francis *et al.*,1980). 1,4-Dioxane was detected (below 1 ppm) between 1983 and 1986 in ground water near a landfill in Ontario, Canada (NICNAS, 1998). In 1982, the highest level of 1,4-dioxane in the groundwater beneath a landfill in Gloucester, Canada was 500 ppb (NICNAS, 1998). Lesage *et al.* (1990) found 1,4-dioxane in 13% of groundwater samples from an Ontario waste disposal site ranging from approximately 300 ppb to 2,000 ppb. A study in Japan found widespread occurrence of 1,4-dioxane in ground and surface water with the a high correlation between 1,4-dioxane and 1,1,1-trichloroethane in ground water (Abe, 1999). A number of states and national and international agencies have promulgated standards or guidance for 1,4-dioxane in drinking water.

1,4-Dioxane has been detected in surface impoundments associated with highway rest stops (for collecting radiator boil-overs) and airports (from ethylene glycol used in antifreeze and de-icing agents) at over 2,000 ppb (range = ND to 2,300 ppb) in some cases (Hartung, 1989). The 2000 TRI reported that 163,776 pounds of 1,4-dioxane was released to surface water in the United States (USEPA, 2002), and 1,4-dioxane has been detected in the Chicago Sanitary and Ship Channel and in effluents from the North Side and Calumet sewage treatment plants on the Lake Michigan basin at 1 ppb (ATSDR, 2006). River water receiving textile waste discharge in North Carolina was found to contain 1,4-dioxane, but levels were not quantified (Dietrich *et al.*, 1988). In surface water (*i.e.*, creeks, bogs, and lakes) near the Ann Arbor, Michigan plant where 1,4-dioxane was improperly disposed, 1,4-dioxane levels of 10 to 290 ppb were reported, possibly as a result of ground water discharge. Walsom and Tunnicliffe (2002) reported that 1,4-dioxane was detected in groundwater at former industrial site in Ontario, Canada at concentrations up to 10,000 µg/L, with an average of approximately 1,000 µg/L across the plume. In Japan, 1,4-dioxane has been reported in the landfill leachate from 11 sites with a median concentration of 32 ppb (range = ND to 198 ppb) in one study

(Zenker *et al.*, 2003) and from eight other sites with a median concentration of 3.9 ppb (range = 1.1 to 10.9 ppb) (Yasuhara *et al.*, 1997). An analysis of 19 sites from 11 rivers in the Niigata Prefecture in Japan that are affected by domestic effluent measured 1,4-dioxane at concentrations ranging from <0.03 to 0.39 µg/L (Kawata *et al.*, 2003). In Japan, the mean concentration of 1,4-dioxane in water from two major rivers was 0.11 µg/L in the Agano River and 0.05 µg/L in the Shinano River, while the mean concentration in outflow from four sewage treatment plants along the rivers ranged from 0.19 to 0.39 µg/L (Tanabe *et al.*, 2006).

1,4-Dioxane was also measured in leachate before treatment and effluent after treatment from two landfills in Japan. Concentrations of 1,4-dioxane in the leachate ranged from 0.08 to 0.62 µg/L in the smaller landfill (20,000 m^2) and was 2.05 to 13.8 µg/L in the larger landfill (36,000 m^2). In the treated effluent, 1,4-dioxane concentration ranged from 0.16 to 0.5 µg/L and 0.91 to 10.6 µg/L in the smaller and larger landfills, respectively. After conducting leaching tests, it was determined that the greatest concentration of 1,4-dioxane leached from fly ash, bottom ash, and waste plastics (Yasuhara *et al.*, 2003). 1,4-Dioxane has recently been found in groundwater in several locations throughout California from Silicon Valley to the San Gabriel Basin in Southern California as well as in wastewater from household and industrial wastes sources. Following the discovery of 1,4-dioxane in groundwater, the Tucson (AZ) water utility began monitoring for 1,4-dioxane in the groundwater delivered to the Tucson Airport Area Remediation Project (TARP) TCE Treatment Plant and has also detected minute amounts of the compound. The level of 1,4-dioxane in the water at the treatment plant is approximately 1.5 ppb, only slightly above the detection limit of 1.0 ppb. In addition to testing the wells serving the TARP facility, Tucson Water conducted additional tests within the plume of TCE contamination and found levels of 1,4-dioxane ranging from non-detectable to 12.0 ppb. Because most of the water reaching the TARP Treatment Plant comes from wells with no detectable 1,4-dioxane, the average level of the compound at the plant is approximately 1.5 ppb. The Arizona Department of Environmental Quality has also reported finding a single sample from an area distant from the TARP wells that contained 57 ppb. 1,4-Dioxane has also been reported in groundwater from the Netherlands at concentrations ranging from <0.1 to 14 µg/L (TNO and RIVM, 2002).

1,4-Dioxane has also been reported in US drinking water from monitoring data in older (1970s) data. Kraybill (1978) reported that 1,4-dioxane occurred at 1 µg/L in United States drinking water, but provides no indication of the frequency or distribution of the contamination. 1,4-Dioxane was detected in 2/6 samples of groundwater collected from drinking water wells near a solid waste landfill located 60 miles southwest of Wilmington, DE (ATSDR, 2006). They reported levels of 0.1 and 0.5 µg/L in two supply wells, but 1,4-dioxane was not detected in the finished water. More recently, preliminary testing of 19 public water supply wells in Orange County, California showed levels of 1,4-dioxane ranging from non-detectable to 20 ppb (OCWD, 2002). Testing of Santa Monica wells in May 2002 confirmed the presence of 1,4-dioxane at 6 and 22 ppb in two California public supply wells.

A Massachusetts drinking water well contained 1,4-dioxane at 2,100 ppb (ATSDR, 2006). The ground water impacted by 1,4-dioxane in Ann Arbor, Michigan resulted in the contamination of private wells ranging from ND to 200 ppm. Five Ohio wells near an infiltration lagoon receiving industrial waste had 1,4-dioxane concentrations ranging from ND (<1ppb) to 360 ppb and one private well contained 120 ppb of 1,4-dioxane (Hartung, 1989). In the Netherlands drinking water containing 0.5 µg/L 1,4-dioxane has been detected (TNO and RIVM, 2002). The situation is complicated by the fact that the aeration techniques typically used to remove the co-contaminants (*i.e.*, solvents) are not effective at removing the 1,4-dioxane. Bench testing has shown that aeration (*i.e.*, air-stripping) alone produced no decrease from the initial 1,4-dioxane concentration, but that 1,4-dioxane concentrations of 1,000 µg/L can be reduced to less than 5 µg/L using an advanced oxidation process with ultra-violet lights.

These findings suggest that 1,4-dioxane may occur in drinking water at low levels, particularly in those affected by chlorinated solvent or glycol contamination, but it has not been regularly detected. Note, however, that it is not always a required analyte. Additionally, traditional or specialized water treatment is ineffective at removal and, given 1,4-dioxane's resistance to degradation, the exposure to consumers seems possible in these cases. A simple rule of thumb may be to assume water exposure to 1,4-dioxane is possible in areas in which drinking water supplies are impacted by chlorinated solvents or glycols (perhaps in the same relative proportion that the 1,4-dioxane existed to the solvents in which it occurred). For purposes of this assessment, it is assumed that 1,4-dioxane exists in drinking water at an average level of 2 ppb with a range of 0.5 to 2,000 ppb and the distribution is triangular. Probabilistic techniques are employed as appropriate along with standard assumptions regarding water ingestion to estimate the 1,4-dioxane dose to children via drinking water.

6.3.2.3 Food

1,4-Dioxane has been reported as a natural constituent of food including shrimp and Krill, coffee, tomatoes, peppers and certain condiments (Chung *et al.*, 1983; Choi *et al.*, 1983; Tang *et al.*, 1983; Sanceda *et al.*, 1984; Chang *et al.*, 1978; Hartung, 1989). Extracts from fresh shrimp contained 2.2% 1,4-dioxane while fermented shrimp contained 0.5% 1,4-dioxane (Choi *et al.*, 1983). The total odor concentrate of Atlantic krill contained 0.4 to 0.8% 1,4-dioxane. It has also been reported as occurring at "high" levels in frying oils as well as in fried foods (*i.e.*, chicken) as the resultant of chemical reactions associated with the process of deep-fat frying. Generally speaking, no concentrations of 1,4-dioxane occurring naturally have been reported in food making an assessment of exposure to this compound difficult, but levels are generally expected to be low. 1,4-Dioxane was found in the atmosphere of walk-in refrigerators in Russia at 17.5% of the allowable concentration by Dmitriev *et al.* (1985), perhaps associated with its occurrence in ethylene glycol. Stored

foods may absorb airborne 1,4-dioxane in such cases, but the relevance of this finding to exposure in the US is unclear. 1,4-Dioxane also appears as an unwanted impurity in certain ethoxylated food additives used as emulsifiers, solubilizers, and stabilizers such as polysorbates. Given the broad range of products, it is assumed virtually everyone has some exposure to 1,4-dioxane in foods. Many of these same products are also used in cosmetics and manufacturers employ steam and vacuum stripping to reduce 1,4-dioxane to the lowest levels feasible in accordance with FDA requirement (<10 ppm). Some of these uses are listed in **Table 6-2**.

Table 6-2. Food Uses of Products Containing 1,4-Dioxane as an Impurity

Product	Use	Allowable amount of product in food (21 CFR)
Choline Bitartrate/Choline Chloride	Dietary supplement in baby formula	generally recognized as safe when used in accordance with good manuf. practices. 21 CFR 182.5250/182.5252
Ethoxylated mono/di-glycerides	Dough Conditioner/emulsifier	not to exceed 0.2% to 0.5% by weight for food uses. 21 CFR 172.834
hydro-∞-hydroxy poly (oxyethylene) poly(oxypropylene) poly(oxyethylene) block copolymer (Poloxamer 331)	Stabilizer/solubilizer in dry gelatin and beverage base and fruit juice drinks	not to exceed the weight of the oils used in flavoring; or when used in combination with dioctyl sodium sulfosuccinate not to exceed 10 ppm for both substances combined in finished dessert or drink; or in baked goods not to exceed 0.5% of flour used. 21CFR 172.808
Hydro-∞-hydroxy poly (oxyethylene) poly(oxypropylene) poly(oxyethylene) block copolymer (Poloxamer 407)	Stabilizer/solubilizer in flavor concentrates containing flavoring oils	to be used in an amount not greater than required to produce the desired effect. 21 CFR 172.210
Polyethylene glycols	coating and adjuvant in non-nutritive sweeteners, citrus, vitamin and mineral supplements	to be used in an amount not greater than required to produce the desired effect. 21 CFR 172.820
Polysorbate 20	Flavoring substances and adjuvants	Not Specified
Polysorbate 60	Emulsifier in desserts, shortenings, non-dairy creamers, edible oils, dough conditioner, surfactant in gelatins, dry drink mixes, desserts, etc.	not to exceed 0.32% to 0.4% of finished toppings; or not to excedd 0.5% of confections; not to exceed 0.05% to 0.5% in nondairy creamers, dough, sugar-based gelatin and pudding mixes, syrups, etc.; or not to exceed 3.6% to 4.5% in artificially favored gelatins and drink mixes 12 CFR 172.836
Polysorbate 65	Emulsifier in ice crean, frozen custard, ice milk and sherbert, desserts, shortenings, non-dairy creamers, edible oils, etc.	Not to exceed 0.1% of finished frozen desserts; or not to exceed 0.32% to 0.4% of finished cakes, toppings, or edible oils. 21CFR 172.838

Polysorbate 80	Emulsifier in ice crean, frozen custard, ice milk and sherbert, desserts and dessert toppings or fillings, shortenings, non-dairy creamers; solulibizer or dispersing agent in edible oils, pickled products, gelatins, barbecue sauces, cottage cheese, etc.	Not to exceed 0.1% in finished frozen desserts or 1% of shortening or oil; 4 ppm in yeast; 500 ppm in pickles; 10 ppm in salt; 30 ppm in canned green beans; 175 to 475 mg/day in vitamin and mineral supplements, or special dietary foods; or 0.005% to 0.008% in cottage cheese or barbecue sauce and 0.4% in whipped oil-based toppings 21 CFR 172.840

It is interesting to note that the majority of the uses of these additives are in desserts, snacks, fruit juices and other food stuffs that are heavily consumed by children. It is unknown if any other food additives contain 1,4-dioxane as an impurity. FDA requires food additives that may contain 1,4-dioxane be able to pass a limit test of 10 ppm.

In the case of choline bitartrate used in baby formulas, the probable daily intake for an infant 0-6 months old is 379.3 mg (Holodnick, 1989). If 1,4-dioxane is present in this additive at the FDA limit, this suggests an intake of 3.8 µg-d of 1,4-dioxane from formula for bottle fed infants. For purposes of exposure assessment, it is assumed that 1,4-dioxane occurs in these additives at an average level of 5 ppm with a range of 0 to 10 ppm and a triangular distribution. It is further assumed that the additives comprise an average of 0.1% of the food stuffs in which they are used with a range of 0.005% to 5% and a triangular distribution. **Table 6-3** lists the average daily consumption of foodstuffs likely to contain these additives. For purposes of conservatism and simplicity, all the foods consumed (in grams) in the categories listed are assumed to contain the additives in the range listed above. Meat, poultry, and fish is the only category excluded from consideration, and the daily intake from males and females is averaged (USEPA, 2006).

Table 6-3. Mean Food Intake (g/kg-day) by Category and Age (0-21 years)

Age	Milk & Milk Products	Grain Products	Vegetables	Fruits	Total
< 1* years	125.1	5	8.4	16	154.5
1-2* years	38	9	9.6	20	76.6
2-3 years	36	13	9.4	18	76.4
3-6 years	21	10	7.3	11	49.3
6-11 years	15	7.5	5.5	5.7	33.7
11-16 years	7.7	5	4.2	3.4	20.3
16-21 years	5.6	5.6	3.6	5.6	20.4

* excludes breast fed infants

It is recognized that assuming all foods in a given category contain the additives in question markedly overstates their occurrence and that of 1,4-dioxane in the diet; however, such an assumption is considered acceptable in the absence of monitoring data and qualitative and quantitative knowledge of the natural occurrence of 1,4-dioxane in foods. These assumptions together with relevant data on children's diet and body weights and an assumption of 100% absorption of ingested 1,4-dioxane are combined in a probabilistic model to estimate 1,4-dioxane dose from the diet.

6.2.3 Dermal

Dermal exposure and absorption is possible from two main sources: 1) bathing or showering in contaminated water; or 2) use of consumer products containing a 1,4-dioxane impurity.

6.2.3.1 Water

Contact with contaminated water may expose children to 1,4-dioxane via the skin or lung. Inhalation exposure is considered subsumed under the dose calculated for exposure to 1,4-dioxane in indoor/outdoor air and is not considered further. Dermal dose from showering or bathing is estimated using the same hypothetical water concentrations considered under the water ingestion scenario. Skin area, body weights, and exposure frequency and duration are chosen consistent with the age range of children in a probabilistic model (USEPA, 2006). The dermal permeability rate established for unoccluded and occluded skin is use to calculate dose (Section 4.2.6.1).

6.2.3.2 Consumer Products

1,4-Dioxane can occur as impurity in other materials, as it is formed as a reaction by-product in the manufacture of ethoxylated substances (particularly surfactants and emulsifiers). The compound is created during the acid or base catalyzed addition of ethylene oxide as part of the ethoxylation steps involved in the manufacture of anionic, cationic, amphoteric, and nonionic surfactants. This covers a large array of substances used in many applications such as food, cosmetic, agricultural and veterinary, therapeutic, household and various industrial products (Hartung, 1989) . For instance, 41 samples of used ethylene glycol anti-freezes contained 3.4$\pm$0.82 mg/L (range = 0.01 to 22 mg/L) of 1,4-dioxane while a sample of 13 de-icing fluids had a mean level of 3.9$\pm$2.04 mg/L (range = <0.01 to 26 mg/L) (Hartung, 1989). A survey undertaken by NICNAS indicated indeed widespread public exposure to 1,4-dioxane from a variety of consumer products including cosmetics/toiletries, household detergents, pharmaceuticals, foods, agricultural and veterinary products, and ethylene glycol based antifreeze coolants (NICNAS, 1998). From the limited quantitative data available on 1,4-dioxane levels in pharmaceuticals (100 to 380 ppm), agricultural and veterinary products (<<10 ppm), and ethylene glycol based antifreeze coolants (0.1 to 22 ppm), and taking into account the use pattern and volatility, it was concluded by NICNAS that consumer exposure from these sources would be negligible (NICNAS, 1998). A recent survey of consumer products in Japan found that 1,4-dioxane concentration ranged from nondetect to 9.5 mg/kg in shampoo, nondetect in liquid soap, nondetect to 51 mg/L in dishwashing liquid, nondetect in laundry detergent, nondetect to 6.4 mg/L in bath detergent, and nondetect to 38 mg/L in car detergent. Exposure of 1,4-dioxane from shampoo use was estimated to be an average of 5.7×10^{-3} µg/kg-d via inhalation and 3.4×10^{-3} µg/kg-d via dermal, while exposure from dishwashing liquid was estimated to be 1.0×10^{-2} µg/kg-d via inhalation and 5.8×10^{-3} µg/kg-d via dermal exposure. It was concluded that there is no significant health risk to consumers from either inhalation or dermal exposure of 1,4-dioxane from use of these consumer products (Makino *et al.*, 2006).

One manufacturer reported ethoxylated surfactants contain levels of 1,4-dioxane at less than 100 ppm w/w for fatty amine polyglycol ethers and less than 10 ppm w/w for alcohol polyglycol ethers. The literature reports on a number of materials containing 1,4-dioxane (as impurity) being used in the construction industry. For instance, 1,4-dioxane is present as a residue in a phosphate alcohol flame retardant at a typical concentration of around 2000 ppm w/w. This product is an ingredient (5%) in a fire resistant caulking agent (*i.e.*, around 100 ppm 1,4-dioxane w/w present in the caulk). Exposure to 1,4-dioxane may occur during application and curing or when grouted surfaces undergo sanding (NICNAS, 1998). 1,4-Dioxane was also detected in 39/270 household aerosol products in Japan (*i.e.*,. water proofing agent, ski wax remover, car bumper cleaner) with concentrations ranging from 0.17 to 2.25% (Mori *et al.*, 1992). Most (60%) of water proofing aerosol products tested

contained 1.4% to 2.2% of 1,4-dioxane (Mori *et al.*, 1993) while 10% of degreasers and 4% of reagents contained an average of 2.7% and 51% 1,4-dioxane (Inoue *et al.*, 1983).

1,4-Dioxane has been found in ethoxylated products used in cosmetics ranging from ND to over 1000 ppm (Hartung, 1989). A more recent survey reports that the concentration of 1,4-dioxane in cosmetic ethoxylated raw materials ranged from 0.6-636 ppm in 1979, 6.3-1410 ppm in 1980, 5-243 ppm in 1993, 20-653 ppm in 1996, and 45-1102 ppm in 1997. In recent years, the concentration of 1,4-dioxane in finished cosmetic products has decreased from 5-141 ppm in 1992 to 6-34 ppm in 1997 (Black *et al.*, 2001). Much of the 1,4-dioxane content can be removed through a 'steam and vacuum stripping' process. Figures suggest that over 80% of the chemical present can be removed this way; however, not all ethoxylated substances are subject to stripping, and the overall effect on 1,4-dioxane presence and content in consumer products currently is unknown. A list of some of these compounds and representative concentrations are presented in **Table 6-4** from FDA analyses (FDA, 1980; 1981).

Table 6-4. Categories of Surfactants Containing 1,4-Dioxane as an Impurity

Compound	1,4-Dioxane Content (ppm)
Laureth Sulfates (ammonium, sodium)	6 - 1282
Myreth Sulfates (ammonium, sodium)	60 - 62
Pareth-25 Sulfates (ammonium, sodium)	74 - 238
Octoxynols	1 - 115
Amphoterics	ND - 115
Polyethylene glycols	ND - 636
Varonics	20 - 353
Polysorbates	ND - 194
Laneths	ND - 20
Oleths	ND - 18
Nonoxynols	1 - 580
Ceteareths	0.8

Analysis of 1,4-dioxane in finished cosmetic products has also been conducted, and 1,4-dioxane has been reported in shampoos, bath gels and foams, lotions, hand and skin

cleansers, and detergents (Beernaert *et al.*, 1987; Hartung , 1989; Italia and Nunes, 1991). 1,4-Dioxane has been reported in regular shampoo ranging from <50 to 487 ppm (Rumenapp and Hild, 1987; TNO and RIVM, 2002), anti-dandruff shampoo from ND to 390 ppm (TNO and RIVM, 2002), hair lotions from 47 to 108 ppm (Scalia and Menegatti, 1991; Scalia, 1992); bath foams from 22 to 41 ppm (Scalia and Menegatti, 1991; Scalia, 1992), douche preparations from 3 to 470 ppm (TNO and RIVM, 2002; NICNAS, 1998), sun screen at 600 ppm (Birkel *et al.*, 1979), and baby lotion at 11 ppm (Scalia, 1992). Some quantitative data are also available for 1,4-dioxane in household detergents. Rumenapp and Hild (1987) detected 1,4-dioxane in hand and dish washing products ranging from <50 to 100 ppm. NICNAS (1998) detected 1,4-dioxane in three hand and dishwashing liquids with concentrations ranging from 29 to 518 ppm. In Germany 1,4-dioxane was detected in hand and dishwashing liquids up to 216 mg/kg (TNO and RIVM, 2002).

This data also largely dates from the 1980s and includes European data as well (reportedly levels of 1,4-dioxane were higher in products of European origin than US). Therefore, the entirety of the database may overstate the occurrence and levels of 1,4-dioxane in current (US) products. In 1980, it was estimated that 1/3 of emulsion-based cosmetics or consumer products contain 1,4-dioxane as an impurity at the time most of this data were generated (Zenker *et al.*, 2003). Since these data were collected, progress has been made in reducing the levels of dioxane in shower-gels, bubble bath products, hair-care agents and similar products that contain ethoxylated surfactants (NICNAS, 1998; TNO and RIVM, 2002). The levels of 1,4-dioxane in ppm from various categories of consumer products from these earlier analyses is contained in **Table 6-5**.

Table 6-5. 1,4-Dioxane Content in Finished Consumer Products (ppm)

Product type	N	Mean	Range
Shampoo	224	88	ND - 670
Bath Gel/Foam	47	66	ND - 264
Lotions	12	14	ND - 75
Cleansers	8	41	ND - 160
Detergents	7	26	ND - 50

Analysis of finished cosmetics by the FDA in 1980 and 1981 reported that 21.3 % (n= 26) exceeded 100 ppm 1,4-dioxane while 37.7% (n= 46) contained 10 to 100 ppm, 20.5% (n= 25) contained and 20.5% (n= 25) were non-detects (FDA, 1980; 1981). In the United States, the Cosmetic Ingredients Review board of the Cosmetic, Toiletry and Fragrance Association proposed a tentative final level of 0.1% 1,4-dioxane in cosmetics while the FDA adopted a

formal policy of no more than 10 ppm of 1,4-dioxane in finished cosmetics in the mid-1980s (50 FR 11575, 1985). In Germany, the Commission for Cosmetic products also set a residual 1,4-dioxane content of 10 mg/kg as a value capable of being attained and a target to be aimed for (NICNAS, 1998; TNO and RIVM, 2002); however, it is unclear to what extent this target has been reached or is monitored. An analysis of finished cosmetics and detergents by Rastogi (1990) reported only 5% of cosmetics and 8% exceeded 50 ppm of 1,4-dioxane while 36% of cosmetics and 15% of detergents had between 20 and 50 ppm, 15% of both cosmetics and detergents had between 11 and 20 ppm, 19% of cosmetics and 46% of detergents had between 0.3 and 10 ppm, and 16% of cosmetics and 15% of detergents were non-detects.

For purposes of exposure assessment, an average 1,4-dioxane level in finished cosmetic products (*i.e*, shampoos, bath preparations and lotions) of 10 ppm in accordance with FDA regulations with a range of 0 to 500 ppm and a log-normal distribution. Most cosmetic (or consumer) products containing 1,4-dioxane are used in the bath and would be applied in relatively small amounts and quickly removed. Some like baby or skin lotions may be applied multiple times during the day and left on for extended periods; however, 1,4-dioxane has been shown to rapidly (85% in 15 minutes) evaporate from the skin or non-absorbent surfaces in lotions or other vehicles (Section 4.2.6.1). For purposes of estimating exposure, a daily average amount of 20 ml of the product is applied to the scalp (or skin) with a range of 0 to 50 ml in a triangular distribution. The area of the skin and length of exposure varies by age and activity. Some of the skin area of an infant may be covered repeatedly with lotion between cleanings during the course of the day while for older children, the extent of exposure may be a daily 15 minute shower using shampoo and soap. For infants, a 4 hour exposure per day is assumed for the first year of life taking into account the rapid evaporative loss. For older children, the average length of exposure is 15 minutes per day with a range of 0 to 30 minutes in a triangular distribution. The rate of skin absorption identified in experiments (Section 4.2.6.1) is used along with an assumption that 100% of the skin surface is contacted by the one or more cosmetic preparations during the day. The associated exposure parameters for children are used in a probabilistic model to estimate dermal exposure from this route (USEPA, 2006).

6.3 Environmental Estimates From Fugacity Modeling

As a different approach to defining the potential environmental exposure to 1,4-dioxane, fugacity modeling was also used to estimate what levels of 1,4-dioxane might occur in the various environmental media. Modeling the movement and concentrations of 1,4-dioxane in the environment was accomplished through the use of a fugacity model pioneered by Dr. Donald MacKay and co-workers at the University of Toronto (MacKay *et al.*, 1992). The reader is referred to this paper to gain a more thorough description of fugacity-based modeling than is addressed in this report. In general, there are three levels of modeling (level I, II, and III), each requiring increasing complex and detailed input. All three levels

of the model contain the same environmental compartments: aerosol, air, water, soil, sediment, suspended sediments, and biota (*e.g.*, fish). However, the model levels differ in several ways with respect to (1) treatment of degradative processes that serve to decrease the chemical concentrations within each environmental compartment (incorporated in levels II and III, but not level I): (2) migration from one compartment to another by processes that are not dictated by fugacity (incorporated only in level III); and (3) defining the environmental compartment(s) to which the chemicals are directly released (incorporated only in level III). Based upon a preliminary review of the information available for 1,4-dioxane, there is sufficient information to conduct a level II evaluation. Although some specific information regarding the environmental behavior of 1,4-dioxane is lacking, those values can be estimated based on a consideration of similar chemicals.

In addition to the measured parameters available, a number of model parameters were estimated based on need, to confirm measured data, or resolve discrepancies. The EPIWIN (Estimation Programs Interface for Windows) software package was used for this purpose. EPIWIN is a suite of software programs that are used to estimate the chemical/physical properties of chemicals, and can predict atmospheric degradation rates, bioconcentration factors, Henry's Law constant, aquatic toxicity, hydrolysis rates, and a variety of other chemical/physical properties. Screening level parameter values for chemical/physical properties and chemical half-lives were identified based on a review of a wide variety of information sources. Default model parameters for the level II fugacity model were also identified (Mackay *et al.*, 1992). The output generated from the environmental fate and transport component of the model can be used as input for the risk assessment.

Environmental concentrations were estimated on a mesoscale basis (*i.e.*, the entire continental US) using the total yearly production of the one remaining US producer of 1,4-dioxane averaged over the last five years (since the use of 1,4-dioxane as a chlorinated solvent stabilizer ceased) and assuming that the entire amount is released to the environment. This is a highly conservative assumption since review of the TRI database suggests that most 1,4-dioxane used is recovered on site and recycled or used to produce energy. However, this assumption was employed both to be conservative as well as to address the imported 1,4-dioxane that may be released and is not accounted for in the domestic production.

The results of the Level II model for 1,4-dioxane are summarized in **Table 6-6**.

Table 6-6. Results of Fugacity Modeling of 1,4-Dioxane Using Yearly US Production

Media	1,4-Dioxane Concentration	Percent of total
Air	0.065 ng/m^3 (0.018 ppt)	8.06%
Surface Water	0.37 ng/L (0.37 ppt)	91.9%
Soil	1.6E-6 ng/g (0.0016 ppt)	0.044%
Sediment	3.3E-6 ng/g (0.0033 ppt)	0.001%
Suspended Sediments	1.6E-5 ng/g (0.016 ppt)	0
Biota	9.9E-6 ng/g (0.0099 ppt)	0
Aerosol	1.6E-9 ng/m^3(4.4E-7 ppq)	0
Total	-	100%

The results of the fugacity model indicate that potential exposure to 1,4-dioxane at steady state are very low. Exposure is obviously likely to be higher near source dominated areas, but is still likely to be in the low parts per billion range.

Edwards *et al.* (1999) used 1,4-dioxane as one of 45 compounds modeled in a Level III Fugacity Model. The environmental data was drawn from the 1995 TRI and the results were used in a proposed ranking scheme incorporating environmental concentrations with a measure of the compound's toxicity. Edwards *et al.*, (1999) reported the level of 1,4-dioxane predicted in water and air as 0.65 ng/L and 0.021 ng/m^3, respectively. These results compare favorably to those estimated in the Level II fugacity model described above as well as the Level I fugacity model described in Section 3.0 (NICNAS, 1998).

6.4 Exposure Scenarios

Monte Carlo simulations of the exposures and resultant doses were run using the Decisioneering Inc.'s Crystal Ball 4.0 Software Package. Each simulation had 5000 iterations. The input parameters for each exposure scenario and results are found in Appendix A.

6.4.1 Occupational Exposure

The adult occupational exposure to 1,4-dioxane was evaluated to estimate the dose potentially received during pregnancy to determine if a potential adverse reproductive outcome is anticipated and so addresses a woman exposed to 1,4-dioxane during gestation

via the lungs and skin at work as well as through water, diet, and consumer products. Standard physiologic and exposure parameters along with estimates of inhalation and dermal exposure were combined a in probabilistic model to identify the mean and the upper bound (95^{th} percentile) estimates of the average daily1,4-dioxane dose. The mean total dose for 1,4-dioxane is **0.94 mg/kg-d** while the 95^{th} percentile estimate is **2.4 mg/kg-d**. The results of this modeling are summarized in **Figure 6-1**. The model output and sensitivity analysis for this route of exposure are included as Appendix A.

6.4.2 Children's Exposure

Children's exposure was estimated for a variety of pathways. For infants (age 0 to 1 years), exposure to 1,4-dioxane was estimated for breast feeding infants for an occupationally-exposed mother. This dose was assumed to be greater than breast milk from an environmentally-exposed mother or bottle-fed baby. 1,4-Dioxane exposure from inhalation of indoor air, ingestion of food, and dermal exposure to water and lotions or cleaning materials was also estimated for infants as both an average daily dose and a lifetime average daily dose (**Tables 6-7 and 6-8**).

For older children (ages 2-3, 3-6, and 6-11 years) and youths (ages 11-16 and 16-21 years), 1,4-dioxane exposure was estimated for ingestion of dietary components containing additives that may have 1,4-dioxane as an impurity and for water, inhalation of 1,4-dioxane for indoor and ambient air was estimated, and dermal exposure was estimated from household water contact as well as use of consumer products containing 1,4-dioxane. Similar to the infant estimates, age-specific physiologic and exposure parameters along with estimates of inhalation ingestion, and dermal exposure were combined a in probabilistic model to identify best and upper bound estimates of 1,4-dioxane dose for each age group as an average daily dose (ADD) and a lifetime average daily dose (LADD). The results of this modeling are summarized in **Table 6-7** and **6-8** and **Figures 6-2** and **6-3**. Model output and sensitivity analysis are included as Appendix A.

Table 6-7. Mean (and 95th Percentile) Average Daily Dose Estimate For 1,4-Dioxane Exposure in Fetuses, Infants, Children, and Youths

Exposed Age Group	Ingestion Dose (mg/kg-d)	Inhalation Dose (mg/kg-d)	Dermal Dose (mg/kg-d)
Pregnant Worker (Fetus)	1.7E-2 (4.0E-2)	4.1E-01(1.1E+0)	6.9E-1(2.0E+0)
Infants (0-1 years)	1.1E-2 (2.5E-2)	1.0E-3 (2.7E-3)	3.3E-2 (1.1E-1)
Children (1-2 years)	2.6E-2 (6.8E-2)	1.0E-3 (2.8E-3)	2.7E-3 (8.7E-3)
Children (2-3 years)	2.4E-2 (6.1E-2)	8.4E-4 (2.2E-3)	2.7E-3 (8.7E-3)
Children (3-6 years)	2.1E-2 (5.5E-2)	6.0E-4 (1.6E-3)	2.7E-3 (8.7E-3)
Children (6-11 years)	1.4E-2 (3.7E-2)	3.6E-4 (9.9E-4)	2.7E-3 (8.7E-3)
Youths (11-16 years)	1.1E-2 (2.8E-2)	2.3E-4 (6.4E-4)	2E-3 (6.4E-3)
Youths (16-21 years)	1.2E-2 (3.1E-2)	2E-4 (5.4E-4)	2.7E-3 (8.7E-3)

Table 6-8. Mean (and 95th Percentile) Lifetime Average Daily Dose Estimate For 1,4-Dioxane Exposure in Infants, Children, and Youths[1]

Exposed Age Group	Ingestion Dose (mg/kg-d)	Inhalation Dose (mg/kg-d)	Dermal Dose (mg/kg-d)
Infants (0-1)	1.5E-4 (3.6E-4)	1.4E-5 (3.9E-5)	4.7E-4 (1.6E-3)
Children (1-2 years)	3.7E-4 (9.7E-4)	1.5E-5 (4E-5)	3.8E-5 (1.2E-4)
Children (2-3 years)	3.4E-4 (8.8E-4)	1.2E-5 (3.2E-5)	3.8E-5 (1.2E-4)
Children (3-6 years)	9E-4 (2.4E-3)	2.6E-5 (6.8E-5)	1.1E-4 (3.7E-4)
Children (6-11 years)	9.9E-4 (2.6E-3)	2.6E-5 (7.1E-5)	1.9E-4 (6.2E-4)
Youths (11-16 years)	7.7E-4 (2E-3)	1.7E-5 (4.5E-5)	1.4E-4 (4.6E-6)
Youths (16-21 years)	8.2E-4 (2.2E-3)	1.4E-5 (3.8E-5)	1.9E-4 (6.2E-4)

[1] Note: LADDs were not appropriate for fetal exposures and therefore not calculated.

Figure 6-1. Pregnant Worker (Fetus) Calculated ADD Values

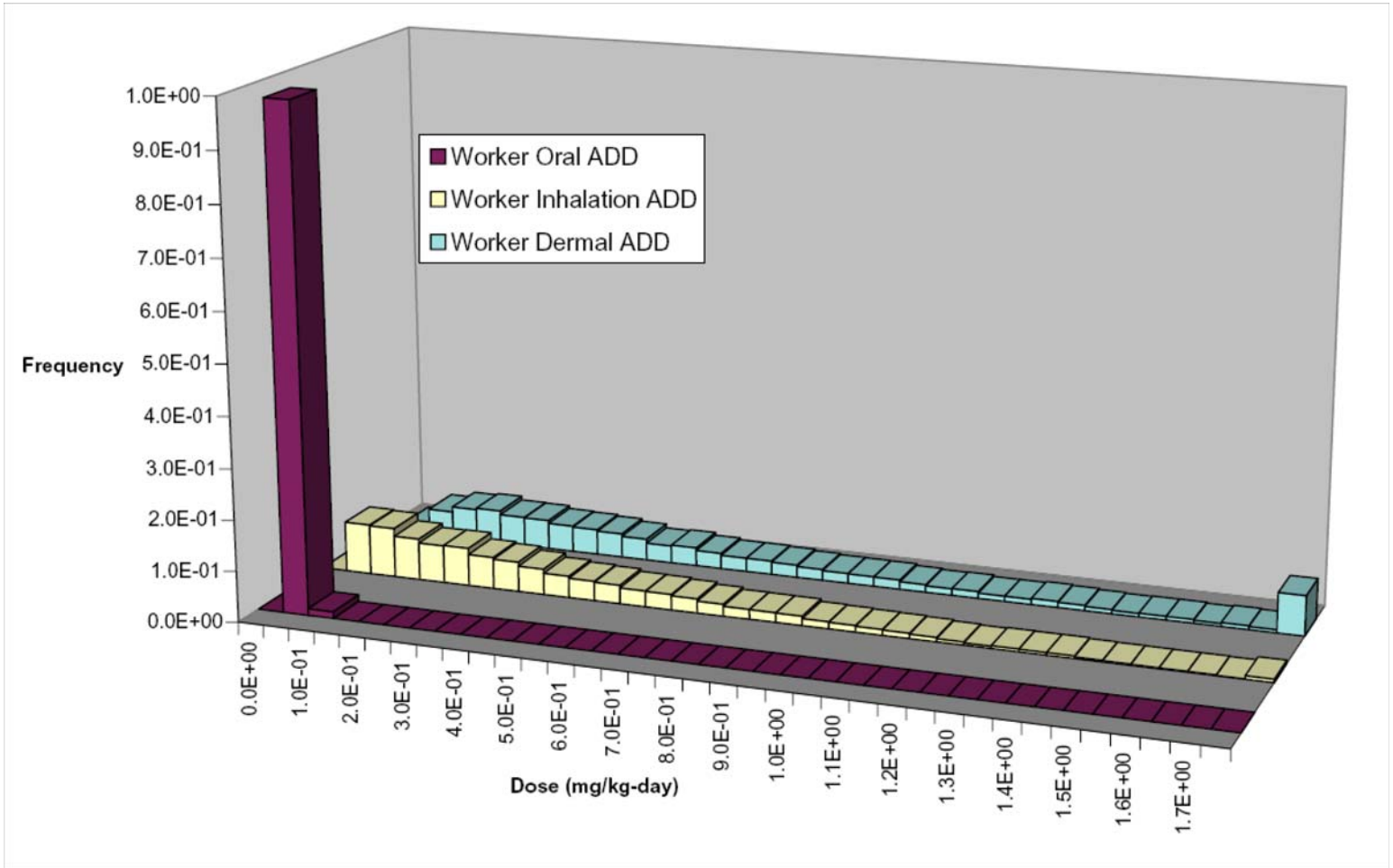


Figure 6-2. Child/Youth Calculated ADD Values

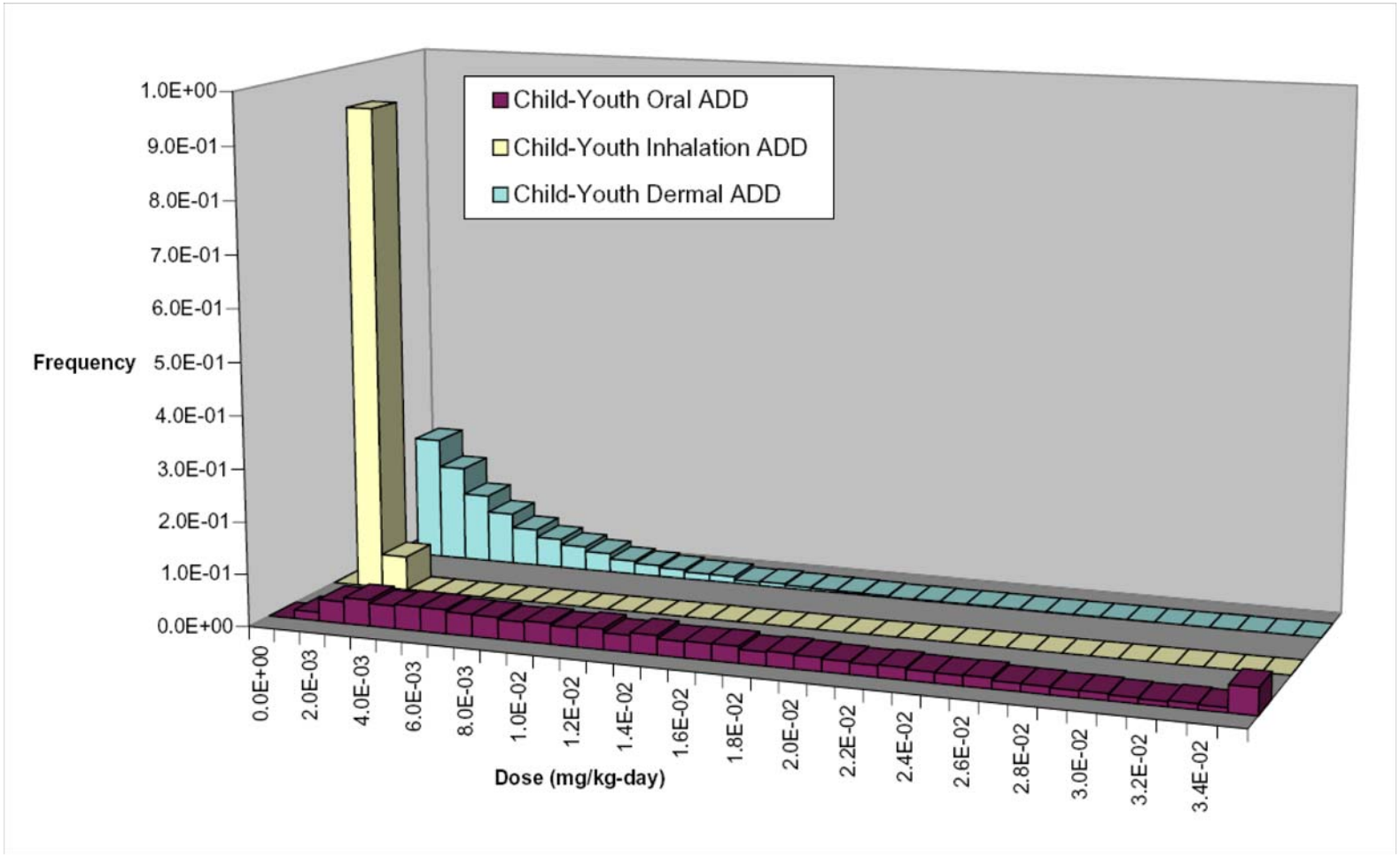
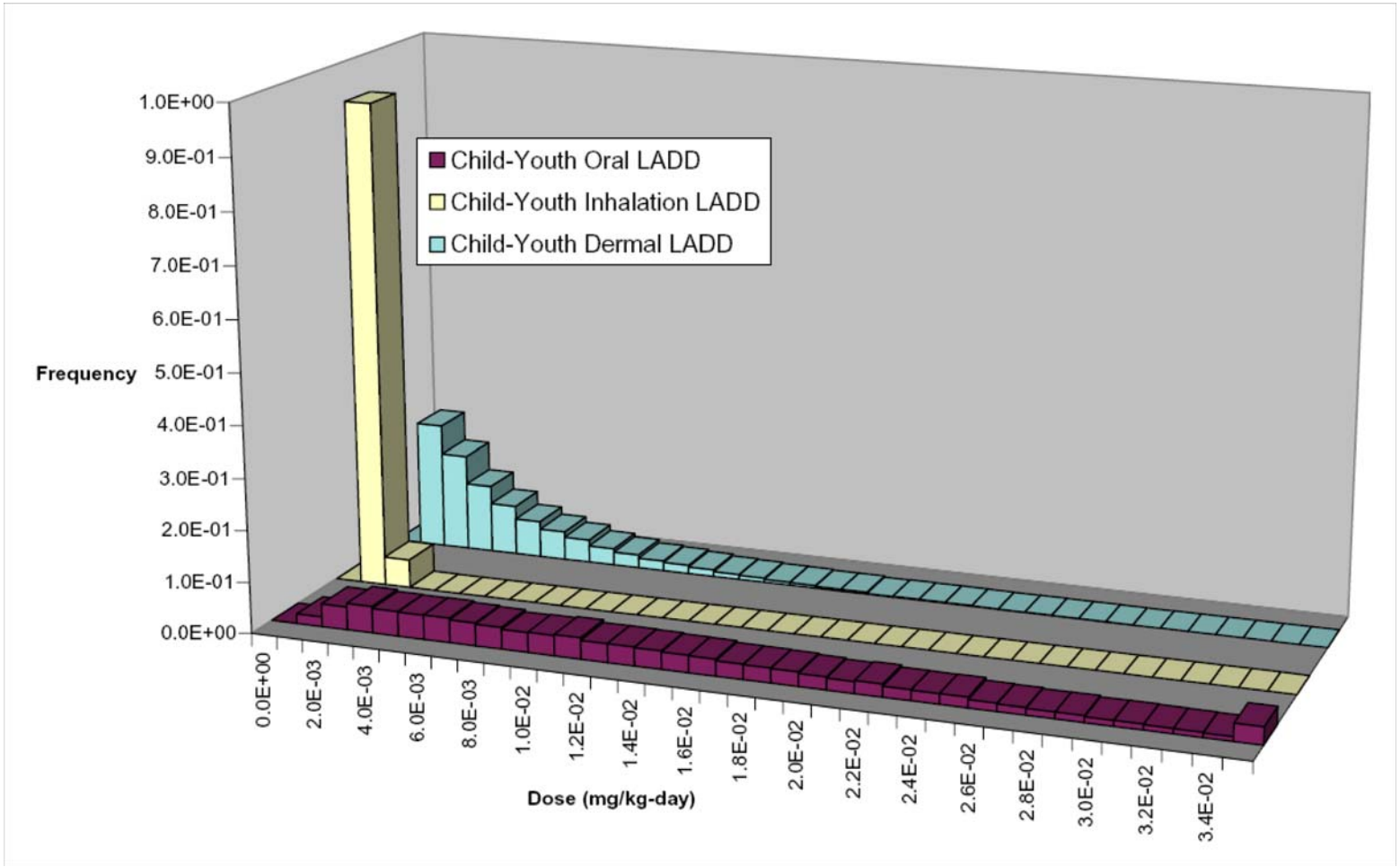


Figure 6-3. Child/Youth Calculated LADD Values



6.5 Exposure Potential Summary

Children's exposure to 1,4-dioxane is possible *in utero* and *ex utero* ingested in breast milk, food, water, inhaled in air, and absorbed through the skin in water and from consumer products. The exposure pathways considered for the different life-stages of a child are listed in **Table 6-9**. Conservative estimates of the occurrence of 1,4-dioxane in various environmental media and consumer products were made based on data from the 1980s for the most part. In many cases, these estimates overstate the occurrence of 1,4-dioxane and hence over-estimate current exposure. These data were combined with child specific exposure factors (USEPA, 2006) and used in probabilistic (*i.e.*, Monte Carlo) modeling to develop best and upper bound estimates on the dose (SPC, 1997). The ADD is the appropriate dose metric to assess non-cancer risks and is typically compared to the RfDs. For infants, the ADD is 4.5E-2 mg/kg-d (mean) and 1.4E-1 mg/kg-d (95^{th} percentile). For the child age 1 to 2 years, the total average daily dose is 3E-2 mg/kg-d (mean) and 7.9E-2 mg/kg-d (95^{th} percentile), 2.7E-2 mg/kg-d (mean) and 7.2E-2 mg/g-d (95^{th} percentile) for child age 2 to 3 years, 2.4E-2 mg/kg-d (mean) and 6.5E-2 mg/kg-d (95^{th} percentile) for child age 3 to 6 years, and 1.7E-2 mg/kg-d (mean) and 4.6E-2 mg/kg-d (95^{th} percentile) for child age 6 to 11 years. For the youth age 11 to 16 years, the total average daily dose is 1.3E-2 mg/kg-d (mean) and 3.5E-2 mg/kg-d (95^{th} percentile) and 1.4E-2 mg/kg-d (mean) and 4E-2 mg/kg-d (95^{th} percentile) for youth age 16 to 21 years. The LADDs would be the appropriate dose metric to assess the added cancer risk from exposure to 1,4-dioxane during these life-stages. The LADDs are infants: 6.4E-4 mg/kg-d (mean) and 2E-3 mg/kg-d (95^{th} percentile); child age 1-2 years: 4.2E-4 mg/kg-d (mean) and 1.1E-3 mg/kg-d (95^{th} percentile); child age 2-3 years: 3.9E-4 mg/kg-d (mean) and 1E-3 mg/kg-d (95^{th} percentile); child age 3-6 years: 1E-3 mg/kg-d (mean) and 2.8E-3 mg/kg-d (95^{th} percentile); child age 6-11 years: 1.2E-3 mg/kg-d (mean) and 3.3E-3 mg/kg-d (95^{th} percentile); youth age 11-16 years: 9.3E-4 mg/kg-d (mean) and 2.5E-3 mg/kg-d (95^{th} percentile); and youth age 16-21 years: 1E-3 mg/kg-d (mean) and 2.9E-3 mg/kg-d (95^{th} percentile). For infants, the main driver in the dose estimates is the dermal dose received from lotions and other consumer products containing 1,4-dioxane as an impurity , while for the child and youth, ingestion of foods containing 1,4-dioxane as an impurity is the most important contributor to the dose.

Additionally, fugacity modeling using the five-year average US production of 1,4-dioxane was also performed on a nation-wide basis. Doses derived from fugacity modeling were 2 to 4 orders of magnitude below those estimated using the media specific exposure estimates and probabilistic forecasts.

Table 6-9. Exposure Pathways Considered in Child Exposure Assessment

Life-stage	Ingested Water	Ingested Breast Milk	Ingested Food	Inhaled Air (in/out)	Dermal Contact-Water	Dermal Contact-Consumer	Dermal Contact-Solvent
Pregnant Worker (Fetus)	Yes (mother)	No	Yes (mother)	Yes (mother)	Yes (mother)	Yes (mother)	Yes (mother)
Infant: (0-1 yr)	No	Yes	Yes	Yes	Yes	Yes	No
Child: (1-2 yrs, 2-3 yrs, 3-6 yrs, 6-11 yrs)	Yes	No	Yes	Yes	Yes	Yes	No
Youth: (11-16 yrs, 16-21 yrs)	Yes	No	Yes	Yes	Yes	Yes	No

7.0 Risk Assessment and Characterization

By comparing the child-specific exposure estimates derived in Section 6.0 to the toxicological criteria developed for 1,4-dioxane in Section 5.0, an assessment of the potential hazard associated with 1,4-dioxane exposure to children can be made. In this risk assessment, 1,4-dioxane exposure to the fetus is addressed as well as the risk to neonates, older children, and youths. The overall assessment is based on the assumption that 1,4-dioxane functions as a non-genotoxic carcinogen that has a threshold of effect associated with the development of cytotoxicity in target organs (*i.e*. liver, kidney) due to the saturation of metabolic pathways and accumulation of the parent molecule (or a secondary metabolite) at the site of action. Under this threshold assumption, a toxicological criteria that is protective against cytotoxicity is also protective against the development of neoplasm. It should also be borne in mind that the exposure estimates are conservative and based on data that is in most cases 20 years old. Based on changes in the market and regulations, it is likely that exposure to 1,4-dioxane is lower than assumed in this assessment.

7.1 Fetal Exposure and Hazard from 1,4-Dioxane

The fetus may be exposed *in utero* to 1,4-dioxane as a consequence of the mother's exposure to 1,4-dioxane in the air, water, diet and through dermal contact with contaminated water, consumer products, or the solvent itself. For purposes of this assessment, it was assumed that the mother was also a worker to maximize the exposure to 1,4-dioxane via workplace air and through dermal contact with the solvent. Additional exposure is assumed to occur through dietary exposure to products containing 1,4-dioxane as an impurity, ingestion of contaminated water, dermal contact with contaminated water and consumer products containing 1,4-dioxane as an impurity, and inhalation of 1,4-dioxane contained in ambient and outdoor air. The details of the exposure assessment are contained in Section 6.0 and Appendix A. This information together with physiologic parameters associated with pregnancy were combined in a probabilistic model to arrive at the best and upper bound estimates of fetal exposure. The total fetal 1,4-dioxane dose for all pathways combined is 1.1 mg/kg-d (mean) and 3.2 mg/kg-d (upper-bound). These values are used to compare to the reproductive RfD derived in Section 5.0.

As noted in Section 4.0, 1,4-dioxane is not notable as a reproductive or developmental toxicant; however, this data is somewhat sparse. The two-generation study of Giavani *et al.* (1985) identified a NOAEL of 517 mg/kg-d based on slight maternal and embryo- toxicity, and this NOAEL was used in Section 5.0 to derive a reproductive RfD for 1,4-dioxane by incorporating an uncertainty factor of 100. The RfD used to assess the risk to the developing fetus is 5.2 mg/kg-d.

The approach used to assess the hazard for the exposed fetus is identical to the Hazard Index (HI) approach used for non-cancer risk assessment by USEPA and other authoritative bodies in the environment and workplace. In this approach, the estimated dose is divided by the RfD. A quotient less than unity (1.0) is presumed to carry no risk to the individual exposed. In the case of fetal exposure to 1,4-dioxane, the use of the best estimate of exposure compared to the reproductive RfD is **0.2** while the upper bound estimate of exposure gives a HI value of **0.5**. These results are presented in **Table 7-1.**

Table 7-1. Assessment of 1,4-Dioxane Hazards for Pregnant Workers (Fetus)

Pregnant Worker (Fetus) Exposure	Exposure Dose (mg/kg-d)	Reproductive RfD (mg/kg-d)	Hazard Index	≥1.0
Best Estimate (mean)	1.1	5.2	**0.2**	**No**
Upper Bound (95^{th} percentile)	2.6	5.2	**0.5**	**No**

Based on the results of this evaluation, maternal exposure to 1,4-dioxane does not pose an unacceptable hazard to the pregnant mother or developing fetus.

7.2 Child Exposure and Hazards from 1,4-Dioxane

Exposure of infants (age 0 to 1 year), children (age 1 to 2 years; 2 to 3 years; 3 to 6 years; and 6 to 11 years), and youths (age 11 to 16 years and 16 to 21 years) to 1,4-dioxane occurs primarily via the ingestion of foods containing 1,4-dioxane as an impurity, dermal contact with contaminated water during showering or bathing and through the use of consumer products containing 1,4-dioxane as an impurity, and through inhalation of 1,4-dioxane in ambient and indoor air (assumed to be the same air concentrations). Children and youths are also exposed to 1,4-dioxane through ingestion of contaminated water whereas infants are exposed through contaminated breast milk or formula. In the case of breast milk, the exposed mother was assumed to be a worker and the dose of 1,4-dioxane in breast milk under these circumstances would be greater than and subsume the dose from a solely environmentally exposed mother or through formula. The details of the exposure assessment for the various life-stages of a child are contained in Section 6.0 and Appendix A. This information together with physiologic parameters associated with children (USEPA, 2006) were combined in a probabilistic model to arrive at the best and upper bound estimates of childhood exposure to 1,4-dioxane. The average daily 1,4-dioxane dose (mean and upper bound) for each pathway (*i.e.*, oral, inhalation, and dermal) for each life stage are presented in **Table 6-7**. These values are compared to the oral, inhalation, and dermal RfDs derived for 1,4-dioxane in Section 5.0.

The chronic bioassays of Kociba *et al.* (1974) and Yamazaki *et al.* (1994) identified a NOAEL of 10 mg/kg-d based on liver endpoints (NICNAS, 1998; TNO and RIVM, 2002) and is considered protective against other target organ toxicities and cytotoxicity. The Torkelson *et al.* (1974) study provided a NOAEL for inhalation of 1,4-dioxane of 108 mg/kg-d. These NOAELs were used in Section 5.0 to derive oral and inhalation RfDs for 1,4-dioxane by incorporating an uncertainty factor of 100. An adjusted oral RfD was developed to estimate an absorbed RfD in accordance with USEPA guidance (USEPA, 1992b) to allow the dermal dose to be assessed. The RfD and RfCs used to assess the risk to children are 0.1 mg/kg-d (oral), 1.1 mg/kg-d (inhalation), and 0.1 mg/kg-d (adjusted dermal), respectively.

As with fetal risk, the approach used to assess the risk for the exposed child at various life-stages is the Hazard Index (HI) approach. In this case, the estimated route-specific dose (in mg/kg-d) is divided by the route-specific RfD (in mg/kg-d) and the total HI is summed from each route-specific hazard quotient for each life-stage of the child. A quotient less than unity (1.0) is presumed to carry no risk to the individual exposed by route or life-stage. The results for this evaluation are presented in **Table 7-2.**

Table 7-2. Assessment of 1,4-Dioxane Hazards for Children

Life stage	Oral dose	Oral Rfd	Oral HI	Inhal dose	Inhal RfC	Inhal HI	Dermal Dose	Dermal Rfd	Dermal HI	Total HI	>1
Infant (0-1 years)											
Best Estimate (mean)	0.01	0.1	**0.1**	0.0001	1.1	**0.00009**	0.033	0.1	**0.3**	**0.4**	**No**
Upper Bound (95th percentile)	0.025	0.1	**0.3**	0.0027	1.1	**0.002**	0.11	0.1	**1**	**1**	**No**
Child (1-2 years)											
Best Estimate (mean)	0.026	0.1	**0.3**	0.001	1.1	**0.0009**	0.0027	0.1	**0.03**	**0.3**	**No**
Upper Bound (95th percentile)	0.068	0.1	**0.7**	0.0028	1.1	**0.0003**	0.0087	0.1	**0.09**	**0.8**	**No**
Child (2-3 years)											
Best Estimate (mean)	0.024	0.1	**0.2**	0.00084	1.1	**0.0008**	0.0027	0.1	**0.03**	**0.3**	**No**
Upper Bound (95th percentile)	0.061	0.1	**0.6**	0.0022	1.1	**0.002**	0.0087	0.1	**0.09**	**0.7**	**No**
Child (3-6 years)											
Best Estimate (mean)	0.021	0.1	**0.2**	0.0006	1.1	**0.0005**	0.0027	0.1	**0.03**	**0.2**	**No**
Upper Bound (95th percentile)	0.055	0.1	**0.6**	0.0016	1.1	**0.001**	0.0087	0.1	**0.09**	**0.6**	**No**
Child (6-11 years)											
Best Estimate (mean)	0.014	0.1	**0.1**	0.00036	1.1	**0.0003**	0.0027	0.1	**0.03**	**0.2**	**No**
Upper Bound (95th percentile)	0.037	0.1	**0.4**	0.00099	1.1	**0.0009**	0.0087	0.1	**0.09**	**0.5**	**No**

Life stage	Oral dose	Oral Rfd	Oral HI	Inhal dose	Inhal RfC	Inhal HI	Dermal Dose	Dermal Rfd	Dermal HI	Total HI	>1
Youth (11-16 years)											
Best Estimate (mean)	0.011	0.1	**0.1**	0.00023	1.1	**0.0002**	0.002	0.1	**0.02**	**0.1**	**No**
Upper Bound (95th percentile)	0.028	0.1	**0.3**	0.00064	1.1	**0.0006**	0.0064	0.1	**0.06**	**0.3**	**No**
Youth (16 -21)											
Best Estimate (mean)	0.012	0.1	**0.1**	0.0002	1.1	**0.0002**	0.0028	0.1	**0.03**	**0.1**	**No**
Upper Bound (95th percentile)	0.031	0.1	**0.3**	0.00054	1.1	**0.0005**	0.0087	0.1	**0.09**	**0.4**	**No**

[1] Hazard Indexes were derived using chronic RfDs/RfC while the exposure durations were subchronic. Therefore, these HIs are considered conservative.

Based on the results of this evaluation, exposure to 1,4-dioxane does not pose an unacceptable hazard to the child by exposure route or life-stage.

7.3 Cancer Risk Associated with 1,4-Dioxane

Typically, cancer risk is assessed by combining the dose estimates with the relevant cancer potency factor. The cancer potency factors are derived from the extrapolation of high-dose animal cancer data to the low doses experienced by humans in the workplace or environment. The dose-extrapolation generally relies on the upper-bound predictions of a conservative mathematical model that assumes no threshold for the carcinogenic response. The true added risk is described as being no higher than the estimate, probably lower and maybe zero as a consequence of the numerous conservative assumptions made throughout the process.

In the case of 1,4-dioxane, there are a number of reasons why this approach to assessing cancer risk may not be appropriate. While high, prolonged doses of 1,4-dioxane consistently result in similar tumors in experimental animals, the evidence is equally strong that 1,4-dioxane exerts these effects through a non-genotoxic mechanism, which implies that a threshold for these effects exists. The most likely mechanism for the observed carcinogenic response is that, at high, prolonged doses, the metabolism of 1,4-dioxane becomes saturated. 1,4-Dioxane (or perhaps a secondary metabolite) builds up in the target tissue (*i.e.*, liver) to cytotoxic levels resulting in tissue damage. The biological response is to increase cellular

repair to compensate for this damage and the continuous cellular proliferation that results causes hyperplasia and hypertrophy in the affected tissues. A consequence of this continuous cellular activity is the increased possibility of errors occurring in the translation and transcription process resulting in an altered cell line if these errors are not repaired prior to cellular division, and the possibility of neoplastic growth associated with the altered cell line. The tumors associated with 1,4-dioxane occur only in tissues that are affected by cytotoxicity at or above cytotoxic doses. In the absence of continuous tissue damage, no tumors result. Since the RfD/RFCs derived were based on NOAELs that did not induce cytotoxicity (or tumors) in lifetime cancer bioassays, these exposures below those doses can be considered to carry no significant added cancer risk. Since no total HI was greater than one (Table 7-1), it is concluded that there is no cancer risk from these 1,4-dioxane exposures.

There is precedent for adopting this non-linear, threshold approach in the regulatory record where compounds cause cancer through a non-genotoxic mechanism. For instance, certain compounds that cause thyroid cancer by disrupting the homeostasis of thyroid hormones are assessed as threshold carcinogens. More recently and directly relevant to the situation with 1,4-dioxane, the recent re-evaluation of chloroform identified a similar non-linearity of dose-response also associated with cytotoxicity following metabolic saturation. The recognition that a threshold existed for the carcinogenic effect of chloroform lead to a determination that the levels typically found in drinking water as a consequence of chlorination posed no excess cancer risk to consumers.

If, in spite of the evidence that a threshold for the carcinogenicity of 1,4-dioxane exists, a cancer potency factor approach is desired, there are reasons why the currently available cancer potency factors for 1,4-dioxane should not be used in this evaluation. The USEPA cancer potency factor is over 15 years old and has not been significantly updated since the late 1980s. It is based on nasal tumors in the rat (NCI, 1978), which appear inconsistently in animal studies and have questionable relevance to humans, uses extrapolation methods (*i.e*. scaling factors) that have since been superceded by more recent cancer risk assessment principles, and does not take full advantage of the relevant pharmacokinetic and toxicological data for this compound that would aid in more appropriate dose extrapolation decisions. The CalEPA cancer potency factor is of more recent vintage, but uses the mouse tumor data (NCI, 1978) as the basis when the rat is pharmacokinetically more similar to humans, and otherwise has the same deficiencies as the USEPA cancer potency factor. The PBPK assessments that have been done for 1,4-dioxane are in agreement that the cancer risk estimates using the current cancer potency factors over state the cancer risk by 2 to 4 orders of magnitude as a consequence of ignoring the pharmacokinetic data. This is in addition to the other conservatisms that would remain in the cancer potency factors if this issue was appropriately addressed. As with the recognition that non-genotoxic carcinogens pose thresholds, the USEPA has employed PBPK modeling for estimating both non-cancer risks (*i.e.*, ethylene glycol butyl ether) and cancer risks (*i.e.*, vinyl chloride, trichloroethylene,

methylene chloride). Given the availability of PBPK models for 1,4-dioxane and the database, this application seems appropriate to include in a re-evaluation of the 1,4-dioxane toxicological criteria. The PBPK model would allow additional questions regarding potential route and age influence on the toxicity of 1,4-dioxane to be addressed.

To illustrate the point, the approaches of Reitz *et al.* (1990) were used in this current assessment to develop cancer potency factors for 1,4-dioxane (conservatively assuming a linear low dose cancer response) that take into account the available pharmacokinetic data for 1,4-dioxane. These values are 1.8E-5 $(mg/kg\text{-}d)^{-1}$ for oral doses, 1.3E-5 $(mg/kg\text{-}d)^{-1}$ for inhaled doses, and 1.8E-5 $(mg/kg\text{-}d)^{-1}$ for dermal exposure (adjusted from the oral potency factor for an absorbed dose), based upon a linear extrapolation of the authors 1x10-5 risk specific dose estimates using the weighted average for four data sets (see Section 5.3). These values were combined with the LADD for 1,4-dioxane (**Table 6-8**) to provide an estimate of the added lifetime cancer risk associated with route of exposure and life-stages of a child. This information is presented in **Table 7-3**.

Table 7-3. Cancer Risk Estimates for 1,4-Dioxane Based on Reitz *et al.*, 1990

Life-stage (age)	Oral LADD	Oral Risk	Inhalation LADD	Inhalation Risk	Dermal LADD	Dermal Risk	Total Risk
Infant (0 - 1 yrs)							
Best Estimate (mean)	0.00015 mg/kg-d	2.8E-9	0.00001 mg/kg-d	1.35E-10	0.00047 mg/kg-d	8.7E-9	**1.2E-8**
Upper Bound (95th percentile)	0.00036 mg/kg-d	6.7E-9	0.000039 mg/kg-d	5.3E-10	0.0016 mg/kg-d	2.96E-8	**3.7E-8**
Child (1 - 2 yrs)							
Best Estimate (mean)	0.00037 mg/kg-d	6.8E-9	0.000015 mg/kg-d	2.0E-10	0.000038 mg/kg-d	7.0E-10	**7.75E-9**
Upper Bound (95th percentile)	0.00097 mg/kg-d	1.8E-7	0.00004 mg/kg-d	5.4E-10	0.00012 mg/kg-d	2.2E-9	**1.8E-7**
Child (2 - 3 yrs)							
Best Estimate (mean)	0.00034 mg/kg-d	6.3E-9	0.000012 mg/kg-d	1.6E-10	0.000038 mg/kg-d	7.0E-10	**7.15E-9**
Upper Bound (95th percentile)	0.00088 mg/kg-d	1.6E-8	0.000032 mg/kg-d	4.3E-10	0.00012 mg/kg-d	2.2E-9	**1.9E-8**
Child (3 - 6 yrs)							
Best Estimate (mean)	0.0009 mg/kg-d	1.7E-8	0.000026 mg/kg-d	3.5E-10	0.00011 mg/kg-d	2.0E-9	**1.9E-8**
Upper Bound (95th percentile)	0.0024 mg/kg-d	4.4E-8	0.000068 mg/kg-d	9.2E-10	0.00037 mg/kg-d	6.8E-8	**5.2E-8**

Life-stage (age)	Oral LADD	Oral Risk	Inhalation LADD	Inhalation Risk	Dermal LADD	Dermal Risk	Total Risk
Child (6 - 11 yrs)							
Best Estimate (mean)	0.00099 mg/kg-d	1.8E-8	0.000026 mg/kg-d	3.5E-10	0.00019 mg/kg-d	3.5E-9	**2.2E-8**
Upper Bound (95th percentile)	0.0026 mg/kg-d	4.8E-8	0.000071 mg/kg-d	9.6E-10	0.00062 mg/kg-d	1.1E-8	**6.05E-8**
Youth (11-16 yrs)							
Best Estimate (mean)	0.00077 mg/kg-d	1.4E-8	0.000017 mg/kg-d	2.3E-10	0.00014 mg/kg-d	2.6E-9	**1.7E-8**
Upper Bound (95th percentile)	0.002 mg/kg-d	3.7E-8	0.000045 mg/kg-d	6.1E-10	0.00046 mg/kg-d	8.5E-8	**4.6E-8**
Youth (16-21 yrs)							
Best Estimate (mean)	0.00082 mg/kg-d	1.5E-8	0.000014 mg/kg-d	1.9E-10	0.00019 mg/kg-d	3.5E-9	**1.9E-8**
Upper Bound (95th percentile)	0.0022 mg/kg-d	4.1E-8	0.000038 mg/kg-d	5.1E-10	0.00062 mg/kg-d	1.1E-8	**5.3E-8**
Total[1] (0 - 21 yrs)							
Best Estimate (mean)	0.0044 mg/kg-d	8.1E-8	0.00012 mg/kg-d	1.6E-9	0.0012 mg/kg-d	2.2E-8	**1.05E-7**
Upper Bound (95th percentile)	0.010 mg/kg-d	1.85E-7	0.00032 mg/kg-d	4.3E-9	0.0037 mg/kg-d	6.8E-8	**2.6E-7**

[1] Total (0-21 years) exposure was determined by summing the LADD values across age groups within the Monte Carlo simulation

Utilizing these alternate CPFs for comparison purposes only, the added cancer risk (again, assuming no threshold) for 1,4-dioxane is well below the 1×10^{-5} risk level considered

acceptable under the VCCEP. The available USEPA and CalEPA CPFs that do not take into account the pharmacokinetics of 1,4-dioxane are approximately 3 orders of magnitude higher, and the risks calculated using these values would be comparatively higher.

7.4 Uncertainties in the Risk Assessment of 1,4-Dioxane

The largest uncertainty in the risk assessment of 1,4-dioxane lies in the derived toxicologic criteria, particularly the cancer potency factor, as a consequence of the conservative assumption made in extrapolating from animals to man and from high doses to low doses. This source of uncertainty was not addressed using Monte Carlo methods. Some of these uncertainties could be reduced by incorporating all available data into the model selection and development, and revising the toxicological criteria accordingly.

Additional uncertainties are associated with the exposure estimates derived for 1,4-dioxane due to the assumptions that were made to address the data gaps and uncertainty over exposure parameters. The probabilistic model provides a sensitivity analysis of the exposure estimates that can be used to decide where to devote additional resources to reduce uncertainty, and this information is included as **Table 7-4**. In all estimates (fetal, infant, child, and youth) the dermal permeability constant (K_p) contributes approximately 30% of the uncertainty (based on its contribution to variance). For the fetal exposure, the workplace air concentrations and mother's dermal contact with the solvent are the next most important contributors to uncertainty. For the infant, the workplace air concentration (as a contributor to breast milk concentration of 1,4-dioxane), indoor air concentrations, and lotion concentrations of 1,4-dioxane are also important contributors to uncertainty. For both the child and the youth , the water, indoor/outdoor air, and lotion concentrations of 1,4-dioxane are the most important sources of uncertainty in the model.

7.5 Summary and Conclusion

Based on the assumption of 1,4-dioxane being a non-genotoxic carcinogen that possesses a threshold, conservative dose estimates of 1,4-dioxane from various sources of exposure were compared to RfDs that were considered protective of the cytotoxicity associated with both target organ toxicity and carcinogenicity for the various life-stages of a child. In no instance did the estimated exposures exceed the RfDs by route of exposure or life-stage in general. Under these assumptions, exposure to 1,4-dioxane does not pose an unacceptable non-cancer or cancer risk to children.

Table 7-4. Sensitivity Analysis For Exposure Estimates Based Upon Contribution to Variance

Sensitivity Data	Worker Total ADD/ LADD	Infant Oral ADD/ LADD	Infant Inhalation ADD/ LADD	Infant Dermal ADD/ LADD	Youth/ Child Oral ADD/ LADD	Youth/ Child Inhalation ADD/ LADD	Youth/ Child Dermal ADD/ LADD
Workplace Air Concentration (mg/m3)	26.0%	69.9%					
Additive content of food (fraction)		14.6%			8.2%		
Dioxane concentration (mg/kg) in additives		6.2%			2.6%		
Infant Food intakes (g/kg-day)		4.7%					
Breast Milk Dose Adjustment Factor		3.2%					
Child Food Intake (g/kg-day)							
Kp (cm/hr)	30.1%			28.0%			32%
Product/Raw Material Concentration (mg/L)	11.8%						
Ambient Air Concentration (mg/m3)			70.4%			71.4%	
Inhalation Relative Absorption			25.0%			25.2%	
Infant Inhalation Rate (m3/d)			3.2%				
Worker Food Intake (g/kg-day)	0.9%						
Worker Water Ingestion (L/d)	9.1%						
Child/Youth Lotion Exposure Time							5.0%
Water Concentration (mg/L)					86.1%		
Inhalation Relative Absorption	6.9%					96.6%	
Inhalation Rate (m3/d)	1.0%						
Child Body Weight Age 3-6 yrs (kg)					0.2%	0.4%	
Child Water Intake Age 3-6 yrs (L/d)					0.4%		
Child Body Weight Age 6-11 yrs (kg)					0.3%	0.7%	
Child Water Intake Age 6-11 yrs (L/d)					0.7%		
Infant Lotion Exposure Time				19.8%			2.8%
Youth Water Intake Age 11-16 (L/d)					0.5%		
Product/Raw Material Exposure Time	23.5%						
Lotion Concentration (mg/L)				51.4%			59.1%

While the assumed non-genotoxic mode of action for this compound eliminates the need for the traditional cancer risk extrapolation, such an evaluation was conducted using alternate cancer potency factors developed from the PBPK model developed for 1,4-dioxane by Reitz *et al.* (1990). This allows the pharmacokinetics of 1,4-dioxane and the differences in animal and human responses to be included in the dose-response extrapolation. This evaluation similarly found no excess risk from 1,4-dioxane given the assumptions made in the model.

The uncertainties inherent in the decisions made and the models employed were identified through a sensitivity analysis of the model output. These results are discussed further under the data needs assessment (Section 8.0).

8.0 Data Needs Assessment

As part of the VCCEP process, a data needs assessment is required to assist in determining what additional information would be necessary to reduce uncertainty and improve the risk assessment of the candidate compounds.

For 1,4-dioxane, areas considered include toxicity data, dose-response evaluation, and exposure assessment.

8.1 Toxicity Data Needs

The toxicity database for 1,4-dioxane is reasonably robust for most endpoints of interest, including pharmacokinetics, acute, sub-chronic, and chronic toxicity, genotoxicity, and carcinogenicity. Although inhalation and dermal toxicity studies are limited in number, there does not seem to be a significant route-specific difference in the biological responses observed. Additionally, PBPK models such as those developed for 1,4-dioxane can be used for route-to-route extrapolation and are useful to supplement data needs without additional sacrifice of animals or cost and time associated with additional experimentation.

There are no specific immunotoxicity studies available for 1,4-dioxane; however, there is also little indication from available studies that 1,4-dioxane is likely to be an immunotoxicant. Most studies show no histopathology of tissues likely to be involved in the immune response, no changes in clinical biochemistry parameters that are suggestive of an immune response or damage to the immune system, no increase in infectious diseases among exposed animals, and no sensitization potential. There is also an issue in that most immunotoxicity tests are difficult to interpret and the extrapolation to man is uncertain. This calls into question whether a specific immunotoxicity test would be of practical value in addressing 1,4-dioxane hazards. In this light, an additional immunotoxicity test for 1,4-dioxane is not needed.

There is also no specific neurotoxicity studies available for 1,4-dioxane, aside from some acute toxicity and neurochemistry studies. 1,4-Dioxane is a neurotoxicant at high doses, but it displays the non-specific, reversible neurotoxicity observed with other solvents and anesthetics at high doses. This is presumably a concentration-related effect associated with the disruption of the membrane potential of excitable tissues. The fat-solubilizing ability of solvents interferes with ability of the nervous tissues to sustain nerve impulses and causes effects similar to that observed in alcohol intoxication or anesthesia as a consequence. At lower levels of exposure to humans and in sub-chronic and chronic animal bioassays, neurotoxic symptoms have not been generally observed. There is also little evidence from gross pathology or histopathology that the nervous system is a specific target organ for 1,4-

dioxane. Accordingly, a neurotoxicity bioassay for this compound is not recommended at this time.

There is also no specific developmental neurotoxicity study available for 1,4-dioxane. Reproductive and developmental toxicity studies for this compound are limited in number overall. This is obviously of special interest given the intent of the VCCEP. From the data that do exist, 1,4-dioxane does not appear to be a significant reproductive or developmental hazard, and, as noted above, the nervous system does not appear to be a target organ for 1,4-dioxane in adult animals. While an argument can be made for a developmental neurotoxicity test for 1,4-dioxane to fill this gap, the doses likely experienced by exposed fetuses are well below the Reference Doses developed to protect against the critical endpoints, both in terms fo reproductive and chronic toxicity. This presumably extends to issues of developmental neurotoxicity as well. A developmental neurotoxicity study in this light is not a priority.

8.2 Dose-Response Evaluation Needs

The toxicological criteria, RfDs, RfCs and CPFs, used to assess risk from exposure are the largest source of uncertainty in any risk assessment.

In this case, a strong argument can be made that CPFs for 1,4-dioxane are not needed due to the likely existence of a threshold for its effect associated with metabolic saturation and resultant cytotoxicity. The RfDs and RfC developed protect against this cytotoxicity and target organ toxicity, and as a consequence protect against the carcinogenicity associated with this tissue damage. One data need for dose-response could be an evaluation of the animal cancer mode of action information (as well as human relevance) in a format that has been described in the USEPA Cancer Guidance (USEPA, 2005). To the best of our knowledge this has not yet been done for 1,4-dioxane.

If CPFs are viewed as needed, those currently available are suspect for reasons previously discussed and need to be revised in accordance with currently accepted principles of dose-response extrapolation that take into account the appropriate scaling factors, the available pharmacokinetic data, the lack of genotoxicity, and the most appropriate species and tumor to identify the best extrapolation approach.

8.3 Exposure Assessment Needs

The exposure estimates developed for 1,4-dioxane are based on sparse data that dates from 10 to 20 years ago when the use of 1,4-dioxane was more prevalent then it is today. Approximately 90% of the 1,4-dioxane manufactured was used as a stabilizer in chlorinated solvents and that use has now ended. The amount of 1,4-dioxane produced in the US has declined by over 80% from the amount produced in the mid 1980s and this is likely to change

the potential exposure for children as well. **Table 7-4** details the sensitivity analysis of the exposure estimates derived for 1,4-dioxane and identifies areas of greatest uncertainty for each parameter.

8.3.1 Workplace exposure

The number of workers, specifically female workers, exposed to 1,4-dioxane is unknown, but assumed to be lower than that reported by NIOSH in the 1980s. The number of workers, type of work, frequency and duration of exposure, and potential for exposure (use of protective equipment, fume hoods, etc.) needs to be investigated. The workplace air levels at the production facilities are available and current, and allow a reasonable estimate of personal inhalation exposure although potential dermal exposure is uncertain. End-use exposure (inhalation and dermal) to products containing 1,4-dioxane is assumed to be similar to production facilities, but this is more uncertain.

8.3.2 Water Exposure

1,4-Dioxane has been detected in drinking water intermittently and at various concentrations (usually low). Since it is not a regulated contaminant, it is not regularly monitored for in drinking water and so the extent and amount of 1,4-dioxane in public and private supplies is unknown. Given its past association with chlorinated solvents, miscibility and rapid movement in water, resistance to degradation and water treatment, it can be postulated that a significant if unrecognized exposure to 1,4-dioxane may be occurring through drinking water in some areas. USEPA may wish to undertake a survey of water systems affected by chlorinated solvent contamination to determine if this is a potential problem. Based on the findings from such a survey, an estimate of the number of people exposed and the extent of exposure to 1,4-dioxane from this source can be made.

8.3.3 Air Exposure

The levels of 1,4-dioxane in ambient and indoor air were very low in the 1980s when most of the available data was collected, but the data is limited in time and space to a few areas (chiefly California and New Jersey). Inhalation exposure was not a major driver in the exposure or risk assessment for 1,4-dioxane; however, given the number of products in which 1,4-dioxane was used, a significant number of individuals may have been exposed at one time. However, the air levels are the most likely to have been affected by the change in the 1,4-dioxane market, and may have dropped appreciably in the last few years. Given that the TEAM studies are 15 years old, it may be time to re-visit and expand these studies to assess current exposure to 1,4-dioxane and other air contaminants of potential concern.

8.3.4 Food and Consumer Products

The main driver for exposure and risk to 1,4-dioxane was its occurrence in food and consumer products as a consequence of its appearance in various surfactants as an impurity. This assessment was also based on data 10 to 20 years old and changes in production of surfactants and formulation of the finished products may have changed in response to regulatory mandates and customer demands. The FDA and USEPA may wish to undertake a survey to identify the surfactants and other raw materials that contain 1,4-dioxane, the products in which they are used, and the levels of 1,4-dioxane that remain in the finished consumer product. 1,4-Dioxane is also known to occur in foods naturally or as a consequence of the cooking process (*i.e.*, deep fat frying). It would be useful to know the extent and levels of 1,4-dioxane found in foods that do not use surfactants. Additionally, information relevant to exposure should be collected including how much of a specific additive is consumed daily by various age ranges, how much shampoo or lotion is applied to what area of skin for how long and with what frequency, additional information on the permeability of skin to 1,4-dioxane in consumer products would be useful, and so on. This information would be critical in refining the exposure assessment for 1,4-dioxane for children.

8.4 Summary and Conclusion

The toxicological database for 1,4-dioxane is reasonably robust and complete. Deficiencies in the areas of immunotoxicity, neurotoxicity, and developmental neurotoxicity are noted, but not considered high priority or critical to evaluating the potential hazard of 1,4-dioxane to children. The toxicological criteria derived or available for 1,4-dioxane are considered adequate for the purpose of assessing the risk to children under the assumption that 1,4-dioxane is a non-genotoxic carcinogen that has a threshold below which no significant risk exists. Cancer Potency Factors are not necessary under this assumption, and those currently available do not fully take into account all the relevant information and should not be used without significant revision, if at all. The largest area of uncertainty (aside from the toxicity criteria) lies in the exposure estimates. The extent and level of children's exposure to 1,4-dioxane is based on sparse and dated information. Additional sampling of current 1,4-dioxane occurrence in drinking water, ambient and indoor air, and food and consumer products as well as improved understanding of contact frequency and duration would be necessary to improve these estimates and reduce uncertainty in the exposure and risk assessment for this compound.

9.0 References

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Appendix A

EXPOSURE CONCENTRATIONS

TRI DATA - Air

Facility	Location	2000
UCC	TX	7,255
Ferro	LA	2,325
Holman	SC	998
Eastman	SC	780
Env Ser	AR	17
Syntech	OR	26
UOP	CA	97
Onyx	IL	2
Wellman	SC	6,798
Dupont	NC	10,304
Eastman	NY	2,940
Arteva	SC	2,010
Rhodia	IN	5
UCC	WV	116
UCC	WV	787
Arteva	NC	5,571
Rineco	AR	13
Clarksville	VA	9
Aventis	WV	1
Osmonics	MN	1,240
Buckman	MO	55
Hydranautics	CA	33
Holman	MS	20
Holman	MO	20
GL Chem	TN	2,253
Wellman	MS	3,804
Oxid	TX	3,610
Cl Harb	NE	10
Koch	CA	610
Dupont	OH	5,706
BASF	TX	17
Aldrich	WI	10
Eastman	TN	2,780
Cont Cemment	MO	509
Dupont	TX	29
UCC	LA	1,243
DOW	LA	2
Dupont	AL	1,876
Dupont	TN	3,298
Tomah	WI	20
Cognis	SC	369
Lexmark	CO	420
BM Squibb	PR	1,600
DuPont	NC	3,612
Dupont	SC	900
Dupont	SC	502
Arteva	NC	4,058
M&G	WV	2,129
Rhodia	GA	3,086
Albermele	AR	4,995
Wayne Disp	MI	329

Facility	Location	1995
Xerox	NY	5,700
Squibb	PR	30
Mitusbishi	VA	146
Reynolds	VA	10,000
Dupont	AL	5,120
Dupont	NC	6,550
Dupont	NC	18,133
Trevava	NC	7,900
H-C	NC	262
RJR	NC	7,016
Dupont	SC	1,660
Dupont	SC	1,200
Eastman	SC	2,100
Henkel	SC	2,425
Daifoil	SC	2,207
H-C	SC	25,360
Wellman	SC	7,800
Dupont	TN	20,200
GL Chem	TN	2,948
Eastman	TN	6,851
Dow	IL	60
Morton	IL	265
Stepan	IL	2,500
Osmonics	MN	1,028
Dow	LA	30
Ferro	LA	5,451
R-P	LA	4
UCC	LA	25,498
Vulcan	LA	4,160
BASF	TX	4
Dow	TX	540
Oxid	TX	1,820
UCC	TX	6
UCC	TX	12,766
Buckman	MO	779
Whitmire	MO	258
Hexcel	UT	5,876
Thiokol	UT	9,000
EGG	CA	462
Fluid	CA	349
Hydranautics	CA	20
uop	CA	145
Eastman	NY	16,005

Facility	Location	2000	Facility	Location	1995
RJR	NC	1			
RJR	NC	9			
RJR	NC	2			
Mitusibshi	VA	92			
RJR	NC	2,336			
Mitusbshi	SC	2,351			
Stepan	IL	10,967			
Totals		104,957	Totals		220,634

TRI - Water

Facility	Location	2000
UCC	TX	2,617
Ferro	LA	1,747
Eastman	SC	160
Wellman	SC	4,342
Dupont	NC	36
Eastman	NY	5,000
Arteva	SC	131
Arteva	NC	8,790
Buckman	MO	3
Dupont	OH	123
Eastman	TN	35,000
UCC	LA	17,700
Dupont	NC	14,737
Dupont	SC	10,000
Dupont	SC	5,182
Arteva	NC	2,484
M&G	WV	55,724
Totals		163,776

Facility	Location	1995
Dupont	NC	17,273
Dupont	NC	54
Trevava	NC	4,700
H-C	NC	7,240
Dupont	SC	6,978
Dupont	SC	14,100
Eastman	SC	250
Diafoil	SC	2,896
H-C	SC	500
Wellman	SC	30,891
Eastman	TN	65,000
Stepan	IL	1
Ferro	LA	4,974
UCC	LA	4,350
Eastman	TX	1,459
Eastman	NY	56,023
Totals		216,689

TRI - Land

Facility	Location	2000	Facility	Location	1995
Synetch	OR	8	Diafoul	SC	22
Arteva	NC	2700	Stepan	IL	4
Wayne Disp	MI	15,420			
Stepan	IL	3			
Totals		18131	Totals		26

TRI - POTW

Facility	Location	2000
Ferro	LA	27,568
UOP	CA	400
Wellman	SC	499
Eastman	NY	4
UCC	WV	1
Arrteva	NC	1
Rineco	AR	84,891
Osmonics	MN	1,229
Buckman	MO	75,066
hydranuatics	CA	2,000
GL Chem	TN	136,000
Wellman	MS	499
Oxid	TX	332,158
Koch	CA	1,866
Aldrich	WI	5,000
Dupont	AL	2,872
Tomah	WI	664
Cognis	SC	1,937
Dupont	SC	139
Artev	NC	20
Rhodia	GA	8,836
Albermele	AR	772
Mitushibi	VA	6,970
Mitusbhis	SC	467
Stepoan	Il	901,993
Totals		1,591,852

Facility	Location	1995
Dupont	SC	140
Henkel	SC	3,676
G-L Chem	TN	3,900
Osmonics	MN	35,012
Desal	CA	21,003
Fluid	CA	38,267
Hydranautrics	CA	85,224
Totals		187,222

TRI - Transfer

Facility	Location	2000	Facility	Location	1995
Ferro	LA	28,123	Xerox	NY	1,042
Holman	SC	45,000	Schering	PR	8,960
Env Ser	AR	1,010	Mitsubishi	VA	5,200
Syntech	OR	153	Dupont	AL	43,860
UOP	CA	20,006	Dupont	NC	1,020
Onyx	IL	66	Dupont	NC	4,549
Wellman	SC	499	H-C	NC	2
Dupont	NC	9	Dupont	SC	13,467
Eastman	NY	4,300	Eastman	SC	110
Rhodia	IN	1	Diafoil	SC	24,709
UCC	WV	1,617	H-C	SC	4
Arteva	NC	166	G-L Chem	TN	12,000
Rineco	AR	196,783	Dow	IL	17
Osmonic	MN	65,986	Stepan	IL	1,106,016
Hydranuatics	CA	51,214	Osmoinics	MN	1,050
Holman	MO	25	Ferro	LA	37,960
GL Chem	TN	154,500	Oxid	TX	332,526
Wellman	MS	499	Ucc	Tx	80
Oxid	TX	10,727	Buckman	MO	41,876
Koch	CA	72,940	Whitmire	MO	280
UCC	LA	2,011	Hexcel	UT	428
Rhodia	LA	12	Thiokol	UT	9,000
Dupont	AL	181,224	Fluid	CA	3,481
Cognis	SC	4,089	Hydranautics	CA	729
Lexmark	CO	4,500			
BM Squibb	PR	17,623			
Dupont	NC	208			
Dupont	SC	4,593			
Rhodia	GA	11,278			
Albermele	AR	299,572			
Wayne Disp	MI	561			
Mitushbi	SC	17,922			
Totals		1,197,217	Totals		1,648,366

Concentration of 1,4- Dioxane in Cosmetics

Shampoo					Lotion	Bath gel	Soap/Cleanser	Detergent
6	28	57	89	69	50	80	160	50
13	9	32	358	34	75	13	10	40
144	95	68	145	34	10	10	0	30
53	0	10	27	75	4	4	8	28
16	95	70	670	114	0	52	0	27
112	0	221	136	89	0	91	7	6
7	25	81	156	0	0	119	0	0
67	0	36	98	182	0	33	140	181
39	56	56	90	174	0	65	325	
1	8	181	71	17	0	144		
42	0	298	135	487	0	121		
47	59	82	78	250	30	60		
110	45	0	167	253	169	122		
85	0	21	75	344		40		
37	35	60	60	126		15		
37	51	76	40	100		0		
20	52	231	56	59		223		
10	112	8	84	10		54		
10	0	38	85	208		125		
0	0	0	23	213		78		
0	33	7	23	134		58		
95	108	15	12	51		85		
0	11	54	130	341		91		
0	22	0	142	62		264		
13	46	148	57	51		63		
0	0	40	216	38		38		
0	47	45	192	32		29		
0	86	0	284	70		37		
17	13	67	192	66		19		
613	59	94	284	279		0		
100	31	119	192	2		162		
0	42	117	220	33		41		
15	134	99	202			30		
0	47	125	12			23		
31	58	35	82			28		
46	65	160	113			16		
145	88	0	48			13		
52	73	35	144			65		
472	76	66	75			41		
102	35	68	19			216		
490	137	75	22			56		
55	93	20	0			40		
0	115	105	47			8		
154	156	226	119			44		
8	159	64	105			66		
138	212	130	24			36		
73	48	11	65			64		
135	67	121	30			3082		
Mean Concentrations								
87.96429					14.08333	65.57447	40.625	25.85714

EXPOSURE ASSESSMENT ASSUMPTIONS

Toxicity Data

USEPA Oral Cancer Slope Factor	0.011	per mg/kg-day				
USEPA Oral Unit Risk	3.10E-04	per mg/L				
ACGIH TWA	20	ppm	72.1	mg/m3	20.59013	mg/kg-day
CALEPA REL	0.8	ppm	2.9	mg/m3	0.823605	mg/kg-day
CALEPA Oral Cancer Slope Factor	0.027	per mg/kg-day				
CALEPA Inhalation Cancer Slope Factor	0.027	per mg/kg-day				
CALEPA Acute REL	3	mg/m3				
CALEPA No Significant Risk Level	0.03	mg/day				
USEPA 1-day health advisory (child)	4	mg/L	0.4	mg/kg-day		
USEPA 10-day health advisory (child)	0.4	mg/L	0.04	mg/kg-day		

Chemical Data

	HSDB
MW	88.1
BP	101.1 C
MP	11.8 C
Density	1.0337
log Kow	-0.27
Water sol	miscible
Vapor pressure	37 mm hg at 25C 4932.927 Pa
Kp	0.000356

Potential Exposure Scenarios for 1,4-Dioxane

Scenarios	Breast milk	Drinking Water		Soil		Ambient Air	Fruit & Veg	Dairy	Meat	Fish	Cosmetics	Production Materials		Production Air
	Oral	Oral	Dermal	Oral	Dermal	Inhalation	Oral	Oral	Oral	Oral	Dermal	Oral	Dermal	Inhalation
Infant (0-1)	x	x	x			x								
Child (1-2) (2-3) (3-6)		x	x	x	x	x	x	x	x	x				
Adult (20-70)		x	x	x	x	x	x	x	x	x	x			
Worker (20-65)												x	x	x

FUGACITY MODEL - Environmental

Compartment			Air	Water	Soil	Sediment	Susp. Sed	Fish	aerosol
		_1		_2	_3	_4	_5	_6	_7
volume	volume	1.00E+16		2.00E+13	9.00E+11	1.00E+10	1.00E+08	2.00E+07	2.00E+05
depth (m)	depth	1000		20	0.1	0.01			
Area (m^2)	Area	1.00E+13		1.00E+12	9.00E+12	1.00E+12			
Fraction OC	OrgCarb				0.02	0.04	0.2		
Density (kg/m^3)	Density	1.185		1000	2400	2400	1500	1000	2000
Advection residence time (hours)	restime	100		1000		50000			
	Hlife	6.7E+00		1.0E+11	1.0E+11	1.00E+11	1.0E+11	1.0E+11	6.7E+00
U.S. Surface Area	1.0E+13		m2						

FUGACITY MODEL - Chemical

WatSol	1000000	g/m^3			
MolWgt	88.1	g/mol			
VapPress	4.93E+03	Pa			
LKow	-0.27				
Lipid	0.05				
MeltPt	11.8	C			
DataTemp	25	TempK298.15			
GASCNST	8.314				
Density(1)	1.1854132				
EmissRateK	8.109E+01	kg/hrProduction	1.57E+06	lb/year	
		Fraction Released	1		
AdvInflowConcNG_1	0				
AdvInflowConcNG_2	0				

FUGACITY MODEL RESULTS

Media Unit	Air ng/m^3	Water ng/L	Soil ng/g	Sediment ng/g	Susp. Sed ng/g	Fish ng/g	Aerosol ng/m$_3$
Concentration	6.5E-02	3.7E-01	1.6E-06	3.3E-06	1.6E-05	9.9E-06	1.6E-09
%	8.0561	91.8992	0.0437	0.0010	0.0000	0.0000	0.0000

SUMMARY OF EPCs FOR A WORKER
PLANT

	Drinking Water (mg/L)		Soil		Cosmetics		Production Material (mg/L)		Air (mg/m3)		Food	
Parameter	MLE	RME	MLE	RME	MLE	RME	MLE	RME	MLE	RME	MLE	RME
Dioxane, 1,4-	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00	1.0E+00

SUMMARY OF CTVs FOR A WORKER
PLANT

Chemical	Surrogate	WOE	ORfDs	ORfDc	OSF	IRfDs	IRfDc	ISF	AFo	DRfDs	DRfDc	DSF	ABS	Kp
Dioxane, 1,4-	Dioxane, 1,4-	B2	0.04	0.004	0.011	0.82	0.82	0.011	1	0.04	0.004	0.011	0.1	0.000356

SUMMARY OF RBAs FOR A WORKER
PLANT

		ORfDs		ORfDc		OSF		IRfDs		IRfDc		ISF	
Chemical	Surrogate	Aqueous	Solid	Aqueous	Solid	Aqueous	Solid	Vapor	Particulate	Vapor	Particulate	Vapor	Particulate
Dioxane, 1,4-	Dioxane, 1,4-	1	1	1	1	1	1	1	1	1	1	1	1

SUMMARY OF EPVs FOR A WORKER
PLANT

Pathway	Complete	ADD/LADD Equation
Groundwater (ingestion)	FALSE	ADD/LADD = (C*Igw*EF*ED*ADJgw)/(BW*ATn/c)
(dermal contact)	FALSE	ADD/LADD = (C*Kp*SA*EF*ED*ETb*ADJsa*0.001 L/cm3)/(BW*ATn/c)
Soil (ingestion)	FALSE	ADD/LADD = (C*Iso*EF*ED*ADJso*0.000001 kg/mg)/(BW*ATn/c)
(inhalation)	FALSE	ADD/LADD = (C*Ia*EF*ED*ETs*ADJa*1 d/24 hr)/(BW*ATn/c*PEF)
(dermal contact)	FALSE	ADD/LADD = (C*AF*ABS*SA*Fso*EF*ED*ADJdso*0.000001 kg/mg)/(BW*ATn/c)
Cosmetics (ingestion)	FALSE	ADD/LADD = (C*Isd*EF*ED*ADJsd*0.000001 kg/mg)/(BW*ATn/c)
(dermal contact)	FALSE	ADD/LADD = (C*AF*ABS*SA*Fsd*EF*ED*ADJdsd*0.000001 kg/mg)/(BW*ATn/c)
Production Material (ingestion)	TRUE	ADD/LADD = (C*Isw*EF*ED*ETs*ADJsw)/(BW*ATn/c)
(dermal contact)	TRUE	ADD/LADD = (C*Kp*SA*Fsw*EF*ED*ETs*ADJdsw*0.001 L/cm3)/(BW*ATn/c)
Air (inhalation)	TRUE	ADD/LADD = (C*Ia*EF*ED*ETs*ADJa* 1d/24 hr)/(BW*ATn/c)
Food (ingestion)	FALSE	ADD/LADD = (C*Iot*EF*ED*ADJot*0.000001 kg/mg)/(BW*ATn/c)
(inhalation)	FALSE	ADD/LADD = (C*Ia*EF*ED*ETs*ADJa*1 d/24 hr)/(BW*ATn/c*PEF)
(dermal contact)	FALSE	ADD/LADD = (C*AF*ABS*SA*Fot*EF*ED*ADJdot*0.000001 kg/mg)/(BW*ATn/c)

[C=concentration; ABS = chemical-specific absorption; Kp = chemical-specific absorption]

Population name		Adult MLE	Adult RME
Body weight (kg)	BW	70	70
Averaging time, noncancer (d)	ATn	18250	18250
Averaging time, cancer (d)	ATc	25550	25550
Exposure time at Site (hr/d)	ETs	24	24
Exposure time for bathing (hr)	ETb	0	0
Exposure frequency (d/y)	EF	365	365
Exposure duration, (y)	ED	1	1
Particulate emission factor (m3/kg)	PEF	0.0E+00	0.0E+00
Adherence factor (mg/cm2)	AF	0	0
Soil ingestion (mg/d)	Iso	0	0
Soil adjustment factor	ADJso	0	0
Groundwater ingestion (L/d)	Igw	0	0
Groundwater adjustment factor	ADJgw	0	0
Production material ingestion (L/hr)	Isw	0	0
Production material adjustment factor	ADJsw	0	0
Sediment ingestion (mg/day)	Isd	0	0
Sediment adjustment factor	ADJsd	0	0
Food ingestion (mg/d)	Iot	0.00E+0 0	0.00E+0 0

Food adjustment factor	ADJot	0	0
Inhalation rate (m3/d)	Ia	15	20
Inhalation adjustment factor	ADJa	1	1
Total skin surface area (cm2)	SA	18150	18150
Total skin adjustment factor	ADJgw	1	1
Skin Fraction (soil)	Fso	0	0
Skin fraction (soil) adjustment factor	ADJdso	0	0
Skin fraction (SW)	Fsw	0	0
Skin fraction (SW) adjustment factor	ADJdsw	0	0
Skin fraction (Production Materials)	Fsd	0.05	0.1
Skin fraction (Production Material) adjustment factor	ADJdsd	1	1
Skin fraction (other)	Fot	0	0
Skin fraction (other) adjustment factor	ADJdot	0	0
	CONDITIONAL PEF	1.0E+00	1.0E+00
Noncancer = (EF x ED) / (BW x ATn)		0.0003	0.0003
Cancer = (EF x ED) / (BW x ATc)		0.0002	0.0002

[a]Age-adjusted intakes were calculated as: (I1*ED1)/BW1 + (I2*ED2)/BW2

SUMMARY OF ADDs FOR A WORKER
PLANT

Chemical	Groundwater Oral	Groundwater Dermal	Surface Soil Oral	Surface Soil Inhalation	Surface Soil Dermal	Sediment Oral	Sediment Dermal	Surface Water Oral	Surface Water Dermal	Air Inhalation	Total Soil Oral	Total Soil Inhalation	Total Soil Dermal	Chemical-Specific Subtotal
MLE ADDs (mg/kg-day)														
DIoxane, 1,4-										4.3E-03				4.3E-03
Pathway-Specific Subtotal										4.3E-03				**4E-03**
% of Total										100.0%				100.0%
RME ADDs (mg/kg-day)														
DIoxane, 1,4-										5.7E-03				5.7E-03
Pathway-Specific Subtotal										5.7E-03				**6E-03**
% of Total										100.0%				100.0%

SUMMARY OF LADDs FOR A WORKER
PLANT

Chemical	Groundwater Oral	Groundwater Dermal	Surface Soil Oral	Surface Soil Inhalation	Surface Soil Dermal	Sediment Oral	Sediment Dermal	Surface Water Oral	Surface Water Dermal	Air Inhalation	Total Soil Oral	Total Soil Inhalation	Total Soil Dermal	Chemical-Specific Subtotal
MLE LADDs (mg/kg-day)														
Dioxane, 1,4-										3.1E-03				3.1E-03
Pathway-Specific Subtotal										3.1E-03				**3E-03**
% of Total										100.0%				100.0%
RME LADDs (mg/kg-day)														
Dioxane, 1,4-										4.1E-03				4.1E-03
Pathway-Specific Subtotal										4.1E-03				**4E-03**
% of Total										100.0%				100.0%

SUMMARY OF HIs FOR A WORKER
PLANT

Chemical	Groundwater Oral	Groundwater Dermal	Surface Soil Oral	Surface Soil Inhalation	Surface Soil Dermal	Sediment Oral	Sediment Dermal	Surface Water Oral	Surface Water Dermal	Air Inhalation	Total Soil Oral	Total Soil Inhalation	Total Soil Dermal	Chemical-Specific Subtotal	% of Total
MLE HI Estimates															
Dioxane, 1,4-										5.2E-03				5.2E-03	100.0%
Pathway-Specific Subtotal										5.2E-03				**5E-03**	100.0%
% of Total										100.0%				100.0%	
RME HI Estimates															
Dioxane, 1,4-										7.0E-03				7.0E-03	100.0%
Pathway-Specific Subtotal										7.0E-03				**7E-03**	100.0%
% of Total										100.0%				100.0%	

SUMMARY OF RISK FOR A WORKER
PLANT

Chemical	Groundwater Oral	Groundwater Dermal	Surface Soil Oral	Surface Soil Inhalation	Surface Soil Dermal	Sediment Oral	Sediment Dermal	Surface Water Oral	Surface Water Dermal	Air Inhalation	Total Soil Oral	Total Soil Inhalation	Total Soil Dermal	Chemical-Specific Subtotal	% of Total
MLE Risk Estimates															
Dioxane, 1,4-										3.4E-05				3.4E-05	100.0%
Pathway-Specific Subtotal										3.4E-05				**3E-05**	100.0%
% of Total										100.0%				100.0%	
RME Risk Estimates															
Dioxane, 1,4-										4.5E-05				4.5E-05	100.0%
Pathway-Specific Subtotal										4.5E-05				**4E-05**	100.0%
% of Total										100.0%				100.0%	

MONTE CARLO CALCULATIONS

RECOMMENDED VALUES

Exposure Factor	Units	Lower Bound	Mean	Upper Bound
Breast milk intake (1-3 months)	**mL/day**	414	**703**	**992**
Breast milk intake (3-6 months)	**mL/day**	517	**761**	**1005**
Breast milk intake (6-12 months)	**mL/day**	159	**584**	**1009**
Breast milk intake (1-12 months)	**mL/day**	311	**642**	**973**
Drinking water intake (<1 yr)	**L/day**	0.25	**0.5**	**1.3**
Drinking water intake (1-2 yr)	L/day	0.1515	0.303	0.842
Drinking water intake (2-3 yr)	L/day	0.1755	0.351	0.879
Drinking water intake (3-6 yr)	L/day	0.2045	0.409	1.078
Drinking water intake (6-11 yr)	L/day	0.2375	0.475	1.237
Drinking water intake (11-16 yr)	L/day	0.328	0.656	1.619
Drinking water intake (16-21 yr)	L/day	0.4095	0.819	2.299
Drinking water intake (1-21 yr)	**L/day**	0.348667	**0.7**	**1.8**
Total fruit intake (<1 yr)	**g/kg-day**	8	**16**	**40**
Total fruit intake (1-2 yr)	g/kg-day	10	20	69
Total fruit intake (2-3 yr)	g/kg-day	9	18	59
Total fruit intake (3-6 yr)	g/kg-day	5.5	11	33
Total fruit intake (6-11 yr)	g/kg-day	2.85	5.7	19
Total fruit intake (11-16 yr)	g/kg-day	1.7	3.4	13
Total fruit intake (16-21 yr)	g/kg-day	2.3	5.6	8.9
Total fruit intake (1-21 yr)	**g/kg-day**	4.957143	**9.9**	**30.2**
Total vegetable intake (<1 yr)	**g/kg-day**	4.2	**8.4**	**23.3**
Total vegetable intake (1-2 yr)	g/kg-day	4.8	9.6	21
Total vegetable intake (2-3 yr)	g/kg-day	4.7	9.4	26
Total vegetable intake (3-6 yr)	g/kg-day	3.65	7.3	18
Total vegetable intake (6-11 yr)	g/kg-day	2.75	5.5	14
Total vegetable intake (11-16 yr)	g/kg-day	2.1	4.2	9.8
Total vegetable intake (16-21 yr)	g/kg-day	1.8	3.6	12
Total vegetable intake (1-21 yr)	**g/kg-day**	3.5	**7.0**	**18.1**
Total meat intake (<1 yr)	**g/kg-day**	0.65	**1.3**	**5**
Total meat intake (1-2 yr)	g/kg-day	2.1	4.2	10
Total meat intake (2-3 yr)	g/kg-day	2.3	4.6	11
Total meat intake (3-6 yr)	g/kg-day	2.05	4.1	9.4
Total meat intake (6-11 yr)	g/kg-day	1.9	3	4.1
Total meat intake (11-16 yr)	g/kg-day	1.15	2.3	5.2

Exposure Factor	Units	Lower Bound	Mean	Upper Bound
Total meat intake (16-21 yr)	g/kg-day	1.05	2.1	4.4
Total meat intake (1-21 yr)	**g/kg-day**	1.866667	**3.7**	**7.7**
Total dairy intake (<1 yr)	**g/kg-day**	6.9	**125.1**	**243.3**
Total dairy intake (1-2 yr)	g/kg-day	19	38	91
Total dairy intake (2-3 yr)	g/kg-day	18	36	97
Total dairy intake (3-6 yr)	g/kg-day	10.5	21	49
Total dairy intake (6-11 yr)	g/kg-day	7.5	15	35
Total dairy intake (11-16 yr)	g/kg-day	3.85	7.7	20
Total dairy intake (16-21 yr)	g/kg-day	2.8	5.6	16
Total dairy intake (1-21 yr)	**g/kg-day**	9.566667	**19.1**	**47.5**
Total grain intake (<1 yr)	**g/kg-day**	2.5	**5**	**16**
Total grain intake (1-2 yr)	g/kg-day	4.5	9	24
Total grain intake (2-3 yr)	g/kg-day	1	13	25
Total grain intake (3-6 yr)	g/kg-day	5	10	21
Total grain intake (6-11 yr)	g/kg-day	3.75	7.5	16
Total grain intake (11-16 yr)	g/kg-day	2.5	5	11
Total grain intake (16-21 yr)	g/kg-day	2.3	5.6	8.9
Total grain intake (1-21 yr)	**g/kg-day**	4.585714	**9.2**	**18.9**
Fish intake (<1 yr)	**g/kg-day**	0.05	**0.1**	**0.5**
Fish intake (1-2 yr)	g/kg-day	0.175	0.35	2
Fish intake (2-3 yr)	g/kg-day	0.195	0.39	1.6
Fish intake (3-6 yr)	g/kg-day	0.16	0.32	1.7
Fish intake (6-11 yr)	g/kg-day	0.135	0.27	1.6
Fish intake (11-16 yr)	g/kg-day	0.11	0.22	1.2
Fish intake (16-21 yr)	g/kg-day	0.095	0.19	0.7
Fish intake (1-21 yr)	**g/kg-day**	0.162857	**0.3**	**1.7**
Total Food Intake (<1 yr)	**g/kg-day**	77.95	**155.9**	**328.1**
Total Food Intake (1-21 yr)	**g/kg-day**	24.63905	**49.3**	**124.1**
Total Food - (Meat+Fish)	**g/kg-day**	22.60952	**45.219**	**114.7429**

Exposure Factor	Units	Lower Bound	Mean	Upper Bound				
Soil ingestion	mg/day	45	90	236				
						0-1 years		
Inhalation (<1 yr)	m3/day	4.615	8.64	12.665		6.5775	11.02	15.4625
Inhalation (1-2 yr)	m3/day	8.54	13.4	18.26			1-2 years	
Inhalation (2-3 yr)	m3/day	8.935	12.985	17.035		8.7375	13.1925	17.6475
Inhalation (3-6 yr)	m3/day	9.64	12.405	15.17			2-3 years	
Inhalation (6-11 yr)	m3/day	8.795	12.915	17.035		12.93	9.0383333 3	16.82167
Inhalation (11-16 yr)	m3/day	9.45	14.38	19.3			16-21 years	
Inhalation (16-21 yr)	m3/day	9.97	15.4	20.83		14.89	9.6	17.8
Inhalation (1-21 yr)	**m3/day**	11.5619	**17.1**	**22.6**				

			Male	Female	MF	m2/kg	
Surface area (0-2 yr)	m2/kg				0.064		
Surface area (0-2 yr)	m2				0.47		1-11 years
Surface area (1-2 yr)	m2		0.5	0.5		0.037313	0.029314
Surface area (2-3 yr)	m2		0.6	0.6		0.034483	
Surface area (3-6 yr)	m2		0.7	0.7		0.024306	11-21 years
Surface area (6-11 yr)	m2		1.1	1.1		0.021154	0.040967
Surface area (11-16 yr)	m2		1.6	1.5		0.024181	
Surface area (16-21 yr)	m2		1.9	1.7		0.057754	
Surface area (1-21 yr)	**m2**		**1.1**	**1.0**	**1.0**		

Soil adherence (indoor children 1-13 years)	mg/cm2	0.01	0.01	0.01	
Soil adherence in daycare children [indoor and outdoor play] (1-6.5 years)	mg/cm3	0.04	0.04	0.04	
Soil adherence in children playing in dry soil (8 -12 years)	mg/cm4	0.04	0.04	0.04	
Soil adherence in children playing in wet soil (8 -12 years)	mg/cm5	0.2	0.2	0.2	
Soil adherence in children in mud (9 -14 years)	mg/cm6	21	21	21	

		50th	5th	95th	Table 11-8 CEFH 2006
Body weight (<1 yr)	**kg**	**7.4**	4.8	11.2	
Body weight (1-2 yr)	kg	11.3	9.1	14	
Body weight (2-3 yr)	kg	13.4	10.9	16.3	

Exposure Factor	Units	Lower Bound	Mean	Upper Bound	
Body weight (3-5 yr)	kg		17.4	13.5	24.5
Body weight (6-11 yr)	kg		28.8	19.2	49.2
Body weight (11-16 yr)	kg		52	34	81.4
Body weight (16-21 yr)	kg		64.1	48	98.3
Body weight (1-19 yr)	**kg**		**31.2**		

Showering/Bathing	Showering min/day	Bathing Mean (min/day)
birth to <1 year	1	19
1 to <2 years	20	23
2 to < 3 years	22	23
3 to < 6 years	17	24
6 to <11 years	18	24
11 to <16 years	18	25
16 to < 21 years	20	33

Swimming	min/month
birth to <1 year	313
1 to <2 years	251
2 to < 3 years	636
3 to < 6 years	946
6 to <11 years	868
11 to <16 years	667
16 to < 21 years	868

Time indoors	Mean min/day	hours/day
0 to <1 month	1440	24.00
0 to <1 month	1431	23.85
1 to <3 months	1414	23.57
3 to < 6 months	1301	21.68
6 to < 12 months	1132	18.87
1 to <2 years	1112	18.53
2 to < 3 years	1128	18.80
3 to < 6 years	1164	19.40
6 to <11 years	1260	21.00
11 to <16 years	1249	20.82
16 to < 21 years		

PREGNANT WORKER (FETUS)

	ADD (mg/kg-day)			LADD (mg/kg-day)		
Media	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal
Water	4.3E-05		4.5E-09			
Lotion			3.3E-05			
Product/Raw Material			9.3E-02			
Air		1.2E-02				
Food	9.9E-05					
Route Subtotal	1.4E-04	1.2E-02	9.3E-02			
Total	1.0E-01					

Media Concentrations	Water (mg/L)	Cw	0.002	0.0005	2							
	Lotion (mg/L)	Cl	10	0	500							
	Product/Raw Material (mg/L)	Cm	400000	50000	1000000	Professional Judgement						
	Workplace Air (mg/m3)	Cwa	0.54	0	47	Professional Judgement						
	Food (mg/kg)	Cf	0.005			Dioxane concentration (mg/kg) in additive			Additive content of food			
General Parameters	Population name		Pregnant Worker			5	0	10	0.001	0.00005	0.05	
	Body Weight (kg)	BW	60.5	55	66							
	Averaging Time, noncancer (d)	ATn	273.75									
	Averaging Time, cancer (d)	ATc	NA									
	Exposure Time (hr)	ET	8									
	Exposure Time for Bathing (hr)	ETb	0.17	0.08	0.33	EFH (1997)						
	Exposure frequency (d/y)	EF	365									
	Exposure duration, (y)	ED	0.75			9-month gestation period						

Intakes	Water ingestion (L/d)	IW	1.31	0.43	2.19	lactating women mean, 5th, 95th (EFH 1997)			
	Water reduction factor (unitless)	RFw	1			Food intakes (g/kg-day) (EFH, 1997)			
	Food ingestion (mg/d)	IF	1197900			19.8	9.9	62.5	
	Food reduction factor (unitless)	RFf	1						
	Inhalation rate (m3/d)	IA	11.3	9.6	20	EFH (1997)			
	Inhalation reduction factor (unitless)	RFa	0.342	0	0.685	adjusted for relative absorption (0.5,0,1) and EF of 250/365			
	Total skin surface area (cm2)	SA	18573.5	Calculated from body weight using conversion (307 cm2/kg = 16900 cm2/55 kg) obtained from EFH, 1997					
	Total skin reduction factor (unitless)	AF	1						
	Lotion Skin fraction	Fl	1						
	Lotion adjustment factor	AFl	0.031	0	0.125	adjusted for ET (0.25, 0, 1) less than 8 hours			
	Raw material/product Skin fraction	Fm	0.051	0.044	0.054	% for hands only (EFH, 1997)			
	Raw material adjustment factor	AFm	0.043	0	0.170	adjusted for ET (0, 0.5, 2) less than 8 hours, and EF of 250/365			
Chemical-Specific	Permeability Coefficient (cm/hr)	Kp	0.000043	0.0000215	0.00043	Bronough, 1982; max=occluded, mean=occluded/10, min=occluded/20			

Infant (0-1)

	ADD (mg/kg-day)			LADD (mg/kg-day)			
Media	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	
Breastmilk	3.4E-04			4.9E-06			
Water			6.4E-09			9.2E-11	
Lotion			3.8E-04			5.4E-06	
Air		1.5E-04			2.2E-06		
Food	7.7E-04			1.1E-05			
Route Subtotal	1.1E-03	1.5E-04	3.8E-04	1.6E-05	2.2E-06	5.4E-06	
Total	1.6E-03			2.3E-05			
Media Concentrations		Breast Milk (mg Dx/kg Dx)	Cbm	1.00E+06			
		Water (mg/L)	Cw	2.00E-03			
		Lotion (mg/L)	Cl	1.00E+01			
		Ambient Air (mg/m3)	Caa	0.00026	0	0.005	
		Food (mg/kg)	Cf	0.005			
General Parameters		Population name		Infant			
		Body Weight (kg)	BW	7.4	4.8	11.2	CEFH, 2006
		Averaging Time, noncancer (d)	ATn	365			
		Averaging Time, cancer (d)	ATc	25550			
		Exposure Time (hr)	ET	24			
		Exposure Time for Bathing (hr)	ETb	0.17			
		Exposure frequency (d/y)	EF	365			
		Exposure duration, (y)	ED	1			

Intakes	Breast milk Dioxane Intake Calculated from Mothers Inhalation exposure (mg/d)	IBM	0.00336			Calculated from mothers inhalation exposure		
	Breastmilk adjustment factor (variation in intake from Fisher et al., 1997) (unitless)	AFbm	0.75	0.43	1.1	Food intakes (g/kg-day) (CEFH, 2006)		
	Food (mg/d)	IF	1143300			154.5	77.25	322.6
	Food adjustment factor (unitless)	AFf	1					
	Inhalation rate (m3/d)	IA	8.6	4.6	12.7	CEFH, 2006		
	Inhalation reduction factor (unitless)	AFa	0.5	0	1	relative absorption		
	Total skin surface area (cm2)	SA	3.3E+03			Calculated from body weight using conversion (440 cm2/kg) obtained from CEFH, 1999		
	Total skin reduction factor (unitless)	AF	1					
	Lotion Skin fraction	Fl	1					
	Lotion Adjustment factor	AFl	0.083	0	0.25	Adjusting for exposure times (0,2,6hrs) less than 24 hours		
Chemical-Specific	Permeability Coefficient (cm/hr)	Kp	0.000043					

CHILD (1-2)

	ADD (mg/kg-day)			LADD (mg/kg-day)			
Media	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	
Water	5.4E-05		5.6E-09	7.7E-07		7.9E-11	
Lotion			4.1E-05			5.8E-07	
Air		1.5E-04			2.2E-06		
Food	3.8E-04			5.5E-06			
Route Subtotal	4.4E-04	1.5E-04	4.1E-05	6.2E-06	2.2E-06	5.8E-07	
Total	6.3E-04			9.0E-06			
Media Concentrations		Water (mg/L)	Cw	0.002			
		Lotion (mg/L)	Cl	10			
		Ambient Air (mg/m3)	Caa	0.00026			
		Food (mg/kg)	Cf	0.005			
General Parameters		Population name		Child			
		Body Weight (kg)	BW	11.3	9.1	14	CEFH, 2006
		Averaging Time, noncancer (d)	ATn	365			
		Averaging Time, cancer (d)	ATc	25550			
		Exposure Time (hr)	ET	24			
		Exposure Time for Bathing (hr)	ETb	0.17			
		Exposure frequency (d/y)	EF	365			
		Exposure duration, (y)	ED	1			

Intakes	Groundwater ingestion (L/d)	IW	0.303	0.15	0.84			
	Groundwater reduction factor (unitless)	AFw	1			Food intakes (g/kg-day) (CEFH, 2006)		
	Food (mg/d)	IF	865580			76.6	38.3	205.0
	Food reduction factor (unitless)	AFf	1					
	Inhalation rate (m3/d)	IA	13.4	8.5	18.3	CEFH, 2006		
	Inhalation reduction factor (unitless)	AFa	0.5					
	Total skin surface area (cm2)	SA	4294	Calculated from body weight using conversion (380 cm2/kg) obtained from CEFH, 1999				
	Total skin reduction factor (unitless)	AF	1					
	Lotion Skin fraction	Fl	1					
	Lotion skin fraction adjustment factor	AFl	0.0104	0	0.021	adjusted for ET (0,0.25,0.5hrs)less than 24 hours		
Chemical-Specific	Permeability Coefficient (cm/hr)	Kp	0.000043					

CHILD (2-3)

	ADD (mg/kg-day)			LADD (mg/kg-day)			
Media	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	
Water	5.2E-05		5.6E-09	7.5E-07		7.9E-11	
Lotion			4.1E-05			5.8E-07	
Air		1.3E-04			1.8E-06		
Food	3.8E-04			5.5E-06			
Route Subtotal	4.3E-04	1.3E-04	4.1E-05	6.2E-06	1.8E-06	5.8E-07	
Total	6.0E-04			8.6E-06			
Media Concentrations		Water (mg/L)	Cw	0.002			
		Lotion (mg/L)	Cl	10			
		Ambient Air (mg/m3)	Caa	0.00026			
		Food (mg/kg)	Cf	0.005			
General Parameters		Population name		Child			
		Body Weight (kg)	BW	13.4	10.9	16.3	CEFH, 2006
		Averaging Time, noncancer (d)	ATn	365			
		Averaging Time, cancer (d)	ATc	25550			
		Exposure Time (hr)	ET	24			
		Exposure Time for Bathing (hr)	ETb	0.17			
		Exposure frequency (d/y)	EF	365			
		Exposure duration, (y)	ED	1			

Intakes	Groundwater ingestion (L/d)	IW	0.35	0.18	0.88			
	Groundwater reduction factor (unitless)	AFw	1			Food intakes (g/kg-day) (CEFH, 2006)		
	Food (mg/d)	IF	1023760			76.4	38.2	207.0
	Food reduction factor (unitless)	AFf	1					
	Inhalation rate (m3/d)	IA	12.9	8.9	17.0	CEFH, 2006		
	Inhalation reduction factor (unitless)	AFa	0.5					
	Total skin surface area (cm2)	SA	5092	Calculated from body weight using conversion (380 cm2/kg) obtained from CEFH, 2006				
	Total skin reduction factor (unitless)	AF	1					
	Lotion Skin fraction	Fl	1					
	Lotion skin fraction adjustment factor	AFl	0.0104	0	0.021	adjusted for ET (0,0.25,0.5hrs)less than 24 hours		
Chemical-Specific	Permeability Coefficient (cm/hr)	Kp	0.000043					

CHILD (3-6)

	ADD (mg/kg-day)			LADD (mg/kg-day)			
Media	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	
Water	4.7E-05		5.6E-09	2.0E-06		2.4E-10	
Lotion			4.1E-05			1.7E-06	
Air		9.3E-05			4.0E-06		
Food	2.5E-04			1.1E-05			
Route Subtotal	2.9E-04	9.3E-05	4.1E-05	1.3E-05	4.0E-06	1.7E-06	
Total	4.3E-04			1.8E-05			
Media Concentrations		Water (mg/L)	Cw	0.002			
		Lotion (mg/L)	Cl	10			
		Ambient Air (mg/m3)	Caa	0.00026			
		Food (mg/kg)	Cf	0.005			
General Parameters		Population name		Child			
		Body Weight (kg)	BW	17.4	13.5	24.5	CEFH, 2006
		Averaging Time, noncancer (d)	ATn	1095			
		Averaging Time, cancer (d)	ATc	25550			
		Exposure Time (hr)	ET	24			
		Exposure Time for Bathing (hr)	ETb	0.17			
		Exposure frequency (d/y)	EF	365			
		Exposure duration, (y)	ED	3			

Intakes	Groundwater ingestion (L/d)	IW	0.41	0.205	1.08			
	Groundwater reduction factor (unitless)	AFw	1			Food intakes (g/kg-day) (CEFH, 2006)		
	Food (mg/d)	IF	857820			49.3	24.7	121.0
	Food reduction factor (unitless)	AFf	1					
	Inhalation rate (m3/d)	IA	12.4	9.6	15.2	CEFH, 2006		
	Inhalation reduction factor (unitless)	AFa	0.5					
	Total skin surface area (cm2)	SA	6612	Calculated from body weight using conversion (380 cm2/kg) obtained from CEFH, 1999				
	Total skin reduction factor (unitless)	AF	1					
	Lotion Skin fraction	Fl	1					
	Lotion skin fraction adjustment factor	AFl	0.0104	0	0.021	adjusted for ET (0,0.25,0.5hrs)less than 24 hours		
Chemical-Specific	Permeability Coefficient (cm/hr)	Kp	0.000043					

CHILD (6-11)

	ADD (mg/kg-day)			LADD (mg/kg-day)			
Media	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	
Water	3.3E-05		5.6E-09	2.4E-06		4.0E-10	
Lotion			4.1E-05			2.9E-06	
Air		5.8E-05			4.2E-06		
Food	1.7E-04			1.2E-05			
Route Subtotal	2.0E-04	5.8E-05	4.1E-05	1.4E-05	4.2E-06	2.9E-06	
Total	3.0E-04			2.1E-05			
Media Concentrations		Water (mg/L)	Cw	0.002			
		Lotion (mg/L)	Cl	10			
		Ambient Air (mg/m3)	Caa	0.00026			
		Food (mg/kg)	Cf	0.005			
General Parameters		Population name		Child			
		Body Weight (kg)	BW	28.8	19.2	49.2	CEFH, 2006
		Averaging Time, noncancer (d)	ATn	1825			
		Averaging Time, cancer (d)	ATc	25550			
		Exposure Time (hr)	ET	24			
		Exposure Time for Bathing (hr)	ETb	0.17			
		Exposure frequency (d/y)	EF	365			
		Exposure duration, (y)	ED	5			

Intakes	Groundwater ingestion (L/d)	IW	0.48	0.24	1.2			
	Groundwater reduction factor (unitless)	AFw	1			Food intakes (g/kg-day) (CEFH, 2006)		
	Food (mg/d)	IF	970560			33.7	16.9	84.0
	Food reduction factor (unitless)	AFf	1					
	Inhalation rate (m3/d)	IA	12.9	8.8	17.0	CEFH, 2006		
	Inhalation reduction factor (unitless)	AFa	0.5					
	Total skin surface area (cm2)	SA	10944	Calculated from body weight using conversion (380 cm2/kg) obtained from CEFH, 1999				
	Total skin reduction factor (unitless)	AF	1					
	Lotion Skin fraction	Fl	1					
	Lotion skin fraction adjustment factor	AFl	0.0104	0	0.021	adjusted for ET (0,0.25,0.5hrs)less than 24 hours		
Chemical-Specific	Permeability Coefficient (cm/hr)	Kp	0.000043					

YOUTH (11-16)

	ADD (mg/kg-day)			LADD (mg/kg-day)			
Media	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	
Water	2.5E-05		4.1E-09	1.8E-06		2.9E-10	
Lotion			3.0E-05			2.1E-06	
Air		3.6E-05			2.6E-06		
Food	1.0E-04			7.3E-06			
Route Subtotal	1.3E-04	3.6E-05	3.0E-05	9.1E-06	2.6E-06	2.1E-06	
Total	1.9E-04			1.4E-05			
Media Concentrations		Water (mg/L)	Cw	0.002			
		Lotion (mg/L)	Cl	10			
		Ambient Air (mg/m3)	Caa	0.00026			
		Food (mg/kg)	Cf	0.005			
General Parameters		Population name		Youth			
		Body Weight (kg)	BW	52	34	81.4	CEFH, 2006
		Averaging Time, noncancer (d)	ATn	1825			
		Averaging Time, cancer (d)	ATc	25550			
		Exposure Time (hr)	ET	24			
		Exposure Time for Bathing (hr)	ETb	0.17			
		Exposure frequency (d/y)	EF	365			
		Exposure duration, (y)	ED	5			

Intakes	Groundwater ingestion (L/d)	IW	0.66	0.33	1.6	CEFH, 2006		
	Groundwater reduction factor (unitless)	AFw	1			Food intakes (g/kg-day) (CEFH, 2006)		
	Food (mg/d)	IF	1055600			20.3	10.2	53.8
	Food reduction factor (unitless)	AFf	1					
	Inhalation rate (m3/d)	IA	14.4	9.5	19.3	CEFH, 2006		
	Inhalation reduction factor (unitless)	AFa	0.5					
	Total skin surface area (cm2)	SA	14560	Calculated from body weight using conversion (280 cm2/kg) obtained from CEFH, 1999				
	Total skin reduction factor (unitless)	AF	1					
	Lotion skin fraction	Fl	1					
	Lotion skin fraction adjustment factor	AFl	0.0104					
Chemical-Specific	Permeability Coefficient (cm/hr)	Kp	0.000043					

YOUTH (16-21)

	ADD (mg/kg-day)			LADD (mg/kg-day)			
Media	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	
Water	2.6E-05		5.6E-09	1.8E-06		4.0E-10	
Lotion			4.1E-05			2.9E-06	
Air		3.1E-05			2.2E-06		
Food	1.0E-04			7.3E-06			
Route Subtotal	1.3E-04	3.1E-05	4.1E-05	9.1E-06	2.2E-06	2.9E-06	
Total	2.0E-04			1.4E-05			
Media Concentrations		Water (mg/L)	Cw	0.002			
		Lotion (mg/L)	Cl	10			
		Ambient Air (mg/m3)	Caa	0.00026			
		Food (mg/kg)	Cf	0.005			
General Parameters		Population name		Youth			
		Body Weight (kg)	BW	64.1	48	98.3	CEFH, 2006
		Averaging Time, noncancer (d)	ATn	1825			
		Averaging Time, cancer (d)	ATc	25550			
		Exposure Time (hr)	ET	24			
		Exposure Time for Bathing (hr)	ETb	0.17			
		Exposure frequency (d/y)	EF	365			
		Exposure duration, (y)	ED	5			

Intakes	Groundwater ingestion (L/d)	IW	0.82	0.41	2.3			
	Groundwater reduction factor (unitless)	AFw	1			Food intakes (g/kg-day) (CEFH, 2006)		
	Food (mg/d)	IF	1307640			20.4	10.2	45.8
	Food reduction factor (unitless)	AFf	1					
	Inhalation rate (m3/d)	IA	15.4	10.0	20.8			
	Inhalation reduction factor (unitless)	AFa	0.5					
	Total skin surface area (cm2)	SA	24358	Calculated from body weight using conversion (380 cm2/kg) obtained from CEFH, 1999				
	Total skin reduction factor (unitless)	AF	1					
	Lotion Skin fraction	Fl	1					
	Lotion skin fraction adjustment factor	AFl	0.0104	0	0.021	adjusted for ET (0,0.25,0.5hrs)less than 24 hours		
Chemical-Specific	Permeability Coefficient (cm/hr)	Kp	0.000043					

Crystal Ball Report
Simulation started on 2/15/07 at 11:16:39
Simulation stopped on 2/15/07 at 11:17:08

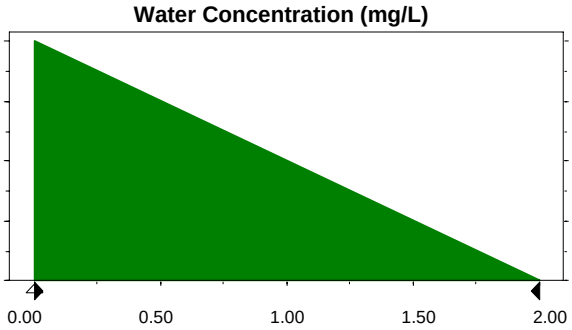
Assumptions

Assumption: Water Concentration (mg/L) **[Exposure_2006.XLS] Pregnant Worker (Fetus) - Cell: E10**

Triangular distribution with parameters:

Minimum	0.00	(=F10)
Likeliest	0.00	(=E10)
Maximum	2.00	(=G10)

Selected range is from 0.00 to 2.00
Mean value in simulation was 0.67

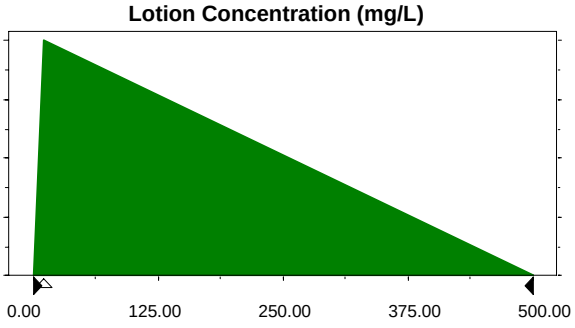


Assumption: Lotion Concentration (mg/L) **[Exposure_2006.XLS] Pregnant Worker (Fetus) - Cell: E11**

Triangular distribution with parameters:

Minimum	0.00	(=F11)
Likeliest	10.00	(=E11)
Maximum	500.00	(=G11)

Selected range is from 0.00 to 500.00
Mean value in simulation was 169.99

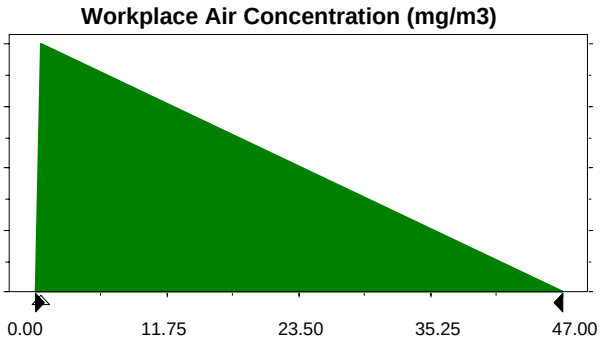


Assumption: Workplace Air Concentration (mg/m3) **[Exposure_2006.XLS] Pregnant Worker (Fetus) - Cell: E13**

Triangular distribution with parameters:

Minimum	0.00	(=F13)
Likeliest	0.54	(=E13)
Maximum	47.00	(=G13)

Selected range is from 0.00 to 47.00
Mean value in simulation was 15.85

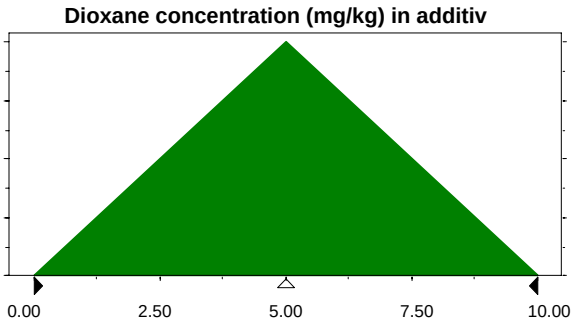


Assumption: Dioxane concentration (mg/kg) in additiv **[Exposure_2006.XLS] Pregnant Worker (Fetus) - Cell: H15**

Triangular distribution with parameters:

Minimum	0.00	(=I15)
Likeliest	5.00	(=H15)
Maximum	10.00	(=J15)

Selected range is from 0.00 to 10.00
Mean value in simulation was 5.00

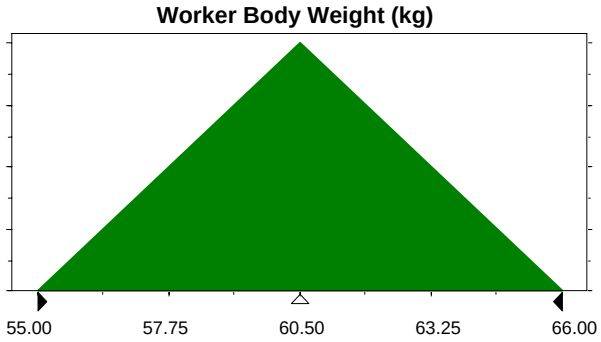


Assumption: Worker Body Weight (kg) **[Exposure_2006.XLS] Pregnant Worker (Fetus) - Cell: E16**

Triangular distribution with parameters:

Minimum	55.00	(=F16)
Likeliest	60.50	(=E16)
Maximum	66.00	(=G16)

Selected range is from 55.00 to 66.00
Mean value in simulation was 60.50

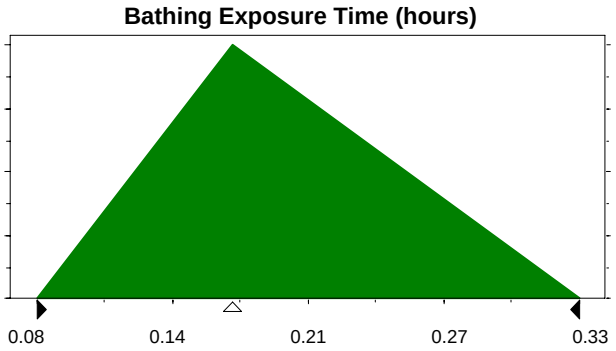


Assumption: Bathing Exposure Time (hours) **[Exposure_2006.XLS] Pregnant Worker (Fetus) - Cell: E20**

Triangular distribution with parameters:

Minimum	0.08	(=F20)
Likeliest	0.17	(=E20)
Maximum	0.33	(=G20)

Selected range is from 0.08 to 0.33
Mean value in simulation was 0.19

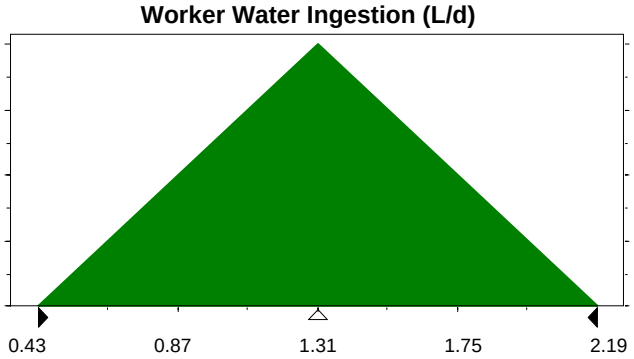


Assumption: Worker Water Ingestion (L/d) **[Exposure_2006.XLS] Pregnant Worker (Fetus) - Cell: E23**

Triangular distribution with parameters:

Minimum	0.43	(=F23)
Likeliest	1.31	(=E23)
Maximum	2.19	(=G23)

Selected range is from 0.43 to 2.19
Mean value in simulation was 1.31

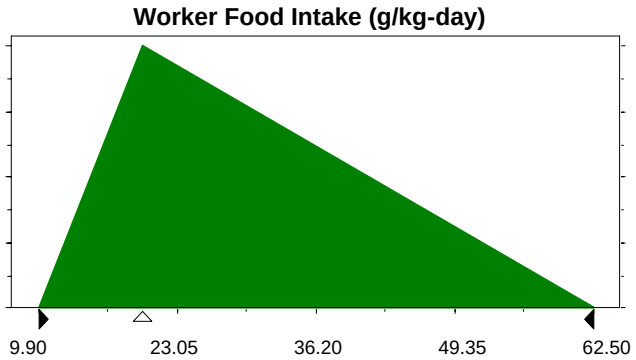


Assumption: Worker Food Intake (g/kg-day) **[Exposure_2006.XLS] Pregnant Worker (Fetus) - Cell: H25**

Triangular distribution with parameters:

Minimum	9.90	(=I25)
Likeliest	19.80	(=H25)
Maximum	62.50	(=J25)

Selected range is from 9.90 to 62.50
Mean value in simulation was 30.73

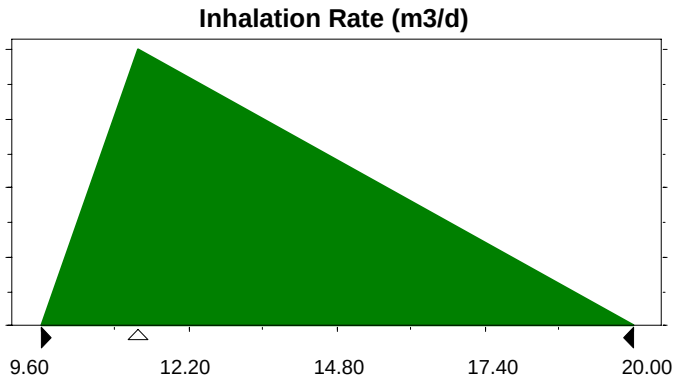


Assumption: Inhalation Rate (m3/d) **[Exposure_2006.XLS] Pregnant Worker (Fetus) - Cell: E27**

Triangular distribution with parameters:

Minimum	9.60	(=F27)
Likeliest	11.30	(=E27)
Maximum	20.00	(=G27)

Selected range is from 9.60 to 20.00
Mean value in simulation was 13.63

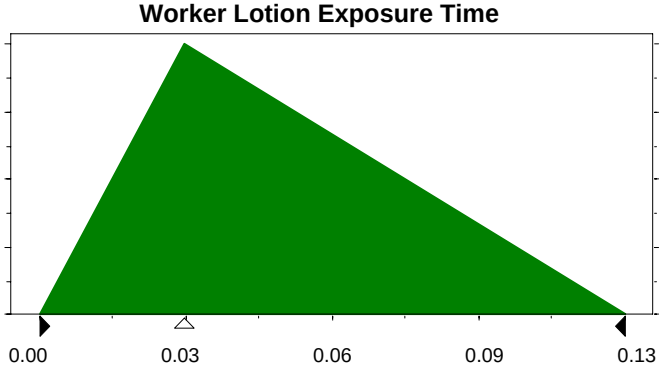


Assumption: Worker Lotion Exposure Time **[Exposure_2006.XLS] Pregnant Worker (Fetus) - Cell: E32**

Triangular distribution with parameters:

Minimum	0.00	(=F32)
Likeliest	0.03	(=E32)
Maximum	0.13	(=G32)

Selected range is from 0.00 to 0.13
Mean value in simulation was 0.05

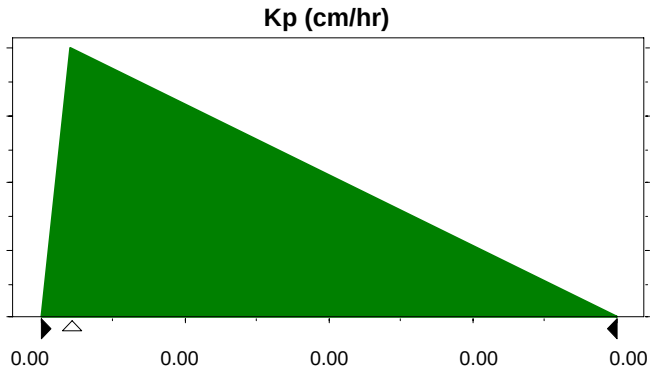


Assumption: Kp (cm/hr) **[Exposure_2006.XLS] Pregnant Worker (Fetus) - Cell: E35**

Triangular distribution with parameters:

Minimum	0.00	(=F35)
Likeliest	0.00	(=E35)
Maximum	0.00	(=G35)

Selected range is from 0.00 to 0.00
Mean value in simulation was 0.00

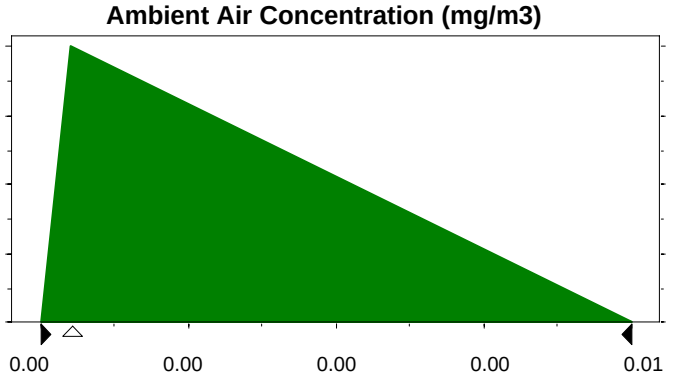


Assumption: Ambient Air Concentration (mg/m3) **[Exposure_2006.XLS] Infant (0-1) - Cell: E13**

Triangular distribution with parameters:

Minimum	0.00	(=F13)
Likeliest	0.00	(=E13)
Maximum	0.01	(=G13)

Selected range is from 0.00 to 0.01
Mean value in simulation was 0.00

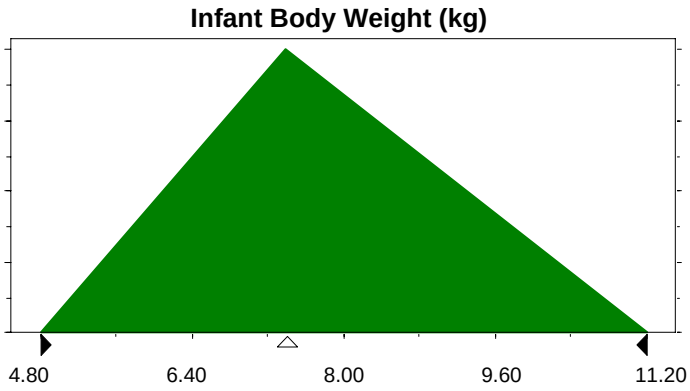


Assumption: Infant Body Weight (kg) **[Exposure_2006.XLS] Infant (0-1) - Cell: E16**

Triangular distribution with parameters:

Minimum	4.80	(=F16)
Likeliest	7.40	(=E16)
Maximum	11.20	(=G16)

Selected range is from 4.80 to 11.20
Mean value in simulation was 7.80

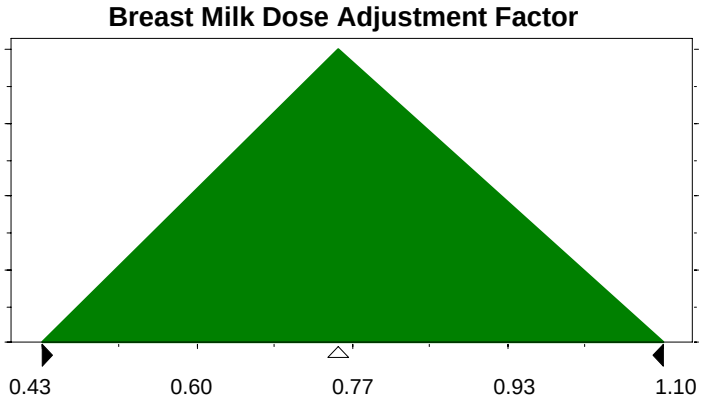


Assumption: Breast Milk Dose Adjustment Factor **[Exposure_2006.XLS] Infant (0-1) - Cell: E24**

Triangular distribution with parameters:

Minimum	0.43	(=F24)
Likeliest	0.75	(=E24)
Maximum	1.10	(=G24)

Selected range is from 0.43 to 1.10
Mean value in simulation was 0.76

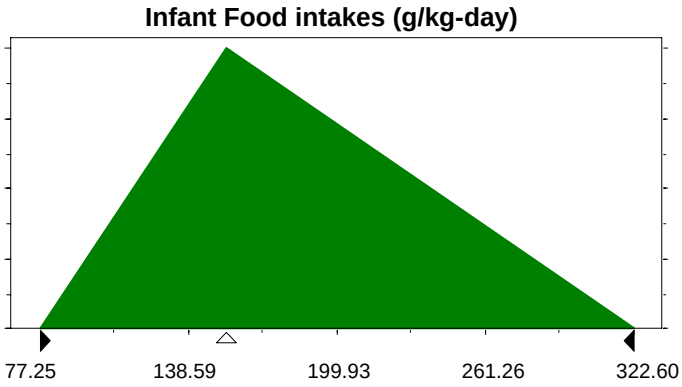


Assumption: Infant Food intakes (g/kg-day) **[Exposure_2006.XLS] Infant (0-1) - Cell: H25**

Triangular distribution with parameters:

Minimum	77.25	(=I25)
Likeliest	154.50	(=H25)
Maximum	322.60	(=J25)

Selected range is from 77.25 to 322.60
Mean value in simulation was 184.78

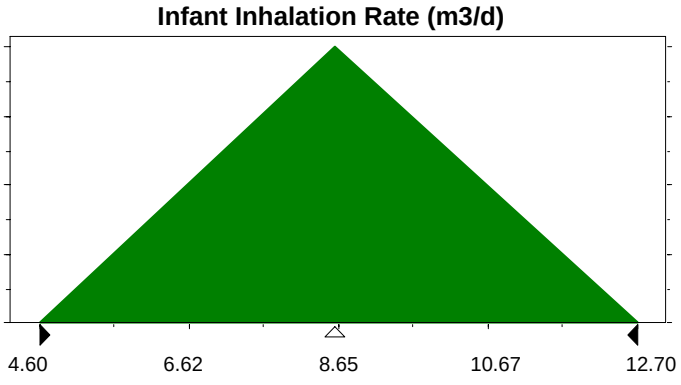


Assumption: Infant Inhalation Rate (m3/d) **[Exposure_2006.XLS] Infant (0-1) - Cell: E27**

Triangular distribution with parameters:

Minimum	4.60	(=F27)
Likeliest	8.60	(=E27)
Maximum	12.70	(=G27)

Selected range is from 4.60 to 12.70
Mean value in simulation was 8.63

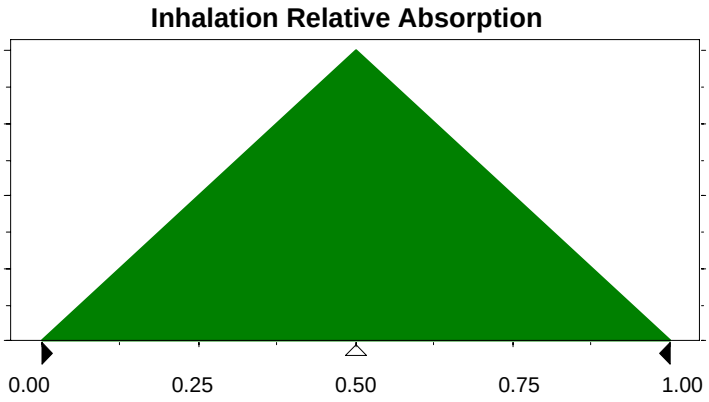


Assumption: Inhalation Relative Absorption **[Exposure_2006.XLS] Infant (0-1) - Cell: E28**

Triangular distribution with parameters:

Minimum	0.00	(=F28)
Likeliest	0.50	(=E28)
Maximum	1.00	(=G28)

Selected range is from 0.00 to 1.00
Mean value in simulation was 0.50

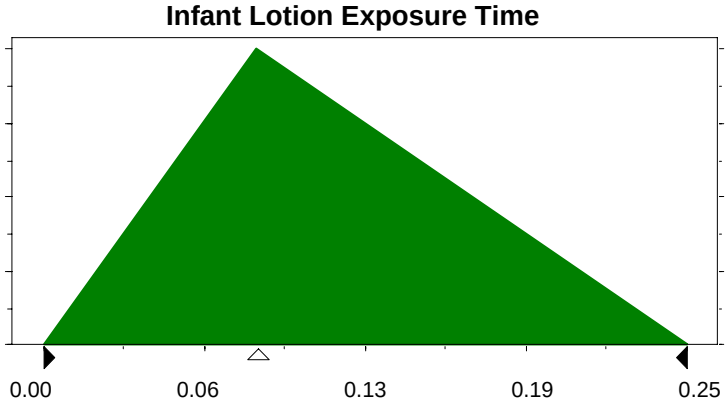


Assumption: Infant Lotion Exposure Time **[Exposure_2006.XLS] Infant (0-1) - Cell: E32**

Triangular distribution with parameters:

Minimum	0.00	(=F32)
Likeliest	0.08	(=E32)
Maximum	0.25	(=G32)

Selected range is from 0.00 to 0.25
Mean value in simulation was 0.11

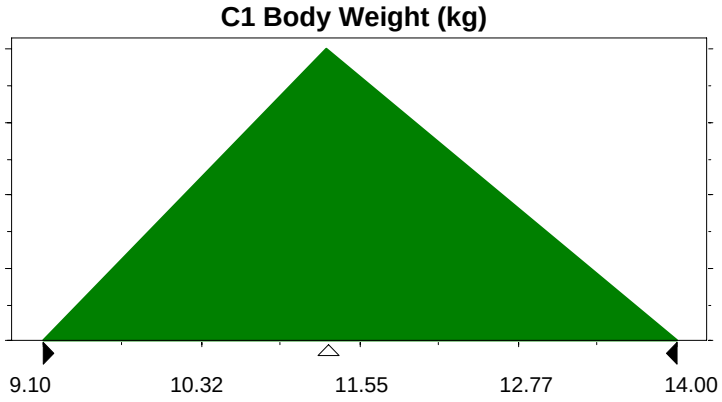


Assumption: C1 Body Weight (kg) **[Exposure_2006.XLS] Child (1-2) - Cell: E15**

Triangular distribution with parameters:

Minimum	9.10 (=F15)	
Likeliest	11.30	(=E15)
Maximum	14.00	(=G15)

Selected range is from 9.10 to 14.00
Mean value in simulation was 11.47

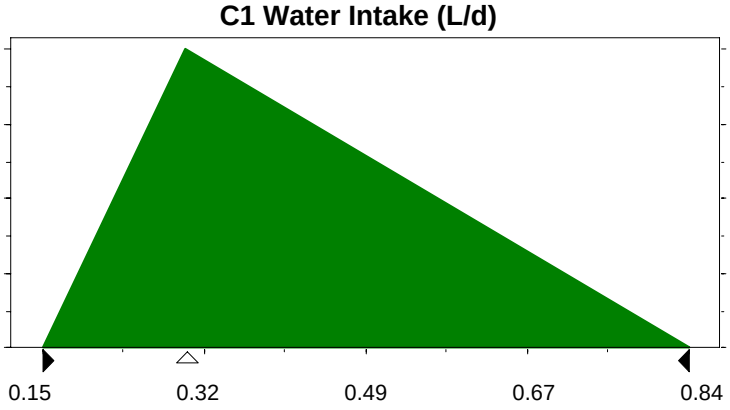


Assumption: C1 Water Intake (L/d) **[Exposure_2006.XLS] Child (1-2) - Cell: E22**

Triangular distribution with parameters:

Minimum	0.15 (=F22)	
Likeliest	0.30	(=E22)
Maximum	0.84	(=G22)

Selected range is from 0.15 to 0.84
Mean value in simulation was 0.43

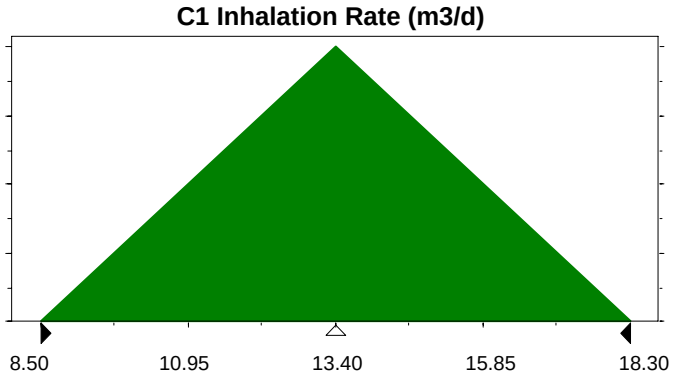


Assumption: C1 Inhalation Rate (m3/d) **[Exposure_2006.XLS] Child (1-2) - Cell: E26**

Triangular distribution with parameters:

Minimum	8.50	(=F26)
Likeliest	13.40	(=E26)
Maximum	18.30	(=G26)

Selected range is from 8.50 to 18.30
Mean value in simulation was 13.40

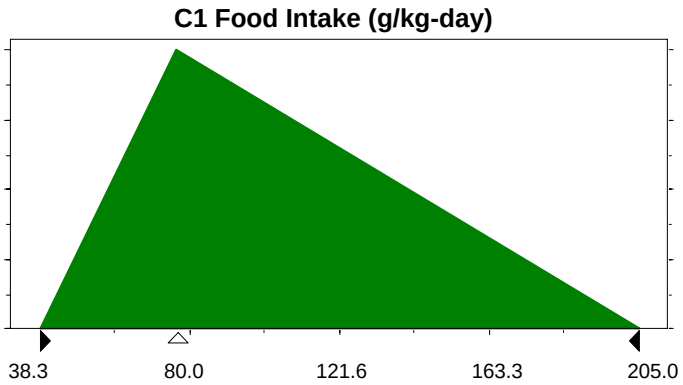


Assumption: C1 Food Intake (g/kg-day) **[Exposure_2006.XLS] Child (1-2) - Cell: H24**

Triangular distribution with parameters:

Minimum	38.3	(=I24)
Likeliest	76.6	(=H24)
Maximum	205.0	(=J24)

Selected range is from 38.3 to 205.0
Mean value in simulation was 106.6

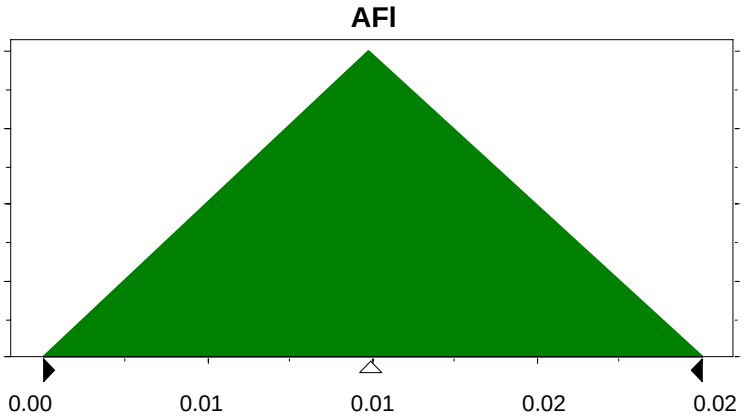


Assumption: AFI **[Exposure_2006.XLS] Child (1-2) - Cell: E31**

Triangular distribution with parameters:

Minimum	0.00	(=F31)
Likeliest	0.01	(=E31)
Maximum	0.02	(=G31)

Selected range is from 0.00 to 0.02
Mean value in simulation was 0.01

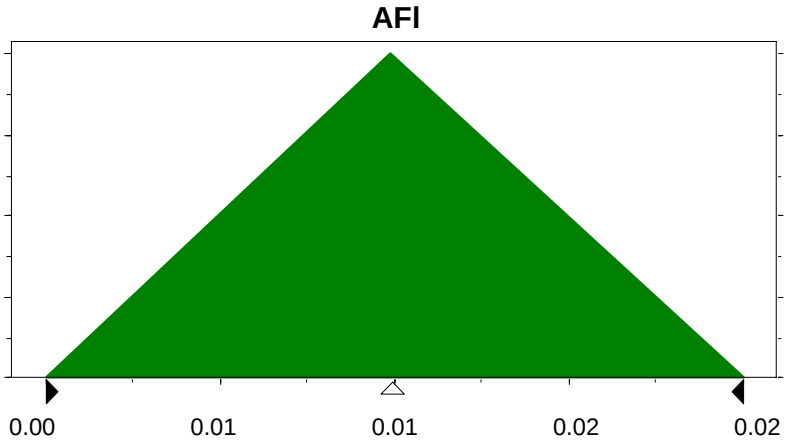


Assumption: AFI **[Exposure_2006.XLS] Child (1-2) - Cell: E31**

Triangular distribution with parameters:

Minimum	0.00	(=F31)
Likeliest	0.01	(=E31)
Maximum	0.02	(=G31)

Selected range is from 0.00 to 0.02
Mean value in simulation was 0.01

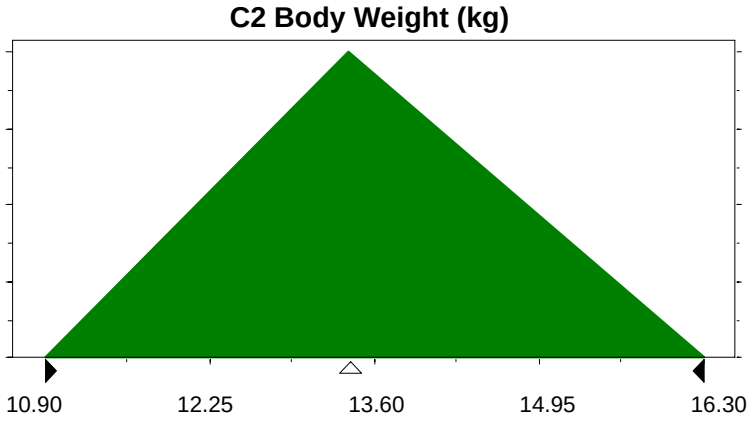


Assumption: C2 Body Weight (kg) **[Exposure_2006.XLS] Child (2-3) - Cell: E15**

Triangular distribution with parameters:

Minimum	10.90	(=F15)
Likeliest	13.40	(=E15)
Maximum	16.30	(=G15)

Selected range is from 10.90 to 16.30
Mean value in simulation was 13.53

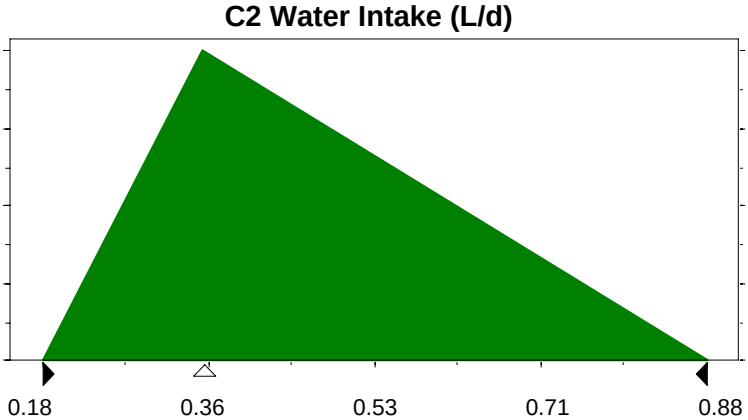


Assumption: C2 Water Intake (L/d) **[Exposure_2006.XLS] Child (2-3) - Cell: E22**

Triangular distribution with parameters:

Minimum	0.18	(=F22)
Likeliest	0.35	(=E22)
Maximum	0.88	(=G22)

Selected range is from 0.18 to 0.88
Mean value in simulation was 0.47

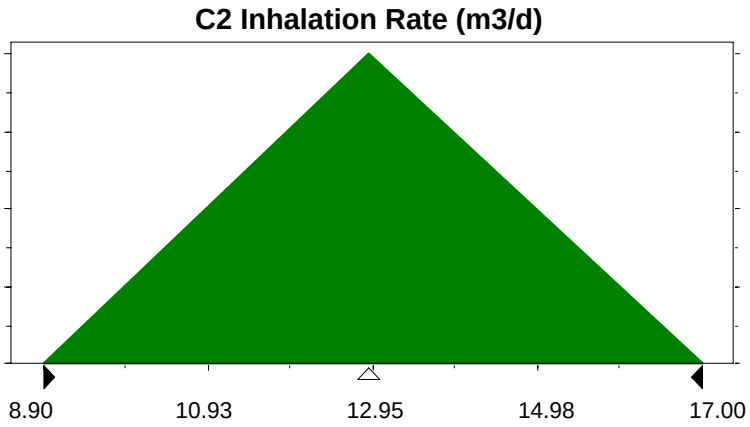


Assumption: C2 Inhalation Rate (m3/d) **[Exposure_2006.XLS] Child (2-3) - Cell: E26**

Triangular distribution with parameters:

Minimum	8.90	(=F26)
Likeliest	12.90	(=E26)
Maximum	17.00	(=G26)

Selected range is from 8.90 to 17.00
Mean value in simulation was 12.93

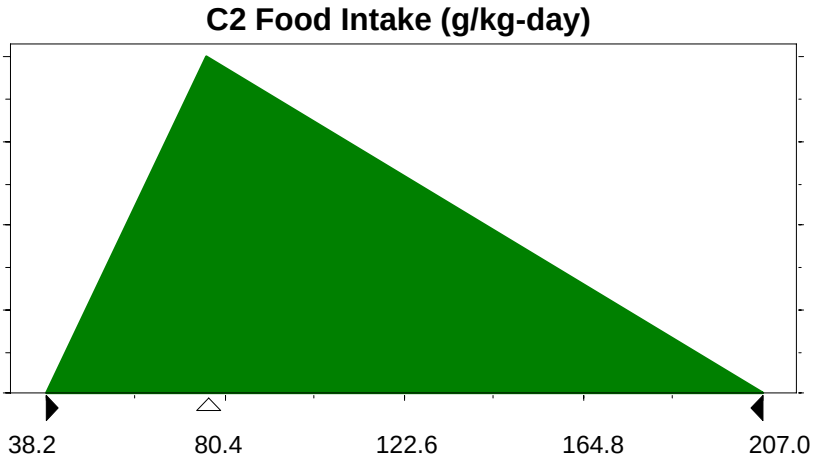


Assumption: C2 Food Intake (g/kg-day) **[Exposure_2006.XLS] Child (2-3) - Cell: H24**

Triangular distribution with parameters:

Minimum	38.2	(=I24)
Likeliest	76.4	(=H24)
Maximum	207.0	(=J24)

Selected range is from 38.2 to 207.0
Mean value in simulation was 107.2

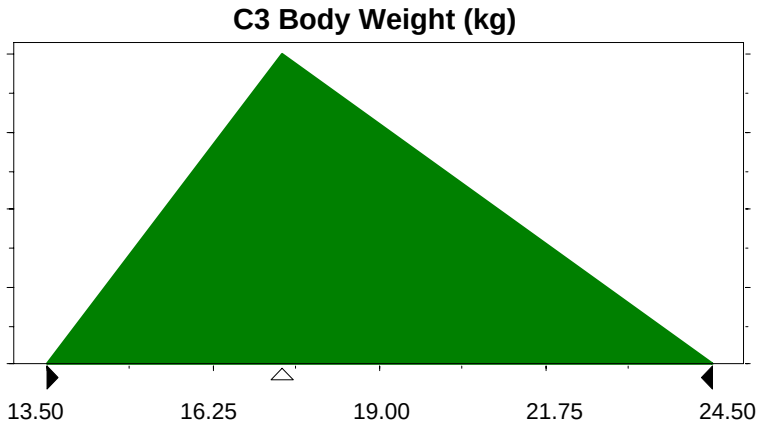


Assumption: C3 Body Weight (kg) **[Exposure_2006.XLS] Child (3-6) - Cell: E15**

Triangular distribution with parameters:

Minimum	13.50	(=F15)
Likeliest	17.40	(=E15)
Maximum	24.50	(=G15)

Selected range is from 13.50 to 24.50
Mean value in simulation was 18.47

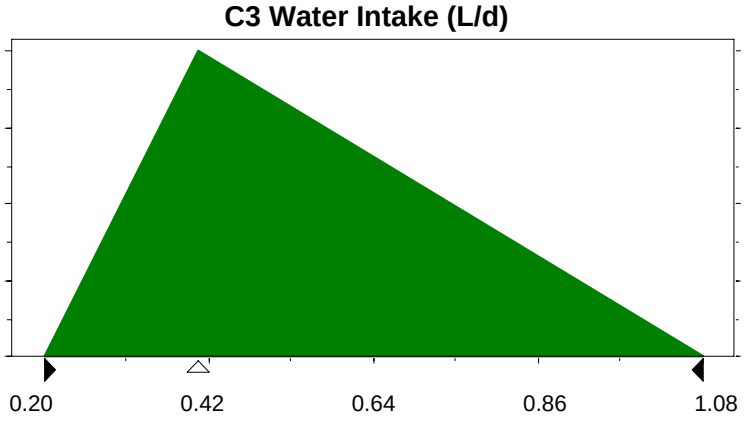


Assumption: C3 Water Intake (L/d) **[Exposure_2006.XLS] Child (3-6) - Cell: E22**

Triangular distribution with parameters:

Minimum	0.21	(=F22)
Likeliest	0.41	(=E22)
Maximum	1.08	(=G22)

Selected range is from 0.20 to 1.08
Mean value in simulation was 0.56

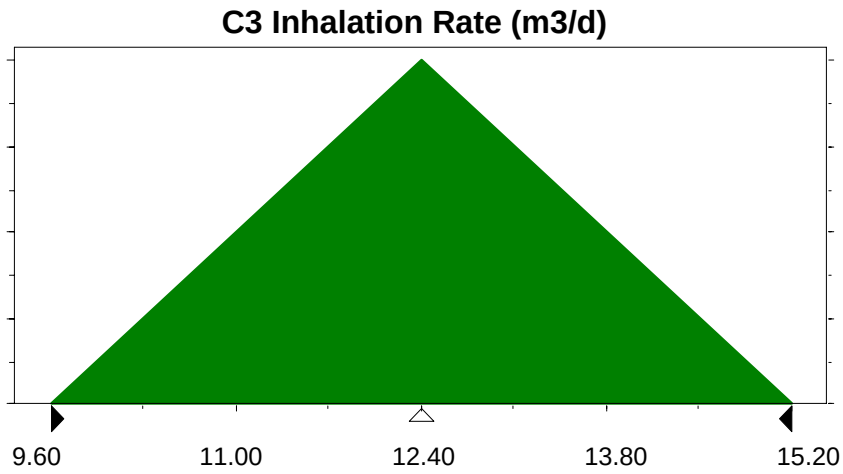


Assumption: C3 Inhalation Rate (m3/d) **[Exposure_2006.XLS] Child (3-6) - Cell: E26**

Triangular distribution with parameters:

Minimum	9.60	(=F26)
Likeliest	12.40	(=E26)
Maximum	15.20	(=G26)

Selected range is from 9.60 to 15.20
Mean value in simulation was 12.40

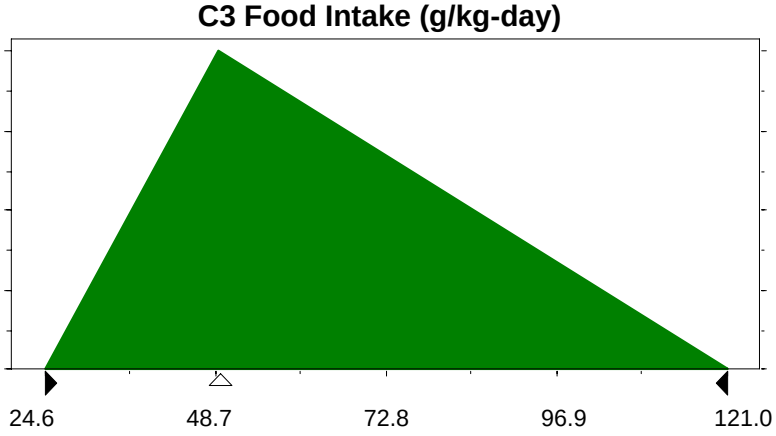


Assumption: C3 Food Intake (g/kg-day) **[Exposure_2006.XLS] Child (3-6) - Cell: H24**

Triangular distribution with parameters:

Minimum	24.7	(=I24)
Likeliest	49.3	(=H24)
Maximum	121.0	(=J24)

Selected range is from 24.6 to 121.0
Mean value in simulation was 65.0

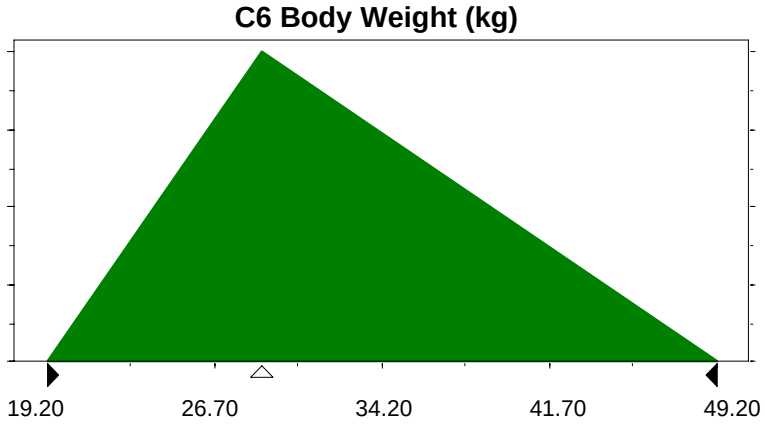


Assumption: C6 Body Weight (kg) **[Exposure_2006.XLS] Child (6-11) - Cell: E15**

Triangular distribution with parameters:

Minimum	19.20	(=F15)
Likeliest	28.80	(=E15)
Maximum	49.20	(=G15)

Selected range is from 19.20 to 49.20
Mean value in simulation was 32.40

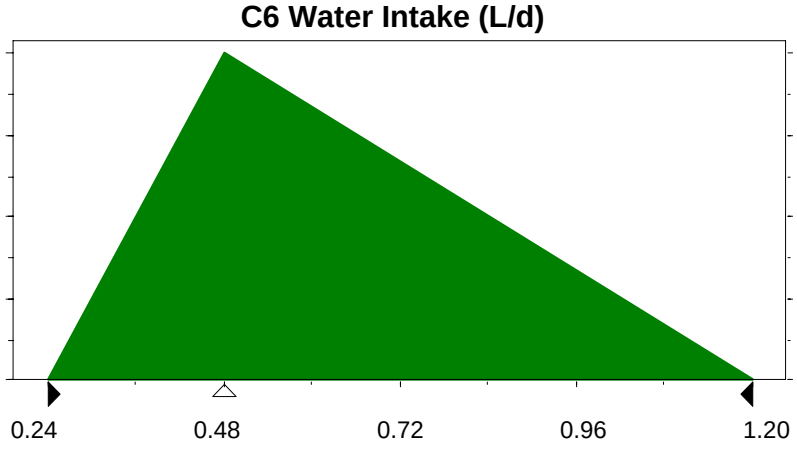


Assumption: C6 Water Intake (L/d) **[Exposure_2006.XLS] Child (6-11) - Cell: E22**

Triangular distribution with parameters:

Minimum	0.24	(=F22)
Likeliest	0.48	(=E22)
Maximum	1.20	(=G22)

Selected range is from 0.24 to 1.20
Mean value in simulation was 0.64

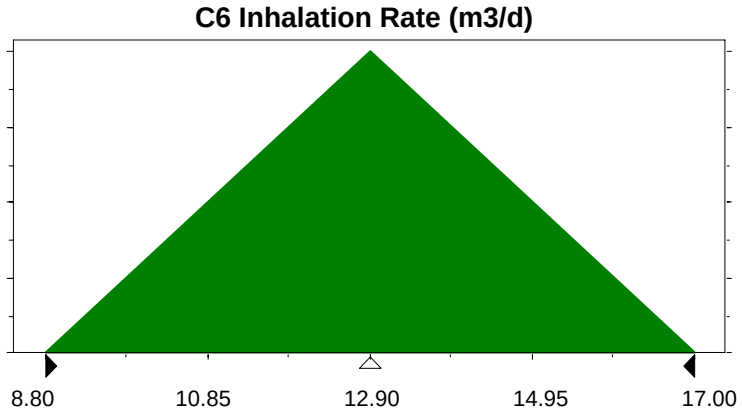


Assumption: C6 Inhalation Rate (m3/d) **[Exposure_2006.XLS] Child (6-11) - Cell: E26**

Triangular distribution with parameters:

Minimum	8.80	(=F26)
Likeliest	12.90	(=E26)
Maximum	17.00	(=G26)

Selected range is from 8.80 to 17.00
Mean value in simulation was 12.90

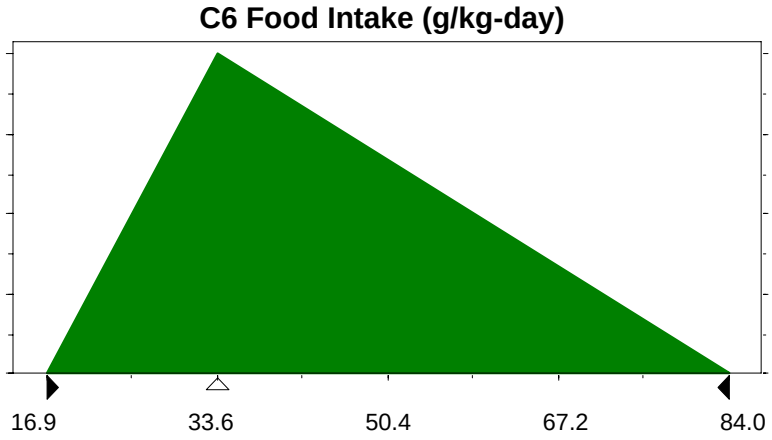


Assumption: C6 Food Intake (g/kg-day) **[Exposure_2006.XLS] Child (6-11) - Cell: H24**

Triangular distribution with parameters:

Minimum	16.9	(=I24)
Likeliest	33.7	(=H24)
Maximum	84.0	(=J24)

Selected range is from 16.9 to 84.0
Mean value in simulation was 44.9

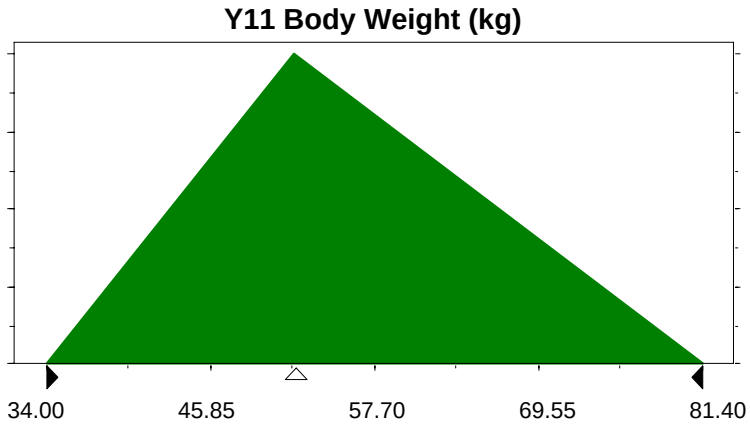


Assumption: Y11 Body Weight (kg) **[Exposure_2006.XLS] Youth (11-16) - Cell: E15**

Triangular distribution with parameters:

Minimum	34.00	(=F15)
Likeliest	52.00	(=E15)
Maximum	81.40	(=G15)

Selected range is from 34.00 to 81.40
Mean value in simulation was 55.80

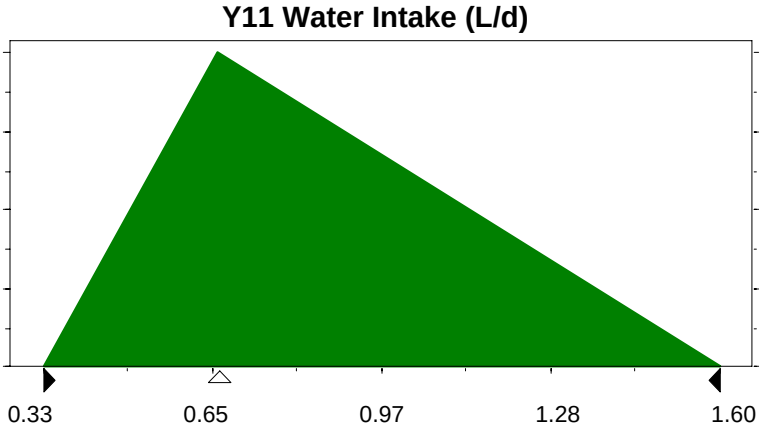


Assumption: Y11 Water Intake (L/d) **[Exposure_2006.XLS] Youth (11-16) - Cell: E22**

Triangular distribution with parameters:

Minimum	0.33	(=F22)
Likeliest	0.66	(=E22)
Maximum	1.60	(=G22)

Selected range is from 0.33 to 1.60
Mean value in simulation was 0.86

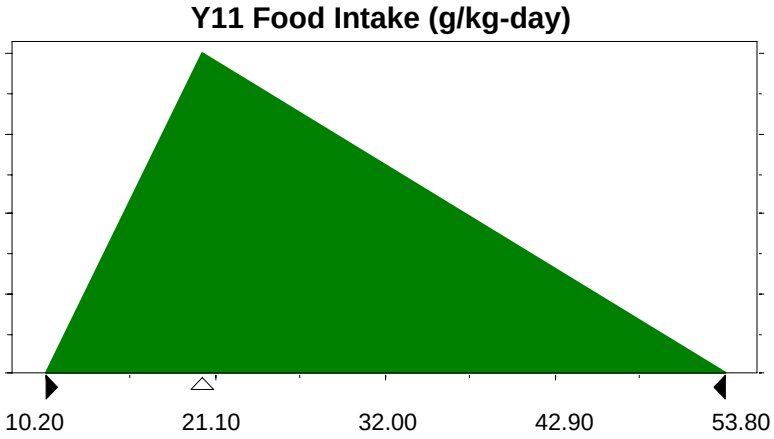


Assumption: Y11 Food Intake (g/kg-day) **[Exposure_2006.XLS] Youth (11-16) - Cell: H24**

Triangular distribution with parameters:

Minimum	10.20	(=I24)
Likeliest	20.30	(=H24)
Maximum	53.80	(=J24)

Selected range is from 10.20 to 53.80
Mean value in simulation was 28.10

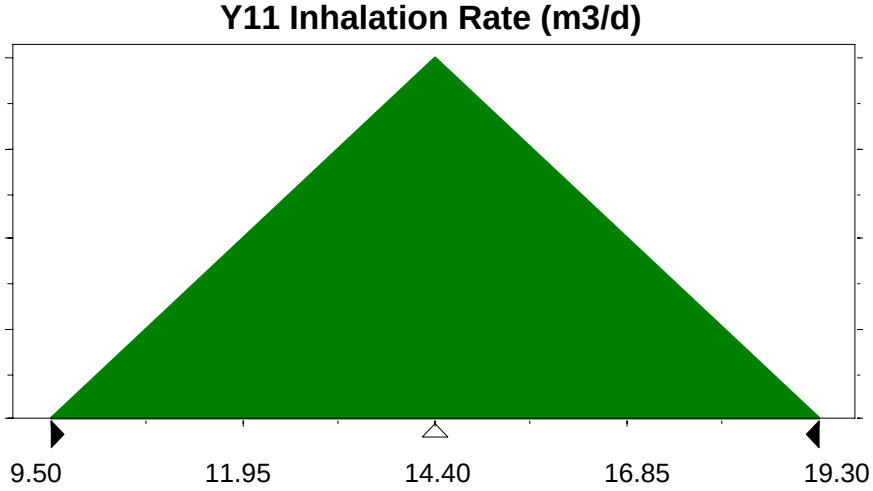


Assumption: Y11 Inhalation Rate (m3/d) **Exposure_2006.XLS] Youth (11-16) - Cell: E26**

Triangular distribution with parameters:

Minimum	9.50	(=F26)
Likeliest	14.40	(=E26)
Maximum	19.30	(=G26)

Selected range is from 9.50 to 19.30
Mean value in simulation was 14.40

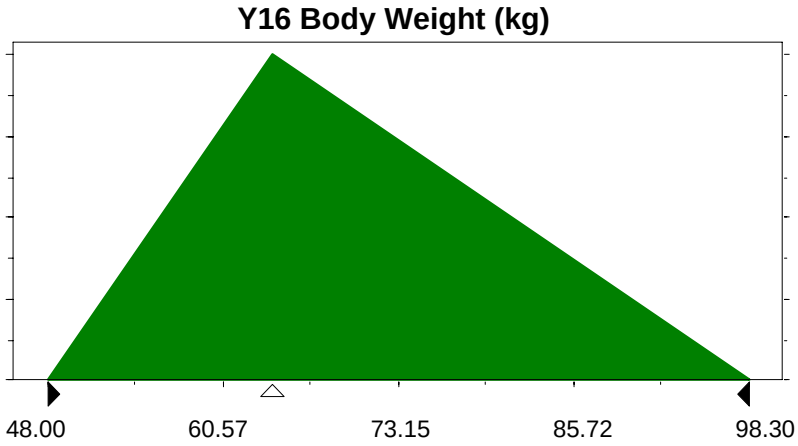


Assumption: Y16 Body Weight (kg) **[Exposure_2006.XLS] Youth (16-21) - Cell: E15**

Triangular distribution with parameters:

Minimum	48.00	(=F15)
Likeliest	64.10	(=E15)
Maximum	98.30	(=G15)

Selected range is from 48.00 to 98.30
Mean value in simulation was 70.13

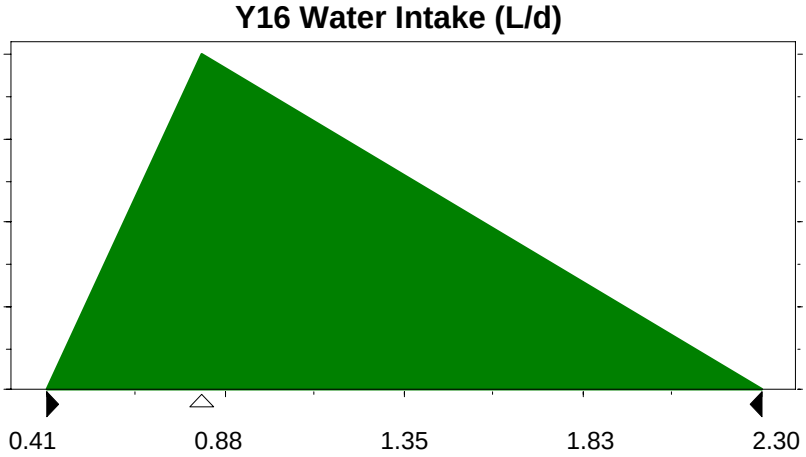


Assumption: Y16 Water Intake (L/d) **[Exposure_2006.XLS] Youth (16-21) - Cell: E22**

Triangular distribution with parameters:

Minimum	0.41	(=F22)
Likeliest	0.82	(=E22)
Maximum	2.30	(=G22)

Selected range is from 0.41 to 2.30
Mean value in simulation was 1.18

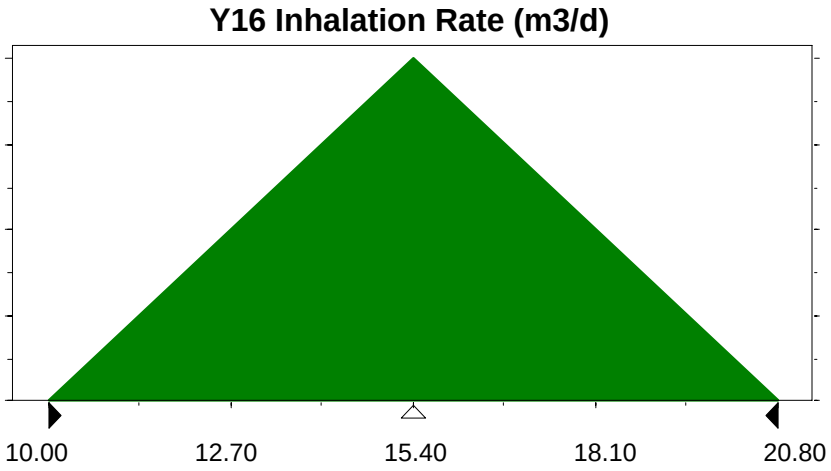


Assumption: Y16 Inhalation Rate (m3/d) **[Exposure_2006.XLS] Youth (16-21) - Cell: E26**

Triangular distribution with parameters:

Minimum	10.00	(=F26)
Likeliest	15.40	(=E26)
Maximum	20.80	(=G26)

Selected range is from 10.00 to 20.80
Mean value in simulation was 15.40

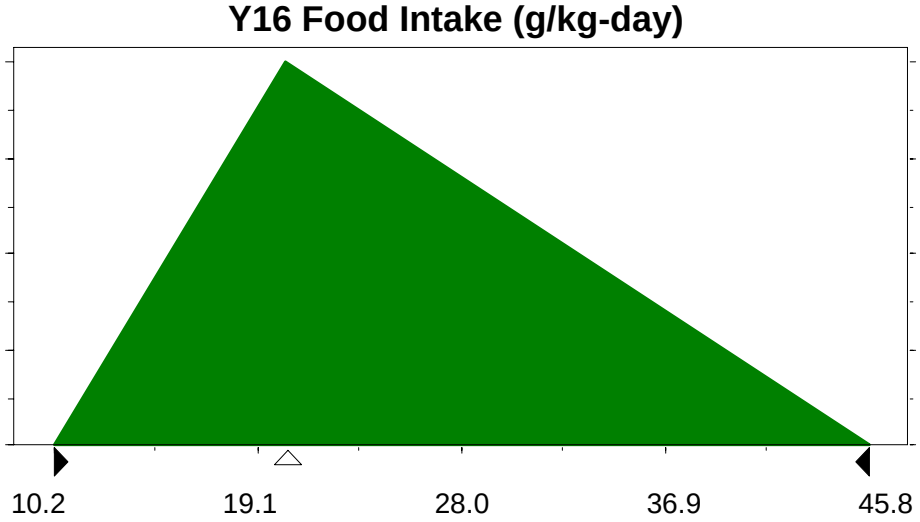


Assumption: Y16 Food Intake (g/kg-day) **[Exposure_2006.XLS] Youth (16-21) - Cell: H24**

Triangular distribution with parameters:

Minimum	10.2	(=I24)
Likeliest	20.4	(=H24)
Maximum	45.8	(=J24)

Selected range is from 10.2 to 45.8
Mean value in simulation was 25.5



Crystal Ball Report
Simulation started on 2/15/07 at 11:16:39
Simulation stopped on 2/15/07 at 11:17:08

Forecast: CY Oral ADD **[Exposure_2006.XLS]Sum - Cell: A4**

Summary:
Display Range is from 0.0E+0 to 4.5E-2 mg/kg-d
Entire Range is from 1.8E-4 to 5.4E-2 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 1.0E-4

Statistics:	Value
Trials	10000
Mean	1.5E-02
Median	1.3E-02
Mode	---
Standard Deviation	1.0E-02
Variance	1.0E-04
Skewness	0.75
Kurtosis	2.92
Coeff. of Variability	0.70
Range Minimum	1.8E-04
Range Maximum	5.4E-02
Range Width	5.3E-02
Mean Std. Error	1.01E-04

Percentiles:

Percentile	mg/kg-d
0.0%	1.8E-04
2.5%	1.2E-03
5.0%	1.8E-03
50.0%	1.3E-02
95.0%	3.4E-02
97.5%	3.7E-02
100.0%	5.4E-02

End of Forecast

Forecast: CY Inhalation ADD **[Exposure_2006.XLS]Sum - Cell: B4**

Summary:

Display Range is from 0.0E+0 to 1.5E-3 mg/kg-d
Entire Range is from 1.8E-6 to 2.2E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 3.4E-6

Statistics:	Value
Trials	10000
Mean	4.1E-04
Median	3.2E-04
Mode	---
Standard Deviation	3.4E-04
Variance	1.1E-07
Skewness	1.23
Kurtosis	4.39
Coeff. of Variability	0.82
Range Minimum	1.8E-06
Range Maximum	2.2E-03
Range Width	2.1E-03
Mean Std. Error	3.35E-06

Percentiles:

Percentile	mg/kg-d
0.0%	1.8E-06
2.5%	2.6E-05
5.0%	4.1E-05
50.0%	3.2E-04
95.0%	1.1E-03
97.5%	1.3E-03
100.0%	2.2E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 1.5E-2 mg/kg-d
Entire Range is from 2.9E-6 to 3.4E-2 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 4.2E-5

Statistics:	Value
Trials	10000
Mean	4.0E-03
Median	2.6E-03
Mode	---
Standard Deviation	4.2E-03
Variance	1.7E-05
Skewness	2.04
Kurtosis	8.42
Coeff. of Variability	1.05
Range Minimum	2.9E-06
Range Maximum	3.4E-02
Range Width	3.4E-02
Mean Std. Error	4.15E-05

Percentiles:

Percentile	mg/kg-d
0.0%	2.9E-06
2.5%	1.3E-04
5.0%	2.3E-04
50.0%	2.6E-03
95.0%	1.2E-02
97.5%	1.6E-02
100.0%	3.4E-02

End of Forecast

Summary:

Display Range is from 0.0E+0 to 1.2E-2 mg/kg-d
Entire Range is from 5.4E-5 to 1.6E-2 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 3.0E-5

Statistics:	Value
Trials	10000
Mean	4.4E-03
Median	3.8E-03
Mode	---
Standard Deviation	3.0E-03
Variance	9.2E-06
Skewness	0.75
Kurtosis	2.92
Coeff. of Variability	0.70
Range Minimum	5.4E-05
Range Maximum	1.6E-02
Range Width	1.6E-02
Mean Std. Error	3.04E-05

Percentiles:

Percentile	mg/kg-d
0.0%	5.4E-05
2.5%	3.7E-04
5.0%	5.4E-04
50.0%	3.8E-03
95.0%	1.0E-02
97.5%	1.1E-02
100.0%	1.6E-02

End of Forecast

Summary:

Display Range is from 0.0E+0 to 4.0E-4 mg/kg-d
Entire Range is from 5.3E-7 to 6.5E-4 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 1.0E-6

Statistics:	Value
Trials	10000
Mean	1.2E-04
Median	9.7E-05
Mode	---
Standard Deviation	1.0E-04
Variance	1.0E-08
Skewness	1.23
Kurtosis	4.39
Coeff. of Variability	0.82
Range Minimum	5.3E-07
Range Maximum	6.5E-04
Range Width	6.4E-04
Mean Std. Error	1.01E-06

Percentiles:

Percentile	mg/kg-d
0.0%	5.3E-07
2.5%	7.9E-06
5.0%	1.2E-05
50.0%	9.7E-05
95.0%	3.2E-04
97.5%	3.9E-04
100.0%	6.5E-04

End of Forecast

Summary:

Display Range is from 0.0E+0 to 4.5E-3 mg/kg-d
Entire Range is from 8.7E-7 to 1.0E-2 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 1.2E-5

Statistics:	Value
Trials	10000
Mean	1.2E-03
Median	7.8E-04
Mode	---
Standard Deviation	1.2E-03
Variance	1.6E-06
Skewness	2.04
Kurtosis	8.42
Coeff. of Variability	1.05
Range Minimum	8.7E-07
Range Maximum	1.0E-02
Range Width	1.0E-02
Mean Std. Error	1.25E-05

Percentiles:

Percentile	mg/kg-d
0.0%	8.7E-07
2.5%	4.0E-05
5.0%	6.9E-05
50.0%	7.8E-04
95.0%	3.7E-03
97.5%	4.7E-03
100.0%	1.0E-02

End of Forecast

Summary:

Display Range is from 0.0E+0 to 1.8E+0 mg/kg-d
Entire Range is from 8.2E-2 to 2.2E+0 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 3.6E-3

Statistics:	Value
Trials	10000
Mean	7.8E-01
Median	7.5E-01
Mode	---
Standard Deviation	3.6E-01
Variance	1.3E-01
Skewness	0.54
Kurtosis	3.02
Coeff. of Variability	0.46
Range Minimum	8.2E-02
Range Maximum	2.2E+00
Range Width	2.1E+00
Mean Std. Error	3.58E-03

Percentiles:

Percentile	mg/kg-d
0.0%	8.2E-02
2.5%	2.1E-01
5.0%	2.6E-01
50.0%	7.5E-01
95.0%	1.4E+00
97.5%	1.6E+00
100.0%	2.2E+00

End of Forecast

Summary:

Display Range is from 0.0E+0 to 3.5E-2 mg/kg-d
Entire Range is from 3.3E-4 to 4.9E-2 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 7.5E-5

Statistics:	Value
Trials	10000
Mean	1.1E-02
Median	9.2E-03
Mode	---
Standard Deviation	7.5E-03
Variance	5.6E-05
Skewness	0.97
Kurtosis	3.72
Coeff. of Variability	0.70
Range Minimum	3.3E-04
Range Maximum	4.9E-02
Range Width	4.9E-02
Mean Std. Error	7.51E-05

Percentiles:

Percentile	mg/kg-d
0.0%	3.3E-04
2.5%	1.3E-03
5.0%	1.7E-03
50.0%	9.2E-03
95.0%	2.5E-02
97.5%	2.9E-02
100.0%	4.9E-02

End of Forecast

Summary:

Display Range is from 0.0E+0 to 3.5E-3 mg/kg-d
Entire Range is from 5.2E-6 to 7.1E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 8.7E-6

Statistics:	Value
Trials	10000
Mean	1.0E-03
Median	7.6E-04
Mode	---
Standard Deviation	8.7E-04
Variance	7.5E-07
Skewness	1.56
Kurtosis	6.24
Coeff. of Variability	0.87
Range Minimum	5.2E-06
Range Maximum	7.1E-03
Range Width	7.1E-03
Mean Std. Error	8.69E-06

Percentiles:

Percentile	mg/kg-d
0.0%	5.2E-06
2.5%	6.0E-05
5.0%	9.2E-05
50.0%	7.6E-04
95.0%	2.7E-03
97.5%	3.2E-03
100.0%	7.1E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 1.5E-1 mg/kg-d
Entire Range is from 6.1E-6 to 3.9E-1 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 3.8E-4

Statistics:	Value
Trials	10000
Mean	3.3E-02
Median	2.0E-02
Mode	---
Standard Deviation	3.8E-02
Variance	1.5E-03
Skewness	2.41
Kurtosis	10.90
Coeff. of Variability	1.16
Range Minimum	6.1E-06
Range Maximum	3.9E-01
Range Width	3.9E-01
Mean Std. Error	3.84E-04

Percentiles:

Percentile	mg/kg-d
0.0%	6.1E-06
2.5%	8.5E-04
5.0%	1.5E-03
50.0%	2.0E-02
95.0%	1.1E-01
97.5%	1.4E-01
100.0%	3.9E-01

End of Forecast

Summary:

Display Range is from 0.0E+0 to 4.5E-4 mg/kg-d
Entire Range is from 4.7E-6 to 7.0E-4 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 1.0E-6

Statistics:	Value
Trials	10000
Mean	1.5E-04
Median	1.3E-04
Mode	---
Standard Deviation	1.1E-04
Variance	1.2E-08
Skewness	0.97
Kurtosis	3.72
Coeff. of Variability	0.70
Range Minimum	4.7E-06
Range Maximum	7.0E-04
Range Width	7.0E-04
Mean Std. Error	1.07E-06

Percentiles:

Percentile	mg/kg-d
0.0%	4.7E-06
2.5%	1.9E-05
5.0%	2.5E-05
50.0%	1.3E-04
95.0%	3.6E-04
97.5%	4.1E-04
100.0%	7.0E-04

End of Forecast

Summary:

Display Range is from 0.0E+0 to 5.0E-5 mg/kg-d
Entire Range is from 7.4E-8 to 1.0E-4 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 1.2E-7

Statistics:	Value
Trials	10000
Mean	1.4E-05
Median	1.1E-05
Mode	---
Standard Deviation	1.2E-05
Variance	1.5E-10
Skewness	1.56
Kurtosis	6.24
Coeff. of Variability	0.87
Range Minimum	7.4E-08
Range Maximum	1.0E-04
Range Width	1.0E-04
Mean Std. Error	1.24E-07

Percentiles:

Percentile	mg/kg-d
0.0%	7.4E-08
2.5%	8.5E-07
5.0%	1.3E-06
50.0%	1.1E-05
95.0%	3.9E-05
97.5%	4.6E-05
100.0%	1.0E-04

End of Forecast

Summary:

Display Range is from 0.0E+0 to 2.0E-3 mg/kg-d
Entire Range is from 8.7E-8 to 5.5E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 5.5E-6

Statistics:	Value
Trials	10000
Mean	4.7E-04
Median	2.8E-04
Mode	---
Standard Deviation	5.5E-04
Variance	3.0E-07
Skewness	2.41
Kurtosis	10.90
Coeff. of Variability	1.16
Range Minimum	8.7E-08
Range Maximum	5.5E-03
Range Width	5.5E-03
Mean Std. Error	5.48E-06

Percentiles:

Percentile	mg/kg-d
0.0%	8.7E-08
2.5%	1.2E-05
5.0%	2.1E-05
50.0%	2.8E-04
95.0%	1.6E-03
97.5%	2.0E-03
100.0%	5.5E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 9.0E-2 mg/kg-d
Entire Range is from 9.4E-5 to 1.3E-1 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 2.1E-4

Statistics:	Value
Trials	10000
Mean	2.6E-02
Median	2.0E-02
Mode	---
Standard Deviation	2.1E-02
Variance	4.5E-04
Skewness	1.26
Kurtosis	4.65
Coeff. of Variability	0.82
Range Minimum	9.4E-05
Range Maximum	1.3E-01
Range Width	1.3E-01
Mean Std. Error	2.11E-04

Percentiles:

Percentile	mg/kg-d
0.0%	9.4E-05
2.5%	1.4E-03
5.0%	2.3E-03
50.0%	2.0E-02
95.0%	6.8E-02
97.5%	8.0E-02
100.0%	1.3E-01

End of Forecast

Summary:

Display Range is from 0.0E+0 to 3.5E-3 mg/kg-d
Entire Range is from 4.2E-6 to 6.6E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 8.7E-6

Statistics:	Value
Trials	10000
Mean	1.0E-03
Median	8.0E-04
Mode	---
Standard Deviation	8.7E-04
Variance	7.5E-07
Skewness	1.39
Kurtosis	5.25
Coeff. of Variability	0.84
Range Minimum	4.2E-06
Range Maximum	6.6E-03
Range Width	6.6E-03
Mean Std. Error	8.67E-06

Percentiles:

Percentile	mg/kg-d
0.0%	4.2E-06
2.5%	6.4E-05
5.0%	9.8E-05
50.0%	8.0E-04
95.0%	2.8E-03
97.5%	3.3E-03
100.0%	6.6E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 1.1E-2 mg/kg-d
Entire Range is from 1.9E-6 to 2.6E-2 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 3.0E-5

Statistics:	Value
Trials	10000
Mean	2.7E-03
Median	1.6E-03
Mode	---
Standard Deviation	3.0E-03
Variance	8.8E-06
Skewness	2.17
Kurtosis	9.20
Coeff. of Variability	1.11
Range Minimum	1.9E-06
Range Maximum	2.6E-02
Range Width	2.6E-02
Mean Std. Error	2.97E-05

Percentiles:

Percentile	mg/kg-d
0.0%	1.9E-06
2.5%	8.0E-05
5.0%	1.4E-04
50.0%	1.6E-03
95.0%	8.7E-03
97.5%	1.1E-02
100.0%	2.6E-02

End of Forecast

Summary:

Display Range is from 0.0E+0 to 1.2E-3 mg/kg-d
Entire Range is from 1.3E-6 to 1.9E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 3.0E-6

Statistics:	Value
Trials	10000
Mean	3.7E-04
Median	2.9E-04
Mode	---
Standard Deviation	3.0E-04
Variance	9.1E-08
Skewness	1.26
Kurtosis	4.65
Coeff. of Variability	0.82
Range Minimum	1.3E-06
Range Maximum	1.9E-03
Range Width	1.9E-03
Mean Std. Error	3.02E-06

Percentiles:

Percentile	mg/kg-d
0.0%	1.3E-06
2.5%	2.0E-05
5.0%	3.2E-05
50.0%	2.9E-04
95.0%	9.7E-04
97.5%	1.1E-03
100.0%	1.9E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 5.0E-5 mg/kg-d
Entire Range is from 6.1E-8 to 9.4E-5 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 1.2E-7

Statistics:	Value
Trials	10000
Mean	1.5E-05
Median	1.1E-05
Mode	---
Standard Deviation	1.2E-05
Variance	1.5E-10
Skewness	1.39
Kurtosis	5.25
Coeff. of Variability	0.84
Range Minimum	6.1E-08
Range Maximum	9.4E-05
Range Width	9.4E-05
Mean Std. Error	1.24E-07

Percentiles:

Percentile	mg/kg-d
0.0%	6.1E-08
2.5%	9.1E-07
5.0%	1.4E-06
50.0%	1.1E-05
95.0%	4.0E-05
97.5%	4.7E-05
100.0%	9.4E-05

End of Forecast

Summary:

Display Range is from 0.0E+0 to 1.5E-4 mg/kg-d
Entire Range is from 2.7E-8 to 3.7E-4 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 4.2E-7

Statistics:	Value
Trials	10000
Mean	3.8E-05
Median	2.4E-05
Mode	---
Standard Deviation	4.2E-05
Variance	1.8E-09
Skewness	2.17
Kurtosis	9.20
Coeff. of Variability	1.11
Range Minimum	2.7E-08
Range Maximum	3.7E-04
Range Width	3.7E-04
Mean Std. Error	4.24E-07

Percentiles:

Percentile	mg/kg-d
0.0%	2.7E-08
2.5%	1.1E-06
5.0%	1.9E-06
50.0%	2.4E-05
95.0%	1.2E-04
97.5%	1.6E-04
100.0%	3.7E-04

End of Forecast

Summary:

Display Range is from 0.0E+0 to 8.0E-2 mg/kg-d
Entire Range is from 1.0E-4 to 1.2E-1 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 1.9E-4

Statistics:	Value
Trials	10000
Mean	2.4E-02
Median	1.9E-02
Mode	---
Standard Deviation	1.9E-02
Variance	3.5E-04
Skewness	1.17
Kurtosis	4.35
Coeff. of Variability	0.79
Range Minimum	1.1E-04
Range Maximum	1.2E-01
Range Width	1.2E-01
Mean Std. Error	1.88E-04

Percentiles:

Percentile	mg/kg-d
0.0%	1.1E-04
2.5%	1.3E-03
5.0%	2.2E-03
50.0%	1.9E-02
95.0%	6.1E-02
97.5%	7.1E-02
100.0%	1.2E-01

End of Forecast

Summary:

Display Range is from 0.0E+0 to 2.8E-3 mg/kg-d
Entire Range is from 3.7E-6 to 5.4E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 7.0E-6

Statistics:	Value
Trials	10000
Mean	8.4E-04
Median	6.5E-04
Mode	---
Standard Deviation	7.0E-04
Variance	4.9E-07
Skewness	1.31
Kurtosis	4.86
Coeff. of Variability	0.83
Range Minimum	3.7E-06
Range Maximum	5.4E-03
Range Width	5.4E-03
Mean Std. Error	7.02E-06

Percentiles:

Percentile	mg/kg-d
0.0%	3.7E-06
2.5%	5.2E-05
5.0%	8.1E-05
50.0%	6.5E-04
95.0%	2.2E-03
97.5%	2.6E-03
100.0%	5.4E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 1.1E-2 mg/kg-d
Entire Range is from 1.9E-6 to 2.6E-2 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 3.0E-5

Statistics:	Value
Trials	10000
Mean	2.7E-03
Median	1.6E-03
Mode	---
Standard Deviation	3.0E-03
Variance	8.8E-06
Skewness	2.17
Kurtosis	9.20
Coeff. of Variability	1.11
Range Minimum	1.9E-06
Range Maximum	2.6E-02
Range Width	2.6E-02
Mean Std. Error	2.97E-05

Percentiles:

Percentile	mg/kg-d
0.0%	1.9E-06
2.5%	8.0E-05
5.0%	1.4E-04
50.0%	1.6E-03
95.0%	8.7E-03
97.5%	1.1E-02
100.0%	2.6E-02

End of Forecast

Summary:

Display Range is from 0.0E+0 to 1.1E-3 mg/kg-d
Entire Range is from 1.5E-6 to 1.7E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 2.7E-6

Statistics:	Value
Trials	10000
Mean	3.4E-04
Median	2.8E-04
Mode	---
Standard Deviation	2.7E-04
Variance	7.2E-08
Skewness	1.17
Kurtosis	4.35
Coeff. of Variability	0.79
Range Minimum	1.6E-06
Range Maximum	1.7E-03
Range Width	1.7E-03
Mean Std. Error	2.69E-06

Percentiles:

Percentile	mg/kg-d
0.0%	1.6E-06
2.5%	1.9E-05
5.0%	3.1E-05
50.0%	2.8E-04
95.0%	8.8E-04
97.5%	1.0E-03
100.0%	1.7E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 4.0E-5 mg/kg-d
Entire Range is from 5.3E-8 to 7.7E-5 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 1.0E-7

Statistics:	Value
Trials	10000
Mean	1.2E-05
Median	9.3E-06
Mode	---
Standard Deviation	1.0E-05
Variance	1.0E-10
Skewness	1.31
Kurtosis	4.86
Coeff. of Variability	0.83
Range Minimum	5.3E-08
Range Maximum	7.7E-05
Range Width	7.7E-05
Mean Std. Error	1.00E-07

Percentiles:

Percentile	mg/kg-d
0.0%	5.3E-08
2.5%	7.4E-07
5.0%	1.2E-06
50.0%	9.3E-06
95.0%	3.2E-05
97.5%	3.7E-05
100.0%	7.7E-05

End of Forecast

Summary:

Display Range is from 0.0E+0 to 1.5E-4 mg/kg-d
Entire Range is from 2.7E-8 to 3.7E-4 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 4.2E-7

Statistics:	Value
Trials	10000
Mean	3.8E-05
Median	2.4E-05
Mode	---
Standard Deviation	4.2E-05
Variance	1.8E-09
Skewness	2.17
Kurtosis	9.20
Coeff. of Variability	1.11
Range Minimum	2.7E-08
Range Maximum	3.7E-04
Range Width	3.7E-04
Mean Std. Error	4.24E-07

Percentiles:

Percentile	mg/kg-d
0.0%	2.7E-08
2.5%	1.1E-06
5.0%	1.9E-06
50.0%	2.4E-05
95.0%	1.2E-04
97.5%	1.6E-04
100.0%	3.7E-04

End of Forecast

Summary:

Display Range is from 0.0E+0 to 7.0E-2 mg/kg-d
Entire Range is from 1.3E-4 to 1.2E-1 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 1.7E-4

Statistics:	Value
Trials	10000
Mean	2.1E-02
Median	1.7E-02
Mode	---
Standard Deviation	1.7E-02
Variance	3.0E-04
Skewness	1.30
Kurtosis	4.90
Coeff. of Variability	0.82
Range Minimum	1.3E-04
Range Maximum	1.2E-01
Range Width	1.2E-01
Mean Std. Error	1.73E-04

Percentiles:

Percentile	mg/kg-d
0.0%	1.3E-04
2.5%	1.0E-03
5.0%	1.8E-03
50.0%	1.7E-02
95.0%	5.5E-02
97.5%	6.6E-02
100.0%	1.2E-01

End of Forecast

Summary:

Display Range is from 0.0E+0 to 2.0E-3 mg/kg-d
Entire Range is from 2.5E-6 to 3.2E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 4.9E-6

Statistics:	Value
Trials	10000
Mean	6.0E-04
Median	4.6E-04
Mode	---
Standard Deviation	4.9E-04
Variance	2.4E-07
Skewness	1.30
Kurtosis	4.82
Coeff. of Variability	0.83
Range Minimum	2.5E-06
Range Maximum	3.2E-03
Range Width	3.2E-03
Mean Std. Error	4.93E-06

Percentiles:

Percentile	mg/kg-d
0.0%	2.5E-06
2.5%	3.8E-05
5.0%	5.8E-05
50.0%	4.6E-04
95.0%	1.6E-03
97.5%	1.8E-03
100.0%	3.2E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 1.1E-2 mg/kg-d
Entire Range is from 1.9E-6 to 2.6E-2 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 3.0E-5

Statistics:	Value
Trials	10000
Mean	2.7E-03
Median	1.6E-03
Mode	---
Standard Deviation	3.0E-03
Variance	8.8E-06
Skewness	2.17
Kurtosis	9.20
Coeff. of Variability	1.11
Range Minimum	1.9E-06
Range Maximum	2.6E-02
Range Width	2.6E-02
Mean Std. Error	2.97E-05

Percentiles:

Percentile	mg/kg-d
0.0%	1.9E-06
2.5%	8.0E-05
5.0%	1.4E-04
50.0%	1.6E-03
95.0%	8.7E-03
97.5%	1.1E-02
100.0%	2.6E-02

End of Forecast

Summary:

Display Range is from 0.0E+0 to 3.0E-3 mg/kg-d
Entire Range is from 5.4E-6 to 5.3E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 7.4E-6

Statistics:	Value
Trials	10000
Mean	9.0E-04
Median	7.3E-04
Mode	---
Standard Deviation	7.4E-04
Variance	5.5E-07
Skewness	1.30
Kurtosis	4.90
Coeff. of Variability	0.82
Range Minimum	5.4E-06
Range Maximum	5.3E-03
Range Width	5.3E-03
Mean Std. Error	7.41E-06

Percentiles:

Percentile	mg/kg-d
0.0%	5.4E-06
2.5%	4.5E-05
5.0%	7.7E-05
50.0%	7.3E-04
95.0%	2.4E-03
97.5%	2.8E-03
100.0%	5.3E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 9.0E-5 mg/kg-d
Entire Range is from 1.0E-7 to 1.4E-4 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 2.1E-7

Statistics:	Value
Trials	10000
Mean	2.6E-05
Median	2.0E-05
Mode	---
Standard Deviation	2.1E-05
Variance	4.5E-10
Skewness	1.30
Kurtosis	4.82
Coeff. of Variability	0.83
Range Minimum	1.1E-07
Range Maximum	1.4E-04
Range Width	1.4E-04
Mean Std. Error	2.11E-07

Percentiles:

Percentile	mg/kg-d
0.0%	1.1E-07
2.5%	1.6E-06
5.0%	2.5E-06
50.0%	2.0E-05
95.0%	6.8E-05
97.5%	7.9E-05
100.0%	1.4E-04

End of Forecast

Summary:

Display Range is from 0.0E+0 to 4.5E-4 mg/kg-d
Entire Range is from 8.1E-8 to 1.1E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 1.2E-6

Statistics:	Value
Trials	10000
Mean	1.1E-04
Median	7.1E-05
Mode	---
Standard Deviation	1.3E-04
Variance	1.6E-08
Skewness	2.17
Kurtosis	9.20
Coeff. of Variability	1.11
Range Minimum	8.1E-08
Range Maximum	1.1E-03
Range Width	1.1E-03
Mean Std. Error	1.27E-06

Percentiles:

Percentile	mg/kg-d
0.0%	8.1E-08
2.5%	3.4E-06
5.0%	5.8E-06
50.0%	7.1E-05
95.0%	3.7E-04
97.5%	4.7E-04
100.0%	1.1E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 4.5E-2 mg/kg-d
Entire Range is from 6.2E-5 to 1.0E-1 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 1.1E-4

Statistics:	Value
Trials	10000
Mean	1.4E-02
Median	1.1E-02
Mode	---
Standard Deviation	1.2E-02
Variance	1.3E-04
Skewness	1.38
Kurtosis	5.36
Coeff. of Variability	0.83
Range Minimum	6.2E-05
Range Maximum	1.0E-01
Range Width	1.0E-01
Mean Std. Error	1.16E-04

Percentiles:

Percentile	mg/kg-d
0.0%	6.2E-05
2.5%	6.8E-04
5.0%	1.2E-03
50.0%	1.1E-02
95.0%	3.7E-02
97.5%	4.3E-02
100.0%	1.0E-01

End of Forecast

Summary:

Display Range is from 0.0E+0 to 1.2E-3 mg/kg-d
Entire Range is from 1.7E-6 to 2.3E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 3.2E-6

Statistics:	Value
Trials	10000
Mean	3.6E-04
Median	2.8E-04
Mode	---
Standard Deviation	3.2E-04
Variance	1.0E-07
Skewness	1.52
Kurtosis	5.80
Coeff. of Variability	0.87
Range Minimum	1.7E-06
Range Maximum	2.3E-03
Range Width	2.3E-03
Mean Std. Error	3.16E-06

Percentiles:

Percentile	mg/kg-d
0.0%	1.7E-06
2.5%	2.2E-05
5.0%	3.4E-05
50.0%	2.8E-04
95.0%	9.9E-04
97.5%	1.2E-03
100.0%	2.3E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 1.1E-2 mg/kg-d
Entire Range is from 1.9E-6 to 2.6E-2 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 3.0E-5

Statistics:	Value
Trials	10000
Mean	2.7E-03
Median	1.6E-03
Mode	---
Standard Deviation	3.0E-03
Variance	8.8E-06
Skewness	2.17
Kurtosis	9.20
Coeff. of Variability	1.11
Range Minimum	1.9E-06
Range Maximum	2.6E-02
Range Width	2.6E-02
Mean Std. Error	2.97E-05

Percentiles:

Percentile	mg/kg-d
0.0%	1.9E-06
2.5%	8.0E-05
5.0%	1.4E-04
50.0%	1.6E-03
95.0%	8.7E-03
97.5%	1.1E-02
100.0%	2.6E-02

End of Forecast

Summary:

Display Range is from 0.0E+0 to 3.5E-3 mg/kg-d
Entire Range is from 4.4E-6 to 7.2E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 8.3E-6

Statistics:	Value
Trials	10000
Mean	9.9E-04
Median	7.9E-04
Mode	---
Standard Deviation	8.3E-04
Variance	6.8E-07
Skewness	1.38
Kurtosis	5.36
Coeff. of Variability	0.83
Range Minimum	4.4E-06
Range Maximum	7.2E-03
Range Width	7.2E-03
Mean Std. Error	8.27E-06

Percentiles:

Percentile	mg/kg-d
0.0%	4.4E-06
2.5%	4.9E-05
5.0%	8.5E-05
50.0%	7.9E-04
95.0%	2.6E-03
97.5%	3.1E-03
100.0%	7.2E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 9.0E-5 mg/kg-d
Entire Range is from 1.2E-7 to 1.7E-4 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 2.3E-7

Statistics:	Value
Trials	10000
Mean	2.6E-05
Median	2.0E-05
Mode	---
Standard Deviation	2.3E-05
Variance	5.1E-10
Skewness	1.52
Kurtosis	5.80
Coeff. of Variability	0.87
Range Minimum	1.2E-07
Range Maximum	1.7E-04
Range Width	1.7E-04
Mean Std. Error	2.25E-07

Percentiles:

Percentile	mg/kg-d
0.0%	1.2E-07
2.5%	1.5E-06
5.0%	2.5E-06
50.0%	2.0E-05
95.0%	7.1E-05
97.5%	8.6E-05
100.0%	1.7E-04

End of Forecast

Summary:

Display Range is from 0.0E+0 to 8.0E-4 mg/kg-d
Entire Range is from 1.4E-7 to 1.9E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 2.1E-6

Statistics:	Value
Trials	10000
Mean	1.9E-04
Median	1.2E-04
Mode	---
Standard Deviation	2.1E-04
Variance	4.5E-08
Skewness	2.17
Kurtosis	9.20
Coeff. of Variability	1.11
Range Minimum	1.4E-07
Range Maximum	1.9E-03
Range Width	1.9E-03
Mean Std. Error	2.12E-06

Percentiles:

Percentile	mg/kg-d
0.0%	1.4E-07
2.5%	5.7E-06
5.0%	9.7E-06
50.0%	1.2E-04
95.0%	6.2E-04
97.5%	7.8E-04
100.0%	1.9E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 3.5E-2 mg/kg-d
Entire Range is from 7.0E-5 to 6.9E-2 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 8.9E-5

Statistics:	Value
Trials	10000
Mean	1.1E-02
Median	8.5E-03
Mode	---
Standard Deviation	8.9E-03
Variance	8.0E-05
Skewness	1.37
Kurtosis	5.40
Coeff. of Variability	0.83
Range Minimum	7.0E-05
Range Maximum	6.9E-02
Range Width	6.9E-02
Mean Std. Error	8.94E-05

Percentiles:

Percentile	mg/kg-d
0.0%	7.0E-05
2.5%	5.1E-04
5.0%	8.4E-04
50.0%	8.5E-03
95.0%	2.8E-02
97.5%	3.3E-02
100.0%	6.9E-02

End of Forecast

Summary:

Display Range is from 0.0E+0 to 8.0E-4 mg/kg-d
Entire Range is from 7.5E-7 to 1.7E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 2.0E-6

Statistics:	Value
Trials	10000
Mean	2.3E-04
Median	1.8E-04
Mode	---
Standard Deviation	2.0E-04
Variance	4.1E-08
Skewness	1.57
Kurtosis	6.29
Coeff. of Variability	0.87
Range Minimum	7.5E-07
Range Maximum	1.7E-03
Range Width	1.7E-03
Mean Std. Error	2.03E-06

Percentiles:

Percentile	mg/kg-d
0.0%	7.5E-07
2.5%	1.4E-05
5.0%	2.2E-05
50.0%	1.8E-04
95.0%	6.4E-04
97.5%	7.7E-04
100.0%	1.7E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 8.0E-3 mg/kg-d
Entire Range is from 1.4E-6 to 1.9E-2 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 2.2E-5

Statistics:	Value
Trials	10000
Mean	2.0E-03
Median	1.2E-03
Mode	---
Standard Deviation	2.2E-03
Variance	4.8E-06
Skewness	2.17
Kurtosis	9.20
Coeff. of Variability	1.11
Range Minimum	1.4E-06
Range Maximum	1.9E-02
Range Width	1.9E-02
Mean Std. Error	2.19E-05

Percentiles:

Percentile	mg/kg-d
0.0%	1.4E-06
2.5%	5.9E-05
5.0%	1.0E-04
50.0%	1.2E-03
95.0%	6.4E-03
97.5%	8.1E-03
100.0%	1.9E-02

End of Forecast

Summary:

Display Range is from 0.0E+0 to 2.5E-3 mg/kg-d
Entire Range is from 5.0E-6 to 4.9E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 6.4E-6

Statistics:	Value
Trials	10000
Mean	7.7E-04
Median	6.1E-04
Mode	---
Standard Deviation	6.4E-04
Variance	4.1E-07
Skewness	1.37
Kurtosis	5.40
Coeff. of Variability	0.83
Range Minimum	5.0E-06
Range Maximum	4.9E-03
Range Width	4.9E-03
Mean Std. Error	6.39E-06

Percentiles:

Percentile	mg/kg-d
0.0%	5.0E-06
2.5%	3.6E-05
5.0%	6.0E-05
50.0%	6.1E-04
95.0%	2.0E-03
97.5%	2.4E-03
100.0%	4.9E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 5.5E-5 mg/kg-d
Entire Range is from 5.4E-8 to 1.2E-4 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 1.5E-7

Statistics:	Value
Trials	10000
Mean	1.7E-05
Median	1.3E-05
Mode	---
Standard Deviation	1.5E-05
Variance	2.1E-10
Skewness	1.57
Kurtosis	6.29
Coeff. of Variability	0.87
Range Minimum	5.4E-08
Range Maximum	1.2E-04
Range Width	1.2E-04
Mean Std. Error	1.45E-07

Percentiles:

Percentile	mg/kg-d
0.0%	5.4E-08
2.5%	1.0E-06
5.0%	1.6E-06
50.0%	1.3E-05
95.0%	4.5E-05
97.5%	5.5E-05
100.0%	1.2E-04

End of Forecast

Summary:

Display Range is from 0.0E+0 to 5.5E-4 mg/kg-d
Entire Range is from 1.0E-7 to 1.3E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 1.5E-6

Statistics:	Value
Trials	10000
Mean	1.4E-04
Median	8.7E-05
Mode	---
Standard Deviation	1.6E-04
Variance	2.4E-08
Skewness	2.17
Kurtosis	9.20
Coeff. of Variability	1.11
Range Minimum	1.0E-07
Range Maximum	1.4E-03
Range Width	1.4E-03
Mean Std. Error	1.56E-06

Percentiles:

Percentile	mg/kg-d
0.0%	1.0E-07
2.5%	4.2E-06
5.0%	7.1E-06
50.0%	8.7E-05
95.0%	4.6E-04
97.5%	5.8E-04
100.0%	1.4E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 4.0E-2 mg/kg-d
Entire Range is from 5.9E-5 to 8.0E-2 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 9.6E-5

Statistics:	Value
Trials	10000
Mean	1.2E-02
Median	9.1E-03
Mode	---
Standard Deviation	9.6E-03
Variance	9.2E-05
Skewness	1.32
Kurtosis	4.99
Coeff. of Variability	0.83
Range Minimum	5.9E-05
Range Maximum	8.0E-02
Range Width	8.0E-02
Mean Std. Error	9.60E-05

Percentiles:

Percentile	mg/kg-d
0.0%	5.9E-05
2.5%	5.4E-04
5.0%	8.9E-04
50.0%	9.1E-03
95.0%	3.1E-02
97.5%	3.6E-02
100.0%	8.0E-02

End of Forecast

Summary:

Display Range is from 0.0E+0 to 7.0E-4 mg/kg-d
Entire Range is from 8.2E-7 to 1.1E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 1.7E-6

Statistics:	Value
Trials	10000
Mean	2.0E-04
Median	1.5E-04
Mode	---
Standard Deviation	1.7E-04
Variance	2.8E-08
Skewness	1.44
Kurtosis	5.42
Coeff. of Variability	0.85
Range Minimum	8.2E-07
Range Maximum	1.2E-03
Range Width	1.2E-03
Mean Std. Error	1.68E-06

Percentiles:

Percentile	mg/kg-d
0.0%	8.2E-07
2.5%	1.2E-05
5.0%	1.8E-05
50.0%	1.5E-04
95.0%	5.4E-04
97.5%	6.3E-04
100.0%	1.2E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 1.1E-2 mg/kg-d
Entire Range is from 1.9E-6 to 2.6E-2 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 3.0E-5

Statistics:	Value
Trials	10000
Mean	2.7E-03
Median	1.6E-03
Mode	---
Standard Deviation	3.0E-03
Variance	8.8E-06
Skewness	2.17
Kurtosis	9.20
Coeff. of Variability	1.11
Range Minimum	1.9E-06
Range Maximum	2.6E-02
Range Width	2.6E-02
Mean Std. Error	2.97E-05

Percentiles:

Percentile	mg/kg-d
0.0%	1.9E-06
2.5%	8.0E-05
5.0%	1.4E-04
50.0%	1.6E-03
95.0%	8.7E-03
97.5%	1.1E-02
100.0%	2.6E-02

End of Forecast

Summary:

Display Range is from 0.0E+0 to 2.8E-3 mg/kg-d
Entire Range is from 4.2E-6 to 5.7E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 6.9E-6

Statistics:	Value
Trials	10000
Mean	8.2E-04
Median	6.5E-04
Mode	---
Standard Deviation	6.9E-04
Variance	4.7E-07
Skewness	1.32
Kurtosis	4.99
Coeff. of Variability	0.83
Range Minimum	4.2E-06
Range Maximum	5.7E-03
Range Width	5.7E-03
Mean Std. Error	6.86E-06

Percentiles:

Percentile	mg/kg-d
0.0%	4.2E-06
2.5%	3.8E-05
5.0%	6.3E-05
50.0%	6.5E-04
95.0%	2.2E-03
97.5%	2.6E-03
100.0%	5.7E-03

End of Forecast

Summary:

Display Range is from 0.0E+0 to 5.0E-5 mg/kg-d
Entire Range is from 5.9E-8 to 8.4E-5 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 1.2E-7

Statistics:	Value
Trials	10000
Mean	1.4E-05
Median	1.1E-05
Mode	---
Standard Deviation	1.2E-05
Variance	1.4E-10
Skewness	1.44
Kurtosis	5.42
Coeff. of Variability	0.85
Range Minimum	5.9E-08
Range Maximum	8.4E-05
Range Width	8.4E-05
Mean Std. Error	1.20E-07

Percentiles:

Percentile	mg/kg-d
0.0%	5.9E-08
2.5%	8.5E-07
5.0%	1.3E-06
50.0%	1.1E-05
95.0%	3.8E-05
97.5%	4.5E-05
100.0%	8.4E-05

End of Forecast

Summary:

Display Range is from 0.0E+0 to 8.0E-4 mg/kg-d
Entire Range is from 1.4E-7 to 1.9E-3 mg/kg-d
After 10,000 Trials, the Std. Error of the Mean is 2.1E-6

Statistics:	Value
Trials	10000
Mean	1.9E-04
Median	1.2E-04
Mode	---
Standard Deviation	2.1E-04
Variance	4.5E-08
Skewness	2.17
Kurtosis	9.20
Coeff. of Variability	1.11
Range Minimum	1.4E-07
Range Maximum	1.9E-03
Range Width	1.9E-03
Mean Std. Error	2.12E-06

Percentiles:

Percentile	mg/kg-d
0.0%	1.4E-07
2.5%	5.7E-06
5.0%	9.7E-06
50.0%	1.2E-04
95.0%	6.2E-04
97.5%	7.8E-04
100.0%	1.9E-03

End of Forecast

ADD Values for All Scenarios						
	Oral		Inhalation		Dermal	
Scenario	Mean	95th Percentile	Mean	95th Percentile	Mean	95th Percentile
Pregnant Worker (Fetus)	1.7E-02	4.0E-02	4.1E-01	1.1E+00	6.9E-01	2.0E+00
Infant (0-1 year)	1.1E-02	2.5E-02	1.0E-03	2.7E-03	3.3E-02	1.1E-01
Child (1-2 years)	2.6E-02	6.8E-02	1.0E-03	2.8E-03	2.7E-03	8.7E-03
Child (2-3 years)	2.4E-02	6.1E-02	8.4E-04	2.2E-03	2.7E-03	8.7E-03
Child (3-6 years)	2.1E-02	5.5E-02	6.0E-04	1.6E-03	2.7E-03	8.7E-03
Child (6-11 years)	1.4E-02	3.7E-02	3.6E-04	9.9E-04	2.7E-03	8.7E-03
Youth (11-16 years)	1.1E-02	2.8E-02	2.3E-04	6.4E-04	2.0E-03	6.4E-03
Youth (16-21 years)	1.2E-02	3.1E-02	2.0E-04	5.4E-04	2.7E-03	8.7E-03
Child-Youth TWA	1.5E-02	3.4E-02	4.1E-04	1.1E-03	4.0E-03	1.2E-02

LADD VALUES FOR INFANT-YOUTH SCENARIOS						
	Oral		Inhalation		Dermal	
Scenario	Mean	95th Percentile	Mean	95th Percentile	Mean	95th Percentile
Infant (0-1 year)	1.5E-04	3.6E-04	1.4E-05	3.9E-05	4.7E-04	1.6E-03
Child (1-2 years)	3.7E-04	9.7E-04	1.5E-05	4.0E-05	3.8E-05	1.2E-04
Child (2-3 years)	3.4E-04	8.8E-04	1.2E-05	3.2E-05	3.8E-05	1.2E-04
Child (3-6 years)	9.0E-04	2.4E-03	2.6E-05	6.8E-05	1.1E-04	3.7E-04
Child (6-11 years)	9.9E-04	2.6E-03	2.6E-05	7.1E-05	1.9E-04	6.2E-04
Youth (11-16 years)	7.7E-04	2.0E-03	1.7E-05	4.5E-05	1.4E-04	4.6E-06
Youth (16-21 years)	8.2E-04	2.2E-03	1.4E-05	3.8E-05	1.9E-04	6.2E-04

Cumulative LADD for Child-Youth Scenario (0-21 years)		
Route	Mean	95th Percentile
Oral	4.4E-03	1.0E-02
Inhalation	1.2E-04	3.2E-04
Dermal	1.2E-03	3.7E-03