t-BUTANOL

CAS # 75-65-0

ORAL RISK ASSESSMENT DOCUMENT

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EXECUTIVE SUMMARY

t-Butanol – Oral Risk Assessment CAS # 75-65-0			
PARAMETER	**LEVEL**	**UNITS**	**DERIVED**
$BMDL_{10}$ (benchmark dose level)	133	mg/kg-day	From a chronic rat study
Oral RfD (oral reference dose)	1	mg/kg-day	From a chronic rat study
TAC (total allowable concentration)	9	mg/L	For a 70 kg adult drinking 2 L/day, with a 20% relative source contribution for water
SPAC (single product allowable concentration)	0.9	mg/L	For a 70 kg adult drinking 2 L/day
STEL (short term exposure level)	40	mg/L	From a subchronic study, for a 10 kg child drinking 1 L/day

KEY STUDY	National Toxicology Program (NTP). 1995. Toxicology and Carcingoenesis Studies of t-Butyl Alcohol (CAS No. 75-65-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). Technical Report Series No. 436.
CRITICAL EFFECT	Absolute and relative kidney weight increases in female rats.
UNCERTAINTY FACTORS	• 10x for interspecies extrapolation because there are differences in rate of elimination and identity and quantity of metabolites between rats and humans • 10x for intraspecies extrapolation • 1x for study duration, as a chronic study was used • 1x for LOAEL-to-NOAEL conversion, as benchmark dose modeling was used • 1x for database deficiency, as the required studies are all available, although the two-generation reproduction study on t-butanol is as a metabolite of methyl t-butyl ether The total uncertainty factor is therefore 100x.
TOXICITY SUMMARY	t-Butanol is a relatively nontoxic compound with acute effects similar to ethanol. Subchronic and chronic studies in rats identified the kidney as the target organ in both males and females, based on organ weight increases and pathology. However, male rat kidney effects were discounted as due to α-2μ-globulin accumulation, an effect that is not relevant to human health. Mice showed hypoactivity due to the high dose levels used, and mild kidney effects in a subchronic study, but showed thyroid follicular cell hyperplasia, adenoma, and a single high-dose carcinoma in a chronic study. The kidney effects in rats resulted in a lower oral RfD than the oral RfD in mice, considering the thyroid effects to have a threshold. Relative female rat kidney weights were used for the risk assessment calculations of both the TAC and the STEL. Studies in rats and mice suggest that t-butanol is not a developmental toxicant. A two-generation reproduction study of methyl t-butyl ether, which is metabolized to t-butanol and formaldehyde, showed no effects that could be attributed to t-butanol. Evidence suggests that t-butanol is not a genotoxic chemical, based on a number of *in vitro* genetic toxicity studies. However, based on the chronic studies in rats and mice, the "*data are inadequate for an assessment of human carcinogenic potential*" of t-butanol. No adequate or reliable human epidemiological study exists. Long-term animal studies in rats produced carcinogenic responses in male rats due to an α-2μ-globulin effect that is of no relevance to human health. Relevant kidney weight and histopathology effects were seen in female rats. Long-term animal studies in mice produced hyperplasia, adenoma, and one high-dose carcinoma of the thyroid, of questionable relevance to human health because rodents are significantly more sensitive than humans to thyroid effects.
CONCLUSIONS	The uncertainty factors for interspecies and intraspecies extrapolation, used in conjunction with the female relative kidney weight $BMDL_{10}$ values, should ensure that the drinking water action levels established in this document are adequately protective of public health.

1.0 INTRODUCTION

This document has been prepared to allow toxicological evaluation of the unregulated contaminant **t-butanol** in drinking water, as an extractant from one or more drinking water system components evaluated under NSF/ANSI 61 (2002), or as a contaminant in a drinking water treatment chemical evaluated under NSF/ANSI 60 (2002). Both non-cancer and cancer endpoints have been considered, and risk assessment methodology developed by the U.S. Environmental Protection Agency (U.S. EPA) has been used.

Non-cancer endpoints are evaluated using the reference dose (RfD) approach (Barnes and Dourson, 1988; Dourson, 1994; U.S. EPA, 1993), which assumes that there is a threshold for these endpoints that will not be exceeded if appropriate uncertainty factors (Dourson et al., 1996) are applied to the highest dose showing no significant effects. This highest dose is derived from human exposure data when available, but more often is derived from studies in laboratory animals. Either the no-observed-adverse-effect level (NOAEL) taken directly from the dose-response data, or the calculated lower 95% confidence limit on the dose resulting in an estimated 10% increase in response (the LED_{10} or BMDL from benchmark dose programs) can be used (U.S. EPA, 2001a). The lowest-observed-adverse-effect level (LOAEL) can also be used, with an additional uncertainty factor, although the benchmark dose approach is preferred in this case. The RfD is expressed in mg/kg-day. It is defined by the U.S. EPA as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime" (Barnes and Dourson, 1988; U.S. EPA, 1993; U.S. EPA, 1999a).

NSF uses the RfD to derive three product evaluation criteria for non-cancer endpoints. The total allowable concentration (TAC), generally used to evaluate the results of extraction testing normalized to static at-the-tap conditions, is defined as the RfD multiplied by the 70 kg weight of an average adult assumed to drink two liters of water per day. A relative source contribution (RSC), to ensure that the RfD is not exceeded when food and other non-water sources of exposure to the chemical are considered, is also applied in calculating the TAC. The relative source contribution should be data derived, if possible. Alternately, a 20% default contribution for water can be used (U.S. EPA, 1991a). The TAC calculation is then as follows:

$$\text{TAC (mg/L)} = \frac{[\text{RfD (mg/kg-day)} \times 70\text{ kg}] - [\text{total contribution of other sources (mg/day)}]}{2\text{L/day}}$$

or

$$\text{TAC (mg/L)} = \frac{\text{RfD (mg/kg-day)} \times 70\text{ kg}}{2\text{L/day}} \times 0.2\text{ (RSC)}$$

The single product allowable concentration (SPAC), used for water treatment chemicals and for water contact materials normalized to flowing at-the-tap conditions, is the TAC divided by the estimated total number of sources of the substance in the drinking water treatment and distribution system. In the absence of source data, a default multiple source factor of 10 is used.

This accounts for the possibility that more than one product in the water and/or its distribution system could contribute the contaminant in question to drinking water.

Finally, a short-term-exposure level (STEL), at a higher level than the TAC, may be calculated for contaminants such as solvents expected to extract at higher levels from new product, but also expected to decay rapidly over time. The STEL is calculated from the NOAEL or the LED_{10} of an animal study of 14- to 90-days duration, with uncertainty factors appropriate to the duration of the study. The contaminant level must decay to the TAC or below under static conditions, or to the SPAC or below under flowing conditions within 90 days, based on the contaminant decay curve generated from over-time laboratory extraction data.

Endpoints related to cancer are evaluated using modeling to fit a curve to the appropriate dose-response data (U.S. EPA, 1996a; U.S. EPA, 1999b). If there is sufficient evidence to use a non-linear model, the LED_{10} or BMDL, divided by the anticipated exposure, is calculated to give a margin of exposure. If there is insufficient evidence to document non-linearity, a linear model drawing a straight line from the LED_{10} or BMDL to zero, is used as a default. If a linear model (generally reflecting a genotoxic carcinogen) is used, a target risk range of 10^{-6} to 10^{-4} is considered by the U.S. EPA to be safe and protective of public health. (U.S. EPA, 1991a). For the purposes of NSF/ANSI 60 (2002) and 61 (2002), the TAC is set at the 10^{-5} risk level, and the SPAC is set at the 10^{-6} risk level. Use of a higher risk level is not ruled out, but would generally require documentation of a benefit to counteract the additional risk.

The RfD, TAC, SPAC, and STEL values derived in this document are based on available health effects data and are intended for use in determining compliance of products with the requirements of NSF/ANSI 60 (2002) and 61 (2002). Application of these values to other exposure scenarios should be done with care, and with a full understanding of the derivation of the values and of the comparative magnitude and duration of the exposures. These values do not have the rigor of regulatory values, as data gaps are generally filled by industry or government studies prior to regulation. Data gaps introduce uncertainty into an evaluation, and require the use of additional uncertainty factors to protect public health.

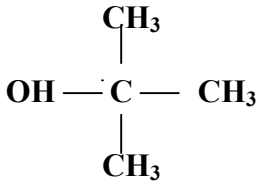
The general guidelines for this risk assessment include those from the National Research Council (1983) and from The Presidential/Congressional Commission on Risk Assessment and Risk Management (1997a, 1997b). Other guidelines used in the development of this assessment may include the following: Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986), proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996a), draft revised Guidelines for Carcinogen Risk Assessment (U.S. EPA 1999b), Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991b), Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996b), Guidelines for Neurotoxicity Risk Assessment (U.S. EPA, 1998a), Recommendations for and Documentation of Biological Values for Use in Risk Assessment (U.S. EPA, 1988), and Health Effects Testing Guidelines (U.S. EPA 1996c; U.S. EPA, 2002a).

The literature search strategy employed for this compound was based on the Chemical Abstract Service Registry Number (CASRN) and at least one common name. As a minimum, the following data banks were searched:

- ChemID Plus
- Registry of Toxic Effects of Chemical Substances (RTECS)
- Hazardous Substances Data Bank (HSDB)
- GENE-TOX
- Environmental Mutagen Information Center (EMIC)
- Developmental and Reproductive Toxicology (DART/ETIC)
- TOXLINE
- Toxicology Literature from Special Sources (TOXLIT)
- CANCERLIT
- Chemical Carcinogenesis Research Information System (CCRIS)
- Medline (via PubMed)
- Integrated Risk Information System (IRIS)
- Syracuse Research Corporation Online Toxic Substance Control Act Database (TSCATS)

2.0 PHYSICAL AND CHEMICAL PROPERTIES

t-Butanol is a branched-chain alcohol with synonyms tert-butanol, tertiary-butanol; t-, tert-, or tertiary butyl alcohol; 2-methyl-2-propanol; 1,1-dimethyl ethanol; trimethyl methanol and trimethyl carbinol. It has the physical and chemical properties listed in Table 1, and the following structure:



t-Butanol tissue/air partition coefficients were determined experimentally by Borghoff et al. (1996a) for blood, liver, kidney, muscle, fat, saline, and oil. The values were 481, 400, 543, 490, 191, 556, and 150, respectively. NihlJn and Johanson (1999) reported blood/air, water/blood, liver/blood, fat/blood, muscle/blood, and rapidly-perfused tissue/blood partition coefficients of 462, 1.31, 1.02, 0.57, 1.03, and 1.05, respectively, for t-butanol.

2.1 Organoleptic Properties

Ruth (1986) reported that t-butanol has a camphor-like odor with an air odor threshold of 219 mg/m^3 (72.3 ppm). A 47 ppm odor threshold for t-butanol in air was reported by Amoore and Hautala (1983). An odor safety factor of 2.1, calculated by dividing the Threshold Limit Value (TLV®) of t-butanol by its odor threshold, put it in a category where less than 50% of distracted persons would be warned by its odor at the 100 ppm TLV® in air. These same authors reported a water odor threshold of 290 ppm (mg/L). That is the concentration of t-butanol in water that would result in the air TLV® concentration of 100 ppm in the headspace of a stoppered flask.

Table 1. The physical and chemical properties of t-butanol

Property	Data	Reference
Empirical Formula	$C_4H_{10}O$	Budavari, 1996
CAS #	75-65-0	Budavari, 1996
Molecular Weight	74.12	Budavari, 1996
Physical State and Color	Colorless liquid which forms rhombic-like crystals.	HSDB, 2002
Melting Point	25.6 °C 25.7 °C	Budavari, 1996 HSDB, 2002
Boiling Point	82.41 °C	HSDB, 2002
Specific Gravity	0.78086	Budavari, 1996
Vapor Pressure	30.6 mm Hg @ 20 °C 42 mm Hg @ 25 °C	Kirk-Othmer, 1985 HSDB, 2002
Water Solubility	Soluble in water.	Budavari, 1996
n-Octanol/WaterPartition Coefficient (log K_{ow})	0.35 (experimental) 0.73 (estimated)	HSDB, 2002 SRC, 2002
Henry's Law Constant (air/water partition)	1.175×10^{-5} atm-m^3/mole (experimental) 9.99×10^{-6} atm-m^3/mole (estimated-bond) 1.04×10^{-5} atm-m^3/mole (estimated-group)	HSDB, 2002 SRC, 2002 SRC, 2002

3.0 PRODUCTION AND USE

3.1 Production

t-Butanol is on the list of high-production volume chemicals in both the United States (U.S. EPA, 2002b) and Europe (OECD, 2000), indicating that at least one million pounds per year are produced or imported. t-Butanol is a synthetic chemical with several available production methods. The first reported production method, from 1867, involved reaction of acetyl chloride with dimethylzinc (HSDB, 2002). It has also been produced from the decomposition of 2-t-butyl-peroxy-2-methyl-propionic acid treated with triethylamine in chlorobenzene, and can be produced by hydrolysis of the sulfuric acid extract obtained during separation of pure isobutylene from mixed butane-butylene streams (HSDB, 2002). Currently, t-butanol is recovered as a by-product from the isobutane oxidation process for the manufacture of propylene oxide (HSDB, 2002; Kirk-Othmer, 1985).

3.2 Use

The principle use for t-butanol is in the coatings industry for nitrocellulose lacquers and latex production (Kirk-Othmer, 1985). t-Butanol is used in a range of other products, such as in paint removers and lacquers as a solvent, and in alcohol as a denaturant. In addition, t-butanol is used in the production of methyl methacrylate and other plastics, as a flotation agent, and as an octane booster in gasoline (HSDB, 2002; Budavari, 1996). Additionally, it is used in perfumes and for water removal in perfume manufacture, and for flavors and fruit essences.

No regulatory acceptance for the use of t-butanol as a flavoring ingredient was located. t-Butanol is acceptable as a defoaming agent used in coatings for articles intended for use in "producing, manufacturing, packaging, processing, preparing, treating, transporting, or holding food" (U.S. FDA, 2002; 21 CFR 176.200), and also as a surface lubricant for metallic utensils or surfaces that contact food (U.S. FDA, 2002: 21 CFR 178.3910).

In products evaluated by NSF, t-butanol has very limited use as a solvent, and is generally not a direct ingredient. It is, however, a decomposition product of some peroxide catalysts.

4.0 ANALYTICAL METHODS

4.1 Analysis in a Water Matrix

t-Butanol can be analyzed by gas chromatography using either a packed or a capillary column, with flame ionization or mass spectrometric detection. At NSF, t-butanol is analyzed by direct injection of a water sample onto the chromatography column, and has a detection limit of 100 ppb (0.1 mg/L) using flame ionization detection.

4.2 Analysis in Biological Matrices

Wood and Laverty (1979) used direct injection packed-column gas chromatographic analysis on a 1:20 dilution of blood taken from the lateral tail veins of rats. Faulkner and Hussain (1989) used packed-column gas chromatography with headspace sampling at 55 °C and flame ionization detection in order to analyze t-butanol in whole blood samples. O'Neal et al. (1996) used both direct injection and headspace sampling with a 160 °C sample loop to analyze ethanol in postmortem blood samples, using t-butanol as an internal standard.

Lee and Weisel (1998) used capillary-column headspace gas chromatography, with mass spectrometric detection, to analyze t-butanol and methyl t-butyl ether in human urine. Purge temperatures of 25, 55, and 90 °C were evaluated. There was no detectable recovery of t-butanol at the lowest purge temperature, but 97.0-100.6% recoveries of t-butanol were obtained from urine samples spiked in the range of 1.57-31.4 μg/L when the purge temperature was 90 °C. This high purge temperature necessitated trapping excess water vapor in a gas-washing tube immersed in an ice bath. Loss of t-butanol in the gas-washing tube was reported to be negligible.

5.0 SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

5.1 Sources of Human Exposure

The major source of human exposure to t-butanol is likely to be occupational, either during production or during use of products containing t-butanol as a solvent, defoamer, or other ingredient. The general population could also be exposed to high concentrations periodically through use of paints, lacquers and other products. Based on its volatility and water solubility, humans may be exposed to small concentrations of t-butanol in air or water because of its discharge into the environment from industrial production and/or use. Exposure to high concentrations is primarily by inhalation, skin, or eye contact.

Exposure by ingestion may occur from alcoholic beverages, because fusel oil, containing the congeners or by-products of the fermentation or distillation process, is 95% amyl, butyl, and propyl alcohols and reaches concentrations as high as 0.25% in liquor (Final Report on the Safety Assessment of t-Butyl Alcohol, 1989). Small ingestion exposures could also occur from residual t-butanol used as a foaming agent in food contact materials (U.S. FDA, 2002; 176.200). t-Butanol has been detected in drinking water and in mother's milk (NTP, 1995; Kool et al., 1982), but no concentrations have been reported. Indirect exposure to t-butanol may also occur as a result of ingestion of methyl t-butyl ether (NihlJn and Johanson, 1998a), ethyl t-butyl ether (NihlJn and Johanson, 1999; NihlJn and Johanson, 1998b), or t-butyl acetate (Groth and Freundt, 1994), as each of these compounds is rapidly metabolized to t-butanol. Methyl t-butyl ether is widely used as a gasoline additive, and exposure to it and its t-butanol metabolite may occur if gasoline is volatilized during refueling or if it migrates to a groundwater source of drinking water (Salvolainen et al, 1985).

t-Butanol is frequently detected as an extractant of products evaluated by NSF under NSF/ANSI 61 (2002) as a result of decomposition of one of the following peroxide initiators: di-t-butyl peroxide; 2,5-dimethyl-2,5-di-t-butyl peroxyhexane; or cumyl t-butyl peroxide. These polymer initiators may be used in polyethylene and cross-linked polyethylene polymers, and also in elastomeric materials such as nitrile butadiene rubber, ethylene propylene diene monomer, and styrene butadiene rubber. Results indicate that the concentration of t-butanol extracted decays over time. Initial concentrations range from 400-50,000 ppb, but then decay to 20-16,000 ppb when extrapolated to day 90 of exposure.

5.2 Sources of Environmental Exposure

Environmental release data from 1997 indicate that the total air release of t-butanol was 573,130 pounds, while the total water release was 29,227 pounds. Total underground injection release was 979,674 pounds, and total land release was 751 pounds. These releases occurred from 86 industrial sites. Total off-site waste transfer was reported to be 13,672,372 pounds (TRI, 2000).

The environmental fate of t-butanol has been estimated based on its physical and chemical properties. t-Butanol discharged to water is expected to volatilize. Aqueous photooxidation of t-butanol by hydroxyl radicals has an estimated half-life of 8.8 years, and the compound is not expected to bioconcentrate in fish. t-Butanol discharged to soil may volatilize, or may readily leach to groundwater. The reaction of atmospheric t-butanol with photochemically generated hydroxyl radicals is estimated to have a half-life of 14.7 days (HSDB, 2002).

6.0 COMPARATIVE KINETICS AND METABOLISM IN HUMANS AND LABORATORY ANIMALS

While studies have been performed on t-butanol alone, several current and definitive studies have also been performed on t-butanol as a metabolite of methyl t-butyl ether. Use of methyl t-butyl ether to oxygenate gasoline has fostered much more research interest in methyl t-butyl ether than in t-butanol. Results of studies where the exposure is to methyl t-butyl ether rather than to t-butanol need to be interpreted here with caution, but provide sufficient supporting information to warrant consideration.

6.1 Absorption

6.1.1 Absorption in Humans

No studies on the absorption of t-butanol in humans were located. However, substantial absorption by the inhalation and oral routes of exposure can be inferred from blood distribution and urinary metabolite studies. A study on male human volunteers, exposed to 5, 25, or 50 ppm methyl t-butyl ether by inhalation for two hours, resulted in detection of methyl t-butyl ether and t-butanol in breath, blood, and urine (NihlJn et al., 1998a). Subjects in this study did light exercise on a computer-controlled bicycle ergometer during exposure. There was at least a two-week interval between exposures. The net respiratory uptake of methyl t-butyl ether (inhaled minus exhaled amount) was approximately 42% at 5 ppm, 32% at 25 ppm, and 41% at 50 ppm.

In a study as yet available only in abstract form, Prah et al. (2000) dosed human subjects with 6.7 μL/250 mL (approximately 4.69 mg or 0.07 mg/kg) methyl t-butyl ether orally in lemon-lime Gatorade. Absorption of methyl t-butyl ether was reported to be slower by the oral route than by inhalation.

6.1.2 Absorption in Laboratory Animals

No studies directly measuring the absorption of t-butanol in laboratory animals were located. However, substantial absorption by the inhalation and oral routes of exposure can be inferred from blood distribution and urinary metabolite studies.

6.2 Distribution

6.2.1 Distribution in Humans

No studies directly measuring the distribution of t-butanol in humans were located, although the two studies summarized below studied the distribution of t-butanol in humans as a metabolite of methyl t-butyl ether

Amberg et al. (1999) studied the kinetics of excretion of methyl t-butyl ether and its metabolites in three male and three female human volunteers exposed by inhalation to nominal 4 ppm or 40 ppm concentrations for four hours. Actual concentrations were reported as 4.5 ± 0.4 and 38.7 ± 3.2 ppm (16 ± 1.4 and 140 ± 12 mg/m^3), and there was a four-week interval between the two exposures. At the low dose, the concentration of t-butanol in blood immediately postexposure was 2.6 ± 0.3 μM and the compound was eliminated with a half-life of 6.5 ± 2.1 hours. At the high dose, the concentration of t-butanol in blood immediately postexposure was 21.8 ± 3.7 μM, and the compound was eliminated with a half-life of 5.3 ± 2.1 hours.

Another study on methyl t-butyl ether, exposing male human volunteers to 5, 25, or 50 ppm (18, 90, or 180 mg/m^3) by inhalation for two hours, followed by analysis of breath, blood, and urine for methyl t-butyl ether and t-butanol, was conducted by NihlJn et al. (1998a). Subjects in this study did light exercise on a computer-controlled bicycle ergometer during exposure. There was at least a two-week interval between exposures. The elimination half-life of t-butanol in blood

was 10 ± 1.8 hours at the 25 ppm (90 mg/m^3) exposure level and 10 ± 2.2 hours at the 50 ppm (180 mg/m^3) exposure level.

In a study as yet available only in abstract form, Prah et al. (2000) dosed human subjects with 6.7 µL/250 mL (approximately 4.69 mg or 0.07 mg/kg) methyl t-butyl ether orally in lemon-lime Gatorade. The half-life in blood of t-butanol as a metabolite of methyl t-butyl ether was reported as 13 hours after oral exposure.

6.2.2 Distribution in Laboratory Animals

6.2.2.1 Studies in Rats

Baker et al. (1982) studied the *in vivo* metabolism of t-butanol in Long-Evans rats of both sexes and in an inbred Sprague-Dawley rat strain. The half-life of t-butanol elimination, as measured by blood levels, was 9.1 hours for rats dosed intraperitoneally at 1g/kg. Elimination of t-butanol was apparently first order.

Groups of four male or three female Fischer 344 rats, approximately 12 weeks old and with cannulae implanted in the right jugular veins for ease of blood sampling, were dosed by intravenous injection with 37.5, 75, 150, or 300 mg/kg t-butanol in sterile saline (Poet et al., 1997). Blood samples were drawn 5, 10, 20, 30, 40, and 60 minutes and 4, 8, 12, 16, and 24 hours after dosing. Samples were analyzed by headspace sampling gas chromatography. The best fit of the data was obtained using a two-compartment model with weighting according to the reciprocals (1/observed y). The distribution half-life of was approximately three minutes. The elimination half-life was approximately 3.8 hours for both male and female rats at doses below 300 mg/kg. At the 300 mg/kg dose, the elimination half-life increased to 5.0 hours in males and 4.3 hours in females. Small values for the volume of the central compartment, and for the volume of distribution at steady state, suggested significant tissue distribution. t-Butanol was restrictively cleared, as the systemic clearance was only about half of the glomerular filtration rate. Both the volume of distribution at steady state and the systemic clearance decreased with increasing dose, and the area under the concentration-time curve ($AUC_{0-\infty}$) increased disproportionately with dose, with males showing the greater effects. Coupled with the increase in elimination half-life at the high dose, the authors suggested that the elimination of t-butanol became saturated at high doses and that binding of t-butanol in tissues may be occurring. Acetone was also detected in the blood in this study, but concentrations were extremely variable.

BeaugJ et al. (1981) studied the effect of t-butanol on liver lipid disposal, in particular to learn if alcohol dehydrogenase involvement was required for the development of fatty liver. Single oral doses of 25 mmol/kg (1.85 g/kg) t-butanol dissolved in water were administered by stomach tube to groups of four to eight female Wistar rats. t-Butanol was eliminated slowly, as indicated by blood concentrations of 13.24 mM at 2 hours, 12.57 mM at 5 hours, and 11.35 mM at 20 hours postexposure. Blood glucose was significantly increased 5 and 20 hours after exposure, while the blood free fatty acids were increased and triacylglycerols were decreased at 5 and at 20 hours, respectively. Neither blood nor liver phospholipid levels were affected. Liver weight was not significantly affected, although liver triacylglycerols were significantly increased at 5 and 20

hours. The increase in liver triacylglycerols at 20 hours was accompanied by an equally significant decrease in blood triacylglycerols.

Liver slices were taken from another group of rats, sacrificed either 5 or 20 hours after t-butanol exposure, and incubated with radiolabeled palmitic acid (BeaugJ et al., 1981). There was no significant difference in carbon dioxide production between liver slices of exposed and control animals. Radiolabeled, albumin-bound palmitic acid was injected intraperitoneally one hour prior to sacrifice of another group of animals. Incorporation into liver was significantly increased at both 5 and 20 hours, while incorporation into blood was decreased by about 50% at both times. The authors concluded that t-butanol induced fatty liver without impairing hepatic fatty acid oxidation, and further suggested this effect may be due to stress-induced hypothermia reported by others or to the stress of slow t-butanol elimination.

Williams and Borghoff (2001) gave six male and six female F-344 rats single 500 mg/kg gavage doses of radiolabeled t-butanol and compared the t-butanol equivalents 12 hours after dosing. In the kidney, liver, and blood males had 19, 86, and 29% more t-butanol, respectively, than females. The increases were significant ($p \leq 0.05$) for the liver and blood, but not significant ($p \leq 0.06$) for kidney at the 95% confidence level.

Aarstad et al. (1985) exposed male Sprague-Dawley rats for six hours to 500 ppm or 2,000 ppm of n-butanol, iso-butanol, sec-butanol, or tert-butanol (1,500 or 6,000 mg/m^3 of t-butanol). Serum concentrations of the alcohols were determined immediately after exposure. The 2,000 ppm (6,000 mg/m^3) exposure resulted in a 9.0 mM t-butanol serum concentration. This was about twice as high as the combined serum concentrations of sec-butanol and its methyl ethyl ketone metabolite (1.3/4.7 mM), but it was about 100 times higher than the serum concentration of n-butanol (0.09 mM) or iso-butanol (0.07 mM).

Borghoff et al. (2001) reported F-344 rat kidney-to-blood ratios of t-butanol after one-day or eight-day six hour/day exposures to 250, 450, or 1,750 ppm (750, 1,350, or 5,250 mg/m^3) t-butanol. Ratios were determined 2, 4, 6, and 16 hours postexposure. Male rat kidney-to-blood ratios were significantly elevated over female ratios at one or more time points at all dose levels and both exposure durations.

Amberg et al. (1999) studied the kinetics of excretion of methyl t-butyl ether and its metabolites in groups of five male and five female Fischer 344 NH rats exposed by inhalation to nominal 4 ppm or 40 ppm concentrations for four hours. Actual concentrations were reported as 4.5 ± 0.4 and 38.7 ± 3.2 ppm (16 ± 1.4 and 140 ± 12 mg/m^3). Concentrations of t-butanol in blood immediately postexposure were 2.9 ± 0.5 μM at the low dose and 36.7 ± 10.8 μM at the high dose, both significantly above the background level.

6.2.2.2 Studies in Mice

McComb and Goldstein (1979a) conducted a series of experiments in male Swiss-Webster mice, to study the physical dependence produced by t-butanol, in comparison with ethanol. A single intraperitoneal dose of 8.1 mmol/kg (600 mg/kg) t-butanol was eliminated in eight to nine hours, while mice inhaling t-butanol vapor at a concentration to maintain the t-butanol blood level at

about 8 mM eliminated the chemical in approximately three hours. An 8.1 mmol/kg (600 mg/kg) intraperitoneal t-butanol dose given four hours after the end of a three-day inhalation exposure was also eliminated from the blood in about three hours. The authors noted that an increased rate of elimination after continuous t-butanol administration could be due to increased glucuronide conjugation.

Faulkner and Hussain (1989) studied the kinetics of elimination of t-butanol after intraperitoneal injection to groups of eight male C57BL/6J mice, at doses of 5.0, 10.0, or 20.0 mmole/kg (370, 740, or 1,480 mg/kg). Blood samples were taken approximately hourly after injection, up to six hours after the lowest dose, up to 10 hours after the middle dose, and up to 17 hours after the high dose. Samples were analyzed for t-butanol by headspace gas chromatography, with the sampling vial at 55 °C. There was a linear relationship between the blood t-butanol concentration and time, except during the first hour postexposure, indicating a zero-order decline in blood concentration over most of the sampling period. The initial sample, at 0.7 hours, did not fit the curve at the two highest doses, suggesting absorption and distribution were not yet complete. These results, along with a disproportionate increase in area under the curve with increasing dose, suggested Michaelis-Menton elimination. The data were, therefore, fitted to a one-compartment Michaelis-Menton model. V_{max} estimates that increased with dose, and V_d estimates that decreased with dose did not support use of this model, which the authors took as an indication of possible multiple elimination pathways or dose-related effects on distribution or elimination.

Groups of six female C57BL/6J mice were given five gavage doses of 10.5 mmol/kg (780 mg/kg) t-butanol or of tap water, with the doses 12 hours apart, in order to ensure that the dose level in the first experiment was sufficient to correspond to the level causing microcephaly in animals exposed to ethanol (Faulkner et al., 1989). Twenty-four hours after the last dose, animals in each group were given a 10.5 mmole/kg (780 mg/kg) gavage dose of t-butanol. Fourteen blood samples were then taken from the infraorbital sinus of each animal over a period of 12 hours. Blood was analyzed by headspace gas chromatography. Mice pretreated with t-butanol showed a statistically insignificant increase in t-butanol elimination in blood three-to-seven hours after the challenge dose of t-butanol. There was no evidence from this study, therefore, that t-butanol was capable of enhancing its own elimination from the blood.

6.3 Metabolism

Numerous *in vivo* and *in vitro* metabolism studies have been done on t-butanol directly or as a metabolite of methyl t-butyl ether. The earliest studies focused on glucuronide conjugation, and on the potential for oxidative metabolism. Early studies attempting to determine whether acetone and formaldehyde were metabolites of t-butanol suffered from both the large variability of these two chemicals in biological systems, and from difficulty in analyzing these volatile, but water soluble, compounds in a complex biological matrix. Later studies identified most major and minor metabolites in both rats and humans.

6.3.1 Metabolism in Humans

Bernauer et al. (1998) exposed one 80 kg male human volunteer to [^{13}C] t-butanol at an oral dose of 5 mg/kg in a gelatin capsule. Urine samples were collected every 12 hours for 48 hours, and

were analyzed by ^{13}C NMR. Metabolites included 2-hydroxyisobutyrate, methyl-1,2-propanediol, t-butyl alcohol glucuronide, t-butanol, and an unknown which could not be identified. The major metabolite was 2-hydroxyisobutyrate.

Amberg et al. (1999) exposed three male and three female human volunteers to nominal 4 or 40 ppm methyl t-butyl ether concentrations for four hours. Actual concentrations were reported as 4.5 ± 0.4 and 38.7 ± 3.2 ppm (16 ± 1.4 and 140 ± 12 mg/m^3), and there was a four-week interval between the two exposures. 2-Hydroxyisobutyrate was the major metabolite, and t-butanol (total conjugated and unconjugated) and methyl-1,2-propanediol were minor metabolites. Pre-test urine samples contained a significant amount of 2-hydroxyisobutyrate, which very likely has a natural source. Lower background levels of t-butanol and methyl-1,2-propanediol were also seen, possibly indicating a food or environmental source of t-butanol.

6.3.2 Metabolism in Laboratory Animals

Kamil et al. (1953) studied a series of aliphatic alcohols, including t-butanol, for their ability to form glucuronic acid conjugates. In a qualitative experiment, six chinchilla rabbits were dosed with 8 mL of t-butanol by stomach tube and 24-hour urine samples were collected. Glucuronide conjugates were precipitated from the urine using basic lead acetate, and then methylated and acetylated. The resulting triacetyl β-1:1-dimethylethyl-D-glucuronide methyl ester was identified by melting point of the recrystallized material. In a quantitative study, three chinchilla rabbits that had been on a limited diet of 75 g/day rat cubes, were each dosed with 4 mmole/kg (300 mg/kg) t-butanol by stomach tube using water as the vehicle. The normal excretion of glucuronic acid by each rabbit was determined in a 24-hour control urine sample prior to dosing. The excess glucuronic acid excreted as a result of the t-butanol exposure was then calculated as the difference in urine concentrations before and after dosing. On average, the excess glucuronic acid excreted was 24.4% of the t-butanol dose. Higher average excess excretion was found from t-amyl alcohol and from t-hexyl alcohol (57.5% and 56.7%, respectively). Of the butyl alcohols, however, t-butanol resulted in the largest excess glucuronic acid excretion, as n-butyl, iso-butyl, and sec-butyl alcohols averaged 1.8, 4.4, and 14.4%, respectively. Finally, no aldehydes or ketones were detected in the expired air of one rabbit dosed orally with 6 mL of t-butanol, suggesting oxidative metabolism did not occur.

Baker et al. (1982) provided *in vivo* evidence for the production of acetone as a t-butanol metabolite. Rats were dosed by intraperitoneal injection at 1 g/kg. Acetone was identified in the blood samples, at 0.5 to 9.5% of the t-butanol dose and with a 12.3-hour half-life, but blood-acetone concentrations were highly variable. Rats dosed with 1.75 g/kg β-[^{14}C] t-butanol produced radioactive acetone and carbon dioxide, providing further evidence of oxidative metabolism. Rats were also treated with 0.5 μCi [U-^{14}C]hexadecanoic acid concurrently with 1.5g /kg t-butanol, to determine whether t-butanol affected fatty acid metabolism. No evidence of radiolabelled acetone was found, thus indicating that hexadecanoic acid was not involved in acetone production**.** In yet another study (Baker et al., 1982) two rats each were dosed with 1.5g/kg of a 1:1 mixture of α-[^{13}C] t-butanol and t-butanol. Only 19 μmol of ^{13}C acetone were recovered from t-butanol, although 91 μmol total acetone were detected. This finding indicated t-butanol metabolism was a minor source of acetone production. Use of phenobarbital to induce the P-450 dependent mixed function oxidase system, and use of amino-1,2,4-triazole to inhibit

catalase, did not appear to affect the amount of acetone recovered from a 1.5 g/kg t-butanol dose. However, results of this study were difficult to interpret because of the wide variations in acetone production.

Aarstad et al (1985) studied cytochrome P-450 enzyme induction following inhalation of t-butanol, as well as other butyl alcohol isomers, on male Sprague-Dawley rats. In the first experiment, rats were exposed for six hours to 500 ppm or to 2,000 ppm of n-butanol, iso-butanol, sec-butanol, or tert-butanol (1,500 or 6,000 mg/m^3 of t-butanol). After exposure to 500 ppm (1,500 mg/m^3) t-butanol for five days, the kidney P-450 concentration was significantly increased. However, the liver and lung P-450 concentrations were not significantly affected. After exposure to 2,000 ppm (6,000 mg/m^3) t-butanol for three days, the liver P-450 concentration was significantly increased, but not the concentration in the kidney or lung. There was no correlation between serum alcohol concentration and P-450 inducing capability, as n-butanol and t-butanol had about the same inducing capabilities, but 100-fold differences in serum concentrations. The authors concluded that this could reflect the inducing capabilities of the butanol isomers, or participation of metabolites in the P-450 induction process. Also, the differences in P-450 induction from kidney to liver in the two experiments may reflect a concentration and time dependence of organ-specific changes.

The biotransformation of unlabeled or ^{13}C-labeled t-butanol, as well as methyl t-butyl ether and ethyl t-butyl ether, was studied by Bernauer et al. (1998) in Fischer 344 rats. Three male rats per experiment were given 250 mg/kg gavage doses of t-butanol in corn oil. Urinary metabolites were identified by ^{13}C NMR as t-butyl alcohol sulfate, 2-hydroxyisobutyrate, 2-methyl-1,2-propanediol, t-butyl alcohol glucuronide, along with unmetabolized t-butanol. The latter two compounds were present in minor amounts. Results for volatile compounds were confirmed by mass spectrometry. Conjugates were confirmed by their disappearance after enzyme or acid cleavage. A minor amount of [^{13}C]acetone was also detected, and attributed to the further oxidation of 2-hydroxybutyrate, confirming results of the earlier studies (Cederbaum et al., 1983; Baker et al., 1982). Similar patterns were seen after inhalation exposures of 2,000 ppm (~ 7,200 mg/m^3) methyl t-butyl ether or ethyl t-butyl ether, a result that was expected since methyl t-butyl ether is metabolized to equimolar amounts of formaldehyde and t-butanol (Brady et al., 1990). These metabolite identifications were also confirmed by GC/MS in a later study by Amberg et al. (1999), in urine samples from F344 NH rats exposed to nominal 4 or 40 ppm (16 ± 1.4 or 140 ± 12 mg/m^3) methyl t-butyl ether concentrations for four hours.

Several effects described as ‘whole body metabolism’ have been attributed to t-butanol. Siviy et al. (1987) compared several measured or calculated parameters for ethanol and t-butanol in groups of 10 adult male Wistar rats dosed with 0.1, 0.2, 0.4, or 0.8 mg/kg t-butanol, or with ethanol doses which gave similar effects on motor activity. The respiratory quotient, as the measured volume of CO_2 produced divided by the measured volume of O_2 consumed, was known to approach 1.0 when carbohydrates were used as the primary energy source, but to decreases as the utilization of lipids increased. The energy expenditure was calculated as a function of the moles of oxygen consumed and the respiratory quotient. Motor activity was measured by placing the metabolic chambers on electronic balances during the 50-minute tests. t-Butanol had no significant effect on energy expenditure, while ethanol increased energy expenditure approximately 14%, suggesting the involvement of oxidative processes. Both

chemicals produced significant dose-dependent decreases in respiratory quotient, suggesting increased use of lipids as an energy source by a mechanism independent of oxidation. Both chemicals also produced significant decreases in motor activity.

6.3.3 *In Vitro* Metabolism Studies

The role of oxidative metabolism in the disposition of t-butanol was of considerable interest, because of the similarity of observed acute toxic effects to ethanol and because glucuronidation did not seem to fully account for the dosed t-butanol in the study of Kamil et al. (1953). As part of a study to characterize rat liver alcohol dehydrogenase (EC 1.1.1.1), Arslanian and Pascoe (1971) tested a series of alcohols for their ability to serve as substrates for this enzyme, which was prepared from Sprague-Dawley rat liver supernatant fraction. t-Butanol was a poor substrate, having only two percent of the activity of the reference compound, pentan-1-ol. Ethanol, propan-1-ol, butan-1-ol, and 2-methylpropan-1-ol all had higher relative activities as substrates (17, 27, 33, and 18% of the reference compound, respectively).

Thurman et al. (1980) showed, using perfused Sprague-Dawley rat liver, that neither oxygen uptake nor the pyridine nucleotide redox state was affected by t-butanol, indicating that oxidation by cytochrome P-450 and a liver alcohol dehydrogenase did not occur.

Cedarbaum and Cohen (1980) studied the reaction of t-butanol with liver microsomes from Sprague-Dawley rats. EDTA and $NADP^+$ were included in the buffered microsomal protein reaction mixture. The reaction was initiated by addition of glucose-6-phosphate and glucose-6-phosphate dehydrogenase, and terminated by the addition of trichloroacetic acid. Aliquots of the supernatant from centrifugation of the mixture were analyzed for formaldehyde. t-Butanol at 35 mM resulted in production of formaldehyde at a concentration of approximately 25 nmoles/mg protein. The addition of azide, which inhibits microsomal catalase and prevents decomposition of hydrogen peroxide, resulted in formaldehyde production of approximately 90 nmoles/mg protein. When hydrogen peroxide was added to the reaction mixture as an electron scavenger, and glucose-6-phosphate was omitted, production of formaldehyde was considerably reduced, indicating a requirement for microsomal electron flow. Mannitol, benzoate, and 2-keto-4-thiomethylbutyrate, competing hydroxyl radical scavengers, also reduced formaldehyde production. Two non-microsomal hydroxyl-radical generating systems were also tested: the oxidation of xanthine by xanthine oxidase, which produces the hydroxyl radical as a byproduct; and an iron-EDTA-ascorbate system. Both of these systems resulted in production of formaldehyde from t-butanol, in a time-dependent manner. The authors concluded that t-butanol was oxidatively demethylated to form formaldehyde, by reaction with hydroxyl radicals generated by the microsomes from hydrogen peroxide.

The study of t-butanol as a scavenger of hydroxyl radicals was extended by these authors (Cederbaum et al., 1983) using iron catalyzed oxidation of ascorbic acid, the Fenton reaction between hydrogen peroxide and iron, the coupled oxidation of xanthine by xanthine oxidase, and NADPH-dependent microsomal electron transfer. In this study, production of both formaldehyde and acetone were measured. Both reaction products were formed using the iron-ascorbate system. Superoxide dismutase had little effect, indicating no role for the superoxide anion in generating hydroxyl radicals by this system, but catalase had a significant inhibitory effect

indicating a major role for hydrogen peroxide. Only acetone was reported as a reaction product of the Fenton system, although generation of formaldehyde was inferred in the discussion. Both products were reported, however, using the xanthine-xanthine oxidase system, but their levels were reduced by catalase and superoxide dismutase, indicating roles for both the superoxide anion and hydrogen peroxide. The Sprague-Dawley rat liver microsomal system also produced both formaldehyde, as previously reported, and acetone. Catalase inhibition by azide again increased formaldehyde and acetone production, while hydroxyl radical scavengers decreased production of both chemicals. Two inhibitors of cytochrome P-450, metyrapone and SKF-525A, had no effect on the production of formaldehyde in the rat liver microsomal system. Likewise, inducers of the microsomal mixed function oxidase system, phenobarbital and 3-methylcholanthrene, had no effect on t-butanol metabolism.

Aarstad et al (1985) showed that the *in vitro* metabolism of n-hexane by rat liver microsomes after three days of exposure to 2,000 ppm (6,000 mg/m^3) t-butanol was increased, but the increase was not statistically significant. There was no correlation between serum alcohol concentration and P-450 inducing capability, as n-butanol and t-butanol had about the same inducing capabilities, but 100-fold differences in serum concentrations. The authors concluded that this could reflect the inducing capabilities of the butanol isomers, or participation of metabolites in the P-450 induction process. Also, the differences in P-450 induction from kidney to liver in the two experiments may reflect a concentration and time dependence of organ-specific changes.

6.4 Elimination/Excretion

6.4.1 Elimination/Excretion in Humans

Human data on the elimination/excretion of t-butanol in humans come from studies of methyl t-butyl ether. A study on male human volunteers exposed to 5, 25, or 50 ppm (18, 90, or 180 mg/m^3) methyl t-butyl ether by inhalation for two hours followed by analysis of breath, blood, and urine for methyl t-butyl ether and t-butanol, was conducted by NihlJn et al. (1998a). Subjects in this study did light exercise on a computer-controlled bicycle ergometer during exposure. There was at least a two-week interval between exposures. t-Butanol excreted in the urine represented approximately 0.49%, 0.74%, and 0.55% of respiratory uptake at the respective exposure concentrations. This most likely represented unconjugated t-butanol, as no mention was made of acid or enzyme treatment to cleave conjugates. The elimination half-life of t-butanol in urine was 8.9 ± 2.7 hours at the 25 ppm (90 mg/m^3) exposure level and 7.4 ± 2.7 hours at the 50 ppm (180 mg/m^3) exposure level. The renal clearance rate was 0.72 ± 0.16 mL/hr-kg at the 25 ppm (90 mg/m^3) exposure level and 0.62 ± 0.15 mL/hr-kg at the 50 ppm (180 mg/m^3) exposure level.

Amberg et al. (1999) studied the kinetics of excretion of methyl t-butyl ether and its metabolites in three male and three female human volunteers exposed by inhalation to nominal 4 ppm or 40 ppm concentrations for four hours. Actual concentrations were reported as 4.5 ± 0.4 and 38.7 ± 3.2 ppm (16 ± 1.4 and 140 ± 12 mg/m^3), and there was a four-week interval between the two exposures. Total 72-hour low-dose urinary excretion of t-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyrate was 3.4 ± 0.8, 16.5 ± 3.0, and 78.9 ± 27.6 μM, with respective half-lives of

12.0 ± 3.2, 7.8 ± 1.8, and 10.4 ± 4.4 hours. Total 72-hour high-dose urinary excretion of t-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyrate was 29.9 ± 9.4, 205.0 ± 15.6, and 943.8 ± 332.3 μM, with respective half-lives of 10.4 ± 1.8, 9.7 ± 1.5, and 17.0 ± 2.5 hours. Total recovery of methyl t-butyl ether as metabolites was 42% at the low dose and 69 % at the high dose.

6.4.2 Elimination/Excretion in Laboratory Animals

Thurman et al. (1980) performed a number of experiments in Sprague-Dawley rats, comparing the effects of ethanol and t-butanol toxicity. Animals pretreated by gavage with t-butanol in saline for one or 2.5 days, and then dosed to a blood level of 125-150 mg%, showed little or no effect on elimination as a result of pretreatment, indicating slow elimination, possibly via glucuronidation. In fact, the elimination of t-butanol occurred only 6.5% as fast as ethanol elimination.

Amberg et al. (1999) studied the kinetics of excretion of methyl t-butyl ether and its metabolites in groups of five male and five female Fischer 344 NH rats exposed by inhalation to nominal 4 ppm or 40 ppm concentrations for four hours. Actual concentrations were reported as 4.5 ± 0.4 and 38.7 ± 3.2 ppm (16 ± 1.4 and 140 ± 12 mg/m^3). Total 72-hour low-dose urinary excretion of t-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyrate was 0.3 ± 0.1, 0.7 ± 0.3, and 1.7 ± 0.3 μM, with respective half-lives of 5.0 ± 3.6, 2.9 ± 1.0, and 4.9 ± 1.3 hours. Total 72-hour high-dose urinary excretion of t-butanol, 2-methyl-1,2-propanediol, and 2-hydroxyisobutyrate was 1.2 ± 0.7, 2.8 ± 1.0, and 11.1 ± 2.8 μM, with respective half-lives of 4.5 ± 1.2, 3.4 ± 1.0, and 4.4 ± 0.9 hours. Total recovery of methyl t-butyl ether in urine as metabolites was 42% at the low dose and 39 % at the high dose.

6.5 Pharmacokinetic Modeling

A physiologically based pharmacokinetic model for methyl t-butyl ether and t-butanol was developed in male Fischer-344 rats (Borghoff et al., 1996a), to form the basis for a human model. Doses and blood levels were taken from several published studies. In one study, the concentrations of methyl t-butyl ether and t-butanol were measured in the plasma of rats exposed nose-only at targeted methyl t-butyl ether concentrations of 400 or 800 ppm (1,440 or 2,880 mg/m^3) for six hours. In another study, methyl t-butyl ether and t-butanol plasma concentrations were measured after intravenous (40 mg methyl t-butyl ether/kg; 1 mL/kg) and oral (40 or 400 mg methyl t-butyl ether/kg; 10 mL/kg) administration.

Two different models developed to describe the dosimetry of t-butanol in the rat were tested for their ability to predict t-butanol blood levels after methyl t-butyl ether exposure. Using a five-compartment model (blood, kidney, liver, muscle, fat) with the measured partition coefficients, the model was able to predict the accumulation of t-butanol in blood but not its clearance. A two-compartment model (total body water and liver) best predicted the accumulation and clearance of t-butanol in rats exposed to 400 ppm (1,440 mg/m^3) methyl t-butyl ether by inhalation, and to 400 or 40 mg/kg methyl t-butyl ether by oral administration. The two-compartment model predicted that a single oral dose of 40 mg/kg methyl t-butyl ether would give a t-butanol blood concentration of 300 μM after two hours, which would drop below 3 μM

within eight hours. Extrapolation resulted in a near-zero blood concentration within 10 hours following a single dose. However, to fit higher or lower t-butanol blood levels, the volume of the total body water compartment needed to be decreased or increased, respectively. The information obtained for t-butanol using both models suggested that clearance of t-butanol from the blood of rats after exposure to methyl t-butyl ether involved processes beyond metabolic elimination and that there were dose-dependent changes in t-butanol kinetics.

6.6 Comparative Metabolism and Kinetics

The results of Bernauer et al. (1998) showed that the sulfate conjugate of t-butanol was a major metabolite in the rat, but was present at only a trace amount in the human. Both species produced the glucuronide conjugate, but it was more significant in humans than in rats. Both species also produced methyl-1,2-propanediol and 2-hydroxyisobutyrate. The authors concluded that there was extensive and similar biotransformation of t-butanol in rats and in humans. The pathway for metabolic transformation of t-butanol, based on current understanding, is shown in Figure 1. Based on the half-lives reported in the Amberg et al. (1999) study, t-butanol was excreted approximately three times more rapidly by rats than by humans, although the major metabolite in both species was 2-hydroxyisobutyrate. This study confirmed the results of Bernauer et al. (1998). Both species again showed extensive biotransformation of t-butanol.

Results of metabolism and pharmacokinetic studies show that t-butanol, whether exposure occurs directly or as a result of metabolism of methyl t-butyl ether, is oxidatively metabolized or conjugated similarly in rats and in humans. Rats appear to eliminate t-butanol and its metabolites more rapidly than humans. Although there is considerable qualitative information regarding metabolites, quantitative data are still lacking. Several conclusions can be still drawn:

- Inhaled or ingested t-butanol is efficiently absorbed by both rats and humans (Borghoff et al., 1996a; Amberg et al., 1999; NihlJn et al., 1998a; Prah et al., 2000).

- A significant portion of a t-butanol dose is eliminated in the urine as a conjugate, of glucuronide in humans and rabbits (Bernauer et al., 1998; Kamil et al., 1953), and of sulfate in rats (Bernauer et al., 1998).

- A significant portion of a t-butanol dose is also oxidatively metabolized to produce 2-hydroxyisobutyrate as the major metabolite, and methyl-1,2-propanediol and acetone as minor metabolites, in both humans and rats (Bernauer et al., 1998, Amberg et al., 1999).

- Involvement of cytochrome P-450 mixed function oxidases in metabolism appears to be minimal (Arslanian et al., 1971; Aarstad et al., 1985).

- Elimination of t-butanol is approximately three-fold slower in humans than in rats (Amberg et al., 1999).

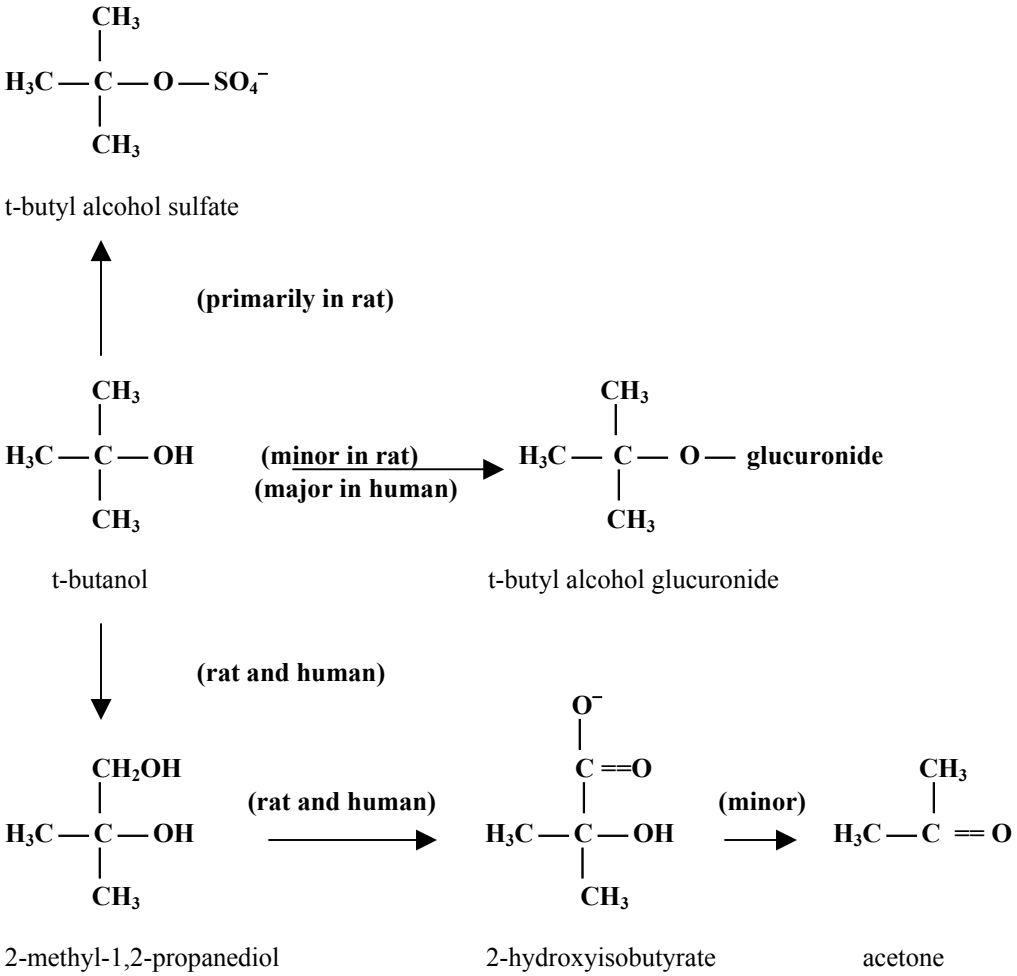


Figure 1. Biotransformation of t-butanol in rats and humans (adapted from ATSDR, 1996; Bernauer et al., 1998, Amberg et al., 1999)

7.0 EFFECTS ON HUMANS

Other than the metabolism studies in Section 6.0, there are few reports on the effects of t-butanol on humans because of its relatively low toxicity. Only one report of a sensitization reaction was located, and sensitization to low molecular weight alcohols was reported to be rare.

7.1 Case Reports

An allergic reaction to t-butanol used as an ethanol denaturant in a sunscreen solvent has been reported (Edwards and Edwards, 1982). Symptoms in the 58-year-old male included widespread pruritic, red, vesicular eruptions at the sites of sunscreen application. The problem was traced to t-butanol through cooperation of the sunscreen manufacturer in providing individual ingredients for patch testing.

NIOSH considers t-butanol to be a skin irritant and recommends prompt flushing with water if skin contact occurs. (ATSDR, 1996).

7.2 Epidemiological Studies

No epidemiological studies on t-butanol were located.

8.0 EFFECTS ON LABORATORY ANIMALS AND *IN VITRO* TEST SYSTEMS

No reports of irritation or sensitization reactions to t-butanol were located, suggesting low potential for these effects. The acute toxicity of t-butanol is in the g/kg range, and its effects include intoxication, development of physical dependence, and cross-tolerance to ethanol. Subchronic and chronic studies in rats show that the kidney is the target organ, although the effects in male rats are attributed to α-2μ-globulin. Subchronic effects in mice included increased kidney weight, and inflammation and transitional epithelial hyperplasia of the urinary bladder. However, these effects occurred at dose levels greater than 1.6 g/kg-day, and were not observed in a chronic study at lower doses. Histopathology effects in mice in the chronic study were seen only in the thyroid gland. There was no two-generation reproduction study on t-butanol, but a study on methyl t-butyl ether did not show fetal effects in the absence of maternal toxicity. Developmental studies of t-butanol in rats and mice showed fetal weight effects in the presence of maternal toxicity, but no statistically significant increases in visceral or skeletal abnormalities. There was no evidence that t-butanol had the potential to cause fetal alcohol syndrome. The similarity of acute neurological effects, and observed cross-tolerance, of t-butanol with ethanol has resulted in the conduct of many studies on the neurotoxic effects of t-butanol.

8.1 Limited-Exposure Effects

8.1.1 Irritation and Sensitization Studies

No studies of irritation or sensitization of laboratory animals by t-butanol were located for review.

8.1.2 Ocular Exposure Studies

t-Butanol vapor and liquid have been reported to be irritating to the eyes (HSDB, 2002; ATSDR, 1996). No specific eye irritation studies were reviewed.

8.2 Single-Exposure Studies

Oral LD_{50} values of 3.5 g/kg in rats and 3.6 g/kg in rabbits have been reported (NTP, 1995). Maickel and McFadden (1979) studied the acute toxicity of t-butanol by intraperitoneal injection in adult Swiss-Webster mice, as part of a study of the acute toxicities of butyl nitrite and butyl alcohol isomers. Ten mice per dose group were used, but specific dose levels were not given. Animals were observed every 30 minutes for two hours after injection and then daily for 10 days. The 30-minute LD_{50} was > 1,000 mg/kg, while the seven-day LD_{50} was 441 mg/kg due to

postexposure deaths. Livers of animals that died showed an abnormal brown color, in the peripheral areas for earlier deaths and uniformly for later deaths.

Walum and Peterson (1983) suggested the use of cultured mouse neuroblastoma C 1300 cells (clone $41A_3$) as an alternative to *in vivo* acute toxicity testing. The endpoint was the concentration of chemical resulting in detachment of 25% of the total cells (the TD_{25}). Reasonable correlation between the TD_{25} and the LD_{50} was believed to result from simplified *in vivo* toxicokinetics at high doses. TD_{25} values for t-butanol and acetone were 18×10^{-2} and 45×10^{-2} M, with LD_{50} values of 3,500 and 9,750 mg/kg, respectively.

Harris and Anders (1980) studied the effects of fasting as well as exposure to ethanol, t-butanol, and other compounds on carbon tetrachloride-induced hepatotoxicity. Intraperitoneal injections of t-butanol in the range of 0 to 20 mmol/kg (0 to ~1,500 mg/kg) were given to groups of four male Sprague-Dawley rats. After 18 hours, animals were given intraperitoneal injections of 4.13 mg/kg (~ 625 mg/kg) carbon tetrachloride, and sacrificed 24 hours later for measurement of serum glutamic pyruvate transaminase (now called alanine aminotransferase) levels. t-Butanol, as well as iso-propanol and methanol, potentiated carbon tetrachloride toxicity as determined by this enzyme measurement, but did not cause loss of body weight and did not lower hepatic glutathione levels. Comparison with results of the ethanol exposure indicated that potentiation was through a different mechanism.

Ray and Mehendale (1990) studied the potentiation of hepatotoxicity of carbon tetrachloride and chloroform by t-butanol (10 mmol/kg or 740 mg/kg) and other alcohols. Plasma aspartate aminotransferase and plasma alanine aminotransferase levels, as well as histopathology, were used to assess liver damage. Dosing with t-butanol, or with methanol, ethanol, isopropanol, or pentanol, followed by dosing with carbon tetrachloride, resulted in increased plasma tramsaminase levels and substantial liver pathology. Dosing with alcohol followed by chloroform produced similar, although less severe, results. Oral administration of t-butanol, followed 18 hours later by administration of either carbon tetrachloride or chloroform, significantly reduced the LD_{50} values of the two halocarbons. Since only half of the alcohols tested potentiated the lethality of carbon tetrachloride, the authors concluded that there were different underlying mechanisms.

8.3 Short-Term Exposure Studies

No short-term exposure studies on t-butanol were located.

8.4 Long-Term and Chronic Exposure Studies

8.4.1 Subchronic Studies

8.4.1.1 Subchronic Studies in Rats

Groups of five or six young male Wistar rats were dosed in their drinking water with 0.5% (v/v) t-butanol for 10 weeks (Acharya et al., 1997). Using the reference body weight and water consumption of male Wistar rats in a subchronic study (U.S. EPA, 1988), the rats received

approximately 575 mg/kg-day t-butanol. The purpose of this study was to compare liver and kidney histopathology from t-butanol exposure with a similar exposure to 25 ppm trichloroacetic acid, and to combined exposure to the two chemicals. Animals were weighed weekly, and food and water consumption were recorded daily, but only histopathology results were reported. In the liver, t-butanol caused periportal proliferation and lymphocytic infiltration, along with centrilobular necrosis, hepatocyte vacuolation, and loss of hepatic architecture. In the kidneys, there was degeneration of the renal tubules, syncitial arrangement of the nucleus in the renal tubular epithelial cells, degeneration of the basement membrane of the Bowman's capsule, and diffused glomeruli with vacuolation. The liver effects seen in this study were not reproduced in other subchronic and chronic studies. Effects of t-butanol and trichloroacetic acid were similar but showed no potentiation.

A subchronic study on t-butanol in Fischer 344/N rats, dosed orally in drinking water, was conducted by Southern Research Institute and reported by Lindamood et al., 1992 (also reported in NTP, 1995). Ten rats per sex per dose level were used, and target drinking water concentrations were 0, 0.25, 0.5, 1, 2, or 4% (w/v). These target concentrations delivered 0, 230, 490, 840, 1,520, or 3,610 mg/kg-day to males and 0, 290, 590, 850, 1,560, or 3,620 mg/kg-day to females (NTP, 1995). Animals were observed twice daily, and weighed and examined weekly. Water consumption was measured weekly at each cage. Blood was collected from the retroorbital sinus of ether-anesthetized animals on Days 15-16 and at terminal euthanasia for analysis of selected clinical chemistry and hematology parameters. Clinical chemistry parameters included bile acids, sorbital dehydrogenase, alanine aminotransferase, gamma-glutamyl transpeptidase, and alkaline phosphatase. Hematology parameters included hematocrit, hemoglobin, erythrocytes, mean cell volume, mean cell hemoglobin, platelets, reticulocytes, leukocytes, segmented neutrophils, lymphocytes, atypical lymphocytes, monocytes, eosinophils, and nucleated erythrocytes. Urine was collected on Days 10-11 and also prior to terminal sacrifice for analysis of pH, volume and specific gravity. At terminal sacrifice, weights of brain, heart, liver, lungs, right kidney, thymus, right ovary of females and right testicle of males were recorded. Complete necropsy was performed, and histopathology was done on control rats and rats from the two highest dose levels. Tissues examined included adrenal gland; bone marrow, femur, and sternum; three sections of brain, clitoral gland, esophagus, heart; cecum, colon, and rectum of the large intestine; duodenum, jejunum, and ileum of the small intestine; kidney, liver, lung, mandibular and mesenteric lymph nodes, mammary gland, three sections of nose, ovary, pancreas, pituitary gland, parathyroid gland, preputial gland, prostate gland, salivary gland, skin, spleen, forestomach and glandular stomach, testis, thymus, thyroid, trachea, urinary baldder, and uterus. This study was conducted in conformance with current regulatory guidelines (U.S. EPA, 1996c; U.S. EPA 2002a).

The earliest clinical signs of t-butanol toxicity were ataxia and hypoactivity. Other signs in males included emaciation, paraphimosis, and blood in the urine. Other signs in females included urine staining of the fur and blood in the urine. All males at the highest dose, as well as six of the females at that dose, died during the study period. The mean terminal body weight of males was reduced 5, 12, and 17% in comparison with controls at the 490, 840, and 1,520 mg/kg-day dose levels, respectively. The mean terminal body weight of females was reduced by 21% compared to controls at the highest dose.

Clinical chemistry effects were somewhat different in males and females, but may have been reflective of mild liver effects. In males, Day 15 alkaline phosphatase levels were significantly decreased at the two highest doses, while at Week 13 sorbital dehydrogenase was increased at the 840 and 1,520 mg/kg-day doses. In females, Day 15 alkaline phosphatase levels were significantly decreased at the 590 and 1,560 mg/kg-day doses and Week 13 alanine aminotransferase levels were significantly increased at the two highest doses.

Hematology effects included significant decreases in hemoglobin and erythrocytes, and increases in platelets, in males at the 840 and 1,520 mg/kg-day doses. The only significant hematology effect in females was a decrease in platelets in high-dose animals at Day 15. Water consumption of both sexes was reduced by half to two-thirds at the three highest dose levels, resulting in significantly reduced urine pH and volume, and increased urine specific gravity values.

Although there were statistically significant changes in absolute and/or relative weights of every organ weighed for both males and females at one or more dose levels, Lindamood et al. (1992) considered them to be secondary to weight loss and/or starvation. However, absolute and relative right kidney weights were significantly increased at all dose levels, with fairly good dose-response relationships at the higher doses, and should be considered relevant, as these weights perhaps mirror the observed kidney pathology. Organ weight effects are shown, along with histopathology findings, in Table 2. Histopathology findings were confined to the kidney and urinary bladder, with males affected to a greater extent than females.

While both lesions are common to both sexes of the F344/N rat, kidney nephropathy is usually more prominent in males and mineralization is usually more prominent in females. There was an apparent dose-response relationship for the severity (males) or incidence (females) of nephropathy starting at the lowest dose level, and for mineralization in the male rat kidney at the three highest dose levels. Crystals seen in the urine of both sexes at the two highest doses were analyzed by light microscopy (Lindamood et al., 1992) and, although not positively identified, were found to match uric acid in crystal structure.

Lindamood et al. (1992) also noted and further studied hyaline droplet formation in the male rats, and found the droplets were associated with crystalline and rhomboid intracytoplasmic protein deposits. At 840 and 1,520 mg/kg-day, a significant increase in PCNA-stained S-phase nuclei were found, indicating enhanced cell proliferation. As will be further discussed below, the male rat kidney effects could have been influenced by hyaline droplet formation and alpha-2μ-globulin accumulation, which does not occur in female rats and is not relevant to human health.

Crystalline, rectangular, and rhomboid forms of the alpha-2μ-globulin protein were identified in a retrospective study (Takahashi et al., 1993). Two studies by CIIT have documented that the lesions seen were due to alpha-2μ-globulin. Borghoff et al. (1996b; 2001) observed protein droplet accumulation and renal cell proliferation in the kidneys of male rats exposed to 1,750 ppm (5,250 mg/m^3) t-butanol six hours per day for 10 consecutive days. The presence of alpha-2μ-globulin was confirmed by immunohistochemical staining, and a statistically significant increase in alpha-2μ-globulin was measured using an enzyme-linked immunosorbent assay. Williams and Borghoff (2001; reviewed by McGregor and Hard, 2001), using radiolabeled t-butanol and gel filtration and anion-exchange chromatography, showed that t-butanol could bind

to alpha-2μ-globulin. Using d-limonene oxide, with a high affinity for this protein, demonstrated that the binding was reversible.

Table 2. Kidney weight and histopathology effects of t-butanol in rats in a subchronic drinking water study (Lindamood et al., 1992; NTP, 1995)

Parameter/ Observation	Dose to Male Rats, mg/kg-day (* signifies a statistically significant result)					
	0	**230**	**490**	**840**	**1,520**	**3,610**
MeanTerminal Body Weight		↓4%*	↓5%*	↓12%*	↓17%*	
Right Kidney Weight Absolute Relative		 ↑12%* ↑19%*	 ↑17%* ↑26%*	 ↑16%* ↑32%*	 ↑26%* ↑54%*	No survivors to study termination
Kidney Mineralization	0/10	0/10	2/10	8/10*	4/10*	4/10*
Kidney Nephropathy[1]	7/10 (1.0)	10/10 (1.6)*	10/10 (2.6)*	10/10 (2.7)*	10/10 (2.6)*	7/10 (1.1)
Kidney Hyaline Droplet Accumulation	0	+	++	++	++	No survivors to study termination
Urinary Bladder Chronic Inflammation[1]	0	Not examined	Not examined	0	1/10 (3.0)	2/10 (1.5)
Urinary Bladder Transitional Epithelial Hyperplasia[1]	0	Not examined	Not examined	0	1/10 (3.0)	7/10* (2.9)
Urinary Bladder Calculi	0	0	0	0	1	6
Parameter/ Observation	**Dose to Female Rats, mg/kg-day (* signifies a statistically significant result)**					
	0	**290**	**590**	**850**	**1,560**	**3,620**
Mean Terminal Body Weight						↓21%*
Right Kidney Weight Absolute Relative		 ↑19%* ↑17%*	 ↑16%* ↑15%*	 ↑29%* ↑28%*	 ↑39%* ↑40%*	 ↑36%* ↑81%*
Kidney Mineralization[1]	10/10 (1.7)	10/10 (2.0)	10/10 (2.0)	10/10 (2.0)	10/10 (2.0)	6/10 (1.2)
Kidney Nephropathy[1]	2/10 (1.0)	3/10 (1.0)	5/10 (1.0)	7/10* (1.0)	8/10* (1.0)	7/10* (1.0)
Urinary Bladder Chronic Inflammation[1]	0	Not examined	Not examined	Not examined	0	1/6 (2.0)
Urinary Bladder Transitional Epithelial Hyperplasia[1]	0	Not examined	Not examined	Not examined	0	3/10 (2.0)

[1] Incidence is given as a ratio, with severity in parentheses

8.4.1.2 Subchronic Study in Mice

A companion subchronic study on t-butanol in $B6C3F_1$ mice, dosed orally in drinking water, was also conducted by Southern Research Institute and reported by Lindamood et al., 1992 (also reported in NTP, 1995). Ten mice per sex per dose level were used, and target drinking water

concentrations were 0, 0.25, 0.5, 1, 2, or 4% (w/v). These target concentrations delivered 0, 350, 640, 1,590, 3,940, or 8,210 mg/kg-day to males and 0, 500, 820, 1,660, 6,430, or 11,620 mg/kg-day to females (NTP, 1995). Animals were observed twice daily, and weighed and examined weekly. Water consumption was measured weekly at each cage. Blood was collected from the retroorbital sinus of ether-anesthetized animals at terminal euthanasia for analysis of selected hematology parameters. Hematology parameters included hematocrit, hemoglobin, erythrocytes, mean cell volume, mean cell hemoglobin, platelets, reticulocytes, leukocytes, segmented neutrophils, bands, lymphocytes, monocytes, and eosinophils. Clinical chemistry and urinalysis were not done on mice. At terminal sacrifice, weights of brain, heart, liver, lungs, right kidney, thymus, and right testicle of males were recorded. Complete necropsy was performed, and histopathology was done on control and high-dose mice, and on male mice at the next lowest dose level. Tissues examined were the same as in the rat study, except that the clitoral gland and preputial gland were omitted and the gallbladder was added. This study was conducted in conformance with current regulatory guidelines (U.S. EPA, 1996c; U.S. EPA, 2002a).

Clinical signs of t-butanol toxicity in mice included ataxia, emaciation, abnormal posture, and hypoactivity. Water consumption was similar to controls except in females at the two highest dose levels. One male control and six high-dose males, one low-dose female, one female at the 6,430 mg/kg-day dose level, and four high-dose females died during the study period. Although food was available *ad libitum* except during urine collection, mean terminal body weights were affected (Table 3). They were suppressed, both statistically and biologically, for males at the two highest doses and for high-dose females. Hematology effects included significant increases in hematocrit, hemoglobin, erythrocytes, and segmented neutrophils in high-dose males, and increases in erythrocytes in high-dose females.

Although statistically significant effects on organ weights were seen at the two highest doses, they lacked clear dose-response relationships and were not considered directly related to t-butanol dosing (Lindamood et al., 1992). There were no gross lesions observed in female mice at any dose level, and treatment-related histopathological findings were limited to the urinary bladder in both sexes, as shown in Table 3.

A NOAEL for male mice at the nominal 0.5% (640 mg/kg-day) dose level, based on hypoactivity observed at the nominal 1% (1,590 mg/kg-day) dose level and a NOAEL for female mice at the nominal 1% (1,660 mg/kg-day) dose level based on weight-gain depression and emaciation observed at the nominal 2% (6,430 mg/kg-day) level were reported.

Table 3. Kidney weight and histopathology effects of t-butanol in mice in a subchronic drinking water study (Lindamood et al., 1992; NTP, 1995)

Parameter/ Observation	Dose to Male Mice, mg/kg-day (* signifies a statistically significant result)					
	0	**350**	**640**	**1,590**	**3,940**	**8,210**
Mean Terminal Body Weight				↓4%	↓14%*	↓24%*
Right Kidney Weight Absolute Relative			 ↑2%	 ↑8%	 ↑22%*	 ↑48%*
Urinary Bladder Inflammation[1]	0	Not examined	Not examined	0	6/10* (1.3)	10/10* (2.3)
Urinary Bladder Transitional Epithelial Hyperplasia[1]	0	Not examined	Not examined	0	6/10* (1.3)	10/10* (2.0)
Parameter/ Observation	**Dose to Female Mice, mg/kg-day (* signifies a statistically significant result)**					
	0	**500**	**820**	**1,660**	**6,430**	**11,620**
Mean Terminal Body Weight					↓7%	↓14%*
Right Kidney Weight Absolute Relative					 ↑15%*	↑12%* ↑35%*
Urinary Bladder Inflammation[1]	0	0	Not examined	Not examined	0	6/9* (1.2)
Urinary Bladder Transitional Epithelial Hyperplasia[1]	0	0	Not examined	Not examined	0	3/9 (2.0)

[1] Incidence is given as a ratio, with severity in parentheses

8.4.2 Chronic Studies

8.4.2.1 Chronic Study in Rats

A two-year toxicology and carcinogenesis bioassay of t-butanol was conducted for the National Toxicology Program (Cirvello et al., 1995; NTP, 1995), using male and female F344/N rats dosed in their drinking water. Water concentrations for male rats were 0, 1.25, 2.5, or 5 mg/mL, which resulted in delivered doses of 0, 90, 200, or 420 mg/kg-day t-butanol. Water concentrations for female rats were 0, 2.5, 5, or 10 mg/mL, which resulted in delivered doses of 0, 180, 330 or 650 mg/kg-day. Groups of 60 rats/sex/dose were allowed *ad libitum* access to the dosed drinking water, as well as food, for 103 weeks. Ten rats per sex per dose group were used for a 15-month interim sacrifice. All rats were observed twice daily, and checked for clinical signs weekly for 13 weeks and monthly thereafter. Water consumption was recorded every four weeks. Blood was collected at the interim sacrifice for analysis of hematology parameters including hematocrit, hemoglobin, erythrocytes, mean cell volume, mean cell hemoglobin concentration, platelets, leukocytes, segmented neutrophils, lymphocytes, atypical lymphocytes, monocytes, and eosinophils. Urine samples were also collected just prior to the interim sacrifice

for analysis of urine volume, pH, appearance, microscopic sediment, and specific gravity. Necropsy was performed on all animals. Weights of the brain, right kidney, and liver were recorded at the 15-month interim sacrifice. Histopathology was performed on all animals and tissues examined included adrenal gland; bone marrow, femur, and sternum; three sections of brain, clitoral gland, esophagus, heart; cecum, colon, and rectum of the large intestine; duodenum, jejunum, and ileum of the small intestine; kidney, liver, lung, mandibular and mesenteric lymph nodes, mammary gland, three sections of nose, ovary, pancreas, pituitary gland, parathyroid gland, preputial gland, prostate gland, salivary gland, skin, spleen, forestomach and glandular stomach, testis, thymus, thyroid, trachea, urinary bladder, and uterus.

Mean survival of control males and males of all dosed groups was approximately 600 days. Only 10 control males, six low-dose males, four mid-dose males and one high-dose male survived to study termination. Mean survival of females of all groups was approximately 650 days. There were 28 control females, 24 low-dose females, 22 mid-dose females, and 12 high-dose females that survived to study termination. There was a dose-related increase in water consumption by males during the second year of the study, while females had a dose-dependent decrease in water consumption at all time points. The only clinical sign was hyperactivity in high-dose females. The mean body weights of dosed animals were at least 95% of control animals except during the second year of the study, when the mean body weight of high-dose males was approximately 14% below the control mean, and the mean body weight of high-dose females was approximately 12% below the control mean. There were no significant hematology findings in either males or females at the 15-month interim sacrifice. Urinalysis parameters in males were not significantly different from controls, although females at the two highest dose levels had significantly reduced urine volume and pH, and significantly increased urine specific gravity. Kidney weights were most affected by t-butanol dosing. Significant nonneoplastic and neoplastic lesions were, as in the subchronic study, concentrated in the kidney. Results are shown in Tables 4a and 4b, using results of an extended pathology evaluation. Male rats were much more severely affected in the chronic study than females, who did not show any evidence of kidney neoplasia.

In males, there was a dose-response for linear mineralization of the kidney, indicative of α-2μ-globulin accumulation; and weaker dose-responses for nephropathy and for transitional epithelial hyperplasia of the kidney. The incidence of renal tubule hyperplasia was significantly increased at the highest dose, while its severity increased but the increase was not statistically significant. Male rats administered 200 mg/kg-day t-butanol had a significantly increased incidence of multiple renal tubule adenoma as well as of combined renal tubule adenoma and carcinoma. Statistically significant effects in female rats included a progressive increase in the severity of nephropathy, suppurative inflammation at the two highest doses, and transitional epithelial hyperplasia at the highest dose. Although there was evidence highly suggestive of hyaline droplet formation in male rat kidneys, hyaline droplets were not positively identified in this study. Hyaline droplet formation is not likely to completely account for the kidney effects, because of the progressive nephropathy seen in female rats.

NTP (1995) concluded that there was some evidence of carcinogenic activity in male F344/N rats based on the increased incidences of renal tubule adenoma or carcinoma (combined); and that there was no evidence of carcinogenic activity in female F344/N rats.

Table 4a. Kidney weight effects at 15-month interim sacrifice and histopathology results at two years in male rats in a chronic drinking water study (Cirvello et al., 1995; NTP, 1995)

Parameter/ Observation	t-Butanol Dose to Male Rats, mg/kg-day (* signifies a statistically significant result)			
	0	90	200	420
Kidney Weight Absolute Relative			↑ 15%*	↑ 20%*
Kidney, Linear Mineralization[1]	0/50	5/50* (1.0)	24/50* (1.2)	46/50* (1.7)
Transitional Epithelial Hyperplasia[1]	25/50 (1.7)	32/50 (1.7)	36/50* (2.0)	40/50* (2.1)
Renal Tubule Hyperplasia[1]	14/50 (2.1)	20/50 (2.3)	17/50 (2.2)	25/50* (2.7)
Renal Tubule Adenoma	7/50	7/50	10/50	10/50
Renal Tubule Adenoma (multiple)	1/50	4/50	9/50*	3/50
Renal Tubule Carcinoma	0/50	2/50	1/50	1/50
Renal Tubule Adenoma and Carcinoma (combined)	8/50	13/50	19/50*	13/50

[1] Incidence is given as a ratio, with severity in parentheses

Table 4b. Kidney weight effects at 15-month interim sacrifice and histopathology results at two years in female rats in chronic drinking water study (Cirvello et al., 1995; NTP, 1995)

Parameter/ Observation	t-Butanol Dose to Female Rats, mg/kg-day (* signifies a statistically significant result)			
	0	180	330	650
Kidney Weight Absolute Relative		↑ 8%* ↑ 14%*	↑ 18%* ↑ 21%*	↑ 22%* ↑ 42%*
Suppurative Inflammation[1]	2/50 (1.0)	3/50 (1.3)	13/50* (1.0)	17/50* (1.1)
Mineralization[1]	49/50 (2.6)	50/50 (2.6)	50/50 (2.7)	50/50 (2.9)
Nephropathy[1]	48/50 (1.6)	47/50 (1.9)	48/50 (2.3)	50/50 (2.9)
Renal Tubule Hyperplasia[1]	0/50	0/50	0/50	1/50 (1.0)
Transitional Epithelial Hyperplasia[1]	0/50	0/50	3/50 (1.0)	17/50* (1.4)

[1] Incidence is given as a ratio, with severity in parentheses

8.4.2.2 Chronic Study in Mice

A two-year toxicology and carcinogenesis bioassay of t-butanol was also conducted for the National Toxicology Program (Cirvello et al., 1995; NTP, 1995), using male and female $B6C3F_1$ mice dosed in their drinking water. Water concentrations for both sexes were 0, 5, 10, or 20 mg/mL, which resulted in delivered doses of 0, 540, 1,040, or 2,070 mg/kg-day t-butanol to males and 510, 1,020, or 2,110 mg/kg-day to females. Groups of 60 mice/sex/dose were allowed *ad libitum* access to the dosed drinking water, as well as food, for 103 weeks. All mice were observed twice daily, and checked for clinical signs weekly for 13 weeks and monthly thereafter. Water consumption was recorded every four weeks. There was no interim sacrifice for mice, and clinical chemistry, hematology, and urinalysis parameters were not measured. Necropsy was performed on all animals. No organ weights were recorded for mice. Histopathology was performed on all animals. Tissues examined were the same as in the rat study, except that the clitoral and preputial glands were omitted and the gallbladder was included.

The mean body weight gain of the high-dose male mice was 5-10% below the control group level for most of the study. Females at all dose levels had dose-dependent mean body weight gains slightly below to 12% below the corresponding control group. Water consumption of all groups was similar to controls. There were no significant dose-related clinical findings. Significant urinary bladder effects in mice were limited to chronic inflammation and transitional epithelial hyperplasia in high dose males and females. It was concluded that the transitional epithelial hyperplasia would not likely result in neoplasia (NTP, 1995), because comparison of the 13-week and 103-week study results did not show significant progression.

The primary effect of t-butanol exposure on male and female mice was seen in the thyroid gland. There was a statistically significant increase in follicular cell hyperplasia of the thyroid in males of all dose groups, and in females at the two highest doses. There was also an increase in follicular cell adenoma or carcinoma (combined) of the thyroid gland in male mice at the two highest doses, and an increase in follicular cell adenoma of the thyroid gland at the highest dose in female mice. However, none of the adenoma and/or carcinoma results achieved statistical significance. NTP (1995) concluded that there was equivocal evidence of carcinogenic activity in male $B6C3F_1$ mice, based on the marginally increased incidences of follicular cell adenoma or carcinoma (combined) of the thyroid gland, and that there was some evidence of carcinogenic activity in female $B6C3F_1$ mice based on increased incidences of follicular cell adenoma of the thyroid gland. Histopathology results are summarized in Table 5.

Table 5. Histopathology effects of t-butanol in mice in a 2-year chronic drinking water study (Cirvello et al., 1995; NTP, 1995)

Parameter/ Observation	t-Butanol Dose to Male Mice, mg/kg-day (* signifies a statistically significant result)			
	0	540	1,070	2,070
Thyroid Follicular Cell Hyperplasia[1]	5/60 (1.2)	18/59* (1.6)	15/59* (1.4)	18/57* (2.1)
Thyroid Follicular Cell Adenoma[2]	1/60 (2%)	0/59 (0%)	4/59 (7%)	1/57 (2%)
Thyroid Follicular Cell Adenoma or Carcinoma[2]	1/60 (2%)	0/59 (0%)	4/59 (7%)	2/57 (4%)
Parameter/ Observation	**t-Butanol Dose to Female Mice, mg/kg-day (* signifies a statistically significant result)**			
	0	**510**	**1,020**	**2,110**
Thyroid Follicular Cell Hyperplasia[1]	19/58 (1.8)	28/60 (1.9)	33/59* (1.7)	47/59* (2.2)
Thyroid Follicular Cell Adenoma[2]	2/58 (3%)	3/60 (5%)	2/59 (3%)	9/59 (15%)

[1] Incidence is given as a ratio, with severity in parentheses
[2] Incidence is given as a ratio, with overall rate as a percentage in parentheses

8.5 Studies of Genotoxicity and Related End-Points

8.5.1 Mutagenicity Assays

Zeiger et al. (1987) reported results of the *Salmonella typhimurium* reverse mutation assay, using the preincubation method, for a number of chemicals including t-butanol. Testing of t-butanol was conducted for the National Toxicology Program (NTP) by Case Western Reserve University, using strains TA98, TA100, TA1535, and TA1537 without metabolic activation and with activation by Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9. These two systems were used to detect species differences in activation. t-Butanol was tested at 0, 100, 333, 1,000, 3,333, and 10,000 μg/plate, the latter dose being the highest tested in the absence of cytotoxicity. Concurrent positive and solvent controls were used. Two separate assays using triplicate plates were performed. t-Butanol was not mutagenic under the conditions of this test.

Two additional *Salmonella*/mammalian-microsome preincubation mutagenicity assays have been performed, on 99.9% pure t-butanol (EG&G Mason, 1981a) and on a commercial t-butanol product called Arconol (EG&G Mason, 1981b). *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 were used, with and without metabolic activation by Aroclor-induced rat liver S9. The 99.9% t-butanol was tested at 100, 500, 2,500, 5,000, or 10,000 μg/plate, using water as the solvent. No significant increase in the number of revertants was seen, although a slight increase in TA1535 revertants was noted with and without metabolic activation (EG&G Mason, 1981a). The Arconol product was tested at 0, 0.1, 0.5, 2.5, 5.0, or 10.0 μL/plate (0, 79, 394, 1,072, 3,944, or 7,887 μg/plate), also using water as the solvent. An increase in TA1535 revertants described as weak but significant was noted with and without

metabolic activation. Interpretation of the latter results is confounded by not knowing the composition and/or purity of the Arconol product (EG&G Mason, 1981b).

The L5178Y tk^+/tk^- mouse lymphoma cell forward mutation assay was also conducted on t-butanol (McGregor et al., 1988), without metabolic activation and with activation by Aroclor 1254-induced F344/N rat liver S9. Methyl methanesulfonate was used as the positive control without activation, and 3-methylcholanthrene was used with activation. In a test without activation at 1,000, 2,000, 3,000, 4,000, or 5,000 μg/mL, a small but significant increase in the mutant fraction (mutant colonies per 10^6 clonable cells) relative to the solvent control was seen at the highest concentration. In a second test at 625, 1,250, or 5,000 μg/mL without activation, no increase in the mutant fraction was seen. With metabolic activation at concentrations of 1,000, 2,000, 3,000, 4,000, or 5,000 μg/mL, and in a second test at all but the 1000 μg/mL concentration, t-butanol produced no evidence of mutagenicity. The authors concluded that t-butanol was not mutagenic under the conditions of this test.

Two ARCO Chemical Company products, 99.9% t-butanol and Arconol, were also tested for mutagenicity using the L5178Y tk^+/tk^- mouse lymphoma cell forward mutation assay, with and without metabolic activation by Aroclor-induced rat liver S9 fraction (EG&G Mason, 1981c). Positive controls were ethylmethanesulfonate without activation and 7,12-dimethybenz(a)anthracene with activation. At least two tests were done without activation, and two tests with activation, on each product. There were at least eight dose levels per test, in the range of 1.2-18 μL/mL (950-14,200 μg/mL). The 99.9% t-butanol was clearly negative. The Arconol product was negative with metabolic activation, but did produce an increase in mutation frequency at the highest doses without activation. There was no dose-response relationship, and the mutation frequency doubled in only one of two tests. The high doses were above those recommended under current guidelines, and the composition/purity of the Arconol product is unknown.

8.5.2 Assays of Chromosomal Damage

An *in vitro* chromosomal aberration assay using Chinese hamster ovary cells was conducted for NTP by Environmental Health Research and Testing, Inc. (NTP, 1995). Two tests without metabolic activation were conducted, the first using t-butanol concentrations of 160, 500, 1,600, or 5,000 μg/mL with an 11.0-hour harvest time; the second using 1,600, 3,000, 4,000, or 5,000 μg/mL with an 11.5-hour harvest time. Cells were incubated with t-butanol for 9-9.5 hours prior to addition of Colcemid®, and incubated for an additional two hours prior to harvesting and preparation for scoring. Two additional tests with metabolic activation by Aroclor 1254-induced male Sprague-Dawley rat liver S9 mix were conducted. Concentrations of t-butanol were the same as in the tests without activation, and harvest times for the two tests were 11.5 and 12.0 hours. In the tests with activation, cells were incubated for two hours with t-butanol and then the treatment medium was replaced with fresh medium for the remainder of the incubation period. Colcemid® was again added two hours prior to harvest. The medium served as the negative control, while mitomycin-C and cyclophosphamide were used as positive controls without and with activation, respectively. One hundred first-division metaphase cells were scored at each dose level in each test, except at the 5,000 μg/mL concentration in the second test with activation where only 13 cells were available due to toxicity. There were no significant increases in the

number of aberrations, aberrations per cell, or the percentage of cells with aberrations. Because of the single harvest time, these must be considered screening tests. Under the conditions of these tests, the ability of t-butanol to induce chromosomal aberrations was not significantly greater than that of the negative control.

At the end of the 13-week repeated dose study summarized above, an *in vivo* mouse peripheral blood micronucleus test was performed (NTP, 1995). Urethane was used for the positive control in three male mice that were not part of the NTP study. Smears were prepared and scored for the frequency of micronuclei in 10,000 normochromatic erythrocytes in up to 10 animals per dose group, using a semi-automated image analysis system. The scoring criteria included a requirement for micronuclei to exhibit characteristic DNA fluorescent emissions. The percentage of polychromatic erythrocytes in the total erythrocyte population was also determined. Neither male nor female mice exhibited any increase in the percentage of micronucleated normochromatic erythrocyte cells or in the percentage of polychromatic erythrocytes.

8.5.3 Other Assays of Genetic Damage

Tests for sister chromatid exchange (SCE) in Chinese hamster ovary cells were also conducted for NTP (1995) by Environmental Health Research and Testing, Inc. Two tests without metabolic activation were conducted, the first using 160, 500, 1,600, or 5,000 μg/mL t-butanol; and the second using 2,000, 3,000, 4,000, or 5,000 μg/mL. Cells were incubated with t-butanol for 26 hours, with bromodeoxyuridine added two hours after culture initiation. The dosing medium was then removed and replaced with fresh medium including bromodeoxyuridine and Colcemid®, incubation continued for an additional two hours, and then cells were harvested and prepared for scoring. Two tests with metabolic activation by Aroclor 1254-induced male Sprague-Dawley rat liver S9 were also conducted. Test concentrations were the same as in the tests without activation. Incubation with t-butanol for two hours in medium free of fetal bovine serum was followed by continued incubation for 26 hours in a replacement medium containing serum and bromodeoxyuridine. Colcemid® was added at 24 hours, and cells were harvested and prepared for scoring at 26 hours. Mitomycin-C was the positive control without activation, and cyclophosphamide was the positive control with activation. Fifty second-division metaphase cells were scored at each concentration in each test. A 20% increase in SCE frequency above the concurrent solvent control was considered positive. The only positive response (20.32% of control) was that observed in cells incubated with 5,000 μg/mL in the absence of S9. Although the authors considered that test to be weakly positive, the second test without activation, as well as both tests with activation, produced negative responses. Therefore, t-butanol was considered negative for induction of SCEs under the conditions of these tests.

SCE assays using Chinese hamster ovary cells, with and without metabolic activation, were also done on 99.9% t-butanol (EG&G Mason, 1981d) and Arconol (EG&G Mason, 1981e), produced by ARCO Chemical Company. Six or seven doses in the range of 0.31-20 μL/mL (245-15,770 μg/mL) were used. Criteria for considering the test positive were either a doubling of SCEs over the solvent control at two dose levels, or a positive dose-response relationship over a minimum of three dose levels. A significant increase in SCEs was produced by 99.9% t-butanol at the highest dose without activation, and at the two highest doses with activation. However, the criteria for a positive test were not met. Arconol produced a marginal increase in SCE frequency,

which also did not meet the criteria for a positive test. The higher doses in these tests were above those considered appropriate under current guidelines (U.S. EPA, 1996c; U.S. EPA, 2002a).

Roots and Okada (1972) studied t-butanol at concentrations of 0.5, 1.0, 2.0, 3.0, or 4.0 M as a scavenger to protect DNA molecules of mouse lymphoma L5178Y cells from radiation-induced single-strand breaks. t-Butanol was shown to protect DNA by acting as a hydroxyl radical scavenger, but protection was only effective up to a t-butanol concentration of 2.5 M. The maximum protection was the same for several alcohols tested, including methanol, ethanol, ethylene glycol, and glycerol. This study supports the studies of Cedarbaum and Cohen (1980) and Cederbaum et al. (1983) in identifying t-butanol as a hydroxyl radical scavenger.

8.6 Reproductive and Developmental Toxicity Studies

8.6.1 Reproductive Toxicity

8.6.1.1 Studies on t-Butanol

There is limited information regarding the effects of t-butanol on reproduction.

The effects of ethanol and t-butanol on the *in vitro* fertilizing ability of mouse spermatoza were reported by Anderson et al., (1982). Oocytes were obtained from superovulated female Swiss-Webster mice, and epididymal spermatoza from males. Sensitive processes were determined by adding the alcohols, in separate experiments, to only the capacitation medium, to only the fertilization medium, or to both. Ethanol added to the capacitation medium, or to both the capacitation and the fertilization media, substantially inhibited fertilization, although the inhibition was reversible. Pyrazol, an alcohol dehydrogenase inhibitor, was protective of the effects of ethanol but acetaldehyde, the major ethanol metabolite, did not inhibit fertilization. t-Butanol, at a concentration of 87 mM, did not have an inhibiting effect on *in vitro* fertilization, indicating the effect of ethanol was more complex than a simple solvent effect.

Nelson et al. (1991) included male reproductive effects in their study of the behavioral teratology of t-butanol. Groups of 18 male Sprague-Dawley rats were exposed by inhalation to 6,000 or 12,000 mg/m^3 (approximately 4,970 or 9,950 mg/kg-day assuming 100% absorption) t-butanol seven hours per day for six weeks. They were then mated to unexposed females. Resulting litters were culled to four males and four females, and these offspring underwent a variety of tests for neuromotor coordination, activity, and learning periodically for up to 90 days. No significant behavioral differences were noted between the control groups and the groups whose parental males had been exposed to t-butanol. Brains from 10 offspring per group were evaluated for protein, acetylcholine, dopamine, norepinephrine, serotonin, met-endorphin, and substance-P. No significant differences between groups were reported.

Evaluation of sperm morphology and vaginal cytology were included for both rats and mice in the subchronic studies previously reviewed (Lindamood et al., 1992; NTP, 1995). Samples were taken from males at terminal sacrifice, while vaginal samples were collected for up to seven days during the twelfth week of the study. No significant differences in sperm morphology or motility were seen, nor were there any differences in the percentage of time spent in the various stages of

the estrous cycle. In mice, but not in rats, the overall length of the estrous cycle was significantly increased at the highest dose.

8.6.1.2 Studies on Methyl t-Butyl Ether

A two-generation reproduction study has been done on methyl t-butyl ether. Since methyl t-butyl ether is rapidly metabolized to equimolar amounts of t-butanol and formaldehyde (Brady et al., 1990), this study should provide relevant, although not definitive, information on t-butanol.

Bevan et al. (1997) exposed groups of 25 Sprague-Dawley rats per sex to 0, 400, 3,000, or 8,000 mg/m^3 methyl t-butyl ether (approximately 330, 2,500, or 6,600 mg/kg-day methyl t-butyl ether; approximately 1.9, 14, or 38 mmol/kg-day or approximately 140, 1,040, or 2,800 mg/kg-day t-butanol assuming 100% absorption) for 10 weeks prior to mating. Animals were mated one male to one female, with females not mating during the first seven days placed with another male for a total mating period of 21 days. Exposure continued for parental females through weaning, on all but the first four days of lactation, and for parental males through delivery of the last litter they sired. One pup per sex per litter from the F_o parental generation was randomly selected for the F_1 parental generation of 25 neonates per sex per group, with exposure starting on postnatal day 28 and continuing for 10 weeks prior to mating, as with the previous generation. All parental animals were observed twice and examined once daily. Body weights of females were recorded weekly before mating, on GD 0, 7, 14, and 20, and on postnatal days 0, 7, 14, 21, and 28. Body weights of males were recorded weekly. Liver weights of F_1 parental animals at all exposure levels were recorded. The pituitary, testes, epididymides, prostate and seminal vesicles, vagina, uterus, ovaries, respiratory tract including nasal turbinates, and all gross lesions of control and high-dose animals were examined microscopically. Live pups were counted, sexed, weighed, and examined for external anomalies on postnatal days 0, 1, 4, 7, 14, 21, and 28, although on postnatal day 4, litters were culled to four males and four females. Culled pups were sacrificed unless external anomalies were noted, in which case a gross postmortem examination was conducted.

Both generations of parental animals showed hypoactivity, ataxia, blepharospasms, and lack of startle reflex at 8,000 mg/m^3 (~ 2,800 mg/kg-day), and hypoactivity, blepharospasms and lack of startle reflex at 3,000 mg/m^3 (~ 1,040 mg/kg-day). There were no symptoms of central nervous system depression at the lowest exposure level. No significant effects on either the F_o or F_1 parental animals were noted in number of sperm-positive females, number of pregnancies, number of live litters, length of gestation, male or female mating index, male or female fertility index, or gestational index. High-dose F_1 males had significantly reduced final body weights. High-dose F_1 males and females had significantly increased absolute and relative liver weights, without any associated histopathology. Liver weights at the middle dose were also increased, although not significantly. No histopathological effects were noted in any of the reproductive organs examined. No significant effects on either the F_1 or F_2 offspring were noted in stillborn animals, mean live pups per litter, 4-day, 7-day, 14-day, 21-day, or 28-day survival index, or lactation index. Body weights of offspring were reduced in both generations through postnatal day 28, attributed by the authors to indirect methyl t-butyl ether exposure. The authors concluded that the NOAEL for both parental and developmental toxicity was 400 mg/m^3 (~ 140

mg/kg-day t-butanol), while the NOAEL for reproductive effects was at least 8,000 mg/m^3 (~ 2,800 mg/kg-day t-butanol).

8.6.2 Developmental Toxicity Studies

The developmental toxicity of three butanol isomers was studied (Nelson et al., 1989) in Sprague-Dawley rats, exposed by inhalation to t-butanol at concentrations of 2,000, 3,500, or 5,000 ppm (6,000, 10,500, or 15,000 mg/m^3; approximately 4,970, 8,700, or 12,430 mg/kg-day assuming 100% absorption) seven hours/day on Gestation Days (GD) 1 to 19. There were 15-18 animals bred, and 13-18 pregnant animals, per dose group. Chamber concentrations were monitored using an infrared analyzer. All exposure concentrations were high enough to impair postexposure locomotor activity. Maternal food intake was significantly reduced during the first two weeks of gestation, but not the third week, at the highest t-butanol exposure level. Maternal weight gain was consequently reduced at this level. Pregnant females were euthanized by carbon dioxide asphyxiation on GD 20, and the fetuses serially removed for examination. There were no significant effects on the number of pregnant animals; corpora lutea, resorptions of live fetuses per litter; sex ratio of fetuses; or visceral malformations. Skeletal variations increased at the two highest doses, but not significantly, and included reduced ossification and rudimentary cervical ribs typically seen in conjunction with fetotoxicity. The primary effect of t-butanol was a dose-dependent decrease in fetal weight for both males and females. Thus, t-butanol did not cause selective developmental effects even at doses that produced maternal and fetal toxicity.

Grant and Samson (1982) reported effects of ethanol and t-butanol on brain growth parameters in neonatal Long Evans rats, reared on an artificial liquid diet on postnatal days 4-18 and exposed to one of the alcohols on postnatal days 4-7 (days 26 through 29). Postnatal dosing of the rats was done because the brain growth spurt in rats occurs during the first two postnatal weeks, while in humans it occurs in the third trimester of gestation. Feeding and alcohol dosing were done by surgically placed cannulae. Doses of t-butanol on the four exposure days were 1.44, 2.16, 0.60, and 2.69 g/kg, and were calculated to correspond to blood-ethanol levels that had previously been shown to result in microcephaly. Animals were tested daily for righting reflex, cliff avoidance, and negative geotaxis. Exposed animals were visibly intoxicated and had difficulty performing these reflex tests. The mean blood t-butanol level of six animals on the last exposure day was 48.2 ± 13.1 mg/100 mL. On day 40, eleven days after the last alcohol dose, animals were decapitated and their brains were removed for examination. Absolute brain weights and brain-to-body weight ratios were reduced in comparison to controls for animals exposed to both ethanol and t-butanol. In the forebrain, the absolute DNA level of the t-butanol group was slightly but not significantly reduced, but absolute protein content was reduced approximately 15%. In the hindbrain, the absolute DNA level of the t-butanol group was significantly reduced. There was no evidence that t-butanol interfered with myelin formation, but it did appear to interfere with cell proliferation during this rapid phase of brain development through its action as a general membrane solvent.

In an *in vitro* study comparing the effect of t-butanol on the cerebral cortex of neonatal rats during the brain growth spurt with its effect in adult rats, Candura et al. (1991) reported that t-butanol was the most effective alcohol in causing inhibition of muscarinic receptor-stimulated inositol metabolism. This site had been proposed as a target for the neurotoxic effects of ethanol during brain development.

In a study only available in abstract form (Abel and Bilitzke, 1992), pregnant Long Evans rats were dosed with 0, 0.65, 1.3, or 10.9% (v/v) t-butanol in a liquid diet. Reported effects included reduced maternal weight gain, reduced litter size, reduced birth weights and weights at weaning, increased perinatal mortality, and increase postnatal mortality up to 100%. The dose levels at which these effects occurred were not specified.

Two developmental studies have also been conducted in mice. In a study of prenatal effects of t-butanol on mice, Faulkner et al. (1989) dosed pregnant CBA/J and C57BL/6J mice by gavage every 12 hours with 10.5 mmol/kg (780 mg/kg) t-butanol in tap water, or with tap water alone as a control, on days 6-18 of gestation. Animals had free access to food and water during the dosing period. The mice were sacrificed by decapitation on GD 18, and the fetuses were removed for examination. Exposure to t-butanol caused a significant increase in resorptions per litter and a significant decrease in live fetuses per litter. There were no effects on soft tissue defects, and only non-significant effects on skeletal defects, including unossified sternebrae and reduced ossification of the supraoccipital bone. No significant strain differences were noted in any parameter.

Daniel and Evans (1982) studied the postnatal development of Swiss-Webster (Cox) mice using the following psychomotor/behavioral tests: righting reflex, negative geotaxis (turning to vertical orientation when placed head down on a slope), open field behavior, cliff avoidance, and roto-rod performance. Groups of 15 pregnant females were dosed with 0, 0.5, 0.75, or 1.0% t-butanol in a liquid diet given *ad libitum* from day 6 to day 20 of gestation. Litter size, weight, and viability were recorded within 24 hours of parturition, and half of the maternal animals were replaced with maternal animals that had delivered within the previous 24 hours but had not been exposed to t-butanol (fostered). Behavioral tests were conducted on four fostered litters and four litters maintained with the maternal animal at each of the two low dose groups, while only three litters each were tested at the high dose. Testing was conducted every other day from day 2 through day 10, except rotorod testing that was conducted every other day from day 14 to day 22.

Slight decreases in maternal diet intake and maternal weight gain were noted. Postparturition data showed significant dose-dependent decreases in the total number of litters, the number of neonates per litter, and fetal weight at day 2 as well as a significant dose-dependent increase in the number of stillborn animals. Dose-dependent declines in performance in the righting reflex, open field behavior, cliff avoidance, and rotorod performance tests were seen. The negative geotaxis test gave unreliable results. Righting reflex was the only test affected by the dam, with the pups raised by the maternal dam taking slightly longer than fostered pups to return to a position of all four feet on the surface after being placed on their backs.

8.7 Studies of Immunological and Neurological Effects

8.7.1 Immunological Effects

No studies on the immunological effects of t-butanol were located.

8.7.2 Neurological Effects

8.7.2.1 General Effects

Several effects described as 'whole body metabolism' have been attributed to t-butanol. Siviy et al. (1987) compared several measured or calculated parameters for ethanol and t-butanol in groups of 10 adult male Wistar rats dosed with 0.1, 0.2, 0.4, or 0.8 g/kg t-butanol, or with ethanol doses which gave similar effects on motor activity. Motor activity was measured by placing the metabolic chambers on electronic balances during the 50-minute tests. Both chemicals produced significant decreases in motor activity.

Atrens et al. (1989) reported that t-butanol, as well as ethanol, decreased body temperature and increased blood glucose levels in exposed Wistar rats. Rats were handled on a regular basis for four weeks prior to testing, to diminish stress as a confounding factor in the results. Each animal in a group of 10 male Wistar rats was dosed sequentially with 0, 1.5, and 3.0 g/kg ethanol, and each animal in a second group was dosed sequentially with 0, 0.3, and 0.6 g/kg t-butanol. All animals in each group received all three doses, separated by four-day intervals. Dosing was by intraperitoneal injection, and temperature and blood glucose measurements were made 1.0, 2.0, and 3.0 hours after dosing. The body temperature profiles, as a function of time, were very similar for the two chemicals. At the middle doses, there was a drop in rectal temperature of 1.5 to 2.0 ^{o}C that persisted and only began to show signs of slow recovery at the 3.0-hour measurement. At the high doses, each chemical exposure resulted in a rectal temperature drop of approximately 3 ^{o}C, and this drop persisted throughout the measurement period. Blood glucose levels increased approximately one mmol/L at the middle doses, and three-to-five mmol/L at the high doses for each chemical. The decrease in body temperature and the increase in blood glucose were highly correlated, and represented large and long-lasting responses. The authors postulated that these effects appeared to be due to direct physico-chemical effects on nerve cell membranes.

HOT mice, selectively bred for resistance to the hypothermic effects of an acute dose of ethanol, and COLD mice selectively bred for sensitivity to this effect, were used by Feller and Crabbe (1991) to study the effects of other alcohols, as well as sedative hypnotics. The lines did not differ in the ability to metabolize ethanol. The dose necessary to produce a 2 °C decrease in body temperature was used for comparison. When measured in mice of the seventh to ninth generations, HOT mice required 0.94 g/kg t-butanol and COLD mice required 0.70 g/kg t-butanol to produce the 2 °C decrease in body temperature. When measured in mice of the eleventh to fifteenth generations, HOT mice required 0.90 g/kg and COLD mice required 0.51 g/kg to produce the same decrease in body temperature. This response was typical of alcohols, with COLD mice significantly more sensitive than HOT mice, and the difference between them increasing with increasing generation. The fact that mice selected for sensitivity to the hypothermic effects of ethanol were also sensitive to the hypothermic effects of other alcohols suggested a common genetic basis, which was supported by the increasing response differential with increasing generation.

8.7.2.2 Studies of Physical Dependence and Withdrawal in Rats

McCreery and Hunt (1978) used the ED_3 for pronounced ataxia (the dose required to produce a score of 3) as a behavioral endpoint to compare with the membrane/buffer partition coefficient, the concentration in the non-aqueous phase of the animal, the volume of the non-aqueous phase occupied, and thermodynamic activity. Some correlation of ataxia with the membrane/buffer partition coefficient, which was taken to be 20% of the n-octanol/water partition coefficient, was seen in compounds with up to 7-9 carbon atoms. No correlation was found with the other parameters studied.

Wood and Laverty (1979) studied the physical dependence produced by prolonged ethanol or t-butanol administration to rats. Groups of 12 young random-bred male albino Wistar rats were maintained on a liquid diet including 87 mL/L ethanol (14.7 g/day) or 20 mL/L t-butanol (3.5 g/kg-day) for 20 days. The alcohol was then isocalorically replaced with sucrose, which was also used in the control diet. Rats were observed for signs of withdrawal for 9 hours, and then at 24, 48, and 72 hours for persistent withdrawal effects. Blood samples were taken for alcohol analysis on days 1, 5, and 10 (4 samples), and hourly after the start of withdrawal. The concentration of t-butanol in the blood fell much more slowly than the blood-ethanol concentration, and was still detectable at the end of the 9-hour observation period. Signs of withdrawal (head bobbing, excessive grooming, and paw shaking, irritability, spontaneous forelimb convulsions, audiogenic convulsions, and death in some cases) were also delayed. Surviving rats showed withdrawal effects up to 72 hours, although with decreasing severity. None of the behavioral changes in the alcohol-treated rats were apparent in the control rats. t-Butanol was seven times more potent than ethanol in its ability to produce physical dependence. t-Butanol completely prevented development of withdrawal in ethanol-treated rats, and ethanol completely prevented development of withdrawal in t-butanol-treated rats. In two separate experiments, the authors showed that dependence could be triggered in as little as four days; and, by using rats pretreated with 6-hydroxydopamine, that catecholamine depletion did not prevent the physical dependence and withdrawal symptoms.

Thurman et al. (1980) scored withdrawal effects using 14 symptoms, which were classified as spontaneous or induced, in female Sprague-Dawley rats dosed with approximately 5 g/kg ethanol or 1.2 g/kg t-butanol. The withdrawal profiles in animals whose blood-alcohol concentrations were maintained for up to six days were nearly identical for the two alcohols, although the t-butanol symptoms occurred at five-fold lower dose levels. Neither ethanol nor t-butanol had an effect at peak withdrawal on cyclic AMP levels in rat brain cortex, cerebellum, mid-brain, or pons-medulla.

LeBlanc and Kalant (1975) studied the intoxicating effects of ethanol, n-propanol, iso-propanol, n-butanol, and t-butanol, along with the effects of ethanol pretreatment, using a moving belt test. Male Wistar rats were trained to walk on a moving belt, and received a shock if they touched an electrical grid under the belt. The amount of time off the belt was integrated as the response. Rats were given intraperitoneal doses of the alcohols dissolved in isotonic saline, calculated so that the molar dose multiplied by the thermodynamic activity coefficient was constant for all the alcohols. Doses calculated in this manner had been shown to result in very similar biological effects. Two groups of 24 animals were tested: four animals at each of six dose levels without

ethanol pretreatment, and four animals at each of six dose levels after gavage doses of 6 g/kg ethanol daily for 20 days. Blood concentrations of the alcohols were not affected by ethanol pretreatment. There was a highly significant dose-dependent effect on time off the belt with each of the alcohols, including t-butanol, before ethanol pretreatment. Ethanol pretreatment caused shifts in each of the dose-response curves such that a much higher dose of the alcohol was required to produce the same time off the belt, indicating significant cross-tolerance with ethanol. The authors felt this cross-tolerance was not due to changes in absorption, distribution, or metabolism, since the maximum effect was seen within 10 minutes of alcohol administration and there was no effect on blood concentrations of the alcohols. They suggested that development of tolerance was a common functional effect rather than an effect of the structures of the individual alcohols.

The effects of chronic ethanol pretreatment on three different measures of performance were studied by Khanna et al. (1997), after additional single intraperitoneal injections of ethanol, t-butanol, or other alcohols and barbiturates. Male Sprague-Dawley rats were used. Chronic ethanol pretreatment was given by gastric intubation at doses of up to 8 g/kg-day. Pretreatment with isocaloric sucrose was used as the control. In each case, the performance or body temperature of the rat prior to the single additional alcohol or drug dose served as the control. There was a negative correlation between the ED_{60} for performance on a rotarod, programmed to accelerate linearly with time, and the log of the octanol/water partition coefficient. This relationship held for four alcohols and three of four barbiturates. Rats dosed for 10 weeks with ethanol, but not those dosed for 10 weeks with sucrose, and then dosed intraperitoneally with 0.3 g/kg t-butanol, showed a significant decrease in impairment on the accelerating rotarod. Likewise, rats chronically exposed to ethanol, but not to sucrose, showed a decrease in hypothermic response when dosed with 0.65 g/kg t-butanol, and showed a decrease in maximum impairment in a tilt-plane test when dosed with 0.3 g/kg t-butanol. Thus, chronic pretreatment with ethanol resulted in tolerance to ethanol, t-butanol, and other alcohols. This study confirmed the cross-tolerance effect using two additional tests, the rotarod and the tilt plane.

Schedule-induced drinking, whereby adult male Long-Evans rats were given access to food and to water dosed with t-butanol during six, one hour feeding sessions, each separated by three hours, was compared with home-cage controls (Grant and Samson, 1981). There were four or five animals per dose group. Four dosing regimens were used with the schedule-induced drinking, and three dosing regimens were used for the home-cage controls. All animals were tested for susceptibility to seizure seven-to-eight hours after replacing the water dosed with t-butanol with plain drinking water. Testing was done by shaking a ring of keys for 30 seconds, at a rapid rate, over the center of the open cage. Although seizure results were rather erratic, with at least one animal from each group having a withdrawal score of zero, other withdrawal symptoms were seen at the 3% t-butanol level regardless of dosing protocol. Animals dosed at the 3% level were described as intoxicated and frequently in a stupor. The daily t-butanol intake during the last 20 days of dosing was significantly correlated with withdrawal severity, as measured by susceptibility to seizures. Part of the difficulty with these tests was that as the water concentration of t-butanol increased, water consumption decreased. t-Butanol did not induce overdrinking, as ethanol does.

8.7.2.3 Studies of Physical Dependence and Withdrawal in Mice

Neurological effects have been extensively studied, as t-butanol exposure produces symptoms of intoxication and physical dependence similar to those produced by ethanol. t-Butanol has been a popular choice in comparative studies attempting to determine whether various symptoms of ethanol intoxication should be attributed directly to ethanol, or to its acetaldehyde metabolite.

McComb and Goldstein (1979a) studied the withdrawal reactions of mice exposed to either t-butanol or ethanol, using convulsions elicited by handling for scoring. Groups of 24 male Swiss-Webster mice were given low or high priming doses of t-butanol by intraperitoneal injection, and then placed in an inhalation chamber for 1, 3, 6, or 9 days. Chamber t-butanol concentrations were adjusted to maintain blood levels of the low-dose mice at 5.0 ± 0.2 mM, and blood levels of the high-dose mice at 8.5 ± 0.2 mM. In the ethanol study, blood concentrations were maintained at 22 or 44 mM.

At the low blood t-butanol level, the withdrawal response was a linear function of days of exposure. At the high blood t-butanol level, however, the withdrawal response became saturated at three days and was approximately the same after six and nine days of exposure. t-Butanol was approximately five times as potent as ethanol in producing physical dependence, and also has an approximately five times greater lipid solubility. The authors concluded that the two alcohols produced physical dependence by the same mechanism, perhaps acting at a site in the lipid bilayers of neuronal membranes.

In an extension of this work, McComb and Goldstein (1979b) used similar blood alcohol concentrations of 5.0 or 8.0 mM for low- and high-dose t-butanol exposures, and 25.2 or 39.6 mM for low- and high-dose ethanol exposures. Groups of 8-24 mice were exposed to sequential three-day exposures to the alcohols. Three days of t-butanol exposure, followed by another three days of t-butanol exposure, showed a slight degree of saturation of response at the low blood t-butanol concentration, and a significant degree of saturation of response at the high blood t-butanol concentration. Three-day exposures to ethanol followed by ethanol, to t-butanol followed by ethanol, or to ethanol followed by t-butanol showed very similar withdrawal responses at both the low and the high blood alcohol concentrations. There were no withdrawal signs when one alcohol was substituted for the other, and each alcohol was able to substitute for the other in the development and maintenance of physical dependence, again suggesting that they shared a common mode of action.

Erwin et al. (1976) determined the 50% effective dose (ED_{50}) for loss or righting reflex, after intraperitoneal exposure to alcohols and other sedatives, in two lines of mice genetically bred for short-sleep time (SS) or long-sleep time (LS) after ethanol administration. After dosing with t-butanol, the ED_{50} values were 1.39 g/kg for the SS line and 0.84 g/kg for the LS line. Ethanol, methanol, and n-butanol also had approximately two-fold differences in ED_{50} values between the two lines, while sodium pentobarbital and diethyl ether did not. The authors had previously shown that there were no differences in ethanol metabolism between the two lines, and suggested that the SS animals must awaken at higher blood alcohol levels than the LS animals. In another experiment, Dudek et al. (1984) showed that L-S and S-L reciprocal F_1 hybrids, having a female LS parent and a female SS parent, respectively, were intermediate in response to loss of righting

reflex to the LS and SS parental genotypes. Both ethanol and t-butanol produced responses intermediate between the two parentals, while acetaldehyde and pentobarbital differed significantly only from the SS parental line. These experiments further illustrated the similarities in response between ethanol and t-butanol.

LJ et al. (1992) pretreated male CD-1 mice daily for at least 23 days with 3.5–4.5 g ethanol by intraperitoneal injection. They then gave single intraperitoneal injections of 3.5, 4.0, or 4.5 g/kg ethanol or 1.2, 1.4, or 1.6 g/kg t-butanol and measured loss of righting reflex. Both chemicals significantly increased the latency to onset of loss of righting reflex, and significantly decreased the duration of the loss.

In an experiment using female HA/ICR Swiss-Webster mice or female MCR(SD) albino rats, Erickson et al. (1980) studied the effects of intracerebroventricular doses of calcium chloride on the loss of righting reflex in animals also dosed by intraperitoneal injection with sedative doses of ethanol or t-butanol. Doses were in the range of 5-20 μmole calcium for mice and 2-8 μmole calcium for rats. While ethanol enhanced the loss of righting reflex in both species, the enhancement was significant for t-butanol only in mice. The results suggested calcium involvement in the central nervous depressant effects of alcohols.

Snell and Harris (1980) used shock avoidance as another measure of latent alcohol effect. Four groups of 10 male DBA/2J mice were given one of the following diets: a liquid diet delivering approximately 15 g/kg-day ethanol, an isocaloric control diet for the ethanol exposed mice, a liquid diet delivering approximately 3.39 g/kg-day t-butanol, or the control diet for t-butanol. The dosing period was seven days. One day after cessation of dosing animals were tested using a shock avoidance procedure, which consisted of a two-compartment cage requiring the animal to move from one compartment to the other after hearing a bell in order to avoid the shock. Both ethanol and t-butanol impaired the acquisition of shock avoidance behavior. Animals dosed with t-butanol were also tested two days after cessation of exposure, and shock avoidance was still impaired, although to a lesser extent. Withdrawal, determined by observation or by measurement of body temperature, lasted less than eight hours, so the effects could not be attributed to the presence of overt withdrawal signs. The authors attributed the results to chronic sedation, and noted that there was no relationship between severity of the alcohol withdrawal signs and shock avoidance impairment, consistent with previous results.

8.7.2.4 Disordering of Brain Membranes

Many studies of physical dependence and withdrawal related observed effects to the lipid solubility of the alcohols, primarily in terms of their oil/water partition coefficients (McCreery and Hunt, 1978; McComb and Goldstein, 1979a, Khanna et al., 1997). Effects were frequently attributed to generalized interaction with the lipid bilayer of the cell membrane. Studies exploring more selective effects have been published.

Lyon et al (1981) measured the hypnotic potencies of a series of alcohols using the ED_{50} for loss of righting reflex, and also measured the perturbation of mouse brain synaptosomal plasma membranes induced by these alcohols. The ED_{50} values were determined in male Swiss-Webster mice injected intraperitoneally with 20% w/v of the alcohols in 0.9% saline. At least five doses

were used for each alcohol, with six mice per dose group. The ED_{50} for t-butanol was 14.6 mmol/kg (1,080 mg/kg), while that for ethanol was 70.1 mmol/kg (320 mg/kg), again reflecting the approximately five-fold greater potency of t-butanol.

Mouse brain synaptosomal plasma membranes were isolated from whole brains of male Swiss-Webster mice and spin-labeled with 5-doxylstearic acid. They were then incubated with buffered solutions of the alcohols for 10 minutes before first derivative electron paramagnetic resonance spectra were recorded. The aqueous concentration required to produce a 0.01 change in order parameter ranged from 0.00018 M for 1-octanol to 0.60 M for ethanol, with the t-butanol concentration at 0.26 M. This disordering ability was related to membrane solubility as determined by published oil/water partition coefficients. The authors noted that the progressive increase in membrane disordering potency was greater than the progressive increase in the potency for loss of righting reflex.

In an extension of this work, Lyon and Goldstein (1983) again used electron paramagnetic resonance with 5-, 12-, and 16-doxylstearic acid spin labeling of brain synaptosomal plasma membranes of male Swiss-Webster mice physically dependent on ethanol. Pyrazole dosing was used to competitively inhibit alcohol dehydrogenase and maintain blood ethanol levels at approximately 2.5 mg/mL, and was also used in the control mice. The different spin labels explored different depths of the membrane bilayer. 'Tolerant' brain synaptosomal plasma membranes from the ethanol-dependent mice, or membranes from pyrazole-treated controls, labeled with the 5-doxylstearic acid spin probe, were exposed *in vitro* to 0 (pyrazole control), 200, 400, or 600 mM ethanol or to 0 (pyrazole control), 200, or 400 mM t-butanol. Although there was no difference in the baseline (pyrazole control) order parameter, the membranes from ethanol-dependent mice required a higher concentration of either ethanol or t-butanol than the membranes from the pyrazole control mice to produce the same change in order parameter. This demonstrated both tolerance (ethanol dependence, ethanol exposure) and cross-tolerance (ethanol dependence, t-butanol exposure). Similar results were obtained with 12-doxylstearic acid, although the baseline order parameter was also higher in membranes from the ethanol-dependent mice using this probe.

Fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene and (Na^+/K^+)ATPase activity in brain synaptosomal membranes were also used to study the effects of alcohols (BeaugJ et al., 1984). Under the experimental protocol, 100 mmol/kg (4.6 g/kg) ethanol, 50 mmol/kg (3 g/kg) isopropanol, 25 mmol/kg (1.85 g/kg) t-butanol, 12.5 mmol/kg (925 mg/kg) t-butanol, or water as the control was given to naive male Sprague-Dawley rats in a single dose by gastric intubation. There were three to six animals per group. At a time after dosing when the blood-alcohol level had dropped to near zero (18 hours for ethanol, 20 hours for isopropanol, 40 hours for high-dose t-butanol, and 24 hours for low-dose t-butanol), the animals were sacrificed and the crude brain synaptosomal fraction was isolated. The basal fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene was first measured, and the polarization was measured again after the *in vitro* addition of 0.350 or 0.700 M ethanol. Likewise, the basal value of (Na^+/K^+)ATPase activity was measured, and was measured again after the *in vitro* addition of 0.175, 0.300, or 0.700 M ethanol. The basal 1,6-diphenyl-1,3,5-hexatriene fluorescence polarization was not affected by *in vivo* administration of any of the alcohols. The change in 1,6-diphenyl-1,3,5-hexatriene fluorescence polarization after the *in vitro* addition of ethanol was significantly increased over

the control values for both ethanol and isopropanol. The basal (Na^+/K^+)ATPase activity was increased after *in vivo* exposure to ethanol and isopropanol, as was its inhibition after *the in vitro* addition of ethanol. No significant changes in either parameter were noted in the t-butanol tests. Since t-butanol behaved differently than ethanol or isopropanol, the authors concluded that sensitization of the synaptosomal membranes to the *in vitro* addition of ethanol after *in vivo* administration of ethanol or isopropanol to naive animals is probably not determined primarily by lipid solubility.

Lasner et al. (1995) studied the *in vitro* effects of several alcohols on membrane-bound and soluble acetylcholinesterase, an important protein in neurotransmission. Ethanol, n-propanol, and t-butanol, but not n-butanol, had biphasic effects on membrane-bound acetylcholinesterase activity with optimal effects in the range of 10-40 mM. These chemicals had little effect on soluble acetylcholinesterase activity, showing a less than 10% decrease over the 0-200 mM concentration range. Membrane fluidity, measured by 1,6-diphenyl-1,3,5-hexatriene fluorescence polarization, decreased slightly with increasing concentrations of ethanol, n-butanol, or t-butanol, but decreased approximately 50% with increasing concentrations of n-propanol, all over a concentration range of 0-80 mM. In addition to lipid solubility, the authors felt that other factors such as the site of action of the alcohol and its geometry played an important role in the membrane effects of alcohols. The relative potencies of various effects did not follow a simple pattern. The order for the activity of membrane-bound acetylcholinesterase suggested that the active site was too small to accommodate a straight-chain alcohol longer than n-propanol. The effect on membrane fluidity was postulated to involve both lipid solubility and molecular size.

9.0 RISK CHARACTERIZATION

9.1 Hazard Assessment

9.1.1 Evaluation of Major Non-Cancer Effects and Mode of Action

At high doses, the effects of t-butanol are similar to, although more potent than, the effects of ethanol. These effects include intoxication, development of physical dependence, withdrawal following abrupt discontinuation of exposure, and cross-tolerance with other alcohols. There was, however, no evidence of selective developmental effects even at doses causing maternal toxicity and reduction in mean fetal weight (Nelson et al., 1989). t-Butanol did not produce microcephaly, or fetal alcohol syndrome, characteristic of high-dose ethanol exposure during pregnancy (Grant and Samson, 1982).

It is clear, from both subchronic (Lindamood et al., 1992; NTP, 1995) and chronic (NTP, 1995; Cirvello et al., 1995) drinking water studies on t-butanol, that the kidney is the major target organ in male and female rats. Males in the subchronic study had significant dose-dependent increases in absolute and relative kidney weight, as well as significant increases in the incidence of kidney mineralization and the severity of nephropathy. These latter lesions historically are seen spontaneously in the F344 rat, with nephropathy more prominent in males (NTP, 1995). Hyaline droplet accumulation was noted in males at all dose levels. Respective absolute and relative kidney weights 12% and 19% greater than controls at the lowest dose level suggest that a NOAEL cannot be determined for male rats from the subchronic study. A LOAEL of 230 mg/kg-day can be identified, but is not relevant to human health because of the hyaline droplet

accumulation, as discussed below. Female rats in the subchronic study also had statistically significant dose-dependent increases in absolute and relative kidney weights at all dose levels, as well as statistically significant increases in the severity of kidney mineralization and the incidence of nephropathy. Mineralization is the more prominent lesion in female rats (NTP, 1995). Respective absolute and relative kidney weights 19% and 17% greater than controls at the lowest dose level suggest that a NOAEL cannot be identified for female rats from the subchronic study. A LOAEL of 290 mg/kg-day can be identified, based on the female rat kidney weight effects, and is relevant to human health risk assessment.

Mice showed fewer effects from t-butanol dosing than rats in the subchronic study (Lindamood et al., 1992; NTP, 1995). Hypoactivity and some urinary bladder effects from t-butanol exposure were observed. No thyroid effects were noted in mice in the subchronic studies, although the thyroid gland was selected for histopathology. From the subchronic study, a NOAEL for male mice of 640 mg/kg-day based on hypoactivity at the next higher dose level can be established. A NOAEL for female mice of 1,660 mg/kg-day, based on weight-gain depression and emaciation observed at the next higher dose level, can also be established. There are some small differences in dose and effect values between the Lindamood et al. (1992) study and the NTP (1995) report. This very likely resulted from recalculations done during the NTP peer review process and does significantly affect this risk assessment, which has used the NTP (1995) values throughout.

Male rats in the chronic study had a significant increase in relative but not absolute kidney weight at the two highest dose levels, as well as a significant and dose-dependent increase in linear mineralization. Because cancerous and pre-cancerous effects observed in male rats in the chronic study were associated with the α-2μ-globulin effect, it would be inappropriate to select NOAEL and LOAEL values. Female rats in the chronic study had increases in absolute and relative kidney weights at all dose levels. Mineralization and nephropathy were more severe in the chronic study than in the subchronic study of female rats, and there was significant transitional epithelial hyperplasia at the high dose. Kidney weight increases with associated histopathology in female rats remained the critical effect in the chronic study, with a LOAEL of 180 mg/kg-day.

Kidney and bladder effects were not significant in either male or female mice in the chronic study. However thyroid effects, including follicular cell hyperplasia, appeared in both sexes of mice, with a LOAEL of 540 mg/kg-day in males and a NOAEL of 510 mg/kg-day and a LOAEL of 1,020 mg/kg-day in females. LOAEL and NOAEL values from the animal studies reviewed are shown in Table 6.

Table 6. Summary of LOAEL and NOAEL values for t-butanol

Study Type (Species)	Route of Exposure	NOAEL mg/kg-day	LOAEL mg/kg-day	Critical Effects	Reference
Subchronic (Fisher 344/N rats)	Oral, drinking water	Not identified (M or F)	Not relevant (M) 290 (F)	Kidney weight increases in both sexes and nephropathy in females.	Lindamood et al., 1992; NTP, 1995
Subchronic ($B6C3F_1$ mice)	Oral, drinking water	640 (M) 1,660 (F)	1,590 (M) 6,430 (F)	Hypoactivity in males and weight gain depression and kidney weight increase in females.	Lindamood et al., 1992; NTP, 1995
Chronic (Fisher 344/N rats)	Oral, drinking water	Not identified (M) 133 ($F\text{-}BMDL_{10}$)	Not identified (M) 180 (F)	Kidney weight increases and nephropathy in females.	Cirvello et al., 1995; NTP, 1995
Chronic ($B6C3F_1$ mice)	Oral, drinking water	Not identified (M) 510 (F)	540 (M) 1,020 (F)	Thyroid follicular cell hyperplasia.	Cirvello et al., 1995; NTP, 1995
2-Generation Reproduction of methyl-t-butyl ether (Sprague-Dawley rats)	Inhalation	140 estimated (parental toxicity) 140 estimated (developmental toxicity) 2,800 estimated (reproductive toxicity)		Neurotoxic effects in parental animals Reduced body weight in fetuses No effects on reproductive parameters	Bevan et al., 1997
Developmental (Sprague-Dawley rats)	Inhalation	Not identified for maternal or fetal effects	4,970 estimated for maternal and fetal effects	Neurotoxic effects in dams and reduced fetal weight	Nelson et al., 1989
Developmental (CBA/J and C57BL/6J mice)	Oral, gavage	Not identified	1,560	Increased resorptions and decreased live fetuses per litter	Faulkner et al., 1989

9.1.2 Weight-of-Evidence Evaluation and Cancer Characterization

Increases in male rat renal tubule hyperplasia, significant at the highest dose, and in renal tubule multiple adenoma and combined adenoma and carcinoma, significant at 200 mg/kg-day, were seen in the chronic study (NTP, 1995; Cirvello et al., 1995). Several kidney effects seen only in male rats dosed with t-butanol, including hyaline droplet formation, linear mineralization in the form of crystalline and rhomboid intracytoplasmic protein deposits, increased PCNA-stained S-phase nuclei indicative of cell proliferation, renal tubule adenoma, and renal tubule carcinoma, suggested that alpha-2μ-globulin accumulation should be considered. Essential criteria for attributing male rat kidney tumors and precursor effects to alpha-2μ-globulin accumulation, an effect unique to the male rat and not relevant to human health, include the following (U.S. EPA, 1991c; U.S. EPA 1991d):

- positive identification of alpha-2μ-globulin in the hyaline droplets
- distinct nephropathy specific to the male rat
- the chemical tested is not mutagenic and does not bind to DNA
- a nephrotoxic response precedes renal tumor formation

- renal tumors are only seen in the male rat, and not in female rats or in any other species tested
- tumors must be in the renal tubules of the male rat
- the mechanism for neoplasia may be promotional in nature

All of the criteria have been met for the t-butanol male rat kidney lesions. Hyaline droplets were first reported by Lindamood et al. (1992), and these authors also reported increased PCNA-stained S-phase nuclei. The interaction of t-butanol with alpha-2μ-globulin has been extensively studied (Williams et al., 2000; Williams and Borghoff, 2001; Borghoff et al., 2001). Alpha-2μ-globulin nephropathy, binding of t-butanol to alpha-2μ-globulin, and induction of cell proliferation in the male rat kidney were documented in these studies.

U.S. EPA science policy (U.S. EPA, 1991c) states the following:

"If a chemical induces alpha-2μ-globulin accumulation in male rats, the associated nephropathy is not used as an endpoint for determining non-carcinogenic hazard. Estimates of non-carcinogenic risk are based on other endpoints" and further that: "Male renal tubule tumors arising as a result of a process involving alpha-2μ-globulin accumulation do not contribute to the qualitative weight of evidence that a chemical poses a human carcinogenic hazard. Such tumors are not included in dose-response extrapolations for the estimation of human carcinogenic risk." Therefore, the male rat kidney lesions were not considered in the risk assessment of t-butanol.

Although mice showed some urinary bladder effects from t-butanol exposure, the target organ in mice was the thyroid gland. No thyroid effects were noted in the subchronic study (Lindamood et al., 1992; NTP, 1995). Effects were noted, however, in the chronic study (NTP, 1995; Cirvello et al., 1995) as shown in Table 5. Significant increases in follicular cell hyperplasia of the thyroid gland were noted in both male and female mice. In male mice, the increase occurred at all doses, while in females it was limited to the two highest doses. There were apparent increases in the overall rate of follicular cell adenoma in both sexes, one of which progressed to a single carcinoma in a high-dose male. These effects did not occur with statistical significance.

The mechanism of formation of these lesions resulting from t-butanol exposure has not been specifically studied. However, it is known that the metabolism of thyroxine (T_4), a major thyroid hormone, involves conjugation with glucuronic acid in the liver and excretion in the bile (Hill et al., 1989). Xenobiotics such as t-butanol that are also eliminated, at least in part, by glucuronide conjugation may, through enzyme induction in the liver, enhance elimination of T_4. Through a negative feedback loop, the pituitary gland increases or decreases the output of thyroid stimulating hormone (TSH) based on circulating levels of thyroid hormones. Sustained increases in TSH result in hypertrophy, or increased size, of the thyroid gland; or in hyperplasia, an increase in the number of thyroid follicular cells. Sustained thyroid follicular cell proliferation may eventually result in progression of hyperplasia to adenoma and carcinoma (U.S. EPA, 1998b). A study by McClain (1989) supports that altered thyroid function would cause follicular cell hypertrophy and hyperplasia. In fact, the induction of microsomal enzymes may be the cause for increased pituitary TSH secretion and promote thyroid gland carcinogenicty. There are, however, significant differences between humans and rodents in susceptibility to this effect. In humans, T_4 is bound to a thyroxine-binding globulin, making it less available for metabolism

than rodent T_4, which is not bound or is bound to albumin. The serum half-life of T_4 in humans is 5-9 days, but in rodents is only 0.5-1 day. These differences have led to uncertainty regarding the relevance of rodent thyroid follicular cell effects to human health. By policy (U.S. EPA, 1998c) these effects are considered relevant, and humans are assumed to be equally sensitive, in the absence of chemical-specific data.

No measurements of circulating T_4 or TSH have been made in mice exposed to t-butanol to determine whether the mode of action described above is actually followed. In evaluating the relevance to human health of the thyroid effects seen in mice, several points should be noted. Extremely high doses were used in the mouse studies. Some disruption of homeostasis at these doses is expected. No thyroid effects were seen in the subchronic study, at doses up to approximately 8 g/kg-day in males and over 11 g/kg-day in females. Doses in the chronic study ranged from approximately 0.5 g/kg-day to 2 g/kg-day. The thyroid lesions developed very late in the study. Only the incidence of follicular cell hyperplasia was statistically significant, with a reasonable dose-response in females but not in males. The first incidence of follicular cell adenoma was noted at 616 days in males and 646 days in females. The incidence of the only carcinoma was noted at 580 days. Based on the weight of evidence, t-butanol is not genotoxic. While the thyroid lesions in mice must be considered relevant to human health under U.S. EPA policy, there is sufficient evidence to indicate that the mode of action by which these lesions occur has a threshold, suggesting that a margin of exposure rather than a linear approach be used in their evaluation.

Under the proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999b), it is concluded that the *data are inadequate for an assessment of human carcinogenic potential* of t-butanol. No adequate or reliable human epidemiological study exists, and the only long-term animal studies produced results of no (male rat kidney) or questionable (mouse thyroid hyperplasia, adenoma, and carcinoma) relevance to human health.

9.1.3 Selection of Key Study and Critical Effect

The chronic study (NTP, 1995; Cirvello et al., 1995), showing effects of long-term t-butanol exposure in both rats and mice, is clearly the key study. The critical effects identified are:

- kidney effects in female rats
- thyroid follicular cell hyperplasia in mice, selected because protection against that endpoint should also protect against progression to follicular cell adenoma and/or carcinoma

Kidney effects were critical in rats. Kidney effects in male rats were discounted as not relevant to human health because of alpha-2μ-globulin nephropathy, but female rats are not subject to this effect and their kidney effects are relevant to human health risk assessment. A NOAEL could not be identified for kidney weight increases and nephropathy in female rats, but a 180 mg/kg-day LOAEL was identified.

Thyroid effects were critical in mice, and the lesion most appropriate for human health risk assessment was identified as thyroid follicular cell hyperplasia, because it is a precursor to the

more serious effects of adenoma and carcinoma. A NOAEL for this lesion could not be identified in male mice, although a 540 mg/kg-day LOAEL was identified. This LOAEL was not used because there was no dose-response relationship for thyroid follicular cell hyperplasia in male mice. A 510 mg/kg-day NOAEL was identified for female mice. The critical effects in both rats and mice would be more appropriately evaluated using a benchmark dose approach to identify $BMDL_{10}$ levels, rather than the NOAEL/LOAEL approach. Since the critical effects for rats and mice were quite different, it is not possible to identify the single critical effect (i.e., the most sensitive endpoint) prior to performing the dose-response calculations.

9.1.4 Identification of Susceptible Populations

No data were located identifying a population uniquely susceptible to the toxic effects of t-butanol. The physical dependence and cross-tolerance effects of t-butanol suggest that alcoholics could be more susceptible to doses > 1 g/kg-day (Section 8.7.2), but the drinking water action levels established in this document are well below this level.

9.2 Dose-Response Assessment

9.2.1 Kidney Effects in Female Rats

Absolute and relative kidney weights of the female rats are the most quantifiable endpoints. Mineralization and nephropathy effects are more difficult to model because they include both incidence and severity. Protection against the kidney weight increase is believed to be protective of mineralization and nephropathy. Specifically, the effect selected for dose-response assessment was increased relative kidney weight in the female rat at the 15-month interim sacrifice (organ weights were not reported at terminal sacrifice). With a 14% increase in relative kidney weight, an 8% increase in absolute kidney weight, and a slight but significant increase in nephropathy at the 180 mg/kg-day dose, this dose is borderline between a LOAEL and a NOAEL. Benchmark dose modeling was used to avoid the LOAEL-to-NOAEL uncertainty. Results of three continuous models run are shown in Table 7. The default parameters were used for each model (U.S. EPA, 2001b), except that non-homogeneous variance was used. Although the BMDL values are identical for the three models, the BMDL curve was not plotted for the first two models because the BMDL calculation failed at one or more dose levels.

Table 7. Benchmark dose results for relative female rat kidney weight at the 15-month interim sacrifice in the two-year study (NTP, 1995)

Model	BMD mg/kg-day	$BMDL_{10}$ mg/kg-day	p value for model fit[1]	AIC for model[2]	Comment
Linear	160	133	0.6967	-45.460164	BMDL curve not plotted
Polynomial	158	133	not calculated	-47.629282	BMDL curve not plotted
Power	158	133	0.3354	-47.629195	Model selected

[1] a p value > 0.1 is required for a good fit
[2] generally the lower the AIC value, the better the fit

The BMDL of 133 mg/kg-day will be used with the following uncertainty factors in calculation of the oral RfD:

- **Interspecies Extrapolation = 10x**

Selection of the interspecies variability factor shall be based on the availability of data that allow for quantitative extrapolation of an animal dose to the equivalent human dose for effects of similar magnitude or for a NOAEL. This includes scientifically documented differences or similarities in physiology, metabolism, and toxic response(s) between experimental animals and humans. If sufficient data are available to quantitate the toxicokinetic and toxicodynamic variabilities between experimental animals and humans, factor values of 3 or 1 shall be considered. In the absence of these data, the default value of 10 shall be used (NSF/ANSI 60 and 61 [2002], Annex A.7.3.1.3.2). The 10x default for interspecies extrapolation was selected because there are differences in the rate of elimination as well as in the identity and quantity of metabolites between rats and humans, with rats eliminating the chemical approximately three-fold more rapidly. Half-life data from rat and human blood level measurements of t-butanol were considered for use in deriving the toxicokinetic portion of the uncertainty factor. The half-life of t-butanol in male and female F-344 rats averaged 3.1 hours after intravenous injection of 37.5 mg/kg (Poet et al., 1997). The half-life of t-butanol in three human subjects of each sex averaged 8.5 hours after a 15 mg per 100 mL dose (~ 0.21 mg/kg) and 8.1 hours after a 5 mg per 100 mL (~ 0.07 mg/kg) dose of methyl-t-butyl ether (Amberg et al., 2001). This would suggest a toxicokinetic factor of 2.7. Much of the half-life data on t-butanol comes from its study as a metabolite of methyl or ethyl t-butyl ether. The different routes of administration, the wide range of dose levels, and the fact that t-butanol elimination became saturated at high doses (Poet et al., 1997), suggests that the data-derived uncertainty factor is no more accurate than the default value of three. The default values of 3x for toxicokinetics and 10x overall were therefore selected.

- **Intraspecies Extrapolation = 10x**

Selection of the intraspecies, or human variability factor, shall be based on the availability of data that identify sensitive subpopulations of humans. If sufficient data are available to quantitate the toxicokinetic and toxicodynamic variability of humans, factor values of 3 or 1 shall be considered. In the absence of these data, the default value of 10 shall be used (NSF/ANSI 60 and 61 [2002], Annex A.7.3.1.3.1). The 10x default for intraspecies extrapolation was selected, as no data were located to quantitatively address the differences in sensitivity of human subpopulations to the effects of t-butanol.

- **LOAEL to NOAEL Extrapolation = 1x**

Selection of the LOAEL to NOAEL extrapolation factor shall be based on the ability of the existing data to allow the use of a LOAEL rather than a NOAEL for non-cancer risk estimation. If a well-defined NOAEL is identified, the factor value shall be 1. When the identified LOAEL is for a minimally adverse or reversible toxic effect, a factor value of 3 shall be considered. When the identified LOAEL is for a severe or irreversible toxic effect, a factor value of 10 shall be used (NSF/ANSI 60 and 61 [2002], Annex A.7.3.1.3.5). A 1x uncertainty factor for extrapolation from a LOAEL to a NOAEL was selected, as benchmark dose modeling was used.

- **Extrapolation from Subchronic to Lifetime Exposure = 1x**

Selection of the subchronic to chronic extrapolation factor shall be based on the availability of data that allow for quantitative extrapolation of the critical effect after subchronic exposure to that after chronic exposure. Selection shall also consider whether NOAELs differ quantitatively when different critical effects are observed after subchronic and chronic exposure to the compound. When the critical effect is identified from a study of chronic exposure, the factor value shall be 1. When sufficient data are available to quantitate the difference in the critical effect after subchronic and chronic exposure, or when the principal studies do not suggest that duration of exposure is a determinant of the critical effects, a factor value of 3 shall be considered. In the absence of these data, the default value of 10 shall be used (NSF/ANSI 60 and 61 [2002], Annex A.7.3.1.3.3). A 1x uncertainty factor for study duration was selected, as data from a 15-month interim sacrifice in a chronic study were used in the dose-response calculation.

- **Incomplete Database = 1x**

Selection of the database sufficiency factor shall be based on the ability of the existing data to support a scientific judgment of the likely critical effect of exposure to the compound. When data exist from a minimum of five core studies: two chronic (greater than or equal to 13 weeks) bioassays in different species, one two-generation reproductive study, and two developmental toxicity studies in different species, a factor value of 1 shall be considered. When several, but not all, of the five core studies are available, a factor value of 3 shall be considered. When several of the five core studies are unavailable, the default value of 10 shall be used (NSF/ANSI 60 and 61 [2002], Annex A.7.3.1.3.4). A 1x uncertainty factor for database deficiencies was selected, as the required repeated dose studies in two species, developmental toxicity studies in two species, and two-generation reproduction study were all available. Although the two-generation reproduction study was on t-butanol as a metabolite of methyl t-butyl ether, this study adequately determined the potential reproductive effects of t-butanol when evaluated in conjunction with histopathology performed on the reproductive organs in the repeated-dose studies.

The reference dose is then calculated as follows:

RfD = 133 mg/kg-day x 1/100

= 1.33 mg/kg-day **≅ 1 mg/kg-day (rounded)**

9.2.2 Thyroid Follicular Cell Hyperplasia in Mice

The incidence of thyroid follicular cell hyperplasia in mice is a more quantifiable endpoint than the non-significant incidences of adenoma and carcinoma, and serves as a precursor to those effects. There was no dose-response relationship for hyperplasia in male mice. Although the low male dose of 540 mg/kg-day showed a statistically significant increase in this effect, it is of questionable biological relevance because there was no progression to adenoma or carcinoma at that dose over the lifetime of the animals. Although female mice were slightly less sensitive to hyperplasia, they showed a good dose-response relationship for this effect, and also some

progression to adenoma at the low dose. The benchmark dose approach was again used, with results for the hyperplasia endpoint shown in Table 8a.

Table 8a. Benchmark dose analysis of female mouse follicular cell hyperplasia in the two-year study (NTP, 1995)

Model	BMD mg/kg-day	$BMDL_{10}$ mg/kg-day	p value for model fit[1]	AIC for model[2]	Comments
Gamma	327	154	0.6307	303.063	
Logistic	375	115	0.5194	303.246	
Multistage	269	155	0.8063	302.892	
Probit	388	278	0.4837	303.321	
Quantal Linear	203	151	0.7344	301.453	Best fit model
Quantal Quadratic	628	527	0.4836	302.296	
Weibull	321	154	0.6621	303.023	

[1] $p > 0.1$ is required for a good fit
[2] generally the lower the AIC value, the better the fit

The model best fitting the data in terms of having a p value > 0.1, Chi-square residuals < 2.0 at each data point, and the lowest AIC value is the quantal linear model. Three other models provided BMDL levels of approximately 150 mg/kg-day along with reasonable fit statistics, and BMD and BMDL values within a factor of two. The 150 mg/kg-day value will therefore be used in an alternate oral RfD calculation with the following uncertainty factors:

- **Interspecies Extrapolation = 1x**

Selection of the interspecies variability factor shall be based on the availability of data that allow for quantitative extrapolation of an animal dose to the equivalent human dose for effects of similar magnitude or for a NOAEL. This includes scientifically documented differences or similarities in physiology, metabolism and toxic response(s) between experimental animals and humans. If sufficient data are available to quantitate the toxicokinetic and toxicodynamic variabilities between experimental animals and humans, factor values of 3 or 1 shall be considered. In the absence of these data, the default value of 10 shall be used (NSF/ANSI 60 and 61 [2002], Annex A.7.3.1.3.2). A 1x uncertainty factor for interspecies extrapolation was selected, because policy guidance assumes humans and rodents are equally sensitive to thyroid effects, in the absence of data to the contrary, even though humans are likely to be less sensitive to these effects than rodents (U.S. EPA, 1998c).

- **Intraspecies Extrapolation = 10x**

Selection of the intraspecies, or human variability factor, shall be based on the availability of data that identify sensitive subpopulations of humans. If sufficient data are available to quantitate the toxicokinetic and toxicodynamic variability of humans, factor values of 3 or 1 shall be considered. In the absence of these data, the default value of 10 shall be used (NSF/ANSI 60 and 61 [2002], Annex A.7.3.1.3.1). A 10x uncertainty factor for intraspecies extrapolation was selected, as there are no data to quantitatively address the differences in sensitivity of human subpopulations to the effects of t-butanol.

- **LOAEL to NOAEL Extrapolation = 1x**

Selection of the LOAEL to NOAEL extrapolation factor shall be based on the ability of the existing data to allow the use of a LOAEL rather than a NOAEL for non-cancer risk estimation. If a well-defined NOAEL is identified, the factor value shall be 1. When the identified LOAEL is for a minimally adverse or reversible toxic effect, a factor value of 3 shall be considered. When the identified LOAEL is for a severe or irreversible toxic effect, a factor value of 10 shall be used (NSF/ANSI 60 and 61 [2002], Annex A.7.3.1.3.5). A 1x uncertainty factor for extrapolation from a LOAEL to a NOAEL was selected, as benchmark dose modeling was used.

- **Extrapolation from Subchronic to Lifetime Exposure = 1x**

Selection of the subchronic to chronic extrapolation factor shall be based on the availability of data that allow for quantitative extrapolation of the critical effect after subchronic exposure to that after chronic exposure. Selection shall also consider whether NOAELs differ quantitatively when different critical effects are observed after subchronic and chronic exposure to the compound. When the critical effect is identified from a study of chronic exposure, the factor value shall be 1. When sufficient data are available to quantitate the difference in the critical effect after subchronic and chronic exposure, or when the principal studies do not suggest that duration of exposure is a determinant of the critical effects, a factor value of 3 shall be considered. In the absence of these data, the default value of 10 shall be used (NSF/ANSI 60 and 61 [2002], Annex A.7.3.1.3.3). A 1x uncertainty factor for study duration was selected, as data from a chronic study were used in the dose-response calculation.

- **Incomplete Database = 1x**

Selection of the database sufficiency factor shall be based on the ability of the existing data to support a scientific judgment of the likely critical effect of exposure to the compound. When data exist from a minimum of five core studies: two chronic (greater than or equal to 13 weeks) bioassays in different species, one two-generation reproductive study, and two developmental toxicity studies in different species, a factor value of 1 shall be considered. When several, but not all, of the five core studies are available, a factor value of 3 shall be considered. When several of the five core studies are unavailable, the default value of 10 shall be used [NSF/ANSI 60 and 61 (2002), Annex A.7.3.1.3.4). A 1x uncertainty factor for database deficiencies was selected, as the required repeated dose studies in two species, developmental toxicity studies in two species, and two-generation reproduction study were all available. Although the two-generation reproduction study was on t-butanol as a metabolite of methyl t-butyl ether, this study adequately determined the potential reproductive effects of t-butanol when evaluated in conjunction with histopathology performed on the reproductive organs in the repeated-dose studies.

The reference dose is then calculated as follows:

RfD = 150 mg/kg-day x 1/10

= **15 mg/kg-day**

Since this oral RfD is considerably higher than the oral RfD based on the relative female kidney weights, it will not be used in the calculation of drinking water levels.

Results of modeling the female mouse follicular cell adenoma are also shown for comparison in Table 8b. Prior to performing the calculations, the animal doses of 0, 510, 1,020, or 2,110 mg/kg-day were converted to human equivalent doses of 0, 83, 167, or 345 mg/kg-day for the adenoma endpoint, as follows:

Human Equivalent Dose = dose x $(0.05 \text{ kg wt. mouse}/70 \text{ kg wt. human})^{0.25}$.

Table 8b. Benchmark dose analysis of female mouse follicular cell adenoma in the two-year study (NTP, 1995)

Model	BMD mg/kg-day	$BMDL_{10}$ mg/kg-day	p value for model fit[1]	AIC for model[2]	Comments
Gamma	337	240	0.8766	113.342	Power estimated at a boundary point
Logistic	342	240	0.8775	113.34	Slope estimated at a boundary point
Multistage	327	235	0.7533	113.668	Beta (1,2) estimated at boundary point
Probit	339	243	0.6092	115.34	
Quantal Linear	358	199	0.3589	115.406	Power estimated at a boundary point
Quantal Quadratic	325	239	0.6267	114.121	Power estimated at a boundary point
Weibull	342	241	0.8775	113.34	Power estimated at a boundary point

[1] a p value > 0.1 is required for a good fit
[2] generally the lower the AIC value, the better the fit

All of the models, with the exception of the quantal linear, gave similar BMDLs for the adenoma effect, although all but one model estimated at least one parameter at a boundary point. This reflects the poor dose-response relationship for adenoma. The BMDLs for adenoma were higher than the BMDLs for follicular cell hyperplasia, and also higher than the BMDLs for the female rat relative kidney weights. The 240 mg/kg-day BMDL for adenoma will be used for a margin of exposure comparison with the drinking water level derived from the oral RfD based on relative kidney weights of female rats.

9.3 Exposure Characterization

Human exposure to t-butanol is most likely to arise from solvent and paint remover use, in both occupational and household settings (HSDB, 2002). Inhalation is the most likely route of exposure. The general population may also be exposed small amounts of to t-butanol in air as a result of industrial emissions; in drinking water as a result of surface water or groundwater contamination; and in food as an indirect additive resulting from contact with certain materials.

t-Butanol is frequently detected as an extractant of products evaluated by NSF under NSF/ANSI 61 (2002) as a result of decomposition of one of the following peroxide initiators: di-t-butyl

peroxide; 2,5-dimethyl-2,5-di- t-butyl peroxyhexane; or cumyl t-butyl peroxide. These polymer initiators may be used in polyethylene and cross-linked polyethylene polymers, and also in elastomeric materials such as nitrile butadiene rubber, ethylene propylene diene monomer, and styrene butadiene rubber. Results indicate that the concentration of t-butanol extracted decays over time. Initial concentrations range from 400-50,000 ppb, but then decay to 20-16,000 ppb when extrapolated to day 90 of exposure.

There are insufficient data regarding levels of exposure to t-butanol from food and other sources to calculate a data-derived relative source contribution. Therefore, the default value of 20% (0.2) will be used.

9.4 TAC Derivation

The TAC is calculated based on the 70 kg default body weight and 2 L/day default drinking water consumption of an adult, with the 0.2 relative source contribution factor. There are no data to suggest that children are more sensitive than adults to the effects of t-butanol.

TAC = 1.33 mg/kg-day x 70 kg x 1 day/2 L x 0.2

= 9.3 mg/L

≅ 9 mg/L (rounded)

9.5 STEL Derivation

NSF/ANSI 61 (2002) allows for the use of a short-term exposure level (STEL) for substances that are initially present in potable water at a relatively high concentration but rapidly decline in concentration because they are volatile or water soluble, or because they chemically or biologically degrade. t-Butanol is sufficiently volatile and water soluble to fall into this category. The short-term exception to the TAC/SPAC guidelines permits an initial extrapolated at-the-tap exposure based on the subchronic NOAEL or BMDL with appropriate uncertainty factors. A product can initially contribute up to the STEL if the at-the-tap concentration decreases to below the TAC (static) or the SPAC (flowing) within 90 days.

The relative kidney weight of female rats in the subchronic study (Lindamood et al., 1992; NTP, 1995) was selected as the most sensitive endpoint for use in calculating the STEL. Male rat kidney effects were again discounted, and effects in mice occurred at much higher doses than the chosen effect. Benchmark dose analysis of the data, using default values except for non-homogeneous variance, with BMDL curve calculation, was used with results shown in Table 9. The BMDL curve was not plotted for the first two models because the BMDL calculation failed at one or more dose levels. The power model selected was the only one where the BMDL calculation did not fail for one or more points on the curve. The power model did not meet the standard criteria for fit, in that it did not have a p value > 0.1 and the lowest AIC, but it did have Chi-squared residuals < 2.0 at each of the data points. The supporting linear model resulted in the same BMDL. It did have a p value > 0.1 and the lowest AIC, although Chi-squared residuals were > 2.0 at four of the six doses. In all models for the calculations shown in Table 9, however, the BMD and BMDL were well within a factor of two. The $BMDL_{10}$ of 367 mg/kg-day will be

used in the STEL calculation. This is a reasonable value based on the fact that the kidney weight, although slightly increased at the two lowest doses, only showed a significant dose-response relationship beginning with the 850 mg/kg-day dose.

Table 9. Benchmark dose analysis of relative female rat kidney weights in the (Lindamood et al., 1992; NTP, 1995) subchronic study

Model	BMD mg/kg-day	$BMDL_{10}$ mg/kg-day	p value for model fit[1]	AIC for model[2]	Comment
Linear	445	367	0.1522	-31.544	BMDL curve not plotted Supporting model
Polynomial	338	213	0.06527	-28.793	BMDL curve not plotted
Power	445	367	0.08515	-29.544	Model selected

[1] a p value > 0.1 is required for a good fit
[2] generally the lower the AIC value, the better the fit

The STEL is calculated based on the 10 kg default body weight and 1 L/day water consumption of a child. The same 10x default uncertainty factors for interspecies and intraspecies extrapolation, used in derivation of the TAC, were used in derivation of the STEL.

STEL = 367 mg/kg-day x 1/100 x 10 kg x 1 day/1L

= 36.7 mg/L

≅ **40 mg/L (rounded)**

10.0 RISK MANAGEMENT

10.1 SPAC Derivation

The SPAC is defined as a percentage of the TAC to account for multiple sources of a chemical in a drinking water system. Since a number of different products can contribute significant levels of t-butanol to drinking water, the default value of 10 will be used in deriving the SPAC from the TAC. Therefore:

SPAC = TAC/10 = (9 mg/L)/10

= 0.9 mg/L

11.0 RISK COMPARISONS AND CONCLUSIONS

t-Butanol is a metabolite of methyl t-butyl ether. As a major gasoline additive methyl t-butyl ether has received more regulatory attention than t-butanol. At the present time, the U.S. EPA has published a Consumer Acceptability Advisory for methyl t-butyl ether. They cite a 20-40 µg/L range, with the former value being the odor threshold and the latter value the taste threshold. They further estimate that the MOE at the 20 µg/L level is 40,000 or greater for cancer effects, and 120,000 or greater for non-cancer effects (U.S. EPA, 2000). At a 100-fold MOE, the methyl t-butyl ether health-based values would range from 8-24 mg/L, similar to the SPAC-TAC range calculated for t-butanol in this document. The U.S. EPA (2002c) has also

published robust summaries for t-butanol under the HPV Challenge Program. However, no risk assessment has yet been conducted for t-butanol under this program.

The STEL, TAC and SPAC levels calculated in this document are well below the 290 mg/L water odor threshold reported by Amoore and Hautala (1983). Note that under the general requirements of NSF/ANSI 61 (2002), use of taste and odor criteria are not considered, i.e., "This Standard does not establish performance or taste and odor requirements for drinking water system products, components, or materials."

The World Health Organization (WHO, 1987) has evaluated the toxicological properties of the butanol isomers. However, its evaluation was done before the availability of the chronic animal studies. A safety assessment of t-butanol done by The Cosmetic Ingredient Review Expert Panel, also before the availability of the chronic animal studies, concluded "available data are insufficient to support the safety of t-BuOH as used in cosmetics."

The American Conference of Governmental Industrial Hygienists has documented the basis of its 100 ppm (303 mg/m^3) time-weighted average Threshold Limit Value (TLV®) for workplace exposure to t-butanol (ACGIH, 1996). The TLV® was set to protect against narcosis. This group also determined that t-butanol was "not classifiable as a human carcinogen based on the equivocal evidence from the NTP bioassay study." ACGIH (1996) documentation noted a 30 mg/kg limit for t-butanol in confectionery established by the Council of Europe, and a 10 mg/kg food residue limit established by the European Economic Community.

t-Butanol enters the body either by ingestion of food or drinking water or by inhalation resulting from occupational or household exposure. At doses much higher (> 1 g/kg) than would be found in drinking water, t-butanol behaves with the typical narcotic/intoxicating effects of short-chain alcohols such as ethanol. The intoxicating effects of t-butanol have been reported by numerous authors to be 4-5 times as potent as the effects of ethanol. Motor function is severely affected by both chemicals. t-Butanol does not appear to share the developmental effects of ethanol beyond effects on fetal weight and organ-to-body-weight ratios.

Uncertainty in this risk assessment regarding the relevance to human health of the thyroid effects seen in mice is minimal, because these thyroid effects occurred at higher doses than the female rat kidney effects. While there is the suggestion that these thyroid effects could be discounted as reflecting enhanced glucuronide elimination of T_4, an effect that would not be relevant to humans, there are no specific data on t-butanol to substantiate such a mode of action. Use of the female rat kidney effects provides an adequate margin of exposure to protect against effects in the thyroid. It is clear that t-butanol has pronounced effects on the kidneys of rats, and the effects in female rats are likely to be relevant to human health. Therefore, the RfD value based on these effects should be adequately protective of both kidney and thyroid effects in humans.

There is a need to ensure that the TAC is adequately protective of the nonlinear cancer (nongenotoxic) effects of t-butanol on the thyroid gland at high sustained dose levels, as seen in the chronic mouse study. Although it is unlikely that these effects are relevant to human health, there are no data to discount them under current U.S. EPA guidelines (U.S. EPA, 1998c). An adult of 70 kg standard body weight drinking 2L/day could consume 18 mg/day (twice the TAC

level) or 0.26 mg/kg-day t-butanol. Comparing this with the 240 mg/kg-day BMDL for the female mouse thyroid gland follicular cell adenoma, the following margin of exposure (MOE) results:

MOE = 240 mg/kg-day/0.26 mg/kg-day

= 923

The U.S. EPA would like a minimum 100-fold margin of exposure (Doull et al., 1999), equivalent to the 10-fold uncertainty factors for interspecies and intraspecies extrapolation. The calculated margin of exposure is protective.

Based on effects of t-butanol on laboratory animals, consideration of the relevance of those effects to human health, and uncertainty factors applied to extrapolate from animal studies to humans, the TAC, SPAC, and STEL values in this risk assessment document are adequately protective of public health.

12.0 REFERENCES

Web addresses were current at the time of publication. Although they may change, these addresses should provide some direction in obtaining on-line information.

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13.0 ANNEX A – BENCHMARK DOSE RESULTS

13.1 Model Selected for the TAC Calculation

```
 ======================================================================
        Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
        Input Data File: C:\BMDS\DATA\BUTANOLFRELKIDNEYWT15MO.(d)
        Gnuplot Plotting File:  C:\BMDS\DATA\BUTANOLFRELKIDNEYWT15MO.plt
                                        Mon May 12 14:22:18 2003
 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = MEAN
   Independent variable = COLUMN1
   The power is restricted to be greater than or equal to 1
   The variance is to be modeled as Var(i) = alpha*mean(i)^rho

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



                  Default Initial Parameter Values
                          alpha =     0.116576
                            rho =            0
                        control =         3.49
                          slope =   0.00638713
                          power =     0.838649
           Asymptotic Correlation Matrix of Parameter Estimates

                  alpha          rho      control        slope        power

     alpha            1        -0.99        -0.11       -0.015        0.029

       rho        -0.99            1          0.1       0.0091       -0.025

   control        -0.11          0.1            1        -0.56         0.51

     slope       -0.015       0.0091        -0.56            1           -1

     power        0.029       -0.025         0.51           -1            1
```

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.000151761	0.000342017
rho	4.48011	1.58288
control	3.51491	0.0670748
slope	0.00223054	0.00259181
power	1	0.182783

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	10	3.49	0.253	3.51	0.206	-0.121
180	10	3.99	0.221	3.92	0.262	0.281
330	10	4.21	0.253	4.25	0.315	-0.13
650	10	4.95	0.538	4.96	0.446	-0.0331

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	25.091490	5	-40.182981
A2	30.889419	8	-45.778838
A3	29.278549	6	-46.557098
fitted	28.814598	5	-47.629195
R	-1.208192	2	6.416384

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)

Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	d.f	p-value
Test 1	64.1952	6	<.00001
Test 2	11.5959	3	0.008904
Test 3	3.22174	2	0.1997
Test 4	0.927903	1	0.3354

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is less than .05. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is greater than .05. The modeled variance appears to be appropriate here

The p-value for Test 4 is greater than .05. The model chosen seems to adequately describe the data

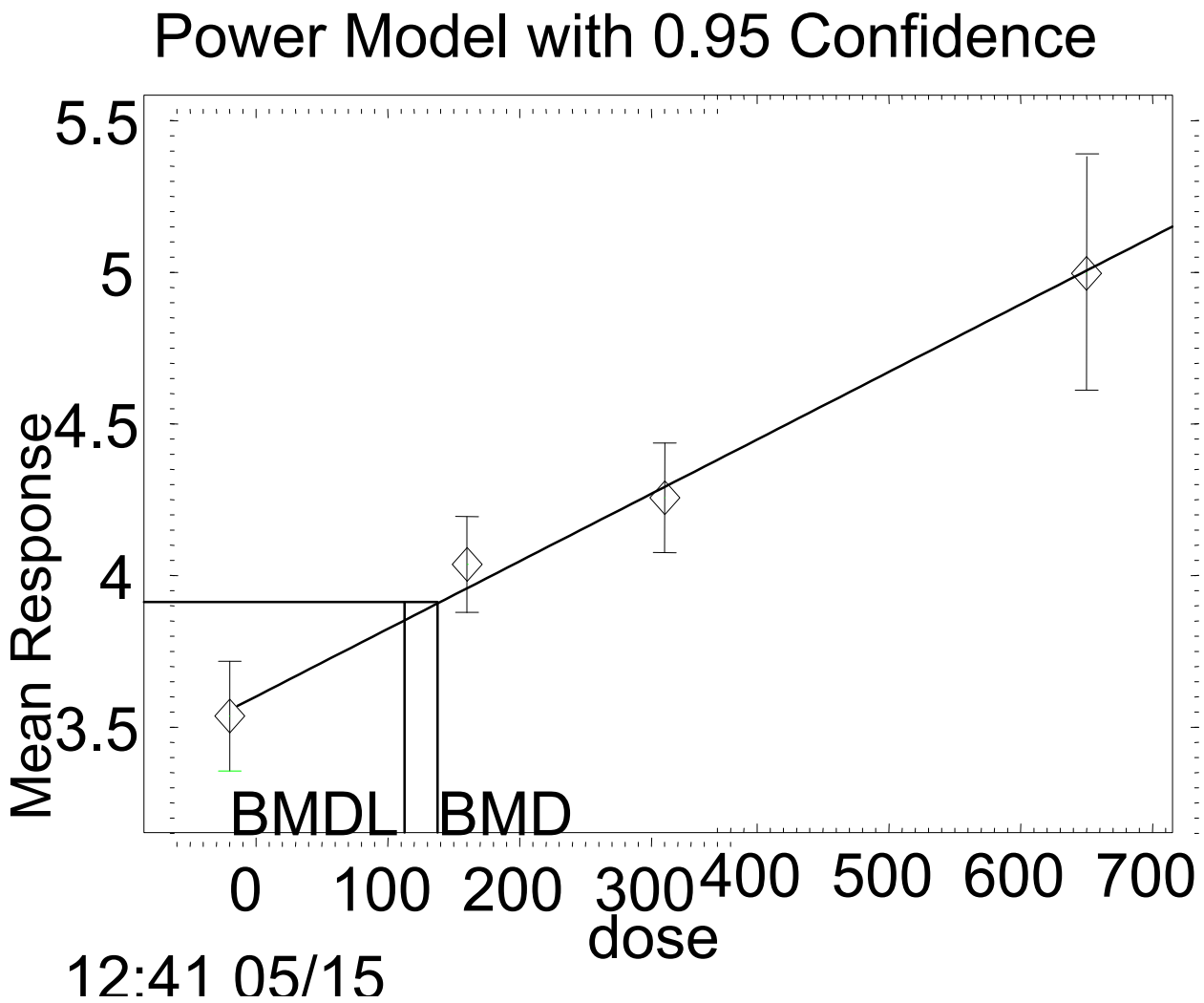
Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 157.581

BMDL = 132.695


Power Model with 0.95 Confidence
Mean Response
5.5
5
4.5
4
3.5
BMDL
BMD
0
100
200
300
400
500
600
700
dose
12:41 05/15

13.2 Model Selected for the STEL Calculation

```
 ======================================================================
        Power Model. $Revision: 2.1 $ $Date: 2000/10/11 20:57:36 $
        Input Data File: C:\BMDS\DATA\BUTANOLKIDWT13WEEK.(d)
        Gnuplot Plotting File:  C:\BMDS\DATA\BUTANOLKIDWT13WEEK.plt
                                        Wed May 14 08:19:49 2003
 ======================================================================

 BMDS MODEL RUN
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = control + slope * dose^power


   Dependent variable = MEAN
   Independent variable = COLUMN1
   The power is restricted to be greater than or equal to 1
   The variance is to be modeled as Var(i) = alpha*mean(i)^rho

   Total number of dose groups = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =     0.283563
                            rho =            0
                        control =         3.62
                          slope =    0.0102004
                          power =     0.678219


           Asymptotic Correlation Matrix of Parameter Estimates

                  alpha          rho      control        slope        power

     alpha            1        -0.99        -0.17        0.085        -0.07

       rho        -0.99            1         0.17       -0.098        0.082

   control        -0.17         0.17            1        -0.79         0.75

     slope        0.085       -0.098        -0.79            1           -1

     power        -0.07        0.082         0.75           -1            1
```

Parameter Estimates

Variable	Estimate	Std. Err.
alpha	0.000759651	0.000974607
rho	3.6464	0.850469
control	3.77381	0.110397
slope	0.000846713	0.00121599
power	1	0.18331

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Obs Std Dev	Est Mean	Est Std Dev	Chi^2 Res.
0	10	3.62	0.47	3.77	0.31	-0.496
290	10	4.23	0.32	4.02	0.348	0.605
590	10	4.17	0.22	4.27	0.389	-0.266
850	10	4.62	0.22	4.49	0.427	0.296
1560	10	5.08	0.19	5.09	0.536	-0.0274
3620	4	6.56	1.78	6.84	0.918	-0.304

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = alpha*(Mu(i))^rho

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	10.208855	7	-6.417711
A2	39.737359	12	-55.474718
A3	23.080666	8	-30.161333
fitted	19.772058	5	-29.544115
R	-21.760729	2	47.521459

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)

Tests of Interest

Test	-2*log(Likelihood Ratio)	d.f	p-value
Test 1	122.996	10	<.00001
Test 2	59.057	5	<.00001
Test 3	33.3134	4	<.00001
Test 4	6.61722	3	0.08515

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels It seems appropriate to model the data

The p-value for Test 2 is less than .05. A non-homogeneous variance model appears to be appropriate

The p-value for Test 3 is less than .05. You may want to consider a different variance model

The p-value for Test 4 is greater than .05. The model chosen seems to adequately describe the data

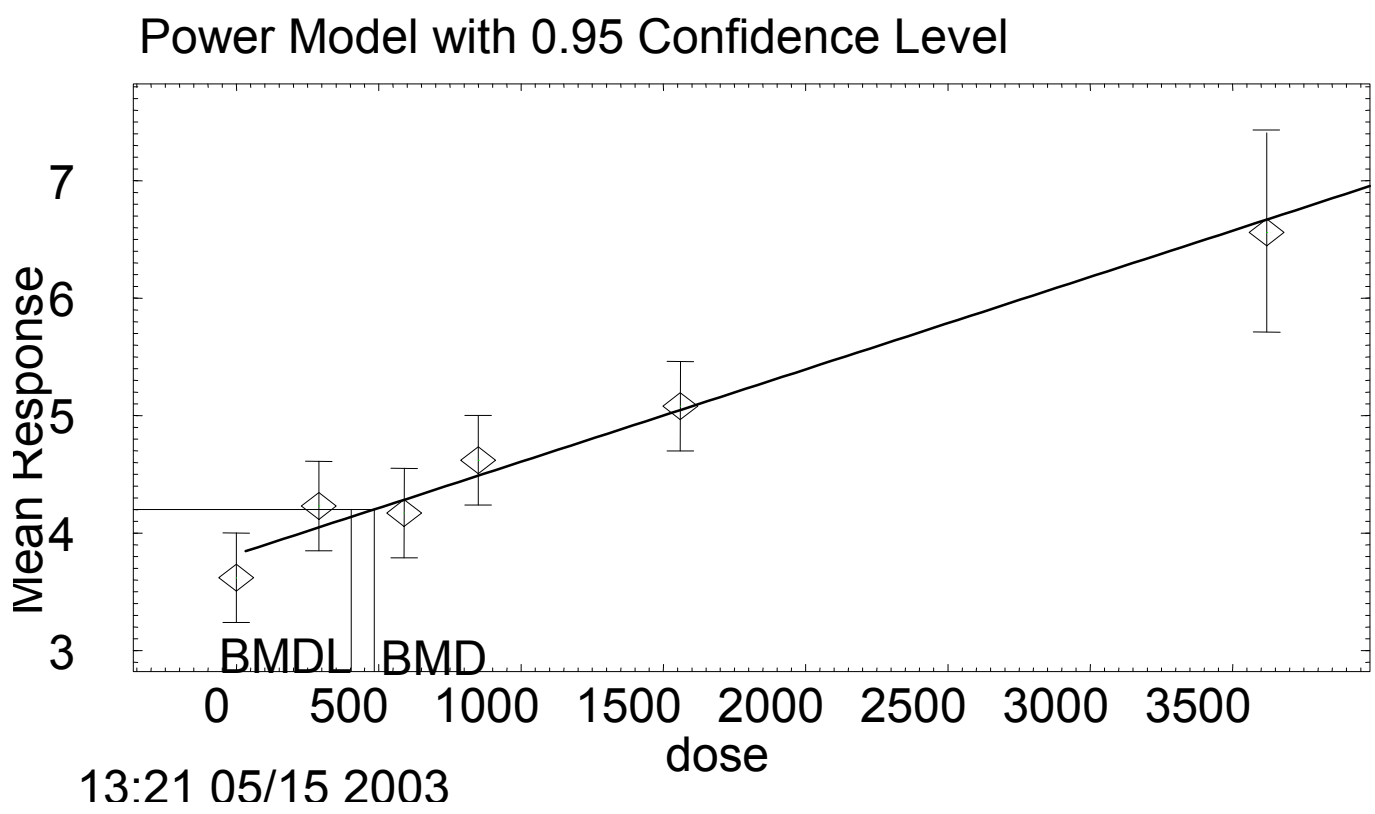
Benchmark Dose Computation
Specified effect = 0.1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 445.701

BMDL = 367.431



14.0 PEER REVIEW HISTORY

Note that section, page, line, and table numbers may refer to a previous version of the document.

Peer Review t-Butanol April 15, 1999

Ms. Rosanna Sohn (NSF, Associate Toxicologist) presented a summary of the t-butanol document.

- A reviewer pointed out that it appears that selective developmental effects and maternal poisoning are occurring at the same t-butanol concentration. He questioned if any of the studies examined the potential of t-butanol to induce fetal alcohol syndrome. He also asked if maternal toxicity was observed at the same concentrations causing late fetal death.

Fetal alcohol syndrome was not specifically addressed, although there are sufficient similarities in neurotoxic effects between t-butanol and ethanol that this effect at dose levels orders of magnitude higher than are found in drinking water cannot be ruled out.

- A reviewer suggested that the other cancers observed with exposure to methyl t-butyl ether should be addressed in addition to the kidney tumors. He continued by noting that if t-butanol is a major metabolite of methyl t-butyl ether, then NSF needs to look at more than the kidney.

The t-butanol studies adequately address the carcinogenicity of that compound.

- Another reviewer disagreed and said that the focus of the paper should be on t-butanol and not methyl t-butyl ether. Another member stated that maybe the document should only mention that t-butanol is a metabolite of methyl t-butyl ether and not go into any detail. A reviewer suggested that there are often problems associated with using other metabolite or parent compound data. There is an inherent issue with whether or not activation of the parent compound is responsible for causing the observed toxic responses. It may be that the parent compound has nothing to do with t-butanol and that the effects observed in the kidney can be attributed to t-butanol. Another reviewer expressed the concern that NSF is trying to "hang their hat" on methyl t-butyl ether.

Methyl t-butyl ether studies were used to provide additional kinetics and metabolism information, but only the two-generation reproduction study on methyl t-butyl ether was used to fill a data gap.

- Dr. Bestervelt noted that there is new information that suggests that t-butanol is <u>not</u> a major metabolite of methyl t-butyl ether. NSF intends to incorporate this new information into the risk assessment document. A reviewer suggested that NSF is only trying to add weight and support the proposed t-butanol mode of action with mode of action from methyl t-butyl ether. Another member suggested that the hazard assessment should be broken up with a section set aside for "supporting evidence". This section could contain the methyl t-butyl ether information without making the conclusions of the document seeming so dependent on the methyl t-butyl ether data.

Only the conclusions regarding reproductive effects are dependent on methyl t-butyl ether data.

- Dr. Ball pointed out that the action level calculations included in the document were based on t-butanol-induced female kidney effects and not on the methyl t-butyl ether data. A reviewer questioned if there was any data on T4 or TSH measurements. Dr. Ball said that nothing was found in the reviewed literature. The reviewer wondered if NTP could have taken enough serum samples to perform these analyses. Another reviewer suggested that the assumption should be made that t-butanol causes a direct effect on the thyroid unless it is shown to be a secondary effect. A reviewer noted that it is inappropriate to discount direct effects because of a lack of data, but only hyperplasia was observed, no adenomas. Therefore, he suggested that the conclusion in the document is correct that carcinogenicity is unlikely. Another reviewer reiterated that it is inappropriate to assume mechanism of action without supporting data.

There are no T4 or TSH measurements. However, it would also be inappropriate to conclude that t-butanol is a carcinogen based on one tumor in a high-dose animal.

- Several reviewers noted that they had difficulty in determining from where the 180 mg/kg/day used in the RfD derivation was chosen. A reviewer questioned what the critical effect was that made NSF chose the 180 mg/kg/day. Dr. Ball explained that the 180 mg/kg/day was the LOAEL from the 15-month interim sacrifice.

Addressed during the meeting.

- Several reviewers questioned why the data at the end of the two-year bioassay was not considered. Dr. Ball explained that the data presented in the most detail was that taken at

the interim sacrifice. The reviewers recommended that the information used in the RfD derivation should be clarified to better explain these issues.

Tables 4a and 4b have been modified to indicate the 15-month interim sacrifice data are presented.

- A reviewer suggested that absolute versus relative kidney weights should be detailed in Table 3. Further explanation of the table might give more weight to the defined LOAEL.

Absolute weights were not affected.

- A reviewer recommended some clarification regarding the observations of hyaline droplets. He noted that on page 18 (paragraph 4) it is stated that hyaline droplets were not positively identified. On page 24 (last paragraph) it is stated that male rats exhibited hyaline droplets. The reviewer asked that NSF clarify in which studies hyaline droplets were observed and which studies evaluated for their presence.

Hyaline droplets have now been positively identified by several groups, and those studies are now included.

- Another reviewer questioned whether a factor needs to be added to the RfD derivation to account for a lack of reproductive data. A reviewer responded that if NSF intends to move away from the "ceilings" currently outlined in the standard with the tiered approach, an additional safety factor of three should be figured into the calculation.

The reproduction study on methyl t-butyl ether was considered adequate, and the 10x uncertainty factor for interspecies extrapolation is probably excessive considering similar profiles in rats and humans. An additional uncertainty factor is not justified.

- For the thyroid dose response, a reviewer suggested that NSF should justify the use of a 3X UF instead of a 10X UF for protection against hyperplasia.

A margin of exposure was calculated.

- In the exposure characterization, it is suggested that the general population may be exposed to t-butanol from surface or ground water contamination. A reviewer asked if this was the result of its presence in gasoline. The reviewer also asked if it was appropriate to make the assumptions made in the exposure characterization regarding sensitive populations. The reviewer cautioned that these could be dangerous statements, especially that addressing the sensitivity of children. The reviewer suggested that the section be written saying that no sensitive populations were identified and stated that unless data exist, sensitive populations should not be included.

A section on susceptible subpopulations has been added.

- A reviewer recommended that if it is decided that a "ceiling" will not be used, this will need to be clearly documented in the report.

Reference to the ceiling has been eliminated, since Annex A is now revised.

Peer Review t-Butanol October 18, 1999

Dr. Ball presented a brief summary of the t-butanol document to ensure that NSF had a clear understanding of the revisions recommended at the previous HAB meeting. After briefly summarizing the female and male rat kidney toxicity and male and female mouse thyroid effects, Dr. Ball requested feedback from the Board on the following t-butanol issues:

- Are the current NSF action levels adequately protective of human health?
- Should t-butanol be evaluated using the linear, non-linear, or RfD approach?
- If the linear approach is justified, what additional data are required to support a non-linear approach?

- A reviewer asked if the authors of any of the studies mentioned hyaline droplets. Dr. Ball indicated that the only requirement under the U.S. EPA guidelines not met to allow for the tumors to be dismissed was the lack of identification of alpha-2μ-globulin. A discussion of the recent CIIT publications on this issue ensued. A reviewer suggested looking at proposed cancer guidelines, and recommended looking at t-butanol with the linear and non-linear approaches. The reviewer suspected that t-butanol is not a linear carcinogen.

The hyaline droplet issue has been addressed by several authors, and is confirmed as a source of the male rat kidney effects.

- Another reviewer asked what the genotoxicity data looked like. Dr. Ball responded that she would not consider t-butanol genotoxic. A reviewer questioned if the Board really recommended that t-butanol should be treated as a linear carcinogen. Dr. Ball responded that at least one of the packets from the last meeting argued that t-butanol should be evaluated as a linear carcinogen. The same reviewer responded that he would certainly not call t-butanol a linear carcinogen. Several reviewers recommended running both the non-linear and RfD approaches. A second reviewer responded that he did not think the models were going to work and recommended trying the margin of exposure approach. Two reviewers requested clarification. The reviewer responded that he did not think a rigorous quantitative fit on the linear model could be achieved given the data available. Dr. Ball noted that she has more data that need to be added to the document.

This was a discussion summary. T-Butanol has not been treated as a linear carcinogen.

- She then asked the Board if anyone had "heartburn" with the STEL. A reviewer responded that some CNS depression may be observed at that level. The reviewer asked how the STEL compares with the methyl t-butyl ether health advisory. Dr. Ball responded that she was not sure. Dr. Ball and the Board agreed that the revised document could be reviewed by mail and finalized prior to the next HAB meeting.

The MTB advisory is based on organoleptic considerations, that are not part of the NSF Standards. Those advisories are 20 and 40 μg/L.

Peer Review t-Butanol (Revisited) April 14, 2000

Dr. Ball introduced Mr. Tej Gill (chemist), Dr. Robert Skoglund (toxicology consultant) and Mr. Richard Houle (product engineer) from Wirsbo Company in attendance for the t-butanol discussion. These individuals were invited to attend, as Wirsbo provided toxicological data and consultant expertise on t-butanol to NSF.

Dr. Ball presented the results of the linear, non-linear (margin of exposure) and RfD approaches for each of the critical effects observed following chronic t-butanol exposure in calculating the TAC. After her presentation, the following issues were discussed by the Board:

- NOAEL versus LOAEL for relative and absolute kidney weight changes: A reviewer asked if hematology and/or clinical chemistry was performed in the key study (NTP). After reviewing the NTP document, a second reviewer indicated that these parameters were not evaluated. The first reviewer suggested that she would be reluctant to call the dose responsible for the marginally biologically significant kidney weight changes (180 mg/kg-day) a LOAEL and argued that more justification would be required for the 3X uncertainty factor for extrapolation from a LOAEL to NOAEL.

The benchmark dose approach has been used.

- Dr. Ball asked if severity could be considered as an endpoint. A reviewer responded that severity could be used as an endpoint, but that the t-butanol dataset would not model well. A second reviewer argued that he would not rest the argument on severity. A reviewer suggested that the 180 mg/kg-day dose should be called an equivocal NOAEL and argued that the 300X uncertainty factor was appropriate. The first reviewer recommended that the uncertainty factor justification section simply needs to be expanded.

All uncertainty factors have been addressed, and the benchmark dose approach was used.

- Choice of uncertainty factors for observed thyroid effects: A reviewer asked what the NTP concluded about the thyroid effects. Dr. Ball responded that NTP concluded that there was some evidence of carcinogenicity in the females (follicular cell adenoma). The reviewer asked if NSF is confident in the conclusions made about the thyroid data and suggested that it seemed reasonable to justify a lower uncertainty factor (less than 10X) for carcinogenic potential should be included in the risk characterization. If the confidence is great, it may be possible to discount the effects entirely. If there is some concern, provide justification for 3X. Dr. Ball responded that she was confident in the conclusions, but had some concern about the U.S. EPA guidelines, which require measurements of thyroid hormone levels, etc… The reviewer suggested that this should be used as the justification for the 3X UF. The reviewer asked if a database deficiency UF was considered. Dr. Ball responded that this UF was not necessary with the reproductive/developmental data (Level III – 0.05 mg/L) restriction in the current Annex A. The reviewer asked what would be used for the database deficiency UF if this limitation were removed from Annex A. Dr. Ball responded that she had not yet considered that option.

The thyroid effects in mice were not selected as critical.

- A reviewer suggested changing the wording in the uncertainty factor justification as it was noted that stating that the maximum total uncertainty factor allowed by the U.S. EPA is 10000X is not correct. Rather, it was recommended that it should be worded to indicate that having an UF of 10000 is understood to be inappropriate. The Board then discussed the current understanding of the areas of uncertainty and the total uncertainty factors associated with increasing areas of uncertainty. A reviewer explained that four areas of uncertainty lead to a total maximum uncertainty factor of 3000X, while five areas result in a total maximum uncertainty factor of 10000X.

This uncertainty factor clarification is now part of Annex A of the NSF Standards.

- Use of Margin of Exposure (MOE)

Dr. Ball explained that NSF has had a difficult time understanding and incorporating the margin of exposure into the risk assessments when appropriate. A reviewer recommended looking at the chloroform document completed for the U.S. EPA as the document has a nice write-up on MOE. A second reviewer suggested that NSF simply state that in the absence of the ability to model the thyroid data, it was not possible to do an MOE. Several sources for MOE examples have been consulted.

- Use of Benchmark dose

A reviewer suggested that if the female mouse thyroid adenomas were modeled with Benchmark dose, the resulting TAC would be greater than all of the other TAC calculated for the female rat kidney effects and male and female thyroid effects. He asked if the extra or added risk model was run. Dr. Ball responded that it was extra risk. The reviewer suggested that NSF verify that extra risk is the appropriate model to use with high background. Extra risk is appropriate.

- A reviewer suggested that a better argument would need to be presented to justify the use of a 3X uncertainty factor for extrapolation from the LOAEL to the NOAEL. A second reviewer asked if use of the Benchmark dose was considered for the STEL. He argued that it would be better than using the standard NOAEL/LOAEL approach because it eliminates the need for justifying the NOAEL/LOAEL uncertainty factor.

The benchmark dose approach has been used for the STEL.

- The chairman asked the Board for their final recommendations for t-butanol. A reviewer noted that she would not discount the MOE as this provides important information for the risk manager. A second reviewer asked what the first reviewer would recommend for the point of departure and argued that the data could not be modeled. The first reviewer suggested looking at the 1999 chloroform non-linear guidance document until the U.S. EPA guidelines are officially available.

- The second reviewer suggested that if the female thyroid adenoma data were used, a MOE model might be possible and the resulting MOE should be meaningful. It is likely that the MOE would be higher than the 1800 calculated for the follicular cell hyperplasia (male mouse thyroid). A third reviewer questioned if it was appropriate to model data that are not statistically significant.

Neither NTP norU.S. EPA generally uses results that are not statistically significant, although NTP may consider them in their conclusion if there is a trend.

- Several reviewers concluded that they believed the kidney effects observed in the female rat was the critical effect on which to perform the risk characterization. A reviewer suggested that use of the Benchmark dose is probably the most appropriate. This would eliminate the need for an uncertainty factor to account for an extrapolation from LOAEL to NOAEL, which allows the reviewer to avoid having to decide if the 180 mg/kg-day dose is more appropriately identified as the LOAEL or the NOAEL.

The female rat kidney results have been used, with the benchmark dose approach.

Peer Review t-Butanol October 19, 2000

The Board reviewed a substantial re-write of the t-butanol document that had been submitted in the meeting package.

- A reviewer noted that the genotoxicity studies were not very robust. While there was one positive sister chromatid exchange study, everything else appeared to be negative. It was suggested that it would be inappropriate to conclude that t-butanol is not genotoxic, but it could be stated that there is no strong evidence to suggest that the compound is genotoxic.

A statement to that effect was included in the Executive Summary.

- Page 18, Line 12: A reviewer suggested that the responses observed in the subchronic rat study (Lindamood, 1992) were not indicative of liver damage. It would be more accurate to state that the clinical chemistry effects may have been reflective mild liver effects.

The sentence has been changed to indicate "effects".

- A reviewer recommended putting the benchmark dose curves in the document as an annex. He also suggested that a comparison of the BMD and BMDL values should be made. If the values are within two-fold of each other then the modeling is fairly robust.

Statements that the values are within a factor of two have been added.

- A reviewer suggested making sure that the discussion on the thyroid (page 40) is consistent with that in the iodine risk assessment document.

The discussion on the thyroid was taken directly from U.S. EPA guidance documents.

- A reviewer recommended re-evaluating the uncertainty factor selected for interspecies extrapolation (Pages 40-41). The argument provided supports the kinetics, but does not address dynamics. There are likely to by dynamic differences between humans and rats. An additional 3X should also be considered for incomplete t-butanol reproductive data. It is possible to argue either way about whether t-butanol induces critical reproductive effects if one considers the methyl t-butyl ether data. The reviewer questioned whether there is any information to suggest that the reproductive responses observed in the methyl t-butyl ether studies are attributable to its other metabolite, formaldehyde. The reviewer recommended writing justification to support a total UF of 100X. Also, if it is determined that the 3X for reproductive effects is not necessary, a discussion will be necessary to indicate why it was not included.

A 100x UF is now used.

Peer Review t-Butanol October 3, 2002

Dr. Ball presented the t-butanol document, which had undergone several previous full or partial reviews. The following comments were offered:

- Dr. Ball noted that the primary remaining issue with this document was the interspecies uncertainty factor, and asked if a compound-specific factor could be used since rats metabolized t-butanol approximately three times faster than humans. The toxicodynamic factor could be either 3x according to U.S. EPA guidelines or 2.5x according to IPCS guidelines. Then, a PBPK model could be used for the toxicokinetic factor. Use the human-to-animal AUC ratio, or the ratio of the half-lives.

A data-derived factor was calculated. However, the default was used because of some discomfort with the comparability of animal and human data in terms of compound studied (much of the t-butanol data comes from studies of methyl t-butyl ether), the route of administration, and the dose levels. Since the default toxicokinetic factor is 3x, and the data-derived toxicokinetic factor was 2.7x, there is little difference for t-butanol.

- A member felt that the metabolic processes between rats and humans was more similar than different. One uses sulfate conjugation and the other glucuronide conjugation, and both species eliminate the compound fairly quickly. Dr. Ball noted that the toxic moiety was difficult to identify from the metabolic pathway.

Comment.

- An increase in water consumption would enhance toxicity, but the females actually drank less (page 25, line 25). Take another look at the data.

Doses were calculated by the study authors on the basis of water consumption, since it was a drinking water study, so differences in water consumption have been taken into consideration.

- In Table 4a, double check the source of "transitional epithelial hyperplasia", as transitional epithelium is present only in the bladder. Change wording to "renal hyperplasia"?

This was checked during the meeting, and the effect was reported (NTP, 1995) under the kidney as transitional epithelial hyperplasia. The study description was retained.

- Is the Prah et al. (2000) study still an abstract? Make sure publication has not occurred, perhaps under junior author names.

The only subsequent Prah study located was on development of a dermal exposure system for pharmacokinetic studies (Prah et al. 2002. J Pharmacol Toxicol Methods 47(3):189-). Since the Prah et al. (2000) study was oral and the Prah et al. (2002) study was dermal, they were clearly different studies. Search of the names of the junior authors in PubMed did not identify a match, although several papers by Borghoff have been cited and are key to this document.

- On page 29, second paragraph, at which concentrations were positive results seen, and how big was the response?

Detail on concentrations and responses was included.

- Include AICs in the benchmark dose tables, as well as footnotes explaining the interpretation of AIC and P values. Include the graph and printout for the model selected. Always include the graph, because statistics can indicate a good fit, but the graph could show a line above the data points in the dose range of interest, which would actually be a bad fit. It is not necessary to include graphs and printouts for models that were not chosen.

Tables of data for all benchmark dose runs have been included in the document, with columns for p value and AIC, and a footnote explaining what is required for a good fit. The printout and graph for the benchmark dose runs used in the TAC and STEL calculations have been included in an annex.

- Several members noted that there were also editorial comments contained within their documents, and one member provided extensive written comments.

These comments have been incorporated.

- Members agreed that the document did not need to be reviewed again, and that once the revisions were made, the document was complete.