

# Amended Final Report of the Safety Assessment of t-Butyl Alcohol as Used in Cosmetics<sup>1</sup>

t-Butyl Alcohol (t-BuOH) is a tertiary aliphatic alcohol that is used as a solvent or an alcohol denaturant and as a perfume carrier in cosmetics. t-BuOH was reported as an ingredient in 32 formulations of eye makeup, fragrance, and shaving preparations, at concentrations ranging from 0.00001% and 0.3%. There is little acute oral toxicity in animals; e.g., the acute oral LD<sub>50</sub> in rats was 3.0 to 3.7 g/kg. In short-term oral studies in rats, t-BuOH at 2% (w/v) or less in drinking water did not cause gross organ or tissue damage in mice, although weight loss was reported and microscopic damage to livers and kidney and alterations such as centrilobular necrosis, vacuolation in hepatocytes, and loss of hepatic architecture were noted. Subchronic oral dosing with t-BuOH increased the mineralization of the kidney, nephropathy, and urinary bladder transitional cell epithelial hyperplasia in rats; and liver damage, chronic inflammation, hyperplasia of transitional cell epithelium urinary, and proliferative changes including hyperplasia and neoplasia in the thyroid in mice. Male rats exposed to t-BuOH were susceptible to  $\alpha$ 2 $\mu$ -globulin nephropathy. t-BuOH (99.9%) was a moderate to severe ocular irritant to rabbits and caused mild to moderate dermal irritation to rabbits. It was not considered to be a primary dermal irritant to rabbits. In animal studies, fetotoxicity generally increased with concentration, and fetal weights were slightly depressed at concentrations of 0.5% to 1% t-BuOH. t-BuOH produced a significant increase in the number of resorptions per litter. There was also a significant decrease in the number of live fetuses per litter. t-BuOH reduced maternal weight gain, litter sizes, birth weights, and weights at weaning, and increased perinatal and post-natal mortality. t-BuOH was not mutagenic in several bacterial and mammalian test systems. The principal effects from 2 years of exposure to t-BuOH in drinking water (up to 10 mg/ml for rats and 20 mg/ml for mice) were proliferative lesions (hyperplasia, adenoma, and carcinoma) in the kidneys of exposed male rats, and nephropathy in all exposed groups of female rats. There was some evidence of carcinogenic activity, but it was not consistent between species, sexes, or doses. A repeat-insult patch test (RIPT) test showed no potential for eliciting either dermal irritation or sensitization by 100% t-BuOH. Dermatitis can result from dermal exposure of humans to t-BuOH. In consideration of these data, it was concluded that t-BuOH was (at most) a weak carcinogen and unlikely to have significant carcinogenic potential as currently used in cosmetic formulations. In addition, the renal tubule effects found in male rats were likely an effect of  $\alpha$ 2 $\mu$ -globulin. In consideration of the reproductive and developmental toxicity data, the increased

incidence of still births occurred at high exposure levels and was likely secondary to maternal toxicity. Based on the available animal and clinical data in this report, it was concluded that t-BuOH is safe as used in cosmetic products.

## INTRODUCTION

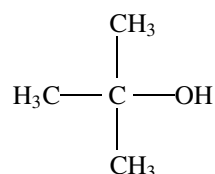
In its initial safety assessment of t-Butyl alcohol (t-BuOH), the Cosmetic Ingredient Review (CIR) Expert Panel concluded that the available data were insufficient to support the safety of this ingredient in cosmetics (CIR 1989). The studies that were needed in order to complete a safety assessment were identified as 90-day oral toxicity, human sensitization, and ultraviolet (UV) absorption. Since then, new human skin sensitization data provided by industry have been reviewed and incorporated into this report. In addition, the Panel considered the findings in a National Toxicology Program (NTP) 2-year carcinogenesis study that was only just underway when the original safety assessment was completed. Other new data published since that original report have also been included.

The Expert Panel is aware that the published literature contains voluminous information on t-Butyl Alcohol dependency and withdrawal. This information is not relevant to the use of t-Butyl Alcohol in cosmetic products and is not reviewed in this report.

## CHEMISTRY

### Definition and Structure

t-BuOH (CAS no. 75-65-0) is a tertiary aliphatic alcohol with the chemical formula (Wimer et al. 1983; Wenninger et al. 2000) shown below



Other names for t-BuOH include tertiary butyl alcohol, tert-butyl alcohol, tertiary butanol, tert-butanol, t-butanol, 2-methyl-2-propanol, and trimethyl carbinol (Wimer et al. 1983; Windholz 1983; Wenninger et al. 2000).

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<sup>1</sup>Reviewed by the Cosmetic Ingredient Review Expert Panel. This report was prepared by Melody Chen, CIR Scientific Analyst and Writer.

## Physical and Chemical Properties

t-BuOH is available in the form of colorless, hygroscopic crystals with a camphoraceous odor. The crystals become a clear liquid above 25.5°C. t-BuOH is soluble in water, alcohol, ether, and other organic solvents (Windholz 1983; CTFA 1985; Hawley 1971; USP 1979; Weast 1982).

t-BuOH is a fire hazard when exposed to heat or flame, and it can react with oxidizing materials. In the form of vapor, t-BuOH is a moderate explosion hazard when exposed to flame. It reacts violently with hydrogen peroxide (Lewis 2000). t-BuOH is stable under typical conditions of cosmetic use (CTFA 1985).

Chemical and physical properties of t-BuOH are presented in Table 1.

## Methods of Production

t-BuOH has been prepared from acetyl chloride and dimethylzinc, by catalytic hydration of isobutylene, by reduction of tert-butyl hydroperoxide, by absorption of isobutene, from cracking petroleum or natural gas, and from sulfuric acid with subsequent hydrolysis by steam. It is purified by distillation (Windholz 1983; CTFA 1985; Hawley 1971).

In the 4th edition of the *Encyclopedia of Chemical Technology*, the butyl alcohol entry states that t-BuOH is produced as a

by-product from the isobutane oxidation process for producing propylene oxide (Kirk-Othmer 1992a). In the butylenes entry, it is further noted that volume quantities of t-BuOH are prepared using the Oxirane process for the manufacture of propylene oxide which produces t-BuOH as a by-product (Kirk-Othmer 1992b).

## Analytical Methods

Qualitative and quantitative determinations of t-BuOH are made by precipitation colorimetry (CFR 1984), gas chromatography (Eiceman and Karasek 1981; Mackison et al. 1978), gas chromatography-mass spectrometry (Eiceman and Karasek 1981), photometry (Zamarakhina 1973), proton magnetic resonance (Muhtadi et al. 1982), and a laser absorption spectrometric method (Green and Steinfeld 1977).

## Impurities

t-BuOH used in cosmetics typically contains 99.5% t-BuOH, a maximum of 0.002% acidity (as acetic acid), a maximum of 0.1% water, and a maximum of 0.001% nonvolatile matter (CTFA 1985).

## USE

### Cosmetic

t-BuOH is used in the manufacture of perfumes (Windholz 1983; Hawley 1971). Its primary use in cosmetics is as an alcohol denaturant, but it is also used as a solvent in hair sprays and aftershave lotions and as a perfume carrier in cosmetics (CTFA 1985, 1999).

Product types and the number of product formulations containing t-BuOH are reported voluntarily by the industry to the Food and Drug Administration (FDA). In 1998, t-BuOH was reported to be an ingredient in 32 formulations of eye makeup, fragrance, and shaving preparations (FDA 1998) as shown in Table 2. The current concentration of use (maximum %) provided by industry is also listed in Table 2.

For historical comparison, in 1986 t-BuOH was reported to be an ingredient in 10 hair and facial skin care preparations and historical concentration of use of t-BuOH ranged from  $\leq 0.1\%$  to between 0.1% and 1% (FDA 1986).

Based on the types of products in which t-BuOH is used, this ingredient may be applied to, or come in contact with, skin, eyes, hair, nails, mucous membranes, and respiratory epithelium. Such products containing t-BuOH may be applied as many as several times a day and may remain in contact with the skin for variable periods following application. Daily or occasional use may extend over many years.

### Noncosmetic

t-BuOH is ubiquitous in the environment, and human exposure is likely. Fusel oil, the congeners or by-products of the fermentation or distillation process in the production of alcoholic

**TABLE 1**  
Chemical and physical properties of t-BuOH

Property	t-BuOH	Reference
Molecular weight	74.12	Windholz 1983
Specific gravity at 20/4°C	0.78581	Windholz 1983
20/4°C	0.7887	Weast 1982
25/4°C	0.78086	Windholz 1983
30/4°C	0.77620	Wimer et al. 1983
Boiling point (°C) at 760 mm Hg	82.41	Windholz 1983
760 mm Hg	82.30	Weast 1982
760 mm Hg	82.50	Wimer et al. 1983
31 mm Hg	20	Weast 1982
Melting point (°C)	25.6	Windholz 1983
	25.5	Weast 1982
	25.5	Wimer et al. 1983
Vapor pressure (mm Hg) at 20°C	30.6	Wimer et al. 1983
Refractive index for D line of the sodium spectrum at 20°C	1.38468	Windholz 1983
20°C	1.3878	Weast 1982
20°C	1.3838	Wimer et al. 1983
25°C	1.38231	Windholz 1983
25°C	1.3811	Wimer et al. 1983
Autoignition temperature (°C)	380	Wimer et al. 1983

**TABLE 2**  
Product formulation data on t-BuOH

Product category (number of formulations reported to FDA) (FDA 1998)	Number of formulations containing ingredient (FDA 1998)	Current concentration of use (CTFA 1999) (%)
Eye brow pencil (91)	—	0.001
Eye makeup remover (84)	1	—
Mascara	—	0.001
Colognes and toilet waters (656)	18	0.001
Perfumes (195)	8	—
Other fragrance preparations (148)	1	—
Hair sprays (aerosol fixatives) (261)	—	0.0001 and 0.5*
Shampoos (noncoloring) (860)	—	0.0001
Tonics, dressings, and other hair-grooming aids (549)	—	0.00001
Blushers (all types) (238)	—	0.0001
Face powders (250)	—	0.0007
Foundations (287)	—	0.0001
Lipstick (790)	—	0.0001
Bath soaps and detergents (385)	—	0.0001
Deodorants (underarm) (250)	—	0.0001
Aftershave lotion (216)	3	0.001 and 0.08*
Other shaving preparation products (60)	1	—
Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	—	0.001
Moisturizing creams, lotions, powders, and sprays	—	0.0001
Night creams, lotions, powders, and sprays	—	0.0001
Skin fresheners	—	0.3*
Other skin care preparations	—	0.001
<b>Total uses and concentration ranges for t-BuOH</b>	<b>32</b>	<b>0.0001–0.5</b>

\*These concentrations are not alcohol denaturant uses.

beverages, is 95% amyl, butyl, and propyl alcohols and has been detected in liquor in a concentration as high as 0.25% (Damrau and Goldberg 1971). t-BuOH has been detected in drinking water (Kool et al. 1982).

As codified in the Code of Federal Regulations (CFR), t-BuOH is permitted as an indirect food additive. It may be used in formulating defoaming agents used in the preparation and application of coatings for paper and paperboard; these coatings may be safely used as components of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food (21CFR176.200).

t-BuOH also may be safely used in surface lubricants employed in the manufacture of metallic articles that contact food; it may be used in surface lubricants used in the rolling of metallic foil or sheet stock, provided that the total residual lubricant remaining on the metallic article in the form in which it contacts food does not exceed 0.015 mg/square inch of metallic food-contact surface (21CFR178.3910).

#### Use as an Alcohol Denaturant

t-BuOH has been used as a denaturant for alcohol in commercial sunscreen preparations (Edwards and Edwards 1982).

t-BuOH has been used as an alcohol denaturant, a flotation agent, a dehydration agent, a solvent, and an octane booster in gasoline. It has been used in paint removers, as a chemical intermediate, and in chemical analyses (Windholz 1983; Hawley 1971). As codified in the CFR, t-BuOH is permitted to be used as a denaturant for the uses described in Table 3.

t-BuOH is a denaturant in SD Alcohols 39, 39-A, 39-B, 40, 40-A, and 40-B at a level of approximately 0.125%, and in 40-C at a level of 3%. All formulas may be used as solvents.

#### GENERAL BIOLOGY

##### Absorption, Distribution, Metabolism, and Excretion

###### Absorption

Nihlén et al. (1995) determined the liquid/air partition coefficient  $\lambda_{\text{blood/air}}$  to be 462 (95% confidence interval 440 to 484) for t-BuOH. The calculated  $\lambda_{\text{water/blood}}$  was 1.31, the  $\lambda_{\text{oil/blood}}$  was 0.363, and the  $\lambda_{\text{oil/water}}$  was 0.278 for t-BuOH.

###### Distribution

t-BuOH moves rapidly from the blood into the tissues. Eleven male Sprague-Dawley rats were cannulated and intravenously

**TABLE 3**  
Allowed uses of t-BuOH as an alcohol denaturant (27CFR 21)

Authorized uses	Formula number						
	39	39-A	39-B	40	40-A	40-B	40-C
Hair and scalp preparations	X	X	X	X	X	X	X
Bay rum	X		X	X	X	X	X
Lotions and creams (hand, face, and body)	X		X	X	X	X	X
Deodorants (body)			X	X	X	X	X
Perfume and perfume tinctures	X		X	X	X	X	X
Toilet waters and colognes	X	X	X	X	X	X	X
Shampoos		X	X	X	X	X	X
Soap and bath preparations			X	X	X	X	X
External pharmaceuticals, not USP or NF			X	X	X	X	X
Disinfectants, insecticides, fungicides, other biocides			X	X	X	X	X
Cleaning solutions (including household detergents)			X	X	X	X	X
Theater sprays, incense, room deodorants			X	X	X	X	X
Miscellaneous solutions							X

given 350 mg/kg [ $^{14}\text{C}$ ]t-BuOH. At numerous times following injection, blood samples were withdrawn and the samples measured for radioactivity. There were two phases in the elimination of  $^{14}\text{C}$ -t-BuOH from the blood. The first was a rapid phase, which probably represented the distribution of [ $^{14}\text{C}$ ]t-BuOH from the blood to other body tissues. The second represented a first-order elimination of radioactivity from the blood with a half-life of approximately 8 h, indicating that [ $^{14}\text{C}$ ]t-BuOH was being eliminated primarily as metabolic product(s) (Arco Chemical Company 1994a).

Male and female F344 rats were intravenously given 37.5, 75, 150, or 300 mg/kg t-BuOH. Four males per dose and three females per dose were used. Blood was drawn at 5, 10, 20, 30, 40, and 60 min and 4, 8, 12, 16, and 24 h after t-BuOH administration. Results confirmed that t-BuOH undergoes a rapid distribution phase followed by a slower elimination phase. The distribution and elimination half-lives were 3 min and 3.8 h, respectively, for doses less than 300 mg/kg in both male and female rats. The elimination half-life increased to 5.0 h for males and 4.3 h in female rats after an injection of 300 mg/kg (Poet et al. 1997).

Beauge et al. (1981) found that t-BuOH is eliminated slowly from the blood of rats. t-BuOH was dissolved in water and a dose of 25 mmol/kg was administered by gastric intubation to female Wistar rats (number unspecified). The t-BuOH blood concentration at 2 h was 13.24 mM, at 5 h it was 12.57 mM, and at 20 h it was 11.35 mM.

A 5.7 (w/v) solution of t-BuOH in saline was administered by gastric intubation to 4 to 6 female Sprague-Dawley rats every 8 h for 1 or 2.5 days; t-BuOH was administered in an amount inversely proportional to the degree of intoxication in order to maintain a uniform blood t-BuOH concentration of 60 to 100 mg % (Thurman et al. 1980). The rats were then given t-BuOH

to elevate their blood concentrations to between 125 and 150 mg %, and blood was taken from the tails and sampled for t-BuOH. Eighteen hours were required to eliminate t-BuOH completely from the blood when the rats were treated for 2.5 days, and 26 h were required when the rats were treated for 1 day; the rate of elimination of 1.2 g/kg t-BuOH was 0.7 mmol/kg rat/h. Acetaldehyde was not detected in the blood or brain of rats treated for 3 days with t-BuOH. t-BuOH did not affect the oxygen uptake or pyridine nucleotide redox state of perfused rat liver.

Two Sprague-Dawley rats were given 1500 mg/kg [ $^{14}\text{C}$ ]t-BuOH by oral gavage. Their blood was sampled at various times following the dosage. The animals were heavily narcosed and did not move about. In addition, their body temperatures were depressed. The radiolabel was eliminated from the blood at a slow rate indicating that a 1500 mg/kg dose had saturated the elimination pathways. In order to investigate the elimination of smaller doses of t-BuOH, three animals were given 500 mg/kg [ $^{14}\text{C}$ ]t-BuOH. There was a half-life of 9 h similar to that seen following intravenous dosing with 350 mg/kg [ $^{14}\text{C}$ ]t-BuOH (Arco Chemical Company 1994a).

Three Sprague-Dawley rats were placed in chambers and exposed to  $1938 \pm 93.4$  ppm [ $^{14}\text{C}$ ]t-BuOH ( $50 \mu\text{Ci}/\text{mmol}$ ) for 6 h. Blood samples were taken at various times during and following exposure. Animals were severely narcosed during the experiment. The results indicated that [ $^{14}\text{C}$ ]t-BuOH is eliminated at approximately the same rate following 6 h of 2000 ppm exposure to t-BuOH vapors as the rate following 1 mg or 500 mg/kg oral gavage dosing (Arco Chemical Company 1994a).

t-BuOH is also slowly eliminated from the blood of mice (McComb and Goldstein 1979). The authors administered a single intraperitoneal dose of 8.1 mmol/kg t-BuOH to nine male Swiss-Webster mice. t-BuOH was eliminated from the blood in 8 to 9 h. The same mice then inhaled t-BuOH vapor for 3 days;

the concentration of t-BuOH vapor administered was that which maintained a mean blood concentration of 8 mM t-BuOH. The researchers found it necessary to raise the t-BuOH vapor concentration progressively to maintain a given concentration of t-BuOH in the blood. t-BuOH was not detected in the blood 3 h after the mice were removed from the vapor chamber.

A single intraperitoneal dose of 8.1 mmol/kg t-BuOH was administered (to an unspecified number of mice) 4 h after the end of a 3-day inhalation period; no t-BuOH was detected in the blood 3 h later. The increased elimination rate of t-BuOH may have been due to metabolic tolerance; more t-BuOH may have been conjugated and eliminated in animals previously exposed to t-BuOH (McComb and Goldstein 1979).

The intragastric administration of t-BuOH to rats increased the rate of elimination of subsequently administered ethanol in comparison with the rate of elimination of ethanol by rats not given t-BuOH (Bleyman and Thurman 1980).

### *Metabolism*

According to Groth and Freundt (1994), t-BuOH is not a substrate for alcohol dehydrogenase or for catalase and has been used as an example of a nonmetabolizable alcohol. Adult female SPF Sprague-Dawley rats (numbers unspecified) inhaled a mixture of tert-butyl acetate with air via a tracheal cannula. Arterial blood was obtained at numerous times and the levels of tert-butyl acetate and t-BuOH determined by gas chromatography. Whereas the concentration of tert-butyl acetate decreased by approximately 50% after the inhalation, t-BuOH levels remained unchanged. The authors explained the accumulation of t-BuOH by invoking a low substrate specificity of rat liver alcohol dehydrogenase that resulted in a low oxidation rate of t-BuOH (Groth and Freundt 1994).

t-BuOH is a hydroxyl radical scavenger; in rat liver microsomes, it can be oxidatively demethylated by hydroxyl radicals generated from NADPH-dependent microsomal electron transfer to yield formaldehyde and acetone (Cederbaum et al. 1983; Cederbaum and Cohen 1980).

Baker et al. (1982) investigated the *in vivo* metabolism of t-BuOH to acetone in Long-Evans rats and inbred Sprague-Dawley rats after intraperitoneal doses of 1 g/kg t-BuOH. t-BuOH concentration in the blood was measured over a 24-h period; the half-life of t-BuOH was 9.1 h. Acetone, produced by the metabolism of t-BuOH, was also detected in the blood. Acetone was slowly eliminated from the blood by excretion in the urine and expired air, but the quantity excreted was highly variable.

These authors also injected two rats with 1.75 g/kg  $\beta$ -[ $^{14}\text{C}$ ]t-BuOH. Over a 24-h period, 68.7% of the total dose was recovered from one rat and 93.2% was recovered from the other rat as  $\text{CO}_2$  and acetone. When the animals were injected with 1.5 g/kg of a 1:1 mixture of  $\alpha$ -[ $^{13}\text{C}$ ] t-BuOH and t-BuOH, more acetone than expected was recovered. t-BuOH was a source of acetone, but also may have stimulated acetone production from other sources. Treatment of rats with U-[ $^{14}\text{C}$ ] hexadecanoic acid and

t-BuOH followed by collection of respiratory gases indicated that t-BuOH did not affect fatty acid synthesis (Baker et al. 1982).

Kamil et al. (1953) administered 12 mmol of t-BuOH by stomach tube to three chinchilla rabbits. t-BuOH was conjugated to a large extent with glucuronic acid, and glucuronides were readily isolated from the rabbit urine; as a percentage of dose, the average extra glucuronic acid excreted over 24 h was 24.4%. The researchers suggested that volatile alcohols might also be eliminated to some extent in an unchanged state by the lungs. No aldehydes or ketones were detected in the expired air of a rabbit given 6 ml t-BuOH (route unspecified).

### **Excretion**

t-BuOH is excreted by rabbits as glucuronide conjugates, but these compounds are not present in dog urine (Derache 1970).

In a study by Arco Chemical Company (1994a), two Sprague-Dawley rats per dose (1, 30, 500 and 1500 mg/kg [ $^{14}\text{C}$ ]t-BuOH) were treated by gavage and placed in metabolism cages where 24-h urines were collected. After 24 h, animals were killed and residual urine was removed from the bladder. For the 1, 30, and 500 mg/kg doses, 23% to 33% of the radioactivity was recovered; however, only 9% of the 1500 mg/kg dose was recovered suggesting the urinary route of elimination is saturated following a 1500 mg/kg dose.

Further analysis showed that excretion saturation was reached at a dose between 500 mg/kg and 1500 mg/kg. Results of reverse-phase high-performance liquid chromatography analyses show that most of the radioactivity recovered was not [ $^{14}\text{C}$ ]t-BuOH, but rather one or more metabolites of [ $^{14}\text{C}$ ]t-BuOH, thus saturation of the elimination of radioactivity in the urine actually results from a saturation of metabolic capacity. It is the metabolite that is usually eliminated in the urine, rather than t-BuOH itself. t-BuOH was presumed to be eliminated from the body in expired air (Arco Chemical Company 1994a).

Three Sprague-Dawley rats were given [ $^{14}\text{C}$ ]t-BuOH (350 mg/kg) by oral gavage. After 24 h, urine and feces were collected. Only about 1% of the administered dose was excreted in the feces. It was concluded that a conjugate of t-BuOH or its metabolites was not excreted to any appreciable extent in the bile (Arco Chemical Company 1994a).

### **Cytotoxicity**

t-BuOH affects the activity of a variety of enzymes and may stabilize or destabilize a variety of biological membranes. These effects vary with concentration and with temperature and may be due to perturbation of protein conformation, structural changes in membrane lipids, or disturbance of lipid-protein interactions (Harris and Schroeder 1981; Hiller et al. 1984; Lyon et al. 1981; Thomas et al. 1980; Baker and Kramer 1999).

t-BuOH has no or only a weak effect on rat hepatic mitochondrial respiration and phosphorylation at concentrations of up to 3% (Thore and Baltscheffsky 1965).

Blood samples from six adult female Dorset sheep and six adult humans (sexes unspecified) were drawn each day (one individual per day). t-BuOH was added to final concentrations of 0.1%, 0.5%, 1%, and 5%. The cells and t-BuOH were incubated for 1 h after which methemoglobin and glutathione concentrations were measured. t-BuOH caused oxidant stress to erythrocytes as measured by either increased methemoglobin formation and/or decreased glutathione concentrations (Gordon and Calabrese 1992).

### Hydroxyl Radical Scavenger

t-BuOH is an hydroxyl radical scavenger that has been shown to protect DNA from the effects of radiation (Lafleur and Loman 1982; Reuvers et al. 1973; Roots and Okada 1972). It has been hypothesized that this action may be due to the scavenging of hydroxyl radicals.

## ANIMAL TOXICOLOGY

### Oral Toxicity

#### *Acute*

The LD<sub>50</sub> of t-BuOH for white rats (unspecified strain) was 3.5 g/kg (details of experiment unspecified) (Schaffarzick and Brown 1952).

Ten to 35 rabbits, weighing 1.5 to 2.5 kg, were given t-BuOH by stomach tube (Munch 1972; Munch and Schwarze 1925). The LD<sub>50</sub> (the quantity that caused death in half of the rabbits within 24 h) was 48 mmol/kg (3.56 g/kg). The ND<sub>50</sub> (the quantity that caused narcosis in half the rabbits) was 19 mmol/kg (1.41 g/kg).

Beauge et al. (1981) administered 25 mmol/kg (1.85 g/kg) t-BuOH as a 25% by volume solution in water by gastric intubation to an unspecified number of female Wistar rats. Control rats received water. t-BuOH concentration in blood dropped only a small amount between 2 and 20 h after dosing. Blood free fatty acid concentration was unchanged at 2 h and increased at 5 h, and triacylglycerol concentration was decreased at 20 h. Hepatic triacylglycerols were increased at 2 and at 5 h. There were no significant changes in hepatic and blood phospholipid concentrations or in the 4 h lactate/pyruvate ratio. Hepatic palmitate uptake into triacylglycerols was increased at 2, 5, and 20 h, and palmitate incorporation into serum triacylglycerols was about 50% of control values at 5 and 20 h. The researchers concluded that t-BuOH induced a fatty liver, but not by impairing fatty acid oxidation.

A group of 12 female Wistar rats was given 4 ml/kg t-BuOH in a single oral dose (Gaillard and Derache 1965, 1966). Seventeen hours later, in comparison with a control group of rats, the relative weight of the liver was significantly increased, but there was no change in the hepatic nitrogen concentration, or in the fatty acid, triglyceride, cholesterol, or phospholipid concentrations in the blood.

A 3 g/kg dose of t-BuOH was administered orally to male Sprague-Dawley rats (unspecified number) (Hunt et al. 1979).

Later (unspecified time but 2 h later is likely), the rats were decapitated and brain homogenate was incubated with choline for 4 min at 37°C. Choline uptake was increased in the caudate nucleus and decreased in the hippocampus in comparison with control rats.

Four male Wistar rats were given a single oral dose of 2.54 g/kg t-BuOH (Videla et al. 1982). Control rats received saline. Six hours after administration, the hepatic reduced glutathione concentration was decreased, although not significantly, and diene conjugate formation was increased, although not significantly, in comparison with the control rats.

The Arco Chemical Company (1994b) gave groups of five male and five female Sprague-Dawley rats a single dose of 1950, 2535, 3296, and 4285 mg/kg undiluted (99.9%) t-BuOH by oral intubation. Piloerection, ataxia, decreased limb tone, and low carriage were observed in all groups. Prostration, impaired righting reflex, bradypnea, hypoactivity, and lacrimation were observed in the 2535, 3296, and 4285 mg/kg groups. Hypothermia and hypopnea were observed in the 3296 and 4285 mg/kg groups. Test article related effects included hemorrhage and congestion in various visceral organs; this was generally found in the 3296 and 4285 mg/kg groups. The calculated LD<sub>50</sub> with 95% confidence limits was 3384 (2975–3848) mg/kg for male rats, 2743 (2470–3045) mg/kg for female rats, and 3046 (2768–3353) mg/kg for combined male and female rats.

In further work, groups of five male and five female Sprague-Dawley rats were dosed by oral gavage with 1500, 1950, 2535, 3296, and 4285 mg/kg gasoline-grade (95%) t-BuOH (G-t-BuOH). The major pharmacotoxic signs observed in all dosage groups were ataxia, piloerection, prostration, bradypnea, decreased limb tone, and hypoactivity. Decreased righting reflex was also observed in the 1500, 1950, 2535, and 3296 mg/kg dosage levels. No significant changes in body weights were observed. There were no macroscopic lesions which could be attributed to the compound. None of the 1500 mg/kg rats died; 3 out of 10 (all female) of the 1950 mg/kg rats died; 5 out of 10 (3 male, 2 female) of the 2535 mg/kg rats died; 5 out of 10 (1 male, 4 female) of the 3295 mg/kg rats died; and all of the 4285 mg/kg rats died. The oral LD<sub>50</sub> of G-t-BuOH was calculated as 3046 mg/kg with 95% confidence limits from 2581 to 3596 mg/kg for male rats; 2298 mg/kg with 95% confidence limits from 1767 to 2987 mg/kg for female rats; and 2733 mg/kg with 95% confidence limits from 2249 to 3320 for combined male and female rats (Arco Chemical Company 1994c).

BASF Corporation (1994) gave groups of five male and five female Wistar rats 1470, 2150, 3160, and 4640 mg/kg t-BuOH by oral gavage. The animals were monitored for 14 days after which they were killed and necropsied. No abnormalities were detected.

Clinical signs for the 1470 mg/kg group (both sexes) were dyspnea, apathy, staggering, piloerection, and erythema. Except for erythema, the 2150 mg/kg group exhibited all the clinical signs of the 1470 mg/kg group in addition to abnormal positions, atonia, and exsiccosis. The male rats in the 3160 mg/kg

group exhibited only staggering and piloerection whereas the females exhibited dyspnea, apathy, abnormal positions, staggering, atonia, paresis, absence of pain reflex, absence of corneal reflex, narcosis, piloerection, and exsiccosis. The male rats for the 4640 mg/kg group exhibited all these clinical signs as well as erythema whereas the females did not exhibit absence of pain reflex, absence of corneal reflex, or narcosis. No animals in the 1470 mg/kg group died; one female in the 2150 mg/kg group died; five females in the 3160 mg/kg group died; and six animals (one male, five female) in the 4640 mg/kg group died.

Based on the results of the study, the oral LD<sub>50</sub> for male rats was >4640 mg/kg, whereas for female rats the LD<sub>50</sub> was interpolated to be about 2380 mg/kg. The LD<sub>50</sub> for male and female rats combined was calculated to be 3720 mg/kg with 95% confidence limits from 2980 to 3720 mg/kg (BASF Corporation 1994).

Williams and Borghoff (2001) dosed Fischer 344 rats (4/group) once with 500 mg/kg t-BuOH, 500 mg/kg [<sup>14</sup>C]t-BuOH, or vehicle (corn oil) by gavage. Rats were killed 12 h after dosing. The liver and kidneys were removed for analysis. Kidneys were minced, homogenized, and frozen. Kidney cytosol was prepared by ultracentrifugation of thawed kidney homogenate. The concentration of  $\alpha 2\mu$ -globulin was measured in kidney cytosol from male rat kidneys using (ELISA). The renal concentration of  $\alpha 2\mu$ -globulin from the kidney cytosol was significantly higher in the t-BuOH treated male rats compared with corn oil treated males.

In addition, kidney cytosol was analysed by gel filtration. The  $\alpha 2\mu$  protein standard coeluted with the low-molecular-weight protein fraction (LMWPF). Analysis of the LMWPF from [<sup>14</sup>C]t-BuOH treated rats demonstrated radioactivity coeluting with the male, but not female LMWPF. To determine indirectly if t-BuOH binds to  $\alpha 2\mu$ -globulin, dialysis of kidney cytosol from [<sup>14</sup>C]t-BuOH treated male rats was performed with *d*-limonene oxide, a chemical with a high affinity for  $\alpha 2\mu$ -globulin. Dialysis with *d*-limonene oxide resulted in the disappearance of radioactivity coeluting with the LMWPF. This demonstrated that *d*-limonene oxide displaced [<sup>14</sup>C]t-BuOH derived radioactivity from the LMWPF and supports the hypothesis that t-BuOH interacts with  $\alpha 2\mu$ -globulin (Williams and Borghoff 2001).

#### Short-Term

Wakabayashi et al. (1991) studied the effects of alkyl alcohols, including t-BuOH, on rat liver function. Fifteen male Wistar rats were given 15% (v/v) t-BuOH in drinking water. Animals were killed after certain periods of time ranging from one week to 3 months. Sections of the liver were examined by a Hitachi HU-12 electron microscope. t-BuOH induced megamitochondria in the rat hepatocytes after 2 to 3 months' treatment. These enlarged mitochondria were rich in cristae and had dense matrices. The hydroxy group is believed to be responsible for the formation of the mega-mitochondria. In addition, proliferation

of smooth-surfaced endoplasmic reticulum and an increase in the number of lysosomes and microbodies were seen.

Elf Atochem North American Incorporated (1994a) conducted a study in which five groups of five male and five female B6C3F<sub>1</sub> mice received 0.125%, 0.25%, 0.50%, 1%, and 2% (w/v) t-BuOH in their drinking water for 14 days. A control group was given tap water. Changes in body weight were difficult to interpret; in both males and females the two high-dose groups outgained the three lower-dose groups. No scientific rationale was provided for these results. Males given 2% t-BuOH consumed 34% less water than the controls in the first week and 28% less than the controls in the second week, whereas the males given 1% t-BuOH consumed 29% less in the first week and 7% less than the controls in the second week. The females given 1% and 2% t-BuOH drank less water in the first week than the controls, but drank 12% and 18% more in the second week.

All the control and treated mice survived the study period and were in good physical condition at termination with the exception of one female mouse (dose unspecified) in which the caudate lobe of the liver was atrophied. No gross pathological changes were found in the treated or control mice. It was therefore concluded that t-BuOH did not cause gross organ or tissue damage at the doses used in this study (Elf Atochem North American Incorporated 1994a).

In a study of interactive toxicity between t-BuOH and trichloroacetic acid, one group of five to six male Wistar rats was given 0.5% (v/v) t-BuOH in water ad libitum (Acharya et al. 1995). A control group received plain water. The study duration was 10 weeks. Compared to the control group, the t-BuOH group showed a significant depression in body weight. There was also an insignificant decrease in the liver triglyceride concentration, an increase in the serum triglyceride and serum glucose concentrations, and a significant decrease in the kidney glutathione concentration. In addition, the terminal body weights of the treated rats were significantly reduced.

In a later study by Acharya et al. (1997), one study group of male Wistar rats (five to six) also received 0.5% (v/v) t-BuOH in water for 10 weeks. After completion of the treatment, rats were anesthetized and the livers and kidneys were removed for microscopic analysis. Alterations such as centrilobular necrosis, vacuolation in hepatocytes, and loss of hepatic architecture were noted. t-BuOH also caused periportal proliferation and lymphocytic infiltration. Degeneration of renal tubules, degeneration of the basement membrane of the Bowman capsule, diffused glomeruli, and vacuolation of the glomeruli were also noted.

#### Subchronic

The preliminary results of a subchronic study of t-BuOH (methods unspecified) found that t-BuOH increased the mineralization of the kidney, nephropathy, and transitional cell epithelial hyperplasia in male and female F344 rats (numbers unspecified). There was a statistically significant trend in the occurrence of renal tubular tumors in male rats for both adenomas and for combined adenomas plus carcinomas. Other tumor rates that were

increased but insignificant were testicular interstitial adenomas and thymomas in males; and lung adenomas and pituitary adenomas/carcinomas in females. The results for B6C3F<sub>1</sub> mice in the same study showed that t-BuOH affected livers in males (fatty changes); urinary bladders (chronic inflammation and hyperplasia of transitional cell epithelium) in both males and females; and thyroids (proliferative changes including hyperplasia and neoplasia) in both males and females (Arco Chemical Company 1992).

As reported in Lindamood et al. (1992) and Takahashi et al. (1993), groups of F344 rats and B6C3F<sub>1</sub> mice (10 male and 10 female) were given 0%, 0.25%, 0.5%, 1%, 2%, and 4% (w/v) t-BuOH in drinking water for 94 to 95 days). All high-dose rats, six male and four female mice given 4%, t-BuOH, died before the end of the study. A significant decrease in body weight occurred in all dose levels of male rats; female rats given 4% t-BuOH; male mice given 1%, 2%, and 4% t-BuOH; and female mice given 2% and 4% t-BuOH. The 0.25% to 2% dose groups showed a statistically significant decrease in body weight gain. Water consumption decreased in the high-dose groups of both sexes and species whereas it increased in the low-dose groups in male rats. Clinical signs for both sexes of rats included emaciation, ataxia, blood in the urine, and hypoactivity. Males also exhibited paraphimosis while females also exhibited urine staining of the fur. In mice, clinical signs included emaciation, ataxia, abnormal posture, and hypoactivity.

Gross lesions in rats were restricted to the urinary tract and included calculi, dilation of the ureter and renal pelvis, or thickening of the urinary bladder mucosa. In mice, gross lesions were thickened urinary bladder walls or plaques on the mucosa. Histological changes in the urinary bladder include hyperplasia and inflammation. At necropsy, rats in the 4% dose group had several atrophic organs and occasional calculi in the urinary bladder and urinary tract. In the kidney there was a treatment associated increase in the number of hyaline droplets and intracytoplasmic deposits (Lindamood et al. 1992 and Takahashi et al. 1993).

Nephropathy was also significantly increased in all treated groups, except for the 4% dose group. Calculated no-effect levels for subchronic toxicity in rodents are less than 0.25% in male rats, 1% in female rats, 0.5% in male mice, and 1% in female mice. No-effect levels for the urinary tract lesions were calculated to be 1% in male rats and mice and 2% for female rats and mice (Lindamood et al. 1992). Takahashi et al. (1993) state that this was consistent with  $\alpha 2\mu$ -globulin deposition.

In a study reported both by Elf Atochem North American Incorporated (1994b) and Amoco Corporation (1994), groups of B6C3F<sub>1</sub> mice and F344 rats (10 male, 10 female) received 0.25%, 0.5%, 1%, 2% or 4% t-BuOH (w/v) in their drinking water for 13 weeks. The control groups received plain tap water. In both species, t-BuOH was found to be more toxic to males than to females.

Among the rats, nine males and two females in the 4% group died between the 4th and 13th weeks. A reduction in growth rate was seen among males in the 1% and higher dose levels with the

controls outgaining them by 16% to 104%. The control females outgained the 2% and 4% groups by 11% and 46%, respectively. Histopathology revealed the presence of papillary hyperplasia of the transitional epithelium of the urinary bladder in five males and two females in the 4% group. This group also had a decrease in the cell population of bone marrow in nine males and three females.

In the mice, four males in the 4% group died and five in the 2% group died. All deaths except one occurred in the first week of the study. Only one female in the control group died. Body weight gains were 11.7% to 32.5% less than their controls for the males except for the 0.25% group. The females outgained their controls except for the 0.5% dose group. Microscopically there was transitional epithelial hyperplasia with cystitis in the urinary bladders of six males and four females in the 4.0% group. Transitional cell hyperplasia was found in the urinary bladders of 5 males in the 2.0% group (Elf Atochem North American Incorporated 1994b; Amoco Corporation 1994).

The NTP (1995) reported a study in which groups of F344 rats and B6C3F<sub>1</sub> mice (10 male, 10 female) were given 0, 2.5, 5, 10, 20, or 40 mg/ml t-BuOH in their drinking water for 13 weeks. Treatment-related mortalities occurred at the highest concentration in male and female rats and mice. In addition, mean body weight gains of these groups were significantly lower than those of the controls. There was decreased water consumption by treated rats and by the 20 and 40 mg/ml groups of mice during the first week indicating decreased palatability of the dosed water. Some liver toxicity was suggested by a slight increase in serum alanine aminotransferase activity in all exposed groups of female rats.

The principal histopathologic findings were in the urinary bladder of rats and mice and in the kidney of rats. Treatment-related lesions in the urinary bladder, consisting of transitional cell hyperplasia and inflammation of the bladder mucosa, were limited to the 20 and 40 mg/ml groups of male rats and mice and the 40 mg/ml groups of female rats and mice. For male rats and mice, the incidence and severity of the urinary bladder lesions were higher than those for females. In addition, calculi were observed in rats but not in mice. Kidney lesions in female rats were limited to an increase in nephropathy in exposed groups while male rats exhibited protein droplets in the kidney and renal tubule epithelial regeneration (NTP 1995).

### *Chronic*

In a 2-year NTP study (1995), groups of 60 male F344 rats were given 1.25, 2.5, or 5 mg/ml (90, 200, 420 mg/kg) t-BuOH; groups of 60 female rats were given 2.5, 5, or 10 mg/ml (180, 330, 650 mg/kg) t-BuOH; and groups of 60 male and 60 female B6C3F<sub>1</sub> mice were given 5, 10, or 20 mg/ml (540, 1040, 2070 mg/kg for males; 510, 1020, 2110 mg/kg for females) t-BuOH in their drinking water. Controls were given untreated water. Survival of the male rats given 5 mg/ml t-BuOH was significantly lower than that of the controls. Survival among exposed female rats was lower than that of controls, especially in

the 10 mg/ml group; however, more than 50% of the females in each group survived through week 85. For rats, water consumption increased with dose for males and decreased with dose for females. For mice, water consumption by exposed groups of males and females was similar to that by controls.

Cirvello et al. (1995) published the results of the NTP study separately. The results of the carcinogenicity study included increased renal lesions in male rats and thyroid lesions in female mice.

#### *Acute Dermal Toxicity*

One group of three male and three female New Zealand albino rabbits received a 0.5 ml application of undiluted (99.9%) t-BuOH for 24 h. Two intact and two abraded sites per rabbit were used. t-BuOH was found to be minimally irritating causing very slight erythema. It was concluded that t-BuOH was not a primary skin irritant (Arco Chemical Company 1994d). Similar results were found when gasoline-grade t-BuOH was studied using the same methods as above. G-t-BuOH was also found to be minimally irritating and not a primary skin irritant (Arco Chemical Company 1994e).

In another study, undiluted (99.9%) t-BuOH (2000 mg/kg) was applied to the abraded skin of five male and five female New Zealand albino rabbits. Animals were observed for 4 days. All of the rabbits exhibited erythema, edema, and desquamation ranging from very slight to moderate, and fissuring ranging from very slight to slight. Some rabbits exhibited coriaceousness and atonia ranging from very slight to slight. Trace or mild acanthosis and hyperkeratosis were observed in the treated skin of 5 rabbits (one male, four female) and mild dermal fibroplasia was observed in the treated skin of four rabbits (one male, three females). None of these symptoms were seen in any of the untreated skins examined. None of the animals died during the experiment. Based on the data obtained, the minimum lethal dose (MLD) for t-BuOH was greater than 2000 mg/kg (Arco Chemical Company 1994f).

Undiluted gasoline grade (approximately 95%) t-BuOH (2000 mg/kg) was applied to the abraded skin of five male and five female New Zealand albino rabbits. Animals were observed for 15 days. Compound-related microscopic changes in the treated skin not found on the untreated sites consisted of hyperkeratosis, acanthosis, increased severity of chronic dermatitis, and hemorrhage. Hyperkeratosis was observed at the treatment site of all males (trace to mild) and all females (trace to moderate) compared to one male (mild) and no females at untreated sites. Acanthosis was present in the treated skin of three males (mild to moderate) and four females (trace to moderate), whereas one male and no females showed mild acanthosis at untreated sites. Chronic dermatitis was present in the treated skin of all males (trace to moderate) and all females (mild to moderate), whereas the severity ranged from trace to mild for all animals at the untreated sites. None of the animals died during the experiment. Based on the results, the acute dermal LD<sub>50</sub> for G-t-BuOH is greater than 2000 mg/kg (Arco Chemical Company 1994g).

## **Inhalation Toxicity**

### *Acute*

Exposure of six nonpregnant Sprague-Dawley rats to 10,000 ppm t-BuOH for 1 day produced severe narcosis in all animals and death in five of the six. Reducing the concentration to 5000 ppm t-BuOH still produced narcosis in all exposed animals. In addition, both 5000 and 3500 ppm t-BuOH produced an unsteady gait at the end of 7 h of exposure. All animals responded to a tap on the cage, but locomotor activity was impaired (Nelson et al. 1989).

A group of five male and five female Sprague-Dawley rats were placed in chambers and exposed for approximately 4 h to 10,000 ppm t-BuOH. The principle signs exhibited during exposure were ocular discharge, dyspnea, and prostration. One rat also exhibited ataxia. Only one rat, a female, died following the exposure period. At necropsy four rats (three male, one female) were observed to have red foci on the lungs. The one female rat was the same one which died (Arco Chemical Company 1994h).

Two groups of albino rats, each consisting of five males and five females, were exposed to vapor atmospheres of 9700 and 14100 ppm gasoline grade t-BuOH for approximately 4 h. All animals exhibited dyspnea and prostration during the exposure. Ocular discharge was observed in all the females. Ataxia and dyspnea were observed for all animals during the post observation period. None of the 9700 ppm animals died, whereas three of the 14100 ppm animals died during the study. In addition, the 14100 ppm animals also exhibited excessive weakness, nasal and/or ocular discharge, and alopecia. Red foci were found in the lungs of both groups of animals (Arco Chemical Company 1994i).

### *Short-Term*

The NTP (1997) reported a study in which groups of five male and five female F344 rats and B6C3F<sub>1</sub> mice were exposed to t-BuOH by whole-body inhalation to target concentrations of 0, 450, 900, 1750, 3500, and 7000 ppm for 6 h plus T<sub>90</sub> per day, 5 days per week, over an 18-day period.

All animals exposed to 7000 ppm died on day 2. Hypoactivity, ataxia, and prostration were also observed at the highest exposure concentration for both rats and mice. Mean body weight gains were significantly lower than those of controls for the male and female rats exposed to 3500 ppm (14% and 13% less, respectively). Ataxia, hyperactivity, and hypoactivity were observed in rats exposed to 900 ppm and higher.

For mice exposed to 3500 ppm, hypoactivity, ataxia, and rapid respiration were observed, whereas hypoactivity, hyperactivity, ataxia, and urogenital wetness occurred in mice exposed to 1750 ppm. The liver weights of male and female mice exposed to 3500 ppm were significantly greater than those of the controls. In addition, thymus weights were significantly less than those of the controls for male and female rats and female mice exposed to 3500 ppm. The results for animals exposed to 450 ppm were not included (NTP 1997).

Borghoff et al. (2001) tried to determine whether t-BuOH induces  $\alpha 2\mu$ -nephropathy and enhanced renal cell proliferation in male, but not female, F344 rats. Eighty male and female rats (5/sex/concentration) were exposed by inhalation to target concentrations of 0, 250, 450, or 1750 ppm t-BuOH for 6 h/day for 10 consecutive days. One day following the final exposure, rats were anesthetized and the kidneys removed, sectioned, and stained for histological analysis. Body, liver, and kidney weights were also evaluated and renal cell proliferation was measured as the percentage of positively stained epithelial cells within the proximal tubules.

A statistically significant decrease in the absolute and relative (expressed as a percentage of body weight) liver weight was observed in male rats exposed to 1750 ppm t-BuOH compared to controls, with no consistent exposure-related change in the liver of female rats. Relative kidney weights were significantly increased in male rats exposed to 1750 ppm and in female rats exposed to 450 and 1750 ppm t-BuOH compared to controls. Although protein droplet accumulation was seen in the proximal tubules in kidney of male rat in the control and t-BuOH-exposed groups, none were observed in any female rats. There was a statistically significant, concentration-dependent positive trend for the accumulation of protein droplets in male rats exposed to t-BuOH.

Likewise,  $\alpha 2\mu$ -immunohistochemical staining only revealed positive staining of protein droplets within the renal proximal tubules of control and t-BuOH-exposed male rats; however, there did not appear to be an exposure-related increase in intensity. When quantification of cell proliferation was restricted to the renal cortex, a concentration-related, statistically significant increase was seen in all groups of t-BuOH exposed male rats as compared to control males. There were no significant differences in cell proliferation observed between t-BuOH exposed and control female rats. The authors concluded that t-BuOH induces  $\alpha 2\mu$ -globulin nephropathy and renal cell proliferation in male, but not female F344 rats. In addition, these effects were due to a male-rat specific mechanism by t-BuOH (Borghoff et al. 2001).

Further studies confirmed that t-BuOH interacts with  $\alpha 2\mu$ -globulin in the male rat kidney following t-BuOH exposure (Williams and Borghoff 2001).

#### *Subchronic*

NTP (1997) reported results of a study in which groups of F344 rats and B6C3F<sub>1</sub> mice (10 male, 10 female) were exposed to t-BuOH for 6 h plus T<sub>90</sub> per day, 5 days per week, for 13 weeks. Actual concentrations used were 135, 270, 540, 1080, and 2100 ppm. The only t-BuOH-related death that occurred was that of a 2100 ppm male mouse. Body weight gains were similar to those of the controls for all exposed rats, but were significantly less for the 135 and 270 ppm male and the 1080 and 2100 ppm female mice. Female rats (2100 ppm) were emaciated and hypoactive.

Other effects of t-BuOHs exposure included slight anemia and decreased serum alkaline phosphatase activity in exposed

male rats and a marked increase in the number of segmented neutrophils in the 2100 ppm male mice. In rats, kidney weights of 1080 ppm males and 2100 ppm males and females were significantly greater than those of the controls. Likewise, liver weights of the 1080 and 2100 ppm female rats and mice were greater than those of the controls (NTP 1997).

#### **Acute Intravenous Toxicity**

Two male Sprague-Dawley rats were injected through cannulas with doses of 60 mg/kg and 350 mg/kg t-BuOH and observed for 24 h. There were no changes in body temperature or behavior (Arco Chemical Company 1994a).

#### **Ocular Irritation**

Rhone-Poulenc (1992) conducted a study in which nine New Zealand white rabbits each received a 0.1 ml drop of a mixture of ethanol and t-BuOH (concentrations unspecified) in one eye. The eyelids were gently held together for 1 s. The untreated eye served as a control. The eyes of 6 animals remained unwashed for 24 h after which the test article was washed out. The eyes of the other three rabbits were irrigated 30 s after dosing. Observations of ocular irritation were made 24, 48, and 72 h after dosing as well as 4 and 7 days if irritation persisted.

For the unrinsed treatment group, ocular effects included increased opacity of the cornea, reduced reaction of the iris to light, extreme redness, chemosis, and discharge. Results were similar for those rabbits which received a rinse though symptoms were less severe. It was concluded that the test article was a severe ocular irritant to rabbits (Rhone-Poulenc 1992).

The Arco Chemical Company (1994j) reported another study of eye irritation in which 0.1 ml of undiluted t-BuOH (99.9%) was administered to the right eye of nine New Zealand albino rabbits (five male, four female). One group (two male, one female) had their eye washed approximately 30 s after instillation for approximately 1 min. The other rabbits (three male, three female) did not have their eyes rinsed. Draize scoring was performed at various times following treatment.

The maximum average scores were 40.6 and 33.2 for the unwashed and washed groups respectively. This occurred at 72 h. After 10 days, the scores were 13.9 and 1.7, and after 25 days the scores were 4.1 and 0 for the unwashed and washed groups, respectively. The study on the washed group terminated on day 25 when the eyes of all three rabbits received scores of 0. The scores for the unwashed group changed very little after day 25 when it was 4.1; at day 34 the group average was 4.4 (minimally irritating). t-BuOH was classified as severely irritating for the unwashed group and moderately irritating in the washed group (Arco Chemical Company 1994j).

In a similar study (Arco Chemical Company, 1994k), 0.1 ml of gasoline-grade t-BuOH was placed in the right eye of nine New Zealand albino rabbits (four male, five female). Three rabbits (one male, two female) had their eyes washed 30 s after

instillation for 1 min. The other rabbits (three male, three female) did not have their eyes washed.

The maximum average Draize score was 41 at 24 h for the unwashed group and 30.3 at 72 h for the washed group. The scores for the unwashed group continually decreased except between days 7 and 10 when the score increased from 0.4 to 5.4. At day 15, the eyes of all six rabbits received scores of 0 and the study was terminated for this group. The scores for the washed group decreased to 0 at day 22 but rose to 2.1 at day 25, then leveled off at 0.8 until day 34. G-t-BuOH was found to be moderately irritating for both test groups. G-t-BuOH was classified as a primary eye irritant for both the washed and unwashed groups (Arco Chemical Company 1994k).

### Dermal Irritation

Renkonen and Tier (1957) conducted an experiment to investigate the intradermal irritation of t-BuOH to rabbits. There were no vehicle controls. Eight rabbits were injected intradermally with t-BuOH (vehicle unspecified). The size of the local skin reaction after injection of 35 mg t-BuOH was 14 mm<sup>2</sup>, and after 10 mg t-BuOH was 43 mm<sup>2</sup>. No explanation of the significance of these results was provided.

Jacobs et al. (1987) tested skin irritation by hydrocarbons including 32 monoalcohols. A Teflon exposure chamber containing a patch soaked with 0.5 ml of test substance was applied to shaved sites on New Zealand white rabbits (one per compound). The exposure time was 4 h, after which the patch was removed and the skin cleaned. The animals were examined for erythema and edema at 1, 24, 48, and 72 h. All the alcohols tested had calculated limit concentrations of 50% (w/w) including 1-Butanol, 1-Methylpropanol, and 2-Methylpropanol, which are structurally similar to t-BuOH. Results indicated that branching in alcohols had no effect on the limit concentrations for the aliphatic isomers. Although t-BuOH was not studied, the results demonstrated that the 50% limit concentration applied to all the alcohols with a molecular weight between that of Ethanol and 1-Undecanol.

In a study by Rhone-Poulenc Inc. (1992), six New Zealand white rabbits each received a single dermal application of 0.5 ml of a mixture of ethanol and t-BuOH (concentrations unspecified). Two 2.5-cm<sup>2</sup> test sites were used, one abraded and one intact. One rabbit exhibited moderate irritation at both the abraded and intact site. Three rabbits exhibited mild irritation at the abraded site, including one which also exhibited mild irritation at the intact site. None of the other four rabbits exhibited any irritation at the intact site. It was concluded that the test article was not a primary dermal irritant to rabbits under the conditions of the study.

Dow Chemical Company (1994) reported that t-BuOH (concentration unspecified) was found to have no irritating effect on the skin of shaved rabbits (strain unspecified) when observed for a period of one week.

### REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Groups of F344 rats and B6C3F<sub>1</sub> mice (10 male, 10 female) were exposed to t-BuOH for 6 h plus T<sub>90</sub> per day, 5 days per week, for 13 weeks. Actual concentrations used were 135, 270, 540, 1080, and 2100 ppm. No significant differences were found in the weight of the testis, epididymis, and cauda; and sperm motility, count, and morphology for males. For females, no significant differences were found in the estrous cycle length or percentage of time spent in the various stages (NTP 1997).

Groups of 60 male F344 rats were given 1.25, 2.5, or 5 mg/ml (90, 200, 420 mg/kg) t-BuOH; groups of 60 female rats were given 2.5, 5, or 10 mg/ml (180, 330, 650 mg/kg) t-BuOH; and groups of 60 male and 60 female B6C3F<sub>1</sub> mice were given 5, 10, or 20 mg/ml (540, 1040, 2070 mg/kg for males; 510, 1020, 2110 mg/kg for females) t-BuOH in their drinking water for 2 years. Controls were given untreated water (NTP 1995). No significant differences in sperm morphology or motility were found for male rats or mice; and for female rats, no significant differences in the length of the estrous cycle or percentage of time spent in the various estrous stages occurred. However, for female mice, estrous cycle length was significantly increased in the highest-dose group whereas percentage of time spent in the various estrous stages did not differ from controls.

Anderson et al. (1982) determined the effect of t-BuOH on in vitro fertilization of Swiss-Webster mice gametes. Capacitated epididymal mouse spermatozoa were added to mouse oocytes with cumulus masses and, after a 24-h incubation, the eggs were examined for fertilization. t-BuOH, at a concentration of 87 mM, was added to both the capacitation and the culture media. It did not affect the in vitro fertilization capacity of spermatozoa.

Daniel and Evans (1982) fed groups of 15 pregnant Swiss-Webster mice liquid diets containing t-BuOH at concentrations of 0.5%, 0.75%, and 1% (w/v) from days 6 to 20 of gestation. Control mice were fed only the liquid diet. The 1% t-BuOH group was fed ad libitum. The other groups were pair-fed based on the consumption of the 1% t-BuOH group.

The average maternal weight gain over the 20 days was 64% for the controls and 62%, 52%, and 51% for the 0.50%, 0.75%, and 1% t-BuOH-fed groups, respectively. Approximately one-half of the maternal animals in each group were replaced with untreated surrogate mothers within 24 h of delivery of litters to determine the role of maternal nutritional and behavioral factors on the young. Length of gestation, gross structural abnormalities, and number of deaths were recorded. Weight measurements, pinna detachment, eye opening, and behavioral test scores for the young were determined various times during days 2 to 22 postparturition.

The total number of litters from 15 animals was 11 (77%) in the control group, 12 (80%) in the 0.5% t-BuOH group, 8 (53%) in the 0.75% group, and 7 (47%) in the 1% group. The average number of neonates per litter was 10.4 in the control group, 10.3 in the 0.5% t-BuOH group, 7.4 in the 0.75% group, and 5.3 in the 1% group. The average fetal weight at day 2 was 1.78 g

in the control group, 1.66 g in the 0.5% t-BuOH group, 1.45 g in the 0.75% group, and 1.10 g in the 1% group. There was a dosage-response relationship between t-BuOH concentration in the diet and total number of stillborns (number of stillborns per litter size not given); there were 3 stillborns in the control group, 6 in the 0.5% t-BuOH group, 14 in the 0.75% group, and 20 in the 1% group.

Pinna detachment occurred between days 6 and 8 in all the groups. Eyes opened in the 1% t-BuOH group at around day 16; this was 2 to 4 days later than in the other groups. Postnatal weight gain was decreased over the first 10 days in the nonfostered 0.75% and 1% groups in comparison to the other groups. There was a general dosage-response relationship between higher t-BuOH exposure in utero and poorer behavioral performance of pups. Fostered pups performed significantly better than nonfostered pups in three of four behavioral tests. All the treated groups did eventually recover and acquire the same level of performance on the behavioral tests (Daniel and Evans 1982).

Virgin female Sprague-Dawley rats (15 to 20) were placed individually with breeder males (Nelson et al. 1989). Females with sperm (day 0 of gestation) were placed in exposure chambers. Exposure consisted of 0, 2000, 3500, or 5000 ppm t-BuOH for 7 h per day. Animals were left in the chambers for degassing for approximately 1/2 hour after exposure. They were then placed in their homecages. On gestation day 20, pregnant females were euthanized and the entire uterus (with ovaries attached) was removed. Fetotoxicity generally increased with concentration, and fetal weights were slightly depressed at all concentrations of t-BuOH. The authors concluded that t-BuOH evidenced developmental toxicity, with effects seen at all concentrations, although these were also associated with maternal toxicity.

Faulker et al. (1989) also studied the effects of prenatal t-BuOH administration. Pregnant CBA/J and C57BL/6J mice (numbers unspecified) were treated by gavage every 12 h with 10.5 mmol/kg of t-BuOH from day 6 through day 18 of gestation. t-BuOH produced a significant increase in the number of resorptions per litter. Eight of the 21 litters in the treated groups had all the fetuses resorbed compared to none in the control groups. There was also a significant decrease in the number of live fetuses per litter and a slight but insignificant decrease in the weight of the surviving fetuses. t-BuOH was not found to have any teratogenic effect on soft tissues. Minor variations in the skull and sternum occurred more frequently but were not significantly different from controls.

In another prenatal exposure study by Abel and Bilitzke (1992), pregnant Long-Evans rats (numbers unspecified) consumed liquid diets containing t-BuOH (0.65%, 1.3%, and 10.9% v/v) beginning on gestation day 8 until parturition. t-BuOH reduced maternal weight gain, litter sizes (from 11 to 3 pups per litter), birth weights, and weights at weaning, and increased perinatal (from 2% to 14%) and postnatal (from 6% to 100%) mortality.

The teratogenic effects of t-BuOH were studied using cells from chicken embryo wing buds (Kulyk and Hoffman 1996). Cultures were fed with medium containing 0.1% to 4% (v/v)

t-BuOH. t-BuOH was effective at enhancing cartilage differentiation. The authors postulated that this could interfere with proper skeletal morphogenesis.

The in vitro effects of short-chain aliphatic alcohols on muscarinic receptor-stimulated phosphoinositide metabolism were studied in cerebral cortical slices from 7-day-old Sprague-Dawley rats. Muscarinic receptor-stimulated phosphoinositide metabolism has been suggested as a possible target for the neurotoxic effects of ethanol during brain development. Out of five alcohols, t-BuOH was the most potent in inhibiting carbachol-stimulated [ $^3$ H]inositol phosphates accumulating in a dose- and time-dependent manner. Similar results were seen when cortical slices from adult animals were used, though the effects were less pronounced (Candura et al. 1991).

An indwelling gastric fistula was surgically implanted 4 days after birth into eight Long-Evans rats from each of six litters to implement an artificial feeding method (Grant and Samson 1982). Four rats from each litter received milk formula containing a mean daily dose of t-BuOH that ranged from 0.60 to 2.69 g/kg on postnatal days 4 through 7 and then received only milk formula for the next 11 days. The remaining 4 rats from each litter received only milk formula. At postnatal day 18, all the rats were decapitated, various organs were weighed, and biochemical analyses were performed.

Only 26 of 48 animals survived the experiment; the major cause of death was a poor fistulation procedure or gastric bloating. Blood concentrations of t-BuOH ranged from 33.0 to 66.0 mg/100 ml of blood during alcohol administration. No differences between groups were observed in emergence of teeth, eye opening, or unfolding of the ears. No significant differences were observed between treated and control rats in body, liver, and heart weights, but the brains weighed significantly less in the treated rats; treated rats had decreased protein in the forebrains and decreased DNA in the hindbrains (Grant and Samson 1982).

## GENOTOXICITY

Lennox and Waldren (1981) and Waldren (1982) stated that t-BuOH was mutagenic to cultured human-Chinese hamster ovary hybrid cells at the mean lethal concentration of 80 mM.

t-BuOH was tested at 6 concentrations (0.625, 1.25, 2.5, 5, 10, and 20  $\mu$ l/ml) using Chinese hamster ovary cells with and without induced rat liver S-9 activation (Arco Chemical Company 1994l). In this study, t-BuOH caused a marginal increase in sister chromatid exchange frequency in treated cultures when compared to controls; however, the increase was insignificant. In another study, t-BuOH was tested at 6 dose levels (0.625, 1.25, 2.5, 5, 10, and 20  $\mu$ l/ml) for 2 h with induced rat liver S-9 activation and 7 dose levels (0.31, 0.625, 1.25, 2.5, 5, 10, and 20  $\mu$ l/ml) for 24 hours without S-9. The results indicate that t-BuOH caused a significant increase in sister chromatid exchanges at the high dose without S-9 and at the two highest doses with S-9 (Arco Chemical Company 1994m).

Using L5178Y mouse lymphoma cells, McGregor et al. (1988) found a small increase in the mutant fraction in one

experiment without S-9 (1.6 times the control value), but this was not reproduced in three other experiments in which concentrations up to 5000  $\mu\text{l}/\text{mg}$  t-BuOH (concentrations unspecified) were used. One of these was conducted without S-9; the remaining two were conducted in the presence of S-9.

t-BuOH (99.9%) and gasoline grade t-BuOH were also tested for mutagenicity effects on L5178Y mouse lymphoma cells. The Arco Chemical Company (1994n) used concentrations of 0.001, 0.01, 0.1, 1, 10, and 100  $\mu\text{l}$  per ml and tests were performed in the presence and absence of induced rat liver S-9. t-BuOH (99.9%) did not induce a significant increase in the mutant frequency in any of the treated cultures in the presence or absence of S-9. G-t-BuOH did not induce an increase in the mutant frequency of cultures in the presence of S-9; however, an increase did occur in the absence of S-9 though this response was not dose-related. t-BuOH does not appear to be a mutagen in L5178Y mouse lymphoma cells.

t-BuOH was nonmutagenic in the *Salmonella*/mammalian microsome mutagenicity test "even at a high concentration" (Ames et al. 1975; Yamaguchi 1980). It was nonmutagenic to *Salmonella typhimurium* in the same test with metabolic activation when the bacterial suspension was preincubated with the chemical (concentrations unspecified) (NTP 1982).

t-BuOH (100 to 10000  $\mu\text{g}/\text{plate}$ ) did not induce mutations in *Salmonella typhimurium* strain TB98, TA100, TA1535, or TA1537 with or without induced rat or hamster liver S-9 in a study by Zeiger et al. (1987).

t-BuOH was also tested by the Arco Chemical Company (1994o) in the *Salmonella*/mammalian microsome mutagenesis assay using strains TA98, TA100, TA1535, TA1537, and TA1538 with and without induced rat liver S-9. The concentrations ranged from 2.9 to 10,000  $\mu\text{g}/\text{plate}$ . The results indicate that t-BuOH did not cause a significant increase in the number of revertants per plate of any of the strains with or without S-9. However, there was a slight increase in TA1535 revertants per plate observed in the presence and absence of S-9. The same study was performed on gasoline grade t-BuOH using the same methods as above (Arco Chemical Company 1994p). In this case, G-t-BuOH caused a weak but significant increase in TA1535 revertants per plate in both the presence and absence of induced rat liver S-9.

t-BuOH, added at a concentration of 1% to medium prior to sterilization by autoclaving, did not increase the incidence of penicillin or streptomycin resistance in *Micrococcus aureus* (Clark 1953). In addition, bacterial cell survival was not affected.

t-BuOH did not induce adenine independence in adenine-dependent *Neurospora crassa* (Dickey et al. 1949). Mutations did not result after exposure to the fungi to a 1.75 mol/L concentration of t-BuOH in water.

Abbondandolo et al. (1980) considered t-BuOH as a possible solvent for water-insoluble chemicals that would be tested for mutagenicity. Because t-BuOH was moderately toxic to V79 Chinese hamster cells at 2% and 5% (v/v), and to the *Schizosaccharomyces pombe* strain of yeast at concentrations of 0.5% to 10% (v/v), the authors did not further pursue its use.

## CARCINOGENICITY

Hoshino et al. (1970) conducted a study in which hair was clipped from the backs close to the base of the tail of female ddN mice, chemicals were applied to their bared skin, and the mice were observed for 450 days. Moribund animals were killed and tissues were examined.

In the first experiment, 0.05 mg 4-nitroquinoline-1-oxide (4NQO) in benzene was applied to the mice 3 times a week for a total of 20 applications. No acute skin damage was observed. In 50 surviving mice, there was 1 small papilloma and no "skin tumors."

In a second experiment, 4NQO was applied as in the first experiment and was followed by applications of 16.6% t-BuOH (actual dosage unspecified) in benzene 6 times a week for a total of 270 applications. No acute skin damage was observed within about 100 days. After 350 days, two "erosions" were produced at the application site and these remained for the duration of the observation period. About 150 days after the start of the experiment and after about 100 applications of t-BuOH, one neoplasm was observed, of which the authors stated: "it developed into squamous cell carcinoma rapidly." About 300 days after the start of the experiment, a subcutaneous granuloma was detected. Fifty mice survived after the appearance of the first tumor in the experiment (Hoshino et al. 1970).

In a 2-year NTP study (1995), groups of 60 male F344 rats were given 1.25, 2.5, or 5 mg/ml (90, 200, 420 mg/kg) t-BuOH; groups of 60 female rats were given 2.5, 5, or 10 mg/ml (180, 330, 650 mg/kg) t-BuOH; and groups of 60 male and 60 female B6C3F<sub>1</sub> mice were given 5, 10, or 20 mg/ml (540, 1040, 2070 mg/kg for males; 510, 1020, 2110 mg/kg for females) t-BuOH in their drinking water. Controls were given untreated water. Because an interim evaluation of 10 rats/sex/dose was performed at 15 months, 50 rats/group completed the study.

The principal effects from 2 years of exposure to t-BuOH in drinking water were proliferative lesions (hyperplasia, adenoma, and carcinoma) in the kidneys of exposed male rats, and nephropathy in all exposed groups of female rats and in males given 5 mg/ml t-BuOH. Female rats in the 5 and 10 mg/ml dose groups also exhibited inflammation of the kidneys. The incidence of follicular cell hyperplasia of the thyroid gland was significantly increased in all exposed groups of male mice and in 10 and 20 mg/ml groups of female mice. The incidence of thyroid follicular cell adenoma was significantly increased in 20 mg/ml females. One thyroid follicular cell carcinoma was observed in a 20 mg/ml male. Effects on the urinary bladders included inflammation and hyperplasia of the transitional epithelium for the 20 mg/ml males and inflammation for the 20 mg/ml females.

Based on increased incidences of renal tubule adenoma or carcinoma, it was concluded that there was "some evidence of carcinogenic activity" of t-BuOH in male F344 rats. There was no evidence of carcinogenic activity in female rats. There was "equivocal evidence of carcinogenic activity" of t-BuOH in male

**TABLE 4**  
Incidence of lesions in male F344/N rats (NTP 1995; Cirvello et al. 1995)

Kidney effects	Dose			
	0 mg/ml	1.25 mg/ml	2.5 mg/ml	5 mg/ml
	Number of kidneys with effect (50 kidneys examined) and severity* (where available)			
Nephropathy	49 with nephropathy; avg. severity* of 3.0	49 with nephropathy; avg. severity* of 3.0 (Cirvello reported an avg. severity* of 3.1)	50 with nephropathy; avg. severity* of 3.1	50 with nephropathy; avg. severity* of 3.3 (Cirvello reported only 49 kidneys)
Transitional epithelium hyperplasia	25 with hyperplasia; avg. severity of 1.7	32 with hyperplasia; avg. severity of 1.7	36 with hyperplasia***; avg. severity of 2.0	40 with hyperplasia***; avg. severity of 2.1
Mineralization	26 with mineralization; avg. severity of 1.0	28 with mineralization; avg. severity of 1.1	35 with mineralization; avg. severity of 1.3	48 with mineralization***; avg. severity of 2.2
Renal tubule hyperplasia	14 with hyperplasia; avg. severity of 2.1	20 with hyperplasia; avg. severity of 2.3	17 with hyperplasia; avg. severity of 2.2	25 with hyperplasia***; avg. severity of 2.7 (Cirvello reported an avg. severity* of 2.8)
Renal tubule adenoma	7 with adenoma	7 with adenoma	10 with adenoma (Cirvello reported only 9 with adenoma)	10 with adenoma (Cirvello reported only 9 with adenoma)
Renal tubule adenoma or carcinoma (comb.)	8 with adenoma or carcinoma	13 with adenoma or carcinoma	19 with adenoma or carcinoma***	13 with adenoma or carcinoma

\* Average severity of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

\*\*p ≤ .05.

\*\*\*p ≤ .01.

**TABLE 5**  
Incidence of kidney lesions in female F344/N rats (NTP 1995; Cirvello et al. 1995)

Kidney effects	Dose			
	0 mg/ml	2.5 mg/ml	5 mg/ml	10 mg/ml
	Number of kidneys with effect (50 kidneys examined) and severity* (where available)			
Inflammation	2 with inflammation; avg. severity of 1.0	3 with inflammation; avg. severity of 1.0	13 with inflammation***; avg. severity of 1.0	17 with inflammation***; avg. severity of 1.1
Mineralization	49 with mineralization; avg. severity of 2.6	50 with mineralization; avg. severity of 2.6	50 with mineralization; avg. severity of 2.7	50 with mineralization; avg. severity of 2.9
Nephropathy	48 with nephropathy; avg. severity of 1.6	47 with nephropathy; avg. severity of 1.9**	48 with nephropathy; avg. severity of 2.3***	50 with nephropathy; avg. severity of 2.9***
Transitional epithelium hyperplasia	0 with hyperplasia	0 with hyperplasia	3 with hyperplasia; avg. severity of 1.0	17 with hyperplasia***; avg. severity of 1.4
Renal tubule hyperplasia	0 with hyperplasia	0 with hyperplasia	0 with hyperplasia	1 with hyperplasia; avg. severity of 1.0

\* Average severity of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

\*\*p ≤ .05.

\*\*\*p ≤ .01.

B6C3F<sub>1</sub> mice due to marginally increased incidences of follicular cell adenoma or carcinoma of the thyroid gland. Due to the increased incidences found in female mice, it was concluded that there was "some evidence of carcinogenic activity" of t-BuOH in female mice (NTP 1995).

Cirvello et al. (1995) published the results of the NTP study separately, with small differences in numbers of animals and severity indices. A summary of the findings of both studies is given for male rats, female rats, and male and female mice in Tables 4, 5, and 6, respectively.

## CLINICAL ASSESSMENT OF SAFETY

### Irritation and Sensitization

A repeat-insult patch test (RIPT) was performed on 119 individuals using 60% ethyl alcohol and 0.125% t-BuOH. A total of 99 individuals completed the study. Those dropping out of the study were for reasons unrelated to the study. No dermal reactions were observed. It was concluded that the test article demonstrated no potential for eliciting either dermal irritation or sensitization (Clinical Research Laboratories, Inc. 1998).

**TABLE 6**  
Incidence of thyroid and bladder lesions in B6C3F<sub>1</sub> mice (NTP 1995; Cirvello et al. 1995)

Thyroid gland effects	Sex	Dose			
		0 mg/ml	5 mg/ml	10 mg/ml	20 mg/ml
Follicular cell hyperplasia		Thyroid glands examined			
	M	60	59	59	57
	F	58	60	59	59
	M	5 with hyperplasia; avg. severity of 1.2	18 with hyperplasia***; avg. severity of 1.6	15 with hyperplasia**; avg. severity of 1.4	18 with hyperplasia***; avg. severity of 2.1
Follicular cell adenoma	F	19 with hyperplasia; avg. severity of 1.8	28 with hyperplasia; avg. severity of 1.9	33 with hyperplasia**; avg. severity of 1.7	47 with hyperplasia***; avg. severity of 2.2
	M	1 with adenoma	0 with adenoma	4 with adenoma	1 with adenoma
Follicular cell adenoma or carcinoma	F	2 with adenoma	3 with adenoma	2 with adenoma	9 with adenoma**
	M	1 with adenoma or carcinoma	0 with adenoma or carcinoma	4 with adenoma or carcinoma	2 with adenoma or carcinoma
	F	Not given	Not given	Not given	Not given
Urinary bladder effects	Sex	Urinary bladders examined			
Inflammation	M	59	59	58	59
	F	59	60	59	57
	M	0 with inflammation	3 with inflammation; avg. severity of 1.7	1 with inflammation; avg. severity of 1.0	37 with inflammation***; avg. severity of 2.4
	F	0 with inflammation	0 with inflammation	0 with inflammation	4 with inflammation**; avg. severity of 2.0
Transitional epithelium, hyperplasia	M	1 with hyperplasia; avg. severity of 2.0	3 with hyperplasia; avg. severity of 1.7	1 with hyperplasia; avg. severity of 1.0	17*** with hyperplasia; avg. severity of 1.8
	F	0 with hyperplasia	0 with hyperplasia	0 with hyperplasia	3 with hyperplasia; avg. severity of 1.0

## Case Reports

A woman who had a positive patch test reaction to ethanol was tested with 100% t-BuOH (Fregert et al. 1963). The alcohol was applied for 48 h and the site was scored at 3, 24, and 48 h after removal of the test material. The woman did not react to t-BuOH. Four female patients were tested on the upper back with 1% and 10% t-BuOH in water (Fregert et al. 1969). The patches were applied for 24 h and reactions were read 24 and 48 h after removal. None of the women had any reaction to t-BuOH.

Edwards and Edwards (1982) described a case of allergic contact dermatitis to the t-BuOH component of SD-40 alcohol in a commercial sunscreen preparation. A man who had a widespread, pruritic, red, vesicular eruption of his face, neck, arms, and chest and who had used a variety of sunscreens was patch-tested with sunscreens and with the individual components of the product to which he reacted. A 70% concentration of t-BuOH was applied to the forearms. At 72 h, erythema was observed and at 96 h, vesiculation was observed. No reactions were observed in two controls who also had applied t-BuOH to their forearms.

Dermatitis has also been observed when t-BuOH is applied to the skin; it caused irritation, moderate hyperemia and erythema, dryness, and vesiculation (ACGIH 2000; Greenberg and Lester 1954; Von Oettingen 1943).

## Occupational Health and Safety

The American Conference of Governmental and Industrial Hygienists (ACGIH) has set a threshold limit value of 100 ppm that is satisfactory to prevent narcosis with t-BuOH (ACGIH 2000). The threshold limit value is the time-weighted average concentration for a normal 8-h workday or 40-h workweek and no adverse effects are expected from it. The short-term exposure limit is that concentration to which workers can be exposed for 15 min without suffering ill effects. Four 15-min periods are permitted per day with at least 60 min between exposure periods. In addition, the daily threshold limit value must not be exceeded (Wimer et al. 1983). National Institute of Occupational Safety and Health (NIOSH) has reported that 8000 ppm t-BuOH is the concentration immediately dangerous to life or health (Mackison et al. 1978).

## SUMMARY

t-BuOH is a tertiary aliphatic alcohol that is used as a solvent or an alcohol denaturant and as a perfume carrier in cosmetics. In 1998, t-BuOH was reported as an ingredient in 32 formulations of eye makeup, fragrance, and shaving preparations. For 1999, the concentration of use in cosmetic products ranged from 0.00001% and 0.3%.

t-BuOH is not a substrate for alcohol dehydrogenase. In rat liver microsomes, t-BuOH can be oxidatively demethylated by hydroxyl radicals to yield formaldehyde. Acetone was found in the blood, urine, and expired air of rats following the intraperitoneal administration of t-BuOH.

In rats, t-BuOH moves rapidly from the blood into the tissues. t-BuOH undergoes a rapid distribution phase followed by a slower elimination phase. In a radiolabel study, t-BuOH was being eliminated primarily as metabolic product(s). t-BuOH is eliminated slowly from the blood of rats and mice.

For rabbits, the excretion saturation was reached at a dose between 500 mg/kg and 1500 mg/kg. Saturation of the elimination of radioactivity in the urine actually results from a saturation of metabolic capacity. As in rats, it is the metabolite that is usually eliminated in the urine, rather than t-BuOH itself.

In sheep and humans, t-BuOH caused oxidant stress to erythrocytes as measured by either increased methemoglobin formation and/or decreased glutathione concentrations. For rats, the oral LD<sub>50</sub> was 3.0 to 3.7 g/kg for t-BuOH and 2.7 g/kg for G-t-BuOH. The oral LD<sub>50</sub> for rabbits was 3.56 g/kg.

In oral studies, t-BuOH at 2% (w/v) or less in drinking water did not cause gross organ or tissue damage in mice when given for 14 days. When given t-BuOH for 10 weeks, male rats showed a significant depression in body weight. Other effects included microscopic damage to livers and kidney. Alterations such as centrilobular necrosis, vacuolation in hepatocytes, and loss of hepatic architecture were noted.

After 2 to 3 months of being given t-BuOH orally, megamitochondria were seen in the rat hepatocytes. In addition, proliferation of smooth-surfaced endoplasmic reticulum and an increase in the number of lysosomes and microbodies were seen.

In a subchronic study (duration unspecified), t-BuOH given orally increased the mineralization of the kidney, nephropathy, and transitional cell epithelial hyperplasia in rats. The results for mice in the same study showed that t-BuOH affected livers in males, urinary bladders (chronic inflammation and hyperplasia of transitional cell epithelium) in both males and females; and thyroids (proliferative changes including hyperplasia and neoplasia) in both males and females.

Male rats were susceptible to  $\alpha$ 2 $\mu$ -globulin nephropathy when exposed to 0, 250, 450, and 1750 ppm t-BuOH for 6 h/day for 10 days. Further experiments using the kidneys of t-BuOH treated male rats confirmed that t-BuOH interacts with the  $\alpha$ 2 $\mu$ -globulin protein.

In a 95-day study, t-BuOH was found to be more toxic to males than to females when given in drinking water at doses of 0%, 0.25%, 0.5%, 1%, 2%, and 4% t-BuOH (w/v) to rats and mice. The principal histopathologic findings were in the urinary bladder of rats and mice and in the kidney of rats. For male rats and mice, the incidence and severity of the urinary bladder lesions were higher than those for females. A significant decrease in body weight occurred in male rats at all dose levels. Clinical signs for both sexes of rats included emaciation, ataxia, blood in the urine, and hypoactivity. In mice, clinical signs included emaciation, ataxia, abnormal posture, and hypoactivity starting at the 1% dose for males and the 2% dose for females. Gross lesions in rats were restricted to the urinary tract. In mice, gross lesions were thickened urinary bladder walls or plaques on the mucosa. Calculated no-effect levels for subchronic toxicity in

rodents are less than 0.25% in drinking water in male rats, 1% in female rats, 0.5% in male mice, and 1% in female mice. No-effect levels for the urinary tract lesions were calculated to be 1% in male rats and mice and 2% for female rats and mice.

In one dermal toxicity study, a dose of 0.5 ml of 99.9% t-BuOH was minimally irritating causing very slight erythema in rabbits. In another study, rabbits experienced erythema, edema, and desquamation ranging from very slight to moderate, and fissuring ranging from very slight to slight when given 2000 mg/kg of 99.9% t-BuOH. Some of the rabbits exhibited coriaceousness and atonia ranging from very slight to slight. Trace or mild acanthosis and hyperkeratosis and mild dermal fibroplasia was also seen. The MLD for t-BuOH was greater than 2.0 g/kg. Using 2000 mg/kg of 99.9% G-t-BuOH, compound-related microscopic changes consisted of hyperkeratosis, acanthosis, increased severity of chronic dermatitis, and hemorrhage. Based on the results, the acute dermal LD<sub>50</sub> for G-t-BuOH was greater than 2.0 g/kg.

In an inhalation study, red foci were found in the lungs of rats exposed to 9700 and 14100 ppm G-t-BuOH for 4 h. An exposure of 5000 ppm t-BuOH for 1 day produced narcosis in rats. When the concentration was lowered to 938 ± 93.4 ppm for 6 h, rats were still severely narcosed. Hypoactivity and ataxia were commonly seen in both rats and mice given 450 to 7000 ppm over an 18-day period.

t-BuOH (99.9%) was a moderate to severe ocular irritant to rabbits and caused mild to moderate dermal irritation to rabbits. It was not considered to be a primary dermal irritant to rabbits.

In an inhalation study where rats and mice were exposed to t-BuOH at concentrations of 135, 270, 540, 1080, and 2100 ppm, no significant differences were found in the weight of the testis, epididymis, and cauda; and sperm morphology motility, count, and morphology for males. For females, no significant differences were found in the estrous length or percentage of time spent in the various estrous stages. However, in a different study where t-BuOH was administered orally, estrous cycle length was significantly increased in female mice given 2110 mg/kg. There were no other significant results for males or females of rats and mice in the other dose levels.

t-BuOH (87 mM) did not affect the in vitro fertilization capacity of mouse spermatozoa. Fetotoxicity generally increased with concentration, and fetal weights were slightly depressed at concentrations of 0.5 to 1% t-BuOH. t-BuOH produced a significant increase in the number of resorptions per litter. There was also a significant decrease in the number of live fetuses per litter. t-BuOH reduced maternal weight gain, litter sizes, birth weights, and weights at weaning, and increased perinatal and postnatal mortality. In addition, the oral administration of t-BuOH to mice during pregnancy resulted in poorer initial behavioral performance of pups. The pups did eventually recover.

t-BuOH was not mutagenic in the *Salmonella*/mammalian-microsome mutagenicity test, did not increase the incidences of penicillin or streptomycin resistance in *Micrococcus aureus*, and did not induce adenine independence in adenine-dependent

*Neurospora crassa*. t-BuOH was mutagenic to cultured human–Chinese hamster ovary hybrid cells at a cytotoxic dose. t-BuOH was not a mutagen in L5178Y mouse lymphoma cells. t-BuOH (100 to 10000 µg/plate) did not induce mutations in *Salmonella typhimurium* strain TB98, TA100, TA1535, TA1537, or TA1538 with or without induced rat or hamster liver S-9. In one study, however, using concentrations ranging from 2.9 to 10,000 µg/plate, gasoline grade t-BuOH caused a weak but significant increase in TA1535 revertants per plate in both the presence and absence of induced rat liver S-9.

The principal effects from 2 years of exposure to t-BuOH in drinking water (up to 10 mg/ml for rats and 20 mg/ml for mice) were proliferative lesions (hyperplasia, adenoma, and carcinoma) in the kidneys of exposed male rats, and nephropathy in all exposed groups of female rats. There was “some evidence of carcinogenic activity” of t-BuOH in male F344 rats. There was “no evidence of carcinogenic activity” in female rats. There was “equivocal evidence of carcinogenic activity” of t-BuOH in the thyroid of male mice; there was “some evidence of carcinogenic activity” of t-BuOH in the thyroid of female mice.

An RIPT test showed no potential for eliciting either dermal irritation or sensitization by 100% t-BuOH. Dermatitis can result from dermal exposure of humans to t-BuOH.

The ACGIH has set a threshold limit value of 100 ppm that is satisfactory to prevent narcosis due to t-BuOH. NIOSH has reported that 8000 ppm t-BuOH is the concentration immediately dangerous to life or health.

## DISCUSSION

In its initial safety assessment, the CIR Expert Panel identified no acute toxicity concerns based on the available data. Overall, however, the available data were insufficient to support the safety of t-BuOH as used in cosmetics. The Panel identified the need for several studies, including 90-day oral toxicity, human sensitization, and UV absorption.

The NTP study provided the oral toxicity data needed by the Panel. Human clinical test data provided by industry demonstrate that t-BuOH (concentration not given) is not an irritant, nor was it a sensitizer. Based on its structure, the CIR Expert Panel does not expect t-BuOH to absorb ultraviolet light at wavelengths of 290 nm or longer.

In the NTP study there was some evidence of carcinogenicity in male rats and female mice. Specifically, NTP found a small increase in renal carcinomas in male rats, but not female rats, and a small increase in thyroid carcinomas in female mice, but not male mice. The CIR Expert Panel considered that this pattern of findings was not consistent between different sexes in different species, and was not likely indicative of a carcinogenic effect of t-BuOH. Perhaps more importantly, the Panel found an absence of a true dose response in the NTP study, further suggesting the absence of a carcinogenic effect. In addition, the Panel concluded that the renal tubule effects found in male rats was likely an effect of  $\alpha 2\mu$ -globulin. Overall, the Panel decided

that the studies on t-BuOH showed that it was a weak carcinogen (at most) and unlikely to have significant carcinogenic potential as currently used in cosmetic formulations.

In its consideration of the reproductive and developmental toxicity data, the Panel noted that maternal toxicity was evident at high doses, suggesting that effects of t-BuOH on development were likely secondary to maternal toxicity. The Panel attributed the effects on learning development to drinking t-BuOH in maternal milk and not to an in utero effect of the t-BuOH treatment.

## CONCLUSION

Based on the available animal and clinical data in this report, the CIR Expert Panel concludes that t-BuOH is safe as used in cosmetic products.

## REFERENCES

- Abbondandolo, A., S. Bonatti, C. Corsi, G. Corti, R. Fiorio, C. Leporini, A. Mazzaccaro, and R. Nieri. 1980. The use of organic solvents in mutagenicity testing. *Mutat. Res.* 79:141–150.
- Abel, E. L., and P. J. Bilitzke. 1992. Effects of prenatal exposure to methanol and t-butanol in Long Evans rats. *Am. J. Obstet. Gynecol.* 166:433.
- Achyara, S., K. Mehta, S. Rodriguez, S. Pereira, S. Krishnan, and C. V. Rao. 1995. Administration of subtoxic doses of t-butyl alcohol and trichloroacetic acid to male Wistar rats to study the interactive toxicity. *Toxicol. Lett. (Shannon)* 80:97–104.
- Achyara, S., K. Mehta, S. Rodriguez, S. Pereira, S. Krishnan, and C. V. Rao. 1997. A histopathological study of liver and kidney in male Wistar rats treated with subtoxic doses of t-butyl alcohol and trichloroacetic acid. *Exp. Toxic. Pathol.* 49:369–373.
- American Conference of Governmental Industrial Hygienists (ACGIH). 2000. *2000 TLVs and BEIs*. Cincinnati, OH:ACGIH.
- Ames, B. N., J. McCann, and M. Yamasaki. 1975. Methods for detecting carcinogens with the *Salmonella/mammalian-microsome mutagenicity test*. *Mutat. Res.* 31:347–364.
- Amoco Corporation. 1994. Subchronic test of t-butanol in B6C3F1 mice and Fischer 344 rats in drinking water with cover letter dated 03/23/94. NTIS Report No. OTS0556795. Springfield, VA: NTIS.
- Anderson, R. A., Jr., J. M. Reddy, C. Joyce, B. R. Willis, H. Van der Ven, and L. J. D. Zaneveld. 1982. Inhibition of mouse sperm capacitation by ethanol. *Biol. Reprod.* 27:833–840.
- Arco Chemical Company. 1992. Initial submission: Letter submitting preliminary results from subchronic toxicity studies of tertiary butyl alcohol in rats and mice dated 10/14/92 and attachments. NTIS Report No. OTS0538283. Springfield, VA: NTIS.
- Arco Chemical Company. 1994a. Toxicologist's report on metabolism and pharmacokinetics studies of radiolabeled TBA 534 tertiary butyl alcohol with cover letter dated 03/24/94. NTIS Report No. OTS0572366. Springfield, VA: NTIS.
- Arco Chemical Company. 1994b. Acute oral toxicity (LD50) study in rats with t-butyl alcohol with cover letter dated 03/24/94. NTIS Report No. OTS0572351. Springfield, VA: NTIS.
- Arco Chemical Company. 1994c. Acute oral toxicity (LD50) study in rats with arconol with cover letter dated 03/24/94. NTIS Report No. OTS0572358. Springfield, VA: NTIS.
- Arco Chemical Company. 1994d. Primary dermal irritation test in rabbits with t-butyl alcohol with cover letter dated 03/24/94. NTIS Report No. OTS0572354. Springfield, VA: NTIS.
- Arco Chemical Company. 1994e. Primary dermal irritation test in rabbits using arconol with cover letter dated 03/24/94. NTIS Report No. OTS0572361. Springfield, VA: NTIS.
- Arco Chemical Company. 1994f. Acute dermal toxicity (LD50) study in rabbits with t-butyl alcohol with cover letter dated 03/24/94. NTIS Report No. OTS0572352. Springfield, VA: NTIS.
- Arco Chemical Company. 1994g. Acute dermal toxicity (LD50) study in rabbits with arconol with cover letter dated 03/24/94. NTIS Report No. OTS0572359. Springfield, VA: NTIS.
- Arco Chemical Company. 1994h. LD<sub>50</sub> acute inhalation toxicity evaluation in rats using t-butyl alcohol with cover letter dated 03/24/94. NTIS Report No. OTS0572353. Springfield, VA: NTIS.
- Arco Chemical Company. 1994i. LC50 acute inhalation toxicity evaluation in rats with arconol with cover letter dated 03/24/94. NTIS Report No. OTS0572360. Springfield, VA: NTIS.
- Arco Chemical Company. 1994j. Eye irritation study in rabbits using t-butyl alcohol with cover letter dated 03/24/94. NTIS Report No. OTS0572355. Springfield, VA: NTIS.
- Arco Chemical Company. 1994k. Eye irritation study in rabbits using arconol with cover letter dated 03/24/94. NTIS Report No. OTS0572362. Springfield, VA: NTIS.
- Arco Chemical Company. 1994l. An in vitro evaluation of t-butyl alcohol—Arconol batch # A209411 to produce sister chromatid exchanges in Chinese hamster ovary cells with cover letter dated 03/24/94. NTIS Report No. OTS0572364. Springfield, VA: NTIS.
- Arco Chemical Company. 1994m. An in vitro evaluation of t-butyl alcohol 99.9% to produce sister chromatid exchanges in Chinese hamster ovary cells with cover letter dated 03/24/94. NTIS Report No. OTS0572357. Springfield, VA: NTIS.
- Arco Chemical Company. 1994n. Evaluation of test article t-butyl alcohol 99.9% (MRI #635) & arconol (MRI#636) for mutagenic potential employing the L5178Y tk +/- mutagenesis assay with cover letter dated 03/24/94. NTIS Report No. OTS0572365. Springfield, VA: NTIS.
- Arco Chemical Company. 1994o. Salmonella/mammalian-microsome preincubation mutagenicity assay with t-butyl alcohol with cover letter dated 03/24/94. NTIS Report No. OTS0572356. Springfield, VA: NTIS.
- Arco Chemical Company. 1994p. Salmonella/mammalian-microsome preincubation mutagenicity assay using arconol with cover letter dated 03/24/94. NTIS Report No. OTS0572363. Springfield, VA: NTIS.
- Baker, R. C., and R. E. Kramer. 1999. Cytotoxicity of short-chain alcohols. *Annu. Rev. Pharmacol. Toxicol.* 39:127–150.
- Baker, R. C., S. M. Sorensen, and R. A. Deitrich. 1982. The in vivo metabolism of tertiary butanol by adult rats. *Alcohol. Clin. Exp. Res.* 6:247–251.
- BASF Corporation. 1994. Letter from BASF Corp. to USEPA regarding submission of health and safety data subject to 8D reporting with attachments, dated 05/05/94 (Sanitized). NTIS Report No. OTS0572606. Springfield, VA: NTIS.
- Beauge, F., M. Clement, J. Nordmann, and R. Nordmann. 1981. Liver lipid disposal following t-butanol administration to rats. *Chem. Biol. Interact.* 38:45–51.
- Bleyman, M. A., and R. G. Thurman. 1980. The swift increase in alcohol metabolism: Comparative studies with other alcohols. *Curr. Alcohol.* 7:115–121.
- Borghoff, S. J., J. S. Prescott, D. B. Janszen, B. A. Wong, and J. I. Everitt. 2001.  $\alpha$ 2 $\mu$ -globulin nephropathy, renal cell proliferation, and dosimetry of inhaled tert-butyl alcohol in male and female F-344 rats. *Toxicol. Sci.* 61:176–186.
- Candura, S. M., W. Balduini, and L. G. Costa. 1991. Interaction of short chain aliphatic alcohols with muscarinic receptor-stimulated phosphoinositide metabolism in cerebral cortex from neonatal and adult rats. *Neurotoxicology* 12:23–32.
- Cederbaum, A. I., and G. Cohen. 1980. Oxidative demethylation of t-butyl alcohol by rat liver microsomes. *Biochem. Biophys. Res. Commun.* 97:730–736.
- Cederbaum, A. I., A. Qureshi, and G. Cohen. 1983. Production of formaldehyde and acetone by hydroxyl radical generating systems during the metabolism of tertiary butyl alcohol. *Biochem. Pharmacol.* 32:3517–3524.

- Cirvello, J. D., A. Radovsky, J. E. Heath, D. R. Farnell, and C. Lindamood III. 1995. Toxicity and carcinogenicity of t-butyl alcohol in rats and mice following chronic exposure in drinking water. *Toxicol. Ind. Health* 11:151–164.
- Clark, J. 1953. The mutagenic action of various chemicals on *Micrococcus aureus*. *Proc. Okla. Acad. Sci.* 34:114–118.
- Clinical Research Laboratories, Inc (CRL). 1998. Final Report: Repeated Insult Patch Test of 60% ethyl alcohol with 0.125% of t-butyl alcohol. Unpublished data submitted by CRL (June 25, 1998).<sup>2</sup>
- Cosmetic Ingredient Review (CIR). 1989. Final report on the safety assessment of t-butyl alcohol. *Journal of the American College of Toxicology* 8:627–641.
- Cosmetic, Toiletry and Fragrance Association (CTFA). August 30, 1985. Submission of unpublished data by CTFA. Cosmetic ingredient chemical description for t-Butyl Alcohol.<sup>2</sup>
- CTFA. 1999. Maximum concentration of use—t-butyl alcohol. Unpublished data received from CTFA.<sup>2</sup>
- Damrau, F., and A. H. Goldberg. 1971. Adsorption of whiskey congeners by activated charcoal: Chemical and clinical studies related to hangover. *Southwest Med.* 5:179–182.
- Daniel, M. A., and M. A. Evans. 1982. Quantitative comparison of maternal ethanol and maternal tertiary butanol diet on postnatal development. *J. Pharmacol. Exp. Ther.* 222:294–300.
- Derache, R. 1970. Toxicology, pharmacology and metabolism of higher alcohols. In *Alcohols and derivatives. Vol. II, International encyclopedia of pharmacology and therapeutics, Sec. 20*, ed. J. Tremolieres, 507–522. London: Pergamon Press.
- Dickey, F. H., G. H. Cleland, and C. Lotz. 1949. Role of organic peroxides in the induction of mutations. *Proc. Natl. Acad. Sci. U. S. A.* 35:581–586.
- Dow Chemical Company. 1994. Report on animal toxicity experiments with ethyl alcohol, tertiary butyl alcohol, and Stoddard's solvent with cover letter dated 03/28/94 (sanitized). NTIS Report No. OTS0572373.
- Edwards, E. K., Jr., and E. K. Edwards. 1982. Allergic reaction to tertiary butyl alcohol in a sunscreen. *Cutis* 29:476–478.
- Eiceman, G. A., and F. W. Karasek. 1981. Identification of residual organic compounds in food packages. *J. Chromatogr.* 210:93–104.
- Elf Atochem North American Incorporated. 1994a. Repeated dose test of t-butanol (C55367) in B6C3F1 mice and Fischer 344 rats with cover letter dated 031594. NTIS Report No. OTS0556768.
- Elf Atochem North American Incorporated. 1994b. Subchronic test of t-butanol (C55367) in B6C3F1 mice and Fischer 344 rats in drinking water with cover letter dated 031594. NTIS Report No. OTS0556767.
- Faulkner, T. P., J. D. Wiechart, D. M. Harman, and A. S. Hussain. 1989. The effects of prenatal tertiary butanol administration in CBA/J and C57BL/6J mice. *Life Sci.* 45:1989–1995.
- Food and Drug Administration (FDA). 1986. Cosmetic product formulation and frequency of use data. *FDA database*. Washington, DC: FDA.
- FDA. 1998. Frequency of use of cosmetic ingredients. *FDA database*. Washington, DC: FDA.
- Fregert, S., O. Groth, N. Hjorth, G. Magnusson, H. Rorsman, and P. Ovrup. 1969. Alcohol dermatitis. *Acta. Derm. Venereol.* 49:493–497.
- Fregert, S., R. Hokanson, H. Rorsman, N. Tryding, and P. Ovrup. 1963. Dermatitis from alcohols. *J. Allergy* 34:404–408.
- Gaillard, D., and R. Derache. 1965. Effect of acute intoxication, by various alcohols, on hepatic lipid fractions in female rats. *C. R. Hebd. Seances Acad. Sci.* 261:3880–3883.
- Gaillard, D., and R. Derache. 1966. Effect of some aliphatic alcohols on the mobilization of various lipid fractions in the rat. *Food Cosmet. Toxicol.* 4:515–520.
- Gordon, D. S., and E. J. Calabrese. 1992. The *in vitro* effect of acetaldehyde and tert-butanol on 1-naphthol-induced oxidant stress in human and sheep erythrocytes. *J. Environ. Sci. Health A* 27:301–316.
- Grant, K. A., and H. H. Samson. 1982. Ethanol and tertiary butanol induced microcephaly in the neonatal rat: comparison of brain growth parameters. *Neurobehav. Toxicol. Teratol.* 4:315–321.
- Green, B. D., and J. I. Steinfeld. 1977. Monitoring complex trace-gas mixtures by long-path laser adsorption spectrometry. *Proc. Soc. Photo-Opt. Instrum. Eng.* 99:32–38.
- Greenberg, L. A., and D. Lester. 1954. *Handbook of Cosmetic Materials*. New York: Interscience Publishers.
- Groth, G., and K. J. Freundt. 1994. Inhaled tert-butyl acetate and its metabolite tert-butyl alcohol accumulate in the blood during exposure. *Hum. Exp. Toxicol.* 13:478–480.
- Harris, R. A., and F. Schroeder. 1981. Effects of ethanol and related drugs on the physical and functional properties of brain membranes. *Curr. Alcohol.* 8:461–68.
- Hawley, G. G., ed. 1971. *The condensed chemical dictionary*, 8th ed. New York: Van Nostrand Reinhold.
- Hiller, J. M., L. M. Angel, and E. J. Simon. 1984. Characterization of the selective inhibition of the delta subclass of opioid binding sites by alcohols. *Mol. Pharmacol.* 25:249–55.
- Hoshino, H., G. Chihara, and F. Fukuoka. 1970. Detection of potential weak carcinogens and procarcinogens. II. Carcinogenicity of tertiary butyl hydroperoxide. *Gann* 61:121–124.
- Hunt, W. A., E. Majchrowicz, and T. K. Dalton. 1979. Alterations in high-affinity choline uptake in brain after acute and chronic ethanol treatment. *J. Pharmacol. Exp. Ther.* 210:259–263.
- Jacobs, G., M. Martens, and G. Mosselmans. 1987. Proposal of limit concentrations for skin irritation with the context of new EEC directive on the classification and labeling of preparations. *Regul. Toxicol. Pharmacol.* 7:370–378.
- Kamil, I. A., J. N. Smith, and R. T. Williams. 1953. Studies in detoxification. 46. The metabolism of aliphatic alcohols. The glucuronic conjugation of acyclic aliphatic alcohols. *Biochem. J.* 53:129–136.
- Kirk-Othmer 1992a. *Encyclopedia of chemical technology*, 4th ed., Butyl Alcohols entry. Volume 4. New York: John Wiley and Sons.
- Kirk-Othmer 1992b. *Encyclopedia of chemical technology*, 4th ed., Butylenes entry, Volume 4. New York: John Wiley and Sons.
- Kool, H. J., C. F. Van Kreijl, and B. C. J. Zoeteman. 1982. Toxicology assessment of organic compounds in drinking water. *Crit. Rev. Environ. Control.* 12:307–57.
- Kulyk, W. M., and L. M. Hoffman. 1996. Ethanol exposure stimulates cartilage differentiation by embryonic limb mesenchyme cells. *Exp. Cell Res.* 223:290–300.
- LaFleur, M. V., and H. Loman. 1982. Influence of anoxic sensitizers on the radiation damage in biologically active DNA in aqueous solution. *Int. J. Radiat. Biol.* 41:295–302.
- Lennox, J. L., and C. A. Waldren. 1981. Measurement of the mutagenic action of alcohols in mammalian cells. *Clin. Res.* 29:36A.
- Lewis, R. J., ed. 2000. *Dangerous Properties of Industrial Materials*, 10th ed., vol. 2, 611–612. New York: John Wiley and Sons.
- Lindamood, C. III; D. R. Farnell, H. D. Giles, J. D. Prejean, J. J. Collins, K. Takahashi, and R. R. Maronpot. 1992. Subchronic toxicity studies of t-butyl alcohol in rats and mice. *Fundam. Appl. Toxicol.* 19:91–100.
- Lyon, R. C., J. A. McComb, J. Schreurs, and D. B. Goldstein. 1981. A relationship between alcohol intoxication and the disordering of brain membranes by a series of short-chain alcohols. *J. Pharmacol. Exp. Ther.* 218:669–675.
- Mackison, F. W., R. S. Stricoff, and L. J. Partridge, Jr. 1978. NIOSH/OSHA Pocket Guide to Chemical Hazards. National Institute for Occupational Safety and Health [NIOSH] and Occupational Safety and Health Administration [OSHA]. DHEW (NIOSH) Publication No. 78-210.
- McComb, J. A., and D. B. Goldstein. 1979. Quantitative comparison of physical dependence on tertiary butanol and ethanol in mice: Correlation with lipid solubility. *J. Pharmacol. Exp. Ther.* 208:113–117.
- McGregor, D. B., A. Brown, P. Cattanch, I. Edwards, D. McBride, and W. J. Caspary. 1988. Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay II: 18 Coded chemicals. *Environ. Mol. Mutagen.* 11:91–118.

<sup>2</sup>Available for review: Director, Cosmetic Ingredient Review, 1101 17th street, NW, suite 310, Washington, DC 2003b, USA.

- Muhtadi, F. J., M. M. A. Hassan, and M. M. Tawakkol. 1982. PMR assay of betalactam antibiotics. I. Assay of cephalosporins. *Spectrosc. Lett.* 15:373–381.
- Munch, J. C. 1972. Aliphatic alcohols and alkyl esters: Narcotic and lethal potencies to tadpoles and to rabbits. *Ind. Med. Surg.* 41:31–33.
- Munch, J. C., and E. W. Schwarze. 1925. Narcotic and toxic potency of aliphatic alcohols upon rabbits. *J. Lab. Clin. Med.* 10:985–996.
- National Toxicology Program (NTP). January, 1982. NTP Technical Bulletin. Issue No. 6. Research Triangle Park, NC.
- NTP. May 1995. NTP Technical Report Series: Toxicology and carcinogenesis studies of t-butyl alcohol (CAS no. 75-65-0) in F344/N rats and B6C3F<sub>1</sub> mice (Drinking water studies). Research Triangle Park, NC.
- NTP. July 1997. NTP Technical Report Series no. 53: NTP technical report on toxicity studies of t-butyl alcohol (CAS no. 75-65-0) administered by inhalation to F344/N rats and B6C3F<sub>1</sub> mice. Research Triangle Park, NC.
- Nelson, B. K., W. S. Brightwell, A. Khan, J. R. Burg, and P. T. Goad. 1989. Lack of selective developmental toxicity of three butanol isomers administered by inhalation to rats. *Fundam. Appl. Toxicol.* 12:469–479.
- Nihlén, A., A. Löf, and G. Johanson. 1995. Liquid/air partition coefficients of methyl and ethyl t-butyl ethers, t-amyl methyl ether, and t-butyl alcohol. *J. Expos. Anal. Environ. Epidemiol.* 5:573–582.
- Poet, T. S., J. L. Valentine, and S. J. Borghoff. 1997. Pharmacokinetics of tertiary butyl alcohol in male and female Fischer 344 rats. *Toxicol. Lett.* 92:179–186.
- Renkonen, K. O., and H. Teir. 1957. Studies on the local reactions of the skin to chemical compounds. *Ann. Med. Exp. Biol. Fenn.* 35:67.
- Reuvers, A. P., C. L. Greenstock, J. Borsa, and J. D. Chapman. 1973. Mechanism of chemical radioprotection by dimethyl sulfoxide. *Int. J. Radiat. Biol.* 24:533–536.
- Rhone-Poulenc Inc. 1992. Primary dermal irritation in rabbits and primary ocular irritation in rabbits with hair spray formula 8758–47-2 with cover letter dated 102692. NTIS Report No. OTS0571816.
- Roots, R., and S. Okada. 1972. Protection of DNA molecules of cultured mammalian cells from radiation-induced single-strand scissions by various alcohols and sulfhydryl compounds. *Int. J. Radiat. Biol.* 21:329–42.
- Schaffarzick, R. W., and B. J. Brown. 1952. Anticonvulsant activity and toxicity of methylparafynol (dormison) and some other alcohols. *Science* 116:663.
- Takahashi, K., C. Lindamood III, and R. R. Maronpot. 1993. Retrospective study of possible  $\alpha$ -2 $\mu$ -globulin nephropathy and associated cell proliferation in male Fischer 344 rats dosed with t-butyl alcohol. *Environ. Health Perspect.* 101:281–286.
- Thomas, M., A. L. A. Boura, and R. Vijayakumar. 1980. Prostaglandin release by aliphatic alcohols from the rat isolated lung. *Clin. Exp. Pharmacol. Physiol.* 7:373–381.
- Thore, A., and H. Baltscheffsky. 1965. Inhibitory effects of lower aliphatic alcohols on electron transport phosphorylation systems. II. Secondary, tertiary, and di-alcohols. *Acta Chem. Scand.* 19:1600–1606.
- Thurman, R. G., K. Winn, and B. Urquhart. 1980. Rat brain cyclic AMP levels and withdrawal behavior following treatment with tert-butanol. *Adv. Exp. Med. Biol.* 126:271–281.
- United States Pharmacopeia (USP) Committee of Revision. 1979. *The United States Pharmacopeia*, 20th revision. Easton, PA: Mack Printing Company.
- Videla, L. A., V. Fernandez, A. De Marinis, N. Fernandez, and A. Valenzuela. 1982. Liver lipoperoxidative pressure and glutathione status following acetaldehyde and aliphatic alcohols pretreatments in the rat. *Biochem. Biophys. Res. Commun.* 104:965–970.
- Von Oettingen, W. F. 1943. The aliphatic alcohols, their toxicity and potential dangers in relation to their chemical constitution and their fate in metabolism. U.S. Public Health Service Public Health Bulletin No. 281. Washington, DC: US Government Printing Office.
- Wakabayashi, T., K. Adachi, and J. Popinigis. 1991. Effects of alkyl alcohols and related chemicals on rat liver structure and function I. Induction of two distinct types of megamitochondria. *Acta Pathol. Jpn.* 41:405–413.
- Waldren, C. 1982. Detection of chromosome deletions and nondisjunction produced by environmental agents in cultured somatic mammalian cells. *Mutat. Res.* 97:234.
- Weast, R. C., ed. 1982. CRC handbook of chemistry and physics, 5th ed. Boca Raton, FL: CRC Press.
- Wenninger, J. A., R. C. Canterbury, and G. N. McEwen, Jr. eds. 2000. *International cosmetic ingredient dictionary and handbook*, 8th ed; Vol. 1–3. Washington, DC: CTFA.
- Williams, T. M., and S. J. Borghoff. 2001. Characterization of tert-butyl alcohol binding to  $\alpha$ -2 $\mu$ -globulin in F-344 rats. *Toxicol. Sci.* 62:228–235.
- Wimer, W. W., J. A. Russell, and H. L. Kaplan. 1983. *Alcohols toxicology*. Park Ridge, NJ: Noyes Data Corporation.
- Windholz, M. (Editor). 1983. *The Merck index*, 10th ed. Rahway, NJ: Merck and Co.
- Yamaguchi, T. 1980. Activation with catalase of mutagenicity of hydroperoxides of some fatty acids and hydrocarbons. *Agric. Biol. Chem.* 44:1989–1991.
- Zamarakhina, L. E. 1973. Determination of tertiary butyl alcohol in the air of industrial rooms. *Gig. Sanit.* 38:72–73.
- Zeiger, E., B. Anderson, S. Haworth, T. Lawlor, K. Mortelmans, and W. Speck. 1987. *Salmonella* mutagenicity tests III. Results from the testing of 255 chemicals. *Environ. Mutagen.* 9:1–110.