Amended Safety Assessment of *t*-Butyl Alcohol as Used in Cosmetics

Status:	Re-Review for Panel Consideration
Release Date:	August 18, 2023
Panel Meeting Date:	September 11-12, 2023

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This safety assessment was prepared by Preethi Raj, M.Sc., Senior Scientific Analyst/Writer, CIR.

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Preethi S. Raj, M.Sc.,
Senior Scientific Analyst/Writer, CIR
Date: August 18, 2023
Subject: Re-Review of the Safety Assessment of *t*-Butyl Alcohol

The Expert Panel for Cosmetic Ingredient Safety (Panel) first published a Final Report on the Safety Assessment of *t*-Butyl Alcohol in 1989, in which the Panel concluded the data were insufficient to support the safety of *t*-Butyl Alcohol in cosmetics (identified in the pdf as *originalreport_t-ButylAlcohol_092023*). Subsequently, data were received that addressed the insufficiencies, and the Panel published an Amended Final Report of the Safety Assessment of *t*-Butyl Alcohol as Used in Cosmetics in 2005 (*2005amendedreport_t-ButylAlcohol_092023*). On the basis of available animal and clinical data in that report, the Panel concluded that this ingredient is safe as used in cosmetic products.

Because it has been at least 15 years since the previous amended safety assessment was published, in accordance with Cosmetic Ingredient Review (CIR) Procedures, the Panel should consider whether the safety assessment of *t*-Butyl Alcohol should be re-opened. In July 2023, an extensive search of the world's literature was performed for studies dated 2000 forward. An historical overview, comparison of original and new use data, the search strategy used, and a synopsis of notable new data are enclosed herein (*newdata_t-Butyl Alcohol_092023*).

Notable new information includes data on dermal absorption, toxicokinetics (in vivo and modeled), oral developmental and reproductive toxicity, inhalation developmental toxicity, in vitro genotoxicity, a guinea pig maximization test, species-specific effects on renal tumor induction, and systemic/margin of exposure calculations. Notably, re-evaluation of the 13-wk NTP carcinogenicity/toxicity studies presented in the previous review were found; the authors concluded that the tumorigenesis process of *t*-Butyl Alcohol in kidneys is species(rat)-specific, and therefore not relevant to human risk.

Also included for your review are current and historical use data (*usetable_t-Butyl Alcohol_092023*). According to 2023 FDA VCRP data, *t*-Butyl Alcohol use has increased to 136 formulations, from 32 reported in 1998. One newly reported use is in a baby product. Reported concentrations of use have increased; for example, the maximum reported use concentration for *t*-Butyl Alcohol in products which come in contact with mucous membranes increased from 0.0001% in to 0.007% in lipsticks. In 2022, the maximum reported concentration of use for *t*-Butyl Alcohol was 0.91% in aftershave lotions, while in 1999, *t*-Butyl Alcohol was reported to be used at a maximum concentration of 0.5% in hair spray aerosol fixatives.

If upon review of the updated data the Panel determines that a re-review is warranted, a Draft Amended Report will be presented at an upcoming meeting.

Re-Review - t-Butyl Alcohol - History and New Data

(Preethi Raj – September 2023 meeting)

Ingredients (1)	Citation	Conclusion	Use - New Data	Results	Use - Existing Data	Results	Notes
***t*-Butyl Alcohol** *Changes to Original List* none	JACT 8:627-41, 1989 IJT 24(S2):1-20, 2005	data insufficient safe as used	frequency of use (2023) conc of use (2022)	136 uses ≤ 0.91%	frequency of use (1998) conc of use (1999)	32 uses ≤ 0.5 %	frequency of use has increased new reported use in baby products (1) concentration of use increased max reported use concentration for *t*-Butyl Alcohol in lipstick increased from 0.0001 to 0.007%

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Chemistry/Natural Occurrence			
VCF (Volatile Compounds in Food): Database/Nijssen, LM; Ingen-Visscher, CA van; Donders, JJH (eds) – Version 15.1 – Zeist (The Netherlands): TNO Triskelion, 1963 – 2014 (as cited in the RIFM 2023 safety assessment)	Natural occurrence	*t*-Butyl Alcohol is reported to naturally occur in the following foods: fresh apple, beef, cheese, chicken, coffee, grape (*Vitis* species), guava and feyoa, *Mangifera* species, walnut (*Juglans* species), and wine.	Yes, natural occurrence is not mentioned in original report
ADME/Toxicokinetics Studies			
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/14112/1/1	ADME- dermal absorption	Male Sprague-Dawley rats (4/group) received a single application of [^{14}C]*t*-Butyl Alcohol to clipped skin (approximate dose: 2 MBq/kg, 7.5 mg/cm^2), which was applied within a silicone rubber saddle to an area of ~ 12 cm for 6 h.[2] Carbon filters were placed into the saddle and covered with stainless steel gauze during exposure. One group of rats was killed after the 6 h exposure, another group was killed after 24 h, and another group was killed after 72 h. Very little dose was absorbed after 6, 24, or 72 h. Less than 1.5% of the applied dose was detected in the excreta and tissues (1%) at all time points. The majority of the dose (group means 84 - 89%) was retained in the carbon filters above the dose site (sampled at 1 h), with an additional 1.1 - 1.2% retained in the filters between 1 and 6 h. The concentration of radioactivity in the blood decreased from 3.25 µg eq/g at 6 h to 0.76 µg eq/g at 72 h. The test material showed a low potential for dermal absorption and bioaccumulation.	Yes; dermal absorption is not in the original report
Bernauer U, et al. Biotransformation of unlabeled and [2-^{13}C] methyl *t*-butyl ether, ethyl *t*-butyl ether, and *t*-Butyl Alcohol in rats: identification of metabolites in urine by [^{13}C] nuclear magnetic resonance and gas chromatography/mass spectrometry. Chem Res Toxicol 1998; 11: 651 - 658	ADME – metabolism, oral	Male Fischer 344 rats (3/group) received a single 250 mg/kg bw dose of either unlabeled *t*-Butyl Alcohol or [^{13}C]*t*-Butyl Alcohol, in corn oil, via gavage. Urine samples were collected in 24-h intervals for 48 h. In [^{13}C]*t*-Butyl Alcohol-dosed rats, [^{13}C]acetone, *t*-Butyl Alcohol, and its glucuronide represented minor metabolites; as with the ethers, 2-methyl-1,2-propanediol, 2-hydroxyisobutyrate, and the presumed *t*-butyl alcohol sulfate were the major metabolites present. Based on these results, *t*-butyl alcohol glucuronide, and *t*-butyl alcohol sulfate, 2-hydroxyisobutyrate and 2-methyl-1,2-propanediol as the major urinary metabolites of *t*-Butyl Alcohol.	no
Bernauer U, et al. Biotransformation of [^{12}C]-and [2-^{13}C]-labeled methyl tert-butyl ether, ethyl tert-butyl ether, and *t*-Butyl Alcohol in rats: identification of metabolites in urine by [^{13}C] nuclear magnetic resonance and gas chromatography/mass spectrometry. Chem Res Toxicol 1998; 11: 651 - 658	ADME – metabolism, oral	One human subject was given 5 mg/kg [^{13}C]*t*-Butyl Alcohol orally as a gel capsule; 2-methyl-1,2-propanediol and 2-hydroxyisobutyrate were the major metabolites in urine detected by ^{13}C NMR analysis. Unconjugated *t*-Butyl Alcohol and *t*-butyl alcohol glucuronide were present as minor metabolites, and traces of *t*-butyl alcohol sulfate were also present.	no

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Leavens TL and Borghoff SJ. Physiologically based pharmacokinetic model of methyl *t*-butyl ether and *t*-Butyl Alcohol dosimetry in male rats based on binding to α2u-globulin. Toxicol Sci 2009; 109(2): 321 - 335	ADME – inhalation, whole body	Male and female Fischer 344 rats (4/group/sex); 250, 450, or 1750 ppm *t*-Butyl Alcohol; 6 h/d, whole-body inhalation exposure, for 1 or 8 d. No controls were used. Rats were killed 2, 4, 6, and 8 h after exposure, for both durations of exposure. Blood, liver, and kidneys were collected for analyses. Concentrations of *t*-Butyl Alcohol were similar in the blood, liver and kidneys of both sexes following a single 6-h exposure but concentrations differed between genders following repeated exposure, possibly corroborating the PBPK model of *t*-Butyl Alcohol binding to α2u-globulin (a protein found in the kidney) in male rats. For both sexes, blood and tissue concentrations of *t*-Butyl Alcohol were lower following repeated exposures.	no
Developmental and Reproductive Toxicity			
Bilitti JE, Faulkner, BC, and Wilson, BW. Absence of acute testicular toxicity of methyl-*t*-butyl-ether and breakdown products in mice. Bull Environ Contam Toxicol 2005; 75: 228 - 235	Reproductive toxicity, oral	After an initial determination of fecal testosterone levels, 4 groups of male CD-1 mice received a single dose of 0, 400, 1000, or 2000 mg/kg *t*-Butyl Alcohol, in canola oil, via gavage and were challenged with hCG (to stimulate testosterone production) the same day and 3 d later. Blood and fecal samples were taken to measure testosterone levels, and histological examination of the testes was performed upon necropsy. Three mice were subcutaneously dosed with cadmium chloride as positive controls. Two animals died in the 400 mg/kg group, due to complications from gavage (non-treatment related). There was no difference in the % change of fecal testosterone or in the serum testosterone of animals treated with *t*-Butyl Alcohol compared to controls. Testis weights of the 1000 and 2000 mg/kg-treated mice averaged 14% higher than the control and 400 mg/kg groups ($p \leq 0.05$) The only significant histological difference in the testes was a higher percentage of tubules from control animals with sloughing ($7 \pm 2\%$, mean SD, $p \leq 0.05$).	Yes, no testicular toxicity data in original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/14112/1/1	Developmental toxicity, oral	OECD TG 421. Male and female albino, Sprague-Dawley rats; F_0: 12 animals/sex/group; F_1: 10 animals/sex/ group. Animals received 64, 160, 400, or 1000 mg/kg bw/d *t*-Butyl Alcohol, in water, via gavage. F_0 males were dosed for 4 wk prior to mating and F_0 females were dosed 4 wk prior to mating through lactation day 21; both F_0 sexes were killed on day 21. F_1 pups received treatment from postnatal day 21 to 27 and were killed on day 28. Maternal effects: no incidences of mortality during the study. Mild central nervous toxicity (characterized by unresponsiveness/lethargy and some ataxia; some animals also exhibited increased vocalization and rapid breathing) appeared 1 – 2 h after dosing in the 1000 mg/kg bw/d group and between the 2nd and 4th wk of dosing in the 400 mg/kg bw/d group; no other significant effects were observed. Embryogenic/fetal effects: There was a significant reduction in the number of live born pups/pregnancy at 1000 mg/kg bw/d and an increase in the number of stillborn pups. The mean litter size for the high-dose group was only 10/litter on postnatal day 1 as compared with 14 or 15 in other groups. Subsequently, there was significantly reduced pup survival at the high dose with only 80% survival to postnatal day 4, and 50% survival to postnatal day 21 as compared with close to 100% in the other groups. Offspring born to dams treated with *t*-Butyl Alcohol at 1000 mg/kg bw/day exhibited lower mean body weight than the control offspring; no effects were observed at lower doses. The NOAEL for developmental/ reproductive effects was determined to be 400 mg/kg bw/d and the NOAEL for overall toxicity was determined to be 160 mg/kg bw/d.	no

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/14112/1/1	Developmental, toxicity, inhalation (paternal exposure)	Groups of male Sprague-Dawley rats (number not specified); whole-body exposure; 6000 or 12,000 mg/m^3 vaporized *t*-Butyl Alcohol (both concentrations administered at different times; approximately 3 mo apart); daily exposure for 6 wk (7h/d, 7d/wk). Controls were used (concurrent vehicle not specified). Due to different timing, comparison between dose groups was not considered appropriate. Exposed males were weighed over the 6 wk study period and mated with non-exposed females. Upon delivery of pups, the offspring were culled to 4 males and 4 females per litter and were fostered to untreated controls, Offspring were weighed each week through 5 wk of age and were observed for behavioral/neurotransmitter effects over 60 d. On postnatal day 10, 1 male and 1 female/litter were randomly assigned to 1 of 4 groups for behavioral testing (including ascent on wire mesh screen, activity in an open field, running wheel activity, avoidance conditioning, and operant conditioning). Additionally, brains from 10 offspring/group were collected on postnatal day 21 for protein and neurotransmitter level analysis in 4 general brain regions (cerebrum, cerebellum, brainstem, and midbrain). Forty pair-wise comparisons were analyzed (20 different postnatal days, using both concentrations, and separate controls). Body weight gain was non-treatment related. In the offspring, 3 pair-wise comparisons in the behavioral tests were statistically significant due to exposure to *t*-Butyl Alcohol: pups sired by 12,000 mg/m^3-exposed males had 20 rpm on the rotorod vs 16 rpm from the concurrent control group, and latency to reach the outer circle on the open field test was 115 sec vs. 210 sec for control-sired pups. In the pups sired by 6000 mg/m^3-exposed males, ascent on the mesh screen was lower than the control group (values not provided); no further pair-wise behavioral differences were observed between treated groups and controls. For the neurotransmitter measurements, 5 pair-wise comparisons were statistically significant. In pups sired by 12,000 mg/m^3-exposed males, the mean concentrations of norepinephrine and β-endophin were reduced in the cerebellum, and the mean concentration of met-enkephalin were reduced in the cerebrum, compared to control pups. In pups sired by 6000 mg/m^3-exposed pups, serotonin in the midbrain and met-enkephalin in the cerebrum were reduced, compared to controls. The lack of a pattern of effects or a dose-response relationship caused the study authors to conclude the few effects observed were unrelated and were likely of little to no biological significance. The following values were determined: LOAEC (paternal body weight and weight gain): ≥ 12,000 mg/m^3 *t*-Butyl Alcohol NOEC (paternal body weight and weight gain): 6000 mg/m^3 *t*-Butyl Alcohol NOAEC (for male and female pups): ≥ 12, 000 mg/m^3 *t*-Butyl Alcohol	Yes, developmental neurotoxicity not in original report

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/14112/1/1	Developmental toxicity, inhalation (maternal exposure)	Pregnant Sprague-Dawley rats (number/group not specified)whole-body exposure; 6000 or 12,000 mg/m^3 vaporized *t*-Butyl Alcohol (both concentrations administered at different times-approximately 3 mo apart) 7 h/d from day 1 to day 20 of gestation. Controls were used (concurrent vehicle not specified). Due to different timing, comparison between dose groups was not considered appropriate. Body weights, feed, and water consumption were collected on gestation days 0, 7, 14, and 21. Upon birth, pups were culled to 4 males and 4 females per litter and were fostered to untreated controls, so that pups received exposure to *t*-Butyl Alcohol only during gestation, Offspring were weighed each week through 5 wk of age and were observed for behavioral/ neurotransmitter effects over 60 d. On postnatal day 10, 1 male and 1 female/litter were randomly assigned to 1 of 4 groups for behavioral testing (including ascent on wire mesh screen, activity in an open field, running wheel activity, avoidance conditioning, and operant conditioning). Additionally, brains from 10 offspring/group were collected on postnatal day 21 for protein and neurotransmitter level analysis (acetylcholine, dopamine, norepinephrine, serotonin, 5-hydroxytryptamine, metenkephalin, β-endorphin, substance P) in 4 general brain regions (cerebrum, cerebellum, brainstem, and midbrain). Forty pair-wise comparisons were analyzed (20 different postnatal days, using both concentrations, and separate controls). In the 12,000 mg/m^3 group, a decrease in body weight during the 1st wk (35%; 8% decrease in mean body weight on gestation day 21) was accompanied by a 39% decrease in feed consumption during the same interval, compared to controls. Water intake was increased by 50% in the 12,000 mg/m^3 group during the 3rd wk of gestation, compared to controls. No changes in body weight or feed or water consumption were seen in the 6000 mg/m^3 group, In the offspring, 2 pair-wise comparisons were statistically significant in the behavioral tests (rotorod in the 12,000 mg/m^3 group and the ascent on the mesh screen in the 6000 mg/m^3 group). For the neurotransmitter measurements, 5 pair-wise comparisons were statistically significant for pups delivered by exposed dams. For pups in the 12,000 mg/m^3 maternally-exposed group, the mean concentration of norepinephrine and β-endorphin were reduced in the cerebellum and the mean concentration of met-enkephalin was reduced in the cerebrum, compared to controls. For pups in the 6000 mg/m^3-maternally exposed group, serotonin was reduced in the midbrain and met-enkephalin was reduced in the cerebrum, compared to controls. Prenatal exposure of up to 12,000 mg/m^3 of *t*-Butyl Alcohol with exposures ending prior to birth did not result in any evidence of a dose-response relationship or discernible pattern of effects of neurotoxicity in the offspring when examined up to 60 days postnatally. Furthermore, the relative severe toxicity observed induced in maternal animals at the 12,000 mg/m^3 exposure concentration suggested that *t*-Butyl Alcohol does not cause developmental neurotoxicity even at maternally toxic exposure concentrations. The NOAEC for male and female pups was determined to be ≥ 12,000 mg/m^3 *t*-Butyl Alcohol	Yes, developmental neurotoxicity not in original report
Genotoxicity Studies			
McGregor DB, et al. The mutagenicity testing of *t*-Butyl Alcohol, *t*-butyl acetate, and methyl *t*-butyl ether in *Salmonella typhimurium*. Mut Res 2005; 565; 181 - 189	Genotoxicity, in vitro	OECD TG 471; Ames test. *Salmonella typhimurium* TA102 was tested with up to 5000 µg/plate *t*-Butyl Alcohol, in DMSO or water, with or without metabolic activation. 2-Aminoanthracene and 1,8-diydroxyanthraquinone were used as positive controls in the presence of metabolic activation, and cumene hydroperoxide and mitomycin C were used as positive controls in the absence of metabolic activation. No statistical or dose-related increase in the number of mutant colonies was observed; the test article was considered non-genotoxic.	no

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/14112/1/1	Genotoxicity, in vitro	OECD TG 471; Ames test. *S. typhimurium* TA102 was tested with 0, 0.75, 1.5, 2.25, 3, and 3.75 mg/plate *t*-Butyl Alcohol, in water, with metabolic activation. Over the dose range tested, the number of revertants/plate reached a maximum of approximately 800 at 2.25 mg/plate compared to 400 for the control. At higher concentrations, the number of revertants/plate decreased in a dose-dependent manner.	no
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/14112/1/1	Genotoxicity, in vitro	OECD TG 479. Sister chromatid exchange assay. Chinese hamster ovary cell lines were treated with up to 5000 µg/ml *t*-Butyl Alcohol, in McCoy's 5A medium, with and without metabolic activation. Mitomycin C was used as the positive control in the absence of metabolic activation at concentrations of 0.001 and 0.010 µg/ml. Weak evidence of mutagenic activity was observed in a trial run, in the absence of metabolic activation (20.32% change of sister chromatid exchanges/chromosome at 5000 µg/ml), but this effect was not reproducible and no effects were seen in the presence of metabolic activation. The test article was considered non-genotoxic.	Yes, in the original report there are positive results for the same type of study
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/14112/1/1	Genotoxicity, in vitro	OECD TG 473; in vitro mammalian chromosome aberration test. Chinese hamster ovary cell lines were treated with up to 5000 µg/ml *t*-Butyl Alcohol, in McCoy's 5A medium, with and without metabolic activation. Cyclophosphamide was used was the positive control in the presence of metabolic activation, at concentrations of 15 and 50 µg/ml; mitomycin C was used as the positive control in the absence of metabolic activation at concentrations of 0.25 and 1 µg/ml. The test article did not induce chromosomal aberrations in treated cells and was considered non-genotoxic.	Yes, no chromosome aberration test in the original report
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/14112/1/1	Genotoxicity, in vitro	Comet assay; DNA damage and/or repair. Human leukemia (HL-60) cells were treated with 1, 5, 10, or 30 mmol/l *t*-Butyl Alcohol (solvent not specified), without metabolic activation, for 1 h; hydrogen peroxide was used as the positive control. Single gel electrophoresis was used to determine DNA damage, while the release of lactate dehydrogenase was used as an indicator of cytotoxicity. *t*-Butyl Alcohol seemed to induce DNA damage in a dose-dependent manner, however, this was partly attributed to cytotoxicity (which had ambiguous results).	no
Carcinogenicity Studies			
Borghoff SJ, et al. Physiologically based pharmacokinetic model for ethyl *t*-butyl ether and *t*-Butyl Alcohol in rats: contribution of binding to α2u-globulin in male rats and high exposure nonlinear kinetics to toxicity and cancer outcomes. J Appl Toxicol 2016; 37(5): 621 - 640	Pharmacokinetic modeling; cancer outcomes	In a PBPK model, the shift from linear to nonlinear kinetics at exposure concentrations below those associated with liver tumors in rats (5000 ppm), following chronic exposure, suggested that the mode of action for liver tumor occurrence in rats is not comparable, or relevant, for assessing human risk.	Yes; renal tumor effects being species-specific is not mentioned in the original report
Hard GC, et al. Renal histopathology in toxicity and carcinogenicity studies with *t*-Butyl Alcohol administered in drinking water to F344 rats: a pathology working group review and re-evaluation. Reg Toxicol Pharmacol 2011; 59 (3) 430 - 436	Re-evaluation of previous toxicity studies	The Pathology working group concluded that both α2u-globulin nephropathy and exacerbated CPN modes of action were operative in *t*-Butyl Alcohol renal tumorigenicity in male rats, neither of which has relevance for human cancer risk.	Yes, this provides further context to studies cited in the original report
Hard GC, et al. Histopathology re-examination of the NTP toxicity/carcinogenicity studies of *t*-Butyl Alcohol to identify renal tumor and toxicity modes of action. Reg Toxicol Pharmacol 2019; 102: 65 - 73	Re-evaluation of previous toxicity studies	Histology slides of male and female rat kidneys from 13-wk NTP drinking water studies (outcomes: 13-wk toxicity and 2-yr carcinogenicity) were reviewed to further elucidate the mode of action for the resulting renal tumors. All of the histopathological changes in the kidney associated with *t*-Butyl Alcohol exposure in the NTP studies (non-neoplastic and neoplastic) can be explained by α2u-globulin nephropathy or enhanced CPN. As neither of these modes of action are relevant to humans, none of the kidney findings in the NTP studies were relevant to human risk assessment.	Yes, this provides further context to studies cited in the original report
McGregor D, et al. Renal tumor induction by *t*-Butyl Alcohol. Toxicol Sci 2001; 61: 1 -3	Opinion on specific research hypothesis	Expounds upon how research done by Borghoff et al (2001), NTP, and others supports the hypothesis that the mechanism of action for *t*-Butyl Alcohol to induce renal tubule tumors is driven by α2u-globulin nephropathy and is species-/strain-specific to male rats.	Yes; renal tumor effects being species-specific is not mentioned in the original report
Api AM, Belsito D, et al. RIFM fragrance ingredient safety assessment, 2-methy-2-propanol, CAS registry number 75-65-0. Food Chem Toxicol 2023; 173: 113512	Carcinogenic risk assessment	Human reference dose RfD was estimated to be 220 µg/kg/d, which is 3500 greater than the total systemic exposure from fragrances (0.062 µg/kg/d), and is therefore considered an adequate MOE.	Yes, there is no human reference dose in the report

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
Other Relevant Studies			
de Peyster A, et al. Responses of the steroidogenic pathway from exposure to methyl-tert-butyl ether and tert-butanol. Toxicol 2014; 314: 23 - 27	Endocrine effects	Androgen receptor binding assay: *t*-Butyl Alcohol solutions (up to 10^{-3} M) were tested in tubes containing androgen receptors isolated from rat prostate tissue in 3 non-concurrent, competitive binding assays. The test article was classified as a "non-binder" (mean specific binding ≥ 50%); the mean relative binding affinity could not be calculated. Steroidogenesis assay: OECD TG 456. H295R cells were treated with 0.001, 0.001, 0.1, 1, 10, and 100 µM *t*-Butyl Alcohol in DMSO, and incubated for 48 h (assays were repeated in triplicates). Testosterone and estradiol levels were measured using HPLC/tandem mass spectrometry with a method detection limit of 100 pg/ml for testosterone and 10 pg/ml for estradiol. No statistically significant changes in estradiol concentrations were observed at any of the exposure concentrations, for all 3 runs of the assay. Statistically significant increases in testosterone were observed in response to 0.1 and 1 µM concentrations of *t*-Butyl Alcohol in 1 out of 3 runs of the assay. Effect on aromatase enzyme: human recombinant microsomes were tested with final concentrations of 10^{-10} – 10^{-3} M *t*-Butyl Alcohol in DMSO (at 1% of the total assay volume) in an aromatase assay. *t*-Butyl Alcohol was classified as a non-inhibitor with a mean aromatase activity of 102.3 (± 1.7%), at the highest tested concentration.	Yes, no information on steroidogenic effects in the original report
Sensitization Studies			
https://echa.europa.eu/da/registration-dossier/-/registered-dossier/14112/1/1	Dermal sensitization, GPMT	OECD TG 406. Female albino guinea pigs; test group: 20 animals; control group: 10 animals. Intradermal injection during induction: - 0.1 ml, FCA and water (1:1) - 0.1 ml, 1% *t*-Butyl Alcohol, in water - 0.1 ml, 1% *t*-Butyl Alcohol in FCA and water (1:1) Epidermal induction and Challenge: *t*-Butyl Alcohol, applied neat (100%) Reactions were scored 24 and 48 h after the challenge application. During the induction phase, strong erythema, edema, and necrosis were observed at the intradermal injection sites using FCA for test and control animals. Since some sites in each animal used FCA, all 30 animals responded. All non-FCA injection sites resulted in no reaction. No reactions were observed either 24 or 48 h after dermal challenge with the undiluted test article. *t*-Butyl Alcohol was considered a non-sensitizer.	Yes, Human sensitization studies are included in the previous report, but animal sensitization studies are not
Risk Assessment (Cosmetic Exposures)			
Api AM, Belsito D, et al. RIFM fragrance ingredient safety assessment, 2-methy-2-propanol, CAS registry number 75-65-0. Food Chem Toxicol 2023; 173: 113512	Safety assessment	Based on the Crème RIFM aggregate exposure model v1.0, the total systemic exposure to *t*-Butyl Alcohol as a fragrance ingredient is 0.000062 mg/kg/d; the majority of which is attributed to inhalation exposure (0.000061 mg/kg/d). This systemic exposure value was used in conjunction with NOAEL values from toxicity studies to calculate 2 MOE values. Namely: MOE for repeated dose toxicity: 195 mg/kg/d ÷ 0.000062 mg/kg/d = 3,145,161 MOE for reproductive toxicity: 160 mg/kg/d ÷ 0.000062 mg/kg/d = 2,580,645	Yes, such information is not present in original report

NOTABLE NEW DATA			
Publication	**Study Type**	**Results – Brief Overview**	**Different from Existing Data?**
National Toxicology Program. 1997. Toxicity studies of *t*-Butyl Alcohol (CAS No. 75-65-0) administered by inhalation to F344/N rats and B6C31 mice. NTP-TOX. No. 53. NIH Publ. No. 97-3942 (As cited in RIFM 2023 safety assessment)	Risk assessment, local respiratory tox	Rats (5/group/sex); whole-body exposure to 0, 409.25, 818.5, 1637.01, 3274.01, or 6366.13 mg/m^3 *t*-Butyl Alcohol for 6 h/d, 5 d/wk for 13 wk. No treatment-related gross pathology or microscopic findings were found in the respiratory tissues of the animals from all exposure groups. The NOAEC for local respiratory effects was determined to be 6366.13 mg/m^3	Yes, this reference is cited in the original report, but this data was not included
Api AM, Belsito D, et al. RIFM fragrance ingredient safety assessment, 2-methy-2-propanol, CAS registry number 75-65-0. Food Chem Toxicol 2023; 173: 113512	Risk assessment, phototox	UV absorption spectra indicate no absorption between 290 and 400 nm and the corresponding molar absorption coefficient is below the benchmark of concern for phototoxicity and photoallergenicity. Based on this lack of absorbance, *t*-Butyl Alcohol does not present a concern for phototoxicity or photoallergenicity.	Yes, such information is not present in original report

Abbreviations: CPN – chronic progressive neuropathy; DMSO – dimethyl sulfoxide; F_0 – first/parental generation; F_1 – second/offspring generation; FCA – Freund's complete adjuvant; GPMT – guinea pig maximization test; hCG- human chorionic gonadotropin; HPLC – high performance liquid chromatography; LOAEL – lowest-observed-adverse-effect-level; MOE – margin of exposure; NOAEC- no-observed-adverse-effect-concentration; NOEC- no-observed-effect-concentration; NOAEL – no-observed-adverse-effect-level; NTP – National Toxicology Program; OECD – Organisation for Economic Cooperation and Development; PBPK – physiologically based pharmacokinetic model; RfD- reference dose; RIFM – Research Institute for Fragrance Materials; TG – test guideline; UV - ultraviolet

Search (from 2000 on)
((((((((((((t-butyl alcohol) OR (75-65-0)) OR (1,1-Dimethylethanol)) OR (2-Methyl-2-Propanol)) OR (2-Methyl-2-propanol)) OR (2-Propanol, 2-MethylTert-Butanol)) OR (tert-Butanol)) OR (Tert-Butyl Alcohol)) OR (Trimethyl Carbinol)) OR (Trimethylmethanol)) OR (tertiary butyl alcohol)) OR (tertiary butanol)) OR (t-butanol)) OR (2-methyl-2-propanol)) OR (trimethyl carbinol)) AND (toxicity) AND (2000:2023[pdat]) – 395,420 hits/ 10 useful

Table 1. Frequency (2023/1998) and concentration (2022/1999) of use of *t*-Butyl Alcohol according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*	
	2023[1]	**1998[2]**	**2022[3]**	**1999[2]**
Totals*	**136**	**32**	**0.00014 – 0.91**	**0.00001 – 0.5**
summarized by likely duration and exposure**				
Duration of Use				
Leave-On	*115*	*30*	*0.003 – 0.91*	*0.00001 – 0.5*
Rinse-Off	*14*	*2*	*0.00014 – 0.16*	*0.0001 – 0.001*
Diluted for (Bath) Use	*7*	*NR*	*NR*	*NR*
Exposure Type				
Eye Area	8	1	0.004 – 0.01	0.001
Incidental Ingestion	19	NR	0.0001 – 0.028	0.0001
Incidental Inhalation-Spray	2; 48[a]; 20[b]	27	0.06 – 0.11; 0.003[a]	0.0001 – 0.5; 0.00001 – 0.3[a]
Incidental Inhalation-Powder	20[b]	NR	0.0054 – 0.05[c]	0.0007
Dermal Contact	110	32	0.003 – 0.91	0.0001 – 0.3
Deodorant (underarm)	1[a]	NR	not spray: 0.89	0.0001[a]
Hair - Non-Coloring	6	NR	0.00014 – 0.11	0.00001 – 0.5
Hair-Coloring	NR	NR	NR	NR
Nail	1	NR	NR	NR
Mucous Membrane	26	NR	0.0001 – 0.16	0.0001
Baby Products	1	NR	NR	NR
as reported by product category				
Baby Products				
Other Baby Products	1	NR	NR	NR
Bath Preparations (diluted for use)				
Bath Oils, Tablets, and Salts	7	NR	NR	NR
Eye Makeup Preparations				
Eyebrow Pencil	NR	NR	NR	0.001
Eye Lotion	3	NR	0.004 – 0.0042	NR
Eye Makeup Remover	NR	1	NR	NR
Mascara	NR	NR	0.01	0.001
Other Eye Makeup Preparations	5	NR	NR	NR
Fragrance Preparations				
Cologne and Toilet Water	1	18	0.097	0.001
Perfumes	1	8	0.096 – 0.11	NR
Other Fragrance Preparation	NR	1	NR	NR
Hair Preparations (non-coloring)				
Hair Conditioner	1	NR	NR	NR
Hair Spray (aerosol fixatives)	NR	NR	0.066 – 0.11	0.0001 and 0.5***
Shampoos (non-coloring)	NR	NR	0.00014	0.0001
Tonics, Dressings, and Other Hair Grooming Aids	4	NR	spray: 0.06	0.00001
Other Hair Preparations	1	NR	NR	NR
Makeup Preparations				
Blushers (all types)	NR	NR	NR	0.0001
Face Powders	NR	NR	NR	0.0007
Foundations	NR	NR	NR	0.0001
Lipstick	17	NR	0.0001 – 0.007	0.0001
Makeup Bases	1	NR	0.006	NR
Other Makeup Preparations	6	NR	NR	NR
Manicuring Preparations (Nail)				
Other Manicuring Preparations	1	NR	NR	NR
Oral Hygiene Products				
Dentifrices	NR	NR	0.028	NR
Other Oral Hygiene Products	2	NR	NR	NR
Personal Cleanliness Products				
Bath Soaps and Detergents	NR	NR	NR	0.0001
Deodorants (underarm)	1	NR	not spray: 0.89	0.0001
Other Personal Cleanliness Products	NR	NR	0.16	NR
Shaving Preparations				
Aftershave Lotion	1	3	0.079 – 0.91	0.001 and 0.08***
Beard Softeners	NR	NR	0.029	NR
Other Shaving Preparations	NR	1	NR	NR
Skin Care Preparations				
Cleansing	7	NR	0.0047 – 0.088	0.001
Face and Neck (exc shave)	16	NR	spray: 0.094 not spray: 0.016 – 0.044	NR
Body and Hand (exc shave)	4	NR	spray: 0.1 not spray: 0.0054 – 0.05	NR
Moisturizing	35	NR	not spray: 0.005 – 0.048	0.0001

Table 1. Frequency (2023/1998) and concentration (2022/1999) of use of *t*-Butyl Alcohol according to likely duration and exposure and by product category

	# of Uses		*Max Conc of Use (%)*	
	2023[1]	**1998[2]**	**2022[3]**	**1999[2]**
Night	5	NR	NR	0.0001
Paste Masks (mud packs)	4	NR	NR	NR
Skin Fresheners	3	NR	0.003	0.3***
Other Skin Care Preparations	8	NR	0.01	0.001
Suntan Preparations				
Indoor Tanning Preparations	1	NR	NR	NR

NR – not reported
*Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types may not equal the sum of total uses.
**likely duration and exposure are derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)
***These concentrations are not alcohol denaturant uses
[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
[c] It is possible these products are powders, but it is not specified whether the reported uses are powders.

REFERENCES

1. U.S. Food and Drug Administration Center for Food Safety & Applied Nutrition (CFSAN). 2023. Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients (VCRP). (Obtained under the Freedom of Information Act from CFSAN; requested as "Frequency of Use Data" January 4, 2023; received February 2, 2023.)

2. Andersen FA (ed.). Amended final report of the safety assessment of t-Butyl Alcohol as used in cosmetics. *Int J Toxicol.* 2005;24 Suppl 2:1-20.

3. Personal Care Products Council. 2022. Concentration of Use by FDA Product Category: t-Butyl Alcohol. (Unpublished data submitted by the Personal Care Products Council on October 31, 2022.)

JOURNAL OF THE AMERICAN COLLEGE OF TOXICOLOGY
Volume 8, Number 4, 1989
Mary Ann Liebert, Inc., Publishers

3

Final Report on the Safety Assessment of *t*-Butyl Alcohol

The safety of this ingredient has not been documented and substantiated. The Cosmetic Ingredient Review Expert Panel cannot conclude that *t*-Butyl Alcohol is safe for use in cosmetic products until such time that the appropriate safety data have been obtained and evaluated. The data that were available are documented in the report as well as the types of data that are required before a safety evaluation may be undertaken.

INTRODUCTION

t-Butyl Alcohol and *n*-Butyl Alcohol were evaluated originally in one report by the Expert Panel. The data were sufficient to reach a safety conclusion for *n*-Butyl Alcohol, but were insufficient for *t*-Butyl Alcohol, and therefore the original report was divided into a report for each ingredient. The Expert Panel concluded that *n*-Butyl Alcohol is safe as presently used in cosmetics. The report for *t*-Butyl Alcohol follows.

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.

CHEMICAL AND PHYSICAL PROPERTIES

t-Butyl Alcohol (CAS No. 75-65-0) (*t*-BuOH) is a tertiary aliphatic alcohol with the chemical formula[1,2] in Figure 1.

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.[1-3]

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.[3-7] Chemical and physical properties of *t*-BuOH are presented in Table 1.

t-BuOH is a fire hazard when exposed to heat or flame, and it can react with oxidizing materials. *t*-BuOH, in the form of vapor, is a moderate explo-

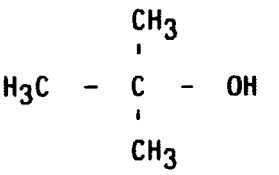


FIG. 1. t-Butyl Alcohol.

sion hazard when exposed to flame. It reacts violently with hydrogen peroxide.[8]

t-BuOH has been prepared from acetyl chloride and dimethylzin, by catalytic hydration of isobutylene, by reduction of tert-butyl hydroperoxide, and by absorption of isobutene, from cracking petroleum or natural gas, and in sulfuric acid with subsequent hydrolysis by steam. It is purified by distillation.[3-5]

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.[4]

Qualitative and quantitative determinations of *t*-BuOH are made by precipitation colorimetry,[9] gas chromatography,[10,11] gas chromatography–mass spectrometry,[10] photometry,[12] proton magnetic resonance,[13] and a laser

TABLE 1. Chemical and Physical Properties of *t*-BuOH

Property	*t-BuOH*	*Reference*
Molecular weight	74.12	
Specific gravity at		
20/4°C	0.78581	3
20/4°C	0.7887	7
25/4°C	0.78086	3
30/4°C	0.77620	2
Boiling point (°C) at		
760 mm Hg	82.41	3
760 mm Hg	82.30	7
760 mm Hg	82.50	2
31 mm Hg	20	7
Melting point (°C)	25.6	3
	25.5	7
	25.5	2
Vapor pressure (mm Hg) at		
20°C	30.6	2
Refractive index for D line		
of the sodium spectrum at		
20°C	1.38468	3
20°C	1.3878	7
20°C	1.3838	2
25°C	1.38231	3
25°C	1.3811	2
Autoignition temperature (°C)	380	2

absorption spectrometric method.[14] *t*-BuOH does not absorb ultraviolet light at wavelengths of 290 nm or longer.[15,16]

USE

Cosmetic Use

t-BuOH is used in the manufacture of perfumes.[3,5] It is used as a solvent or an alcohol denaturant and as a perfume carrier in cosmetics.[4]

Product types and the number of product formulations containing *t*-BuOH are reported voluntarily to the Food and Drug Administration (FDA). Voluntary filing of this information by cosmetic manufacturers, packagers, and distributors conforms to the prescribed format of preset concentration ranges and product types as described in the Code of Federal Regulations.[9] Some cosmetic ingredients are supplied by the manufacturer at less than 100% concentration and, therefore, the value reported by the cosmetic formulator or manufacturer may not necessarily reflect the true concentration of the finished product; the actual concentration in such a case would be a fraction of that reported to FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a 2–10-fold error in the assumed ingredient concentration. In 1986, *t*-BuOH was reported to be an ingredient in 10 hair and facial skin care preparations at concentrations ranging from $\leq 0.1\%$ to between 0.1 and 1%.[17]

Cosmetic products containing *t*-BuOH may be applied to, or come in contact with, skin, eyes, hair, nails, mucous membranes, and respiratory epithelium.[17]

Product formulations 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.[17]

t-BuOH is stable under typical conditions of cosmetic use.[4]

Noncosmetic Use

t-BuOH is permitted as an indirect food additive. *t*-BuOH 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.[9] *t*-BuOH 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.[9]

t-BuOH has been used as a denaturant for alcohol in a commercial sunscreen preparations.[18]

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.[3,5]

BIOLOGY

Effects on Enzymes and Membranes

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.[19–22]

t-BuOH has no or only a weak effect on rat hepatic mitochondrial respiration and phosphorylation at concentrations of up to 3%.[23]

Action as a Hydroxyl Radical Scavenger

t-BuOH is a hydroxyl radical scavenger. *t*-BuOH has been shown to protect DNA from the effects of radiation, and it is hypothesized that this action may be due to the scavenging of hydroxyl radicals.[24–26]

Environmental Occurrence

t-BuOH is ubiquitous in the environment, and human exposure is likely. Fusel oil, the congeners or byproducts of the fermentation or distillation process in the production of alcoholic beverages, is 95% amyl, butyl, and propyl alcohols and has been detected in liquor in a concentration as high as 0.25%.[27] *t*-BuOH has been detected in drinking water.[28]

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

t-BuOH is not a substrate for alcohol dehydrogenase or for catalase and has been used as an example of a nonmetabolizable alcohol. However, the results of recent investigations have indicated that *t*-BuOH is not as inert metabolically as previously assumed.[29,30] *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.[29,31] Baker et al.[32] 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. Baker et al.[32] injected two rats with 1.75 g/kg β-[^{14}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 CO_2 and acetone. When the animals were injected with 1.5 g/kg of a 1:1 mixture of α-[^{13}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}C]hexadecanoic acid and *t*-BuOH followed by collection of respiratory gases indicated that *t*-BuOH did not affect fatty acid synthesis.

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).[33] 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 four to six 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 60–100 mg percent.[34] The rats were then given *t*-BuOH to elevate their blood concentrations to between 125 and 150 mg percent, 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.

t-BuOH is also slowly eliminated from the blood of mice. McComb and Goldstein[35] 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 8–9 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.

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.[36]

Kamil et al.[37] 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 elimi-

nated 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).

t-BuOH is excreted by rabbits as glucuronide conjugates, but these compounds are not present in dog urine.[38]

ANIMAL TOXICOLOGY

Oral Studies

The LD_{50} of *t*-BuOH for white rats (unspecified strain) was 3.5 g/kg (details of experiment unspecified).[39]

Ten to 35 rabbits, weighing 1.5–2.5 kg, were given *t*-BuOH by stomach tube.[40,41] The LD_{50} (the quantity that caused death in half of the rabbits within 24 hours) was 48 mmol/kg (3.56 g/kg). The ND_{50} (the quantity that caused narcosis in half the rabbits) was 19 mmol/kg (1.41 g/kg).

A dose of 25 mmol/kg (1.85 g/kg) *t*-BuOH as a 25% by volume solution in water was administered by gastric intubation to female Wistar rats (unspecified number).[33] 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.[42,43] 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).[44] 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.[30] 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.

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.[45] 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 other 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.

Groups of 10 male and 10 female Fischer-344 rats were given drinking water containing 0, 0.25, 0.5, 1.0, 2.0, and 4.0% *t*-BuOH for 90 days.[46] Average dosages for males were 0. 235.4, 495.8, 803.7, 1598.9, and 3588.5 mg/kg/day, and average dosages for females were 0, 260.6, 510.5, 758.4, 1451.5, and 3500.1 mg/kg/day. All of the male rats and six of the female rats at the highest dosage level died. There was also an absolute body weight loss in the males and a marked weight gain depression in the females. In male rats, there was a dosage-related depression in weight gain at lower dosages. Water consumption decreased in the females that received water containing 1, 2, and 4% *t*-BuOH and in the males that received water containing 4% *t*-BuOH. Water consumption increased in the male rats given water containing 0.25 and 0.5% *t*-BuOH. Ataxia was observed in both sexes, and hypoactivity was observed in male rats. During the study, total bile acid levels in the blood were elevated for all males except those receiving the 4% concentration. At the end of the study, total bile acid levels were elevated only for females receiving the 4% concentration. Urine volume was decreased for all rats at the 1% and greater concentrations. Crystals, presumed to be uric acid based on their size and shape, were observed in the urine in "high incidence" (in up to one-half of the surviving rats) at the 2 and 4% concentrations. At necropsy, gross findings involving the urinary tract, such as calculi, dilatation, and thickening, and those characteristic of inanition, apparently due to low water consumption, were observed. The kidneys, ureters, and urinary bladder were target organs for *t*-BuOH toxicity in the rat. The no-effect concentration was 1% for male rats and 2% for female rats.

Groups of 10 male and 10 female $B6C3F_1$ mice were given drinking water containing 0, 0.25, 0.5, 1.0, 2.0, and 4.0% *t*-BuOH for 90 days.[47] Average dosages for males were 0, 319.3, 726.3, 1565.8, 2838.8, and 6247.2 mg/kg/day, and average dosages for females were 0, 568.3, 941.7, 1731.8, 4362.9, and 7475.8 mg/kg/day. Six of 10 male mice and 4 of 10 female mice died receiving the highest dosage. There was a dosage-related depression in weight gain in the males that received water containing 1, 2, and 4% *t*-BuOH and in the females that received water containing 2 and 4% *t*-BuOH. Hyperplasia of the transitional epithelium of the urinary bladder and inflammation of the urinary bladder were observed. Other pathologic effects were considered secondary to inanition. The no-effect concentration for direct chemical effects was 1% for male mice and 2% for female mice.

Dermal Studies

Renkonen and Tier[48] 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^2, and after 10 mg *t*-BuOH was 43 mm^2. No explanation of the significance of these results was provided.

SPECIAL STUDIES

Animal Reproduction and Teratology

Groups of 15 pregnant Swiss-Webster mice were fed liquid diets containing *t*-BuOH at concentrations of 0.5, 0.75, and 1.0% (w/v) from days 6 to 20 of gestation.[49] Control mice were fed only the liquid diet. The 1.0% *t*-BuOH group was fed ad libitum. The other groups were pair-fed based on the consumption of the 1.0% *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 1.0% *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 2–22 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 1.0% 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 1.0% 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 1.0% 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 1.0% group. Pinna detachment occurred between days 6 and 8 in all the groups. Eyes opened in the 1.0% *t*-BuOH group at around day 16; this was 2–4 days later than in the other groups. Postnatal weight gain was decreased over the first 10 days in the nonfostered 0.75 and 1.0% 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.

Anderson et al.[50] 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.

Mutagenicity

t-BuOH was nonmutagenic in the *Salmonella*/mammalian microsome mutagenicity test "even at a high concentration."[51,52] It was nonmutagenic to *Salmonella typhimurium* in the same test with metabolic activation when the bacterial suspension was preincubated with the chemical (concentrations unspecified).[53]

t-BuOH, added at a concentration of 1% to media prior to sterilization by autoclaving, did not increase the incidence of penicillin or streptomycin resistance in *Micrococcus aureus*.[54] In addition, bacterial cell survival was not affected.

t-BuOH did not induce adenine independence in adenine-dependent *Neurospora crassa*.[55] Mutations did not result after exposure to the fungi to a 1.75 mol/L concentration of *t*-BuOH in water.

t-BuOH was considered as a solvent for water-insoluble chemicals to be tested for mutagenicity.[56] *t*-BuOH was moderately toxic to the yeast, *Schizosaccharomyces pombe*, at concentrations of 0.5–10.0% (v/v) and to V79 Chinese hamster cells at 2.0 and 5.0% (v/v) and, therefore, it was not further considered.

t-BuOH was mutagenic to cultured human–Chinese hamster ovary hybrid cells at the mean lethal concentration of 80 mM.[57,58]

Carcinogenicity

Hair was clipped from the backs close to the base of the tail of female ddN mice, chemicals were applied to this bared skin, and the mice were observed for 450 days.[59] 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 and "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.

t-BuOH is currently under test in the NTP carcinogenicity bioassay program.[60] It is being administered in drinking water to rats and mice.

CLINICAL ASSESSMENT OF SAFETY

A woman who had a positive patch test reaction to ethanol was tested with 100% *t*-BuOH.[61] 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 had a negative reaction to *t*-BuOH.

Four female patients were tested on the upper back with 1 and 10% *t*-BuOH in water.[62] 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[18] 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 slight pain, moderate hyperemia and erythema, dryness, and vesiculation.[63-65]

The ACGIH has set a threshold limit value of 100 ppm and a short-term exposure limit of 150 ppm that is satisfactory to prevent narcosis with *t*-BuOH.[63] 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.[2] NIOSH has reported that 8000 ppm *t*-BuOH is the concentration immediately dangerous to life or health.[11]

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. *t*-BuOH absorbs ultraviolet light at 275 nm and does not absorb at any longer wavelength. In 1986, *t*-BuOH was reported as an ingredient in 10 hair and facial skin care preparations at concentrations ranging from $\leq 0.1\%$ to between 0.1 and 1%.

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. *t*-BuOH was slowly eliminated from the blood of rats and mice; elimination is more rapid in animals previously exposed to *t*-BuOH.

The single oral dose LD_{50} of *t*-BuOH for rats was 3.5 g/kg. The addition of *t*-BuOH to the drinking water of rats and mice for 90 days resulted in gross lesions predominantly involving the urinary tract and those characteristic of inanition. The kidneys, ureters, and urinary bladder were target organs for *t*-BuOH toxicity in the rat. The no-effect concentrations for *t*-BuOH in the drinking water of rats were 1% in males and 2% in females. The urinary bladder was the target organ for *t*-BuOH toxicity in the mouse. The no-effect concentrations for direct chemical effects for *t*-BuOH in the drinking water of mice were 1% in males and 2% in females.

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 did not affect the in vitro fertilization capacity of mouse spermatozoa.

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 in benzene was applied to the skin of 50 mice 6 times a week for a total of 270 applications after the application of 4NQO 3 times a week for a total of 20 applications. One squamous cell carcinoma was observed after 100 applications of *t*-BuOH. Based on this experiment, *t*-BuOH was inactive on mouse skin as a complete carcinogen or as a tumor promoter. *t*-BuOH is currently under test in the NTP carcinogenicity bioassay program. It is being administered in drinking water to rats and mice.

Dermatitis can result from dermal exposure of humans to *t*-BuOH.

The ACGIH has set a threshold limit value of 100 ppm and a short-term exposure limit of 150 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

The Expert Panel is aware that data on the ocular irritation of *t*-BuOH in animals are lacking. These data are not required by the Panel. *t*-BuOH is expected to be a severe eye irritant.

Section 1, paragraph (p) of the CIR Procedures states that "A lack of information about an ingredient shall not be sufficient to justify a determination of safety." In accordance with Section 30(j)(2)(A) of the CIR Procedures, the Panel informed the public of its decision that the data on *t*-BuOH are insufficient to determine whether this ingredient, under each relevant condition of use, is either safe or unsafe. The Panel released a Notice of Insufficient Data Announcement on September 23, 1986 outlining the data needed to

assess the safety of *t*-BuOH. The types of data required included:

1. Data from a 90-day oral study.
2. Data on human sensitization.
3. An ultraviolet absorbance spectrum: if absorbance was observed at greater than 290 nm, then photosensitization data would be required.

Data from a 90-day oral study and an ultraviolet spectrum were received. Data on human sensitization were not received within an appropriate time period.

t-BuOH does not absorb ultraviolet light at wavelengths of 290 nm or longer; it is not expected to be a photosensitizer. The animal and human sensitization data available for *n*-BuOH cannot be used to make a determination of the safety of *t*-BuOH. The Expert Panel has determined that *n*-BuOH is safe as presently used in cosmetics.

The Panel will issue the Final Report in accordance with Section 45 of the CIR Procedures. When new data are available, the Panel will reconsider the Final Report in accordance with Section 46 of the CIR Procedures, Amendment of a Final Report.

CONCLUSION

The CIR Expert Panel concludes that the available data are insufficient to support the safety of *t*-BuOH as used in cosmetics.

ACKNOWLEDGMENT

Karen R. Brandt, Scientific Analyst and writer, prepared the literature review and technical analysis for this report.

REFERENCES

1. ESTRIN, N.F., CROSLEY, P.A., and HAYNES, C.R. (Editors). (1982). *CTFA Cosmetic Ingredient Dictionary*, 3rd ed. Washington, DC: Cosmetic, Toiletry and Fragrance Association.
2. WIMER, W.W., RUSSELL, J.A., and KAPLAN, H.L. (1983). *Alcohols Toxicology*. Park Ridge, NJ: Noyes Data Corporation.
3. WINDHOLZ, M. (Editor). (1983). *The Merck Index*, 10th ed. Rahway, NJ: Merck and Co., Inc.
4. COSMETIC, TOILETRY AND FRAGRANCE ASSOCIATION (CTFA). (August 30, 1985). Submission of unpublished data by CTFA. Cosmetic ingredient chemical description for *t*-Butyl Alcohol.*
5. HAWLEY, G.G. (Editor). (1971). *The Condensed Chemical Dictionary*, 8th ed. New York: Van Nostrand Reinhold.
6. UNITED STATES PHARMACOPEIA (USP) COMMITTEE OF REVISION. (1979). *The United States Pharmacopeia*, 20th revision. Easton, PA: Mack Printing Company.

*Available for review: Director, Cosmetic Ingredient Review, 1110 Vermont Ave., N.W., Suite 810, Washington, DC 20005.

7. WEAST, R.C. (Editor). (1982). *CRC Handbook of Chemistry and Physics*, 5th ed. Boca Raton, FL: CRC Press.
8. SAX, N.I. (1979). *Dangerous Properties of Industrial Materials*, 5th ed. New York: Van Nostrand Reinhold.
9. CODE OF FEDERAL REGULATIONS (CFR). (1984). Title 21, Parts 176.200, 178.3910, 720.4: Title 27, Part 212.73.
10. EICEMAN, G.A., and KARASEK, F.W. (1981). Identification of residual organic compounds in food packages. J. Chromatogr. **210**(1), 93–104.
11. MACKISON, F.W., STRICOFF, R.S., and PARTRIDGE, L.J., 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.
12. ZAMARAKHINA, L.E. (1973). Determination of tertiary butyl alcohol in the air of industrial rooms. Gig. Sanit. **38**(5), 72–73.
13. MUHTADI, F.J., HASSAN, M.M.A., and TAWAKKOL, M.M. (1982). PMR assay of betalactam antibiotics. I. Assay of cephalosporins. Spectrosc. Lett. **15**(5), 373–81.
14. GREEN, B.D., and STEINFELD, J.I. (1977). Monitoring complex trace-gas mixtures by long-path laser adsorption spectrometry. Proc. Soc. Photo-Opt. Instrum. Eng. **99**, 32–38
15. HOFFMANN, D.K.. American Health Foundation, Valhalla, NY, personal communication, June 22, 1987.
16. SHANK, R.C. University of California at Irvine, personal communication, April 7, 1987.
17. FOOD AND DRUG ADMINISTRATION (FDA). (1986). Cosmetic product formulation data. Washington, DC.
18. EDWARDS, E.K., JR., and EDWARDS, E.K. (1982). Allergic reaction to tertiary butyl alcohol in a sunscreen. Cutis **29**(5), 476–78.
19. HARRIS, R.A., and SCHROEDER, F. (1981). Effects of ethanol and related drugs on the physical and functional properties of brain membranes. Curr. Alcohol. **8**, 461–68.
20. HILLER, J.M., ANGEL, L.M., and SIMON, E.J. (1984). Characterization of the selective inhibition of the delta subclass of opioid binding sites by alcohols. Mol. Pharmacol. **25**(2), 249–55.
21. LYON, R.C., McCOMB, J.A., SCHREURS, J., and GOLDSTEIN, D.B. (1981). A relationship between alcohol intoxication and the disordering of brain membranes by a series of short-chain alcohols. J. Pharmacol. Exp. Ther. **218**(3), 669–75.
22. THOMAS, M., BOURA, A.L.A., and VIJAYAKUMAR, R. (1980). Prostaglandin release by aliphatic alcohols from the rat isolated lung. Clin. Exp. Pharmacol. Physiol. **7**(4), 373–81.
23. THORE, A., and BALTSCHEFFSKY, H. (1965). Inhibitory effects of lower aliphatic alcohols on electron transport phosphorylation systems. II. Secondary, tertiary, and di-alcohols. Acta Chem. Scand. **19**(7), 1600–6.
24. LAFLEUR, M.V., and LOMAN, H. (1982). Influence of anoxic sensitizers on the radiation damage in biologically active DNA in aqueous solution. Int. J. Radiat. Biol. **41**(3), 295–302.
25. REUVERS, A.P., GREENSTOCK, C.L., BORSA, J., and CHAPMAN, J.D. (1973). Mechanism of chemical radioprotection by dimethyl sulfoxide. Int. J. Radiat. Biol. **24**(5), 533–36.
26. ROOTS, R., and OKADA, S. (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**(4), 329–42.
27. DAMRAU, F., and GOLDBERG, A.H. (1971). Adsorption of whiskey congeners by activated charcoal: Chemical and clinical studies related to hangover. Southwest Med. **52**(9), 179–82.
28. KOOL, H.J., VAN KREIJL, C.F., and ZOETEMAN, B.C.J. (1982). Toxicology assessment of organic compounds in drinking water. Crit. Rev. Environ. Control. **12**(4), 307–57.
29. CEDERBAUM, A.I., QURESHI, A., and COHEN, G. (1983). Production of formaldehyde and acetone by hydroxyl radical generating systems during the metabolism of tertiary butyl alcohol. Biochem. Pharmacol. **32**(23), 3517–24.
30. VIDELA, L.A., FERNANDEZ, V., DE MARINIS, A., FERNANDEZ, N., and VALENZUELA, A. (1982). Liver lipoperoxidative pressure and glutathione status following acetaldehyde and aliphatic alcohols pretreatments in the rat. Biochem. Biophys. Res. Commun. **104**(3), 965–70.
31. CEDERBAUM, A.I., and COHEN, G. (1980). Oxidative demethylation of *t*-Butyl alcohol by rat liver microsomes. Biochem. Biophys. Res. Commun. **97**(2), 730–36.
32. BAKER, R.C., SORENSEN, S.M., and DEITRICH, R.A. (1982). The *in vivo* metabolism of tertiary butanol by adult rats. Alcoholism Clin. Exp. Res. **6**(2), 247–51.

33. BEAUGE, F., CLEMENT, M., NORDMANN, J., and NORDMANN, R. (1981). Liver lipid disposal following t-butanol administration to rats. Chem. Biol. Interact. **38**(1), 45–51.
34. THURMAN, R.G., WINN, K., and URQUHART, B. (1980). Rat brain cyclic AMP levels and withdrawal behavior following treatment with tert-butanol. Adv. Exp. Med. Biol. **126**, 271–81.
35. McCOMB, J.A. and GOLDSTEIN, D.B. (1979). Quantitative comparison of physical dependence on tertiary butanol and ethanol in mice: Correlation with lipid solubility. J. Pharmacol. Exp. Ther. **208**(1), 113–17.
36. BLEYMAN, M.A., and THURMAN, R.G. (1980). The swift increase in alcohol metabolism: Comparative studies with other alcohols. Curr. Alcohol. **7**, 115–21.
37. KAMIL, I.A., SMITH, J.N., and WILLIAMS, R.T. (1953). Studies in detoxification. 46. The metabolism of aliphatic alcohols. The glucuronic conjugation of acyclic aliphatic alcohols. Biochem. J. **53**(1), 129–36.
38. DERACHE, R. (1970). Toxicology, pharmacology and metabolism of higher alcohols. pp. 507–22. In: *Alcohols and Derivatives*. Vol. II, International Encyclopedia of Pharmacology and Therapeutics, Sec. 20. Edited by J. Tremolieres. London: Pergamon Press.
39. SCHAFFARZICK, R.W., and BROWN, B.J. (1952). Anticonvulsant activity and toxicity of methylparafynol (dormison) and some other alcohols. Science **116**, 663.
40. MUNCH, J.C. (1972). Aliphatic alcohols and alkyl esters: Narcotic and lethal potencies to tadpoles and to rabbits. Ind. Med. Surg. **41**(4), 31–33.
41. MUNCH, J.C., and SCHWARZE, E.W. (1925). Narcotic and toxic potency of aliphatic alcohols upon rabbits. J. Lab. Clin. Med. **10**, 985–96.
42. GAILLARD, D., and DERACHE, R. (1965). Effect of acute intoxication, by various alcohols, on hepatic lipid fractions in female rats. C.R. Hebd. Seances Acad. Sci. **261**(19), 3880–83.
43. GAILLARD, D., and DERACHE, R. (1966). Effect of some aliphatic alcohols on the mobilization of various lipid fractions in the rat. Food Cosmet. Toxicol. **4**(5), 515–20.
44. HUNT, W.A., MAJCHROWICZ, E., and DALTON, T.K. (1979). Alterations in high-affinity choline uptake in brain after acute and chronic ethanol treatment. J. Pharmacol. Exp. Ther. **210**(2), 259–63.
45. GRANT, K.A., and SAMSON, H.H. (1982). Ethanol and tertiary butanol induced microcephaly in the neonatal rat: comparison of brain growth parameters. Neurobehav. Toxicol. Teratol. **4**(3), 315–21.
46. NATIONAL TOXICOLOGY PROGRAM (NTP). (June 5, 1986). Draft subchronic toxicity report on t-butyl alcohol (C55367B) administered by dosed water to Fischer-344 rats. Research Triangle Park, NC.
47. NTP. (June 26, 1986). Draft subchronic toxicity report on t-butyl alcohol (C55367B) administered by dosed water to B6C3F_1 mice. Research Triangle Park, NC.
48. RENKONEN, K.O., and TEIR, H. (1957). Studies on the local reactions of the skin to chemical compounds. Ann. Med. Exp. Biol. Fenn. **35**, 67.
49. DANIEL, M.A., and EVANS, M.A. (1982). Quantitative comparison of maternal ethanol and maternal tertiary butanol diet on postnatal development. J. Pharmacol. Exp. Ther. **222**(2), 294–300.
50. ANDERSON, R.A., JR., REDDY, J.M., JOYCE, C., WILLIS, B.R., VAN DER VEN, H., and ZANEVELD, L.J.D. (1982). Inhibition of mouse sperm capacitation by ethanol. Biol. Reprod. **27**(4), 833–40.
51. AMES, B.N., McCANN, J., and YAMASAKI, M. (1975). Methods for detecting carcinogens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutat. Res. **31**, 347–64.
52. YAMAGUCHI, T. (1980). Activation with catalase of mutagenicity of hydroperoxides of some fatty acids and hydrocarbons. Agric. Biol. Chem. **44**, 1989–91.
53. NTP. (January, 1982). NTP Technical Bulletin. Issue No. 6. Research Triangle Park, NC.
54. CLARK, J. (1953). The mutagenic action of various chemicals on *Micrococcus aureus*. Proc. Okla. Acad. Sci. **34**, 114–18.
55. DICKEY, F.H., CLELAND, G.H., and LOTZ, C. (1949). Role of organic peroxides in the induction of mutations. Proc. Natl. Acad. Sci. USA. **35**, 581–86.
56. ABBONDANDOLO, A., BONATTI, S., CORSI, C., CORTI, G., FIORIO, R., LEPORINI, C., MAZZACCARO, A., and NIERI, R. (1980). The use of organic solvents in mutagenicity testing. Mutat. Res. **79**(2), 141–50.
57. LENNOX, J.L., and WALDREN, C.A. (1981). Measurement of the mutagenic action of alcohols in mammalian cells. Clin. Res. **29**, 36A.
58. WALDREN, C. (1982). Detection of chromosome deletions and nondisjunction produced by environmental agents in cultured somatic mammalian cells. Mutat. Res. **97**(3), 234.
59. HOSHINO, H., CHIHARA, G., and FUKUOKA, F. (1970). Detection of potential weak carcinogens and procarcinogens. II. Carcinogenicity of tertiary butyl hydroperoxide. Gann **61**(2), 121–24.
60. NTP. (February, 1984). Annual Plan for Fiscal Year 1984 (NTP-84-023). Research Triangle Park, NC.

61. FREGERT, S., HOKANSON, R., RORSMAN, H., TRYDING, N., and OVRUM, P. (1963). Dermatitis from alcohols. J. Allergy **34**, 404–8.
62. FREGERT, S., GROTH, O., HJORTH, N., MAGNUSSON, G., RORSMAN, H., and OVRUM, P. (1969). Alcohol dermatitis. Acta Derm. Venereol. **49**, 493–97.
63. AMERICAN CONFERENCE OF GOVERNMENTAL INDUSTRIAL HYGIENISTS (ACGIH). (1980). *Documentation of the Threshold Limit Values*, 4th ed. Cincinnati, OH: ACGIH.
64. GREENBERG, L.A., and LESTER, D. (1954). *Handbook of Cosmetic Materials*. New York: Interscience Publishers.
65. 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 Serv., Public Health Bull. No. 281. Washington, DC: U.S. Govt. Printing Office.

International Journal of Toxicolgy, 24(Suppl. 2):1–20, 2005

ISSN: 1091-5818 print / 1092-874X online
DOI: 10.1080/10915810590953833

Amended Final Report of the Safety Assessment of t-Butyl Alcohol as Used in Cosmetics[1]

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_{50} 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 irriation 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 postnatal 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

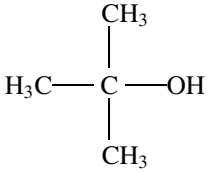
In its initial safety assessment of t-Butyl alcohol (t-BuOH), the Cosmetic Ingredient Review (CIR) Expert Panel concluded that the available data were insufficient to support the safety of this ingredient in cosmetics (CIR 1989). The studies that were needed in order to complete a safety assessment were 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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[1]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).

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

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 ≤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 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) (%)
Eyebrow 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
Total uses and concentration ranges for t-BuOH	**32**	0.0001–0.5

*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 $\lambda_{blood/air}$ to be 462 (95% confidence interval 440 to 484) for t-BuOH. The calculated $\lambda_{water/blood}$ was 1.31, the $\lambda_{oil/blood}$ was 0.363, and the $\lambda_{oil/water}$ 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)

	Formula number						
Authorized uses	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}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}C-t-BuOH from the blood. The first was a rapid phase, which probably represented the distribution of [^{14}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}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}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}C]t-BuOH. There was a half-life of 9 h similar to that seen following intravenous dosing with 350 mg/kg [^{14}C]t-BuOH (Arco Chemical Company 1994a).

Three Sprague-Dawley rats were placed in chambers and exposed to 1938 $\pm$ 93.4 ppm [^{14}C]t-BuOH (50 μC$_i$/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}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 β-[^{14}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 CO_2 and acetone. When the animals were injected with 1.5 g/kg of a 1:1 mixture of α-[^{13}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}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}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}C]t-BuOH, but rather one or more metabolites of [^{14}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}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_{50} 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_{50} (the quantity that caused death in half of the rabbits within 24 h) was 48 mmol/kg (3.56 g/kg). The ND_{50} (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_{50} 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_{50} 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_{50} for male rats was >4640 mg/kg, whereas for female rats the LD_{50} was interpolated to be about 2380 mg/kg. The LD_{50} 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 [^{14}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 [^{14}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 [^{14}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 [^{14}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$_1$ 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 trichloracetic 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 $B6C3F_1$ 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 $B6C3F_1$ 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 $B6C3F_1$ 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 $B6C3F_1$ 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 $B6C3F_1$ 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_{50} 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 $B6C3F_1$ 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_{90} 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 $B6C3F_1$ mice (10 male, 10 female) were exposed to t-BuOH for 6 h plus T_{90} 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^2, and after 10 mg t-BuOH was 43 mm^2. 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^2 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 $B6C3F_1$ mice (10 male, 10 female) were exposed to t-BuOH for 6 h plus T_{90} 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 $B6C3F_1$ 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 mmoles/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 μl/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 μl/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 μl/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 μl/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 μl 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 μg/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 μg/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 $B6C3F_1$ 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)

	Dose			
Kidney effects	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	48with 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.
**$p \leq .05$.
***$p \leq .01$.

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

	Dose			
Kidney effects	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.
**$p \leq .05$.
***$p \leq .01$.

$B6C3F_1$ 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 indecies . 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 $B6C3F_1$ mice (NTP 1995; Cirvello et al. 1995)

Thyroid gland effects	Sex	Dose			
		0 mg/ml	5 mg/ml	10 mg/ml	20 mg/ml
		Thyroid glands examined			
	M	60	59	59	57
	F	58	60	59	59
Follicular cell hyperplasia	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
	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
Follicular cell adenoma	M	1 with adenoma	0 with adenoma	4 with adenoma	1 with adenoma
	F	2 with adenoma	3 with adenoma	2 with adenoma	9 with adenoma**
Follicular cell adenoma or carcinoma	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			
	M	59	59	58	59
	F	59	60	59	57
Inflammation	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 Hygenists (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_{50} was 3.0 to 3.7 g/kg for t-BuOH and 2.7 g/kg for G-t-BuOH. The oral LD_{50} 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_{50} 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 $\pm$ 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 by100% 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 unIikely 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. Patholol.* 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 032394. 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 101492 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_{50} 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 phosphoinoisitide 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).[2]

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.[2]

CTFA. 1999. Maximum concentration of use—t-butyl alcohol. Unpublished data received from CTFA.[2]

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*, edi 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. Ovrum. 1969. Alcohol dermatitis. *Acta. Derm. Venereol.* 49:493–497.

Fregert, S., R. Hokanson, H. Rorsman, N. Tryding, and P. Ovrum. 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-napthol-induced oxidant stress in human and sheep erythrocytes. *J. Environ. Sci. Health A* 27:301–316.

[2]Available for review: Director, Cosmetic Ingredient Review, 1101 17th street, NW, suite 310, Washington, DC 2003b, USA.

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. Cattanach, 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.

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_1 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_1 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 α-2μ-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. Canterbery, 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 α-2μ- 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.