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

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*All interested persons are provided 60 days from the above release date (i.e., **July 14, 2024**) to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party, and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth.*

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ABBREVIATIONS

ACGIH	American Conference of Governmental and Industrial Hygienists
CAS	Chemical Abstracts Service
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
CTFA	Cosmetic, Toiletry, and Fragrance Association
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI)
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
ECHA	European Chemicals Agency
EPA	Environmental Protection Agency
F_0	first/parental generation
F_1	second/offspring generation
FCA	Freund's complete adjuvant
FDA	Food and Drug Administration
hCG	human chorionic gonadotropin
HRIPT	human repeated insult patch test
LOAEC	lowest-observed-adverse-effect concentration
LOAEL	lowest-observed-adverse-effect level
LD	lethal dose
MoE	margin of exposure
MoS	margin of safety
NADPH	nicotinamide adenine dinucleotide phosphate
ND	narcosis dose
NIOSH	National Institute of Occupational Safety and Health
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NoG	Notes of Guidance
NTP	National Toxicology Program
4NQO	4-nitroquinoline-1-oxide
OECD	Organisation for Economic Co-operation and Development
OPPTS	Office of Prevention, Pesticides, and Toxic Substances
Panel	Expert Panel for Cosmetic Ingredient Safety
RfD	reference dose
RIFM	Research Institute for Fragrance Materials
SCCS	Scientific Committee on Consumer Safety
SD	standard deviation
SED	systemic exposure dose
STEL	short-term exposure limit
T_{90}	time required for concentration to decrease to 90% of its initial value after exposure has ended
TG	test guideline
TLV	threshold limit value
TWA	time-weighted average
US	United States
UV	ultraviolet
VCRP	Voluntary Cosmetic Registration Program

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of *t*-Butyl Alcohol, which is reported to function as a denaturant, fragrance ingredient, and solvent in cosmetic products. The Panel reviewed the available data to determine the safety of this ingredient. The Panel concluded that *t*-Butyl Alcohol is safe in cosmetics in the present practices of use and concentration described in this safety assessment.

INTRODUCTION

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), *t*-Butyl Alcohol is reported to function as a denaturant, fragrance ingredient, and solvent.[1] This ingredient was first reviewed by the Expert Panel for Cosmetic Ingredient Safety (Panel) in a safety assessment that was published in 1989, in which the Panel concluded the data were insufficient to support the safety of *t*-Butyl Alcohol in cosmetics.[2] 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.[3] On the basis of the available animal and clinical data in that amended report, the Panel concluded that this ingredient is safe as used as cosmetic products.

In accordance with its Procedures, the Panel evaluates the conclusions of previously issued reports approximately every 15 yr, and it has been at least 15 yr since this assessment was issued. At its September 2023 meeting, the Panel determined that this safety assessment should be re-opened to evaluate developmental and reproductive toxicity effects seen at 1% in a liquid diet, to update their evaluation of previously reviewed carcinogenicity studies in rats, and to rectify the test concentration stated in a previously reviewed human repeat insult patch test (HRIPT). Additionally, the Panel noted an increase in reported uses and use concentrations, as well as a newly reported use of *t*-Butyl Alcohol in other baby products.

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last conducted February 2024. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Some of the data included in this safety assessment was found on the European Chemicals Agency (ECHA) website.[4] Please note that the ECHA website provides summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when ECHA is cited.

The original 1989 report did not include data required for the Panel to make a safety determination, including human sensitization data and results from a 90-d oral toxicity study. Therefore, summarized excerpts from the previous amended report on *t*-Butyl Alcohol, which include this information, as well as data from the original report, are disseminated throughout this document and are identified by *italicized text*.[3] (This information is not included in the tables or the summary section.)

CHEMISTRY

Definition and Structure

According to the *Dictionary*, *t*-Butyl Alcohol (CAS No. 75-65-0) is the aliphatic alcohol that conforms to the structure in Figure 1.[1]

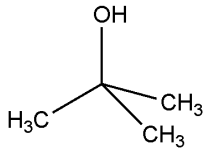

Figure 1. *t*-Butyl Alcohol

Chemical Properties

t-Butyl Alcohol is available in the form of colorless, hygroscopic crystals with a camphoraceous odor and is reported to have a molecular weight of 74.12 g/mol.[3] In the form of vapor, t-Butyl Alcohol is a moderate explosion hazard when exposed to flame and reacts violently with hydrogen peroxide. t-Butyl Alcohol is stable under typical conditions of cosmetic use. The estimated log K_{ow} of *t*-Butyl Alcohol is 0.35.[5] Additional chemical properties of *t*-Butyl Alcohol are presented in Table 1.

Method of Manufacture

t-Butyl Alcohol has been prepared from acetyl chloride and dimethylzinc, by catalytic hydration of isobutylene, via reduction of t-butyl hydroperoxide, by absorption of isobutene, from cracking petroleum or natural gas, and from sulfuric acid with subsequent hydrolysis by steam.[3] Following these steps, it is purified by distillation. t-Butyl Alcohol is also produced as a by-product from the isobutane oxidation process for producing propylene oxide.

Impurities

t-Butyl Alcohol used in cosmetics typically contains 99.5% t-Butyl Alcohol, a maximum of 0.002% acidity (as acetic acid), a maximum of 0.1% water, and a maximum of 0.001% nonvolatile matter.[3]

Natural Occurrence

The presence of t-Butyl Alcohol is ubiquitous in the environment.[3] *Fusel oil, the congeners or by-products 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%. t-Butyl Alcohol has been detected in drinking water.*

t-Butyl Alcohol is reported to naturally occur in foods.[6] A few dietary sources of *t*-Butyl Alcohol include fresh apple, beef, cheese, chicken, coffee, grape (*Vitis* species), guava and feyoa, *Mangifera* species, walnut (*Juglans* species), and wine.

USE

Cosmetic

The safety of the cosmetic ingredient reviewed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of this ingredient in cosmetics and does not cover its use in airbrush delivery systems. Data included herein were obtained from the FDA's Voluntary Cosmetic Registration Program (VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data were provided by cosmetic product categories, based at that time on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 VCRP survey data, *t*-Butyl Alcohol was reported to be used in 136 formulations, 115 of which were in leave-on formulations; in 1998, 32 uses were reported (Table 2).[7] The results of the concentration of use survey conducted by the Council in 2022 indicate that the highest reported maximum concentration of use for *t*-Butyl Alcohol was at up to 0.91% in aftershave lotions.[8] In 1999, the highest reported frequency of use for *t*-Butyl Alcohol was at up to 0.5% in hair spray aerosol fixatives. *t*-Butyl Alcohol had 1 reported use in other baby products (concentration of use was not provided).

t-Butyl Alcohol is used in products which are used near the eye (at up to 0.01%, in mascaras) and in those which may be incidentally ingested (e.g., at up to 0.028% in dentifrices). Additionally, *t*-Butyl Alcohol is reported to be used at up to 0.11% in perfumes, and in several cosmetic formulations that could possibly be in spray or powder form. In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.

Although products containing this ingredient may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of this ingredient (and without consumer habits and practices data or particle size data related to this use technology), the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

t-Butyl Alcohol is not restricted from use in any way under the rules governing cosmetic products in the European Union.[9]

Non-Cosmetic

t-Butyl Alcohol has been used as a flotation agent, a dehydration agent, a solvent, a chemical intermediate, an octane booster in gasoline, and in paint removers.[3] *Additionally, t-Butyl Alcohol has been used as a denaturant for alcohol in commercial sunscreen preparations.*

t-Butyl Alcohol is used as a defoaming agent for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food (27CFR176.200). *t*-Butyl Alcohol is used in surface lubricants employed in the manufacture of metallic articles that contact food (21CFR178.3910). *t*-Butyl Alcohol is also authorized for use as an alcohol denaturant in various cosmetic and non-cosmetic formulations (27CFR21.101; 27CFR21.151). The authorized amount of *t*-Butyl Alcohol used to denature 100 gallons of SD 39-B alcohol (27CFR21.71), SD 40 alcohol (27CFR21.74), SD 40-A alcohol (27CFR21.75), and SD 40-B alcohol (27CFR21.76) is 1/8 gallon, and 3 gallons for SD 40-C alcohol (27CFR21.77).

TOXICOKINETIC STUDIES

Dermal Absorption

Animal

Male Sprague-Dawley rats received a single topical application of undiluted [^{14}C]*t*-Butyl Alcohol to clipped skin (approximate dose: 2 MBq/kg, 7.5 mg/cm^2) in a dermal absorption study, performed in accordance with Organisation for Economic Cooperation and Development (OECD) test guideline (TG) 417.[4] The test material was applied within a silicone rubber saddle to an area of ~ 12 cm^2 for 6 h. Carbon filters were placed into the saddle and were covered with stainless steel gauze during exposure. Rats were killed in groups of 4, after exposure for 6, 24, and 72 h. For each of the sacrifice times, less than 1% of the applied dose was recovered in the tissues; less than 1.5% of the applied dose was detected in the excreta. Most 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 low potential for dermal absorption and bioaccumulation.

Absorption, Distribution, Metabolism, and Excretion

t-Butyl Alcohol is a hydroxyl radical scavenger.[3] In rat liver microsomes, it can be oxidatively demethylated by hydroxyl radicals generated from nicotinamide adenine dinucleotide phosphate (NADPH)-dependent microsomal electron transfer to yield formaldehyde and acetone. Additionally, t-Butyl Alcohol is not a substrate for alcohol dehydrogenase or for catalase. In experimental data, the partition coefficient $\lambda_{blood/air}$ for t-Butyl Alcohol was determined to be 462 (95% confidence interval: 440 - 484), the calculated $\lambda_{water/blood}$ was 1.31, the $\lambda_{oil/blood}$ was 0.363, and the $\lambda_{oil/water}$ was 0.278.

Animal

Oral

The elimination of t-Butyl Alcohol from rat blood has been shown to be slow.[3] Female Wistar rats (number unspecified) received t-Butyl Alcohol (25 mmol/kg) dissolved in water, via gavage. The t-Butyl Alcohol blood concentration was 13.24 mM at 2 h, 12.57 mM at 5 h, and 11.35 mM at 20 h. Female Sprague-Dawley rats received a 5.7 (w/v) solution of t-Butyl Alcohol, in saline, every 8 h for 1 or 2.5 d, in order to maintain a uniform blood concentration of 60 – 100 mg %. Blood was sampled after t-Butyl Alcohol administration was increased to elevate blood concentrations to 125 – 150 mg %. Rats treated for 2.5 d took 18 h to eliminate t-Butyl Alcohol completely from the blood, while rats treated for 1 d took 26 h. The elimination rate for 1200 mg/kg t-Butyl Alcohol was 0.7 mmol/kg rat/h. Two Sprague-Dawley rats were given 1500 mg/kg [^{14}C]t-Butyl Alcohol, via gavage; blood samples were obtained at various times. The slow rate at which the radiolabel was eliminated from the blood indicated that 1500 mg/kg t-Butyl Alcohol had saturated the elimination pathways. A half-life of 9 h was observed when 3 animals were given 500 mg/kg [^{14}C]t-Butyl Alcohol.

Groups of Sprague-Dawley rats (2/group) were treated by gavage with 1, 30, 500, or 1500 mg/kg [^{14}C]t-Butyl Alcohol and placed in metabolism cages. Results of reverse-phase high-performance liquid chromatography analyses showed that most of the radioactivity recovered was of t-Butyl Alcohol metabolites, rather than t-Butyl Alcohol itself. It was presumed that metabolites were mostly excreted in the urine and t-Butyl Alcohol was eliminated from the body in expired air. Three Sprague-Dawley rats were given [^{14}C]t-Butyl Alcohol (350 mg/kg), via gavage. Urine and feces were collected after 24 h; only about 1% of the administered dose was excreted in the feces. It was concluded that a conjugate of t-Butyl Alcohol or its metabolites was not excreted to any appreciable extent in the bile. Three chinchilla rabbits had 12 mmol of t-Butyl Alcohol administered, via gavage; t-Butyl Alcohol was conjugated to a large extent with glucuronic acid, and glucuronides are excreted in urine. As a percentage of dose, the average extra glucuronic acid excreted over 24 h was 24.4%.

Groups of 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.[10] Urine samples were collected at 24-h intervals for 48 h. The major urinary metabolites identified in [^{13}C]*t*-Butyl Alcohol-dosed rats were *t*-butyl alcohol sulfate, 2-hydroxyisobutyrate, and 2-methyl-1,2-propanediol. [^{13}C]Acetone, *t*-Butyl Alcohol, and its glucuronide represented minor metabolites in rat urine.

Inhalation

Three Sprague-Dawley rats were placed in chambers and exposed to 1938 ± 93.4 ppm [^{14}C]t-Butyl Alcohol (50 µCi/mmol) for 6 h.[3] Results indicated that [^{14}C]t-Butyl Alcohol is eliminated at approximately the same rate following 6 h of 2000 ppm exposure to t-Butyl Alcohol vapors as the rate following oral dosing with 1 mg or 500 mg/kg t-Butyl Alcohol.

In a pharmacokinetics study, groups of male and female Fischer 344 rats (4/sex/group) were subjected to a whole-body inhalation exposure of 250, 450, or 1750 ppm *t*-Butyl Alcohol, 6 h/d, for 1 or 8 d, in glass chambers.[11] No controls were used. Rats were killed 2, 4, 6 and 8 h after the final exposure, for both durations of exposure. Blood, liver, and kidneys were collected for analyses. For both sexes, concentrations of *t*-Butyl Alcohol were similar in the blood, liver, and kidneys following a single 6-h exposure, and blood and tissue concentrations of *t*-Butyl Alcohol were lower following repeated exposures. However, concentrations differed between males and females following the repeated, 8-d exposure. The researchers stated this finding possibly corroborated the pharmacokinetic model of *t*-Butyl Alcohol binding to α2u-globulin (a protein found in the kidney) in male rats.

Multiple Exposure Routes

t-Butyl Alcohol was shown to be slowly eliminated from the blood of mice.[3] Nine male Swiss-Webster mice first received a single i.p. dose of 8.1 mmol/kg t-Butyl Alcohol; the test article was eliminated from the blood in 8 to 9 h. After receiving the i.p. dose, mice were exposed to t-Butyl Alcohol vapor for 3 d; t-Butyl Alcohol was not detected in the blood 3 h after mice were removed from the vapor chamber. Similar results were obtained in another study using mice (unspecified number) in which a single i.p. dose of t-Butyl Alcohol (8.1 mmol/kg) was administered 4 h after exposing mice to 3-d inhalation of t-Butyl Alcohol (amount not specified). The researchers surmised that the increased elimination rate may have been due to increased conjugation and elimination in mice previously exposed to t-Butyl Alcohol.

Human

Oral

In a metabolism study, a male subject weighing 80 kg was given 5 mg/kg [^{13}C]*t*-Butyl Alcohol orally as a gel capsule.[10] Urine was collected in 12-h intervals for 48 h; 2-methyl-1,2-propanediol and 2-hydroxyisobutyrate were the major metabolites detected in urine via ^{13}C NMR analysis. Unconjugated *t*-Butyl Alcohol and *t*-butyl alcohol glucuronide were present as minor metabolites; traces of *t*-butyl alcohol sulfate were also present.

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Dermal

In a study in which 2000 mg/kg t-Butyl Alcohol (99.9% pure) was applied neat to the abraded skin of 5 male and 5 female New Zealand albino rabbits, the minimum lethal dose for t-Butyl Alcohol was determined to be > 2000 mg/kg.[3] Undiluted gasoline grade t-Butyl Alcohol (~95% pure; amount not specified) was applied to the abraded skin of 5 male and 5 female New Zealand albino rabbits; the acute dermal LD_{50} for gasoline-grade t-Butyl Alcohol was determined to be > 2000 mg/kg.

Oral

The acute oral LD_{50} of t-Butyl Alcohol in white rats (sex and number not specified) was determined to be 3500 mg/kg.[3] The calculated oral LD_{50} values for groups of male and female Sprague-Dawley rats (5/sex/group) that received a single dose of 1950, 2535, 3296, or 4285 mg/kg undiluted (99.9%) t-Butyl Alcohol, via gavage, were 3384 mg/kg for males, 2743 mg/kg for females, and 3046 mg/kg for both sexes (combined). The calculated oral LD_{50} values for gasoline-grade t-Butyl Alcohol (95%), when administered in a single oral dose of 1500, 1950, 2535, 3296, or 4285 mg/kg to male and female Sprague Dawley rats were 3046 mg/kg for males, 2298 mg/kg for females, and 2733 mg/kg for both sexes (combined). Groups of male and female Wistar rats (5/sex/group) received a single dose of 1470, 2150, 3160, or 4640 mg/kg t-Butyl Alcohol, via gavage. The acute oral LD_{50} values were determined to be: >4640 mg/kg for males, ~ 2380 mg/kg for females, and 3720 mg/kg for both sexes (combined). The LD_{50} and ND_{50} values for t-Butyl Alcohol were determined to be 3560 mg/kg and 1410 mg/kg, respectively, in an acute oral toxicity study using 10 – 35 rabbits.

Inhalation

The acute exposure of 6 Sprague-Dawley rats to 10,000 ppm t-Butyl Alcohol for 1 d produced severe narcosis in all animals and death in 5 animals.[3] Reducing the concentration to 5000 ppm t-Butyl Alcohol still produced narcosis in all exposed animals. In another acute inhalation study, groups of male and female Sprague-Dawley rats (5/sex/group) were placed in chambers and exposed to 10,000 ppm t-Butyl Alcohol for approximately 4 h. The principal signs exhibited during exposure were ocular discharge, dyspnea, and prostration. One female rat died; 1 rat also exhibited ataxia. Upon necropsy, 4 rats (3 males and the female that died) were observed to have red foci on the lungs. Two groups of albino rats (5/sex/ group) were exposed to vapor atmospheres of 9700 or 14,100 ppm gasoline-grade t-Butyl Alcohol for approximately 4 h. None of the animals in the 9700 ppm group died, while all animals in the 14,100 ppm group died during the study. Red foci were found in the lungs of both groups of animals.

Short-Term Toxicity Studies

Oral

Groups of male and female $B6C3F_1$ mice (5/sex/group) received 0, 0.125, 0.25, 0.5, 1, or 2% (w/v) t-Butyl Alcohol in drinking water for 14 d. Upon study termination, the caudate liver of 1 treated female (dose unspecified) was atrophied; all other control and treated mice survived the study period and were in good physical condition at study termination. It was therefore concluded that t-Butyl Alcohol did not cause gross organ or tissue damage at the tested doses. Male Wistar rats (n= 5 – 6) were given 0.5% (v/v) t-Butyl Alcohol in water for 10 wk; controls received plain water. Treated rats showed significant decreases in body weight and kidney glutathione concentrations, an insignificant decrease in the liver triglyceride concentration, and an increase in serum triglyceride and serum glucose concentrations, compared to controls. In another 10-wk study, the alterations such as centrilobular necrosis, vacuolization in hepatocytes, loss of hepatic architecture, periportal proliferation, lymphocytic infiltration, degeneration of renal tubules, degeneration of the basement membrane of the Bowman capsule, diffused glomeruli, and vacuolation of glomeruli were noted.

Inhalation

Groups of male and female B6C3F$_1$ mice and F344 rats (5/sex/group) were exposed via whole body inhalation to 0, 450, 900, 1750, 3500, or 7000 ppm t-Butyl Alcohol for 6 h plus the time required for t-Butyl Alcohol concentration to decrease to 90% of its initial value after exposure had ended (T_{90}) per day, 5 d/wk, over an 18-d period.[3] All animals in the 7000 ppm group died on day 2. 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). The liver weights of male and female mice exposed to 3500 ppm were significantly greater than those of the controls. Also, thymus weights were significantly less than those of the controls for male and female rats and female mice exposed to 3500 ppm t-Butyl Alcohol. Male and female F344 rats (5/sex/group) received a 6 h/d inhalation exposure to 0, 250, 450, or 1750 ppm t-Butyl Alcohol for 10 d. A statistically significant decrease in the absolute and relative liver weight was observed in males in the 1750 ppm group, compared to controls. Relative kidney weights were significantly increased in 1750 ppm males and 450 and 1750 ppm females, compared to controls. A α2u-globulin-immunohistochemical staining revealed positive staining of protein droplets within the renal proximal tubules in only control and treated male rats. No significant differences in renal cell proliferation were observed in control and treated female rats; these results suggested that t-Butyl Alcohol interacts with α2u-globulin in the male rat kidney.

Subchronic Toxicity Studies

Oral

t-Butyl Alcohol was administered to male and female B6C3F$_1$ mice and F344 rats (route of oral administration, duration, dose, and number of animals unspecified) in a subchronic oral toxicity study.[3] For mice, fatty changes in the liver were observed in males, chronic inflammation and hyperplasia of transitional cell epithelium of the bladder, and hyperplasia and neoplasia of the thyroid were seen in both sexes. Mineralization of the kidney, nephropathy, and transitional cell epithelial hyperplasia were observed in both male and female rats. 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. Statistically insignificant increased tumor rates included testicular interstitial adenomas and thymomas in male rats and increased lung adenomas and pituitary adenomas/carcinomas in female rats. Groups of B6C3F$_1$ mice and F344 rats (10/sex/group) received 0.25, 0.5, 1, 2, or 4% t-Butyl Alcohol (w/v) in drinking water for 13 wk. In mice, 4 male mice in the 4% group died and 5 male mice in the 2% group died. One female in the control group died; all deaths, except 1, occurred in the first week of the study. Body weight gains were 11.7 – 32.5% less than controls for the male mice, except for the 0.25% group. Female mice outgained their controls except for the 0.5% group. Microscopically, there was transitional epithelial hyperplasia with cystitis in the urinary bladders of 6 male mice and 4 female mice in the 4% group. Transitional cell hyperplasia was found in the urinary bladders of 5 male mice in the 2% group. In rats, 9 males and 2 females in the 4% group died between wk 4 and wk 13. A reduction in growth rate was seen in males in the 1% and higher dose levels; controls outgained them by 16 to 104% and control females outgained the 2 and 4% groups by 11 and 46%, respectively. Papillary hyperplasia of the transitional epithelium of the urinary bladder in 5 males and 2 females in the 4% group; a decrease in the cell population of bone marrow was also seen in 9 males and 3 females from this group. In another 13-wk study, B6C3F$_1$ mice and F344 rats (10/sex/group) were given 0, 2.5, 5, 10, 20, or 40 mg/ml t-Butyl Alcohol in drinking water. Treatment-related mortality occurred at the highest concentration in male and female mice and rats, mean body weight gains were significantly lower in treated groups compared to controls, and there was decreased water consumption in all treated rats and in 20 and 40 mg/ml mice. Transitional cell hyperplasia and inflammation of the bladder mucosa were considered treatment-related, and were limited to the 20 and 40 mg/ml groups of male mice and rats and the 40 mg/ml groups of female mice and rats. For male mice and rats, the incidence and severity of the urinary bladder lesions were higher than those for females. 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. In a 95-d study, B6C3F$_1$ mice and F344 rats (10/sex/groups) were given 0, 0.25, 0.5, 1, 2, or 4% (w/v) in drinking water. All high-dose rats and 6 male and 4 female high-dose mice died before the end of the study. Gross lesions in mice included thickened urinary bladder walls or plaques on the mucosa, while 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. Nephropathy was significantly increased in all treated groups, except for the 4% dose group. Calculated no-effect-levels for subchronic toxicity in rodents are less than 0.5% in male mice, 1% in female mice, 0.25% in male rats, and 1% in female rats. No-effect levels for the urinary tract lesions were calculated to be 1% in male mice and rats and 2% for female mice and rats.

Inhalation

In a subchronic study, groups of B6C3F$_1$ mice and F344 rats (10/sex/group) were exposed via inhalation to 135, 270, 540, 1080, or 2100 ppm t-Butyl Alcohol for 6 h plus T_{90} per day, 5 d/wk, for 13 wk.[3] One male mouse from the 2100 ppm group died. Body weight gains were similar to those of the controls for all treated rats but were significantly less for males from the 135 and 270 ppm male groups and from the 1080 and 2100 ppm female mice groups. Kidney weights of 1080 ppm males rats and 2100 ppm male and female rats were significantly greater than those of the controls. Similarly, liver weights in the 1080 and 2100 ppm female mice and rats were greater than those of the controls.

The local respiratory effects of *t*-Butyl Alcohol were evaluated in a subchronic inhalation toxicity study.[12] Rats (5/group/sex) were exposed, whole-body, 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 no-observed-adverse-effect concentration (NOAEC) for local respiratory effects was determined to be 6366.13 mg/m^3.

Chronic Toxicity Studies

Oral

In a 2-yr study, groups of male and female B6C3F$_1$ mice (60/sex/group) were given t-Butyl Alcohol, in drinking water, at doses of 0, 540, 1040, or 2070 mg/kg for males and 0, 510, 1020, or 2110 mg/kg for females.[3] Concurrently, groups of 60 male F344 rats were given 0, 90, 200, or 420 mg/kg t-Butyl Alcohol in drinking water; groups of 60 female 344 rats were given 0, 180, 330, or 650 mg/kg t-Butyl Alcohol in drinking water. For mice, water consumption was similar in exposed and control groups; water consumption increased with increasing dosage for male rats and decreased with increasing dosage for female rats. Survival of the male rats from the high-dose group (420 mg/kg) was significantly lower than that of controls. Survival among exposed female rats was lower than that of controls, especially in the high-dose (650 mg/kg) group; however, more than 50% of the females in each group survived through wk 85.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

In Vitro

The effect of t-Butyl Alcohol on the in vitro fertilization of Swiss-Webster mice gametes was evaluated.[3] Capacitated epididymal mouse spermatozoa were added to mouse oocytes with cumulus masses, and after a 24-h incubation, the eggs were examined for fertilization. The addition of 87 mM t-Butyl Alcohol to both the capacitation and the culture media did not affect the in vitro fertilization capacity of spermatozoa. The teratogenic effects of t-Butyl Alcohol were studied using cells from chicken embryo wing buds. Cultures were fed with medium containing 0.1 to 4% Butyl Alcohol; t-Butyl Alcohol was effective at enhancing cartilage differentiation. The researchers postulated that this effect could interfere with proper skeletal morphogenesis.

Animal

Oral

Groups of pregnant Swiss-Webster mice (n = 15/group) were fed a liquid diet containing t-Butyl Alcohol at concentrations of 0.5, 0.75, or 1% (w/v) from day 6 to 20 of gestation.[3] Controls were fed only a liquid diet. The average maternal weight gain over the 20 d was 64% for the controls and 62, 52, and 51% for the low-, mid-, and high-dose groups, respectively. Within 24-h of delivery, approximately one-half of maternal animals in each group were replaced with untreated surrogate dams to determine the role of maternal nutritional and behavioral factors on the young. A dose-response relationship was observed between dietary t-Butyl Alcohol and the total number of stillbirths; there were 3 stillborns in the control group, 6 in the 0.5% group, 14 in the 0.75% group, and 20 in the 1% group. Postnatal weight gain was decreased over the first 10 d in the non-fostered 0.75 and 1% groups in comparison to the other groups. A general dose-response relationship between higher in utero exposure to t-Butyl Alcohol and poorer behavioral performance of pups was observed. Fostered pups performed significantly better than non-fostered pups in 3 of 4 behavioral tests; all tested groups did eventually recover and acquire the same level of performance. In a 2-yr study, male and female B6C3F$_1$ mice and male and female F344 rats were administered t-Butyl Alcohol in drinking water. No significant differences were found in the weight of the testis, epididymis, and cauda or sperm motility, count, and morphology of male B6C3F$_1$ mice dosed with up to 2070 mg/kg t-Butyl Alcohol and male F344 rats dosed with up to 420 mg/kg t-Butyl Alcohol for 2 yr in drinking water, compared to controls. No significant differences were found in the estrous cycle length or percentage of time spent in the various estrous stages of female B6C3F$_1$ mice dosed with up to 2110 mg/kg t-Butyl Alcohol and female F344 rats dosed with up to 650 mg/kg t-Butyl Alcohol for 2 yr in drinking water, compared to controls. The estrous cycle length of female mice in the high-dose group was significantly increased; the length of various estrous stages was not different from controls. In a prenatal exposure study, pregnant CBA/J and C57BL/6J mice (numbers unspecified) were treated by gavage every 12 h with 10.5 mmol/kg t-Butyl Alcohol from day 6 through day 18 of gestation. Eight of the 21 litters in the treated groups had all the fetuses resorbed compared to none in controls. There was also a significant decrease in the number of live fetuses/litter and a slight but insignificant decrease in the weight of the surviving fetuses. Reduced maternal weight gain, litter sizes, birth weights, and weights at weaning, and increased peri-natal mortality (from 2 to 14%) and post-natal mortality (from 6 to 100%) were observed in the pups of pregnant Long-Evans rats exposed to liquid diets containing up to 10.9% (v/v) t-Butyl Alcohol from gestation day 8 until parturition. In a fetal toxicity study, Long Evans rat pups received milk formula containing a mean daily dose of 600 – 2690 mg/kg t-Butyl Alcohol, fed through an implanted gastric fistula, on postnatal days 4 through 7 (followed by milk formula) for the next 11 d. Only 26 of 48 animals survived the experiment; the major cause of death was a poor fistulation procedure or gastric bloating. No significant developmental differences were observed between treated pups and controls. Brains of treated pups weighed significantly less than that of controls. Treated pups had decreased protein in the forebrains and decreased deoxyribonucleic acid (DNA) in the hindbrains.

Details of the oral developmental and reproductive toxicity studies summarized below can be found in Table 3.

The acute testicular toxicity of *t*-Butyl Alcohol was evaluated in male CD-1 mice.[13] After an initial determination of testosterone levels, animals (5/group) were given 0, 400, 1000, or 2000 mg/kg *t*-Butyl Alcohol, in canola oil, via gavage.

Two non-treatment related deaths occurred in the 400 mg/kg group (complications from gavage). No difference was observed in the % change of fecal testosterone or in the serum testosterone of animals treated with *t*-Butyl Alcohol, compared to controls. Testis weights of mice in the 1000 and 2000 mg/kg groups averaged 14% higher than the control and 400 mg/kg groups ($p \leq 0.05$); the only significant histological difference was a higher percentage of tubules in the testes, compared to control animals, along with sloughing ($7 \pm 2\%$, mean SD, $p \leq 0.05$). A developmental and reproductive toxicity study was performed using male and female albino Sprague-Dawley rats (12/sex/group); animals received 0, 64, 160, 400, or 1000 mg/kg bw/d *t*-Butyl Alcohol, in water, via gavage, in accordance with OECD TG 421.[4] No mortality occurred in the parent generation; mild central nervous toxicity appeared 1 – 2 h after dosing in the 1000 mg/kg bw/d group and between the second and fourth wk of dosing in the 400 mg/kg group; no other significant parental effects were observed. There was a significant reduction in the number of live born pups/pregnancy for dams in the 1000 mg/kg group. Survival reduced to 80% on postnatal day 4 and 50% on postnatal day 21 in pups treated with 1000 mg/kg compared to 100% survival in other treatment groups. Offspring born to 1000 mg/kg bw/d dams exhibited lower mean body weights than control offspring; no effects were observed at lower doses. The no-observed-adverse-effect level (NOAEL) values were determined to be 400 mg/kg bw/d for developmental/reproductive effects and 160 mg/kg bw/d for overall toxicity.

Inhalation

Groups of B6C3F$_1$ mice and F344 rats (10/sex/group) received inhalation exposure to 135, 270, 540, 1080, or 2100 ppm t-Butyl Alcohol for 6 h plus T$_{90}$/d, 5 d/wk, for 13 wk.[3] No significant differences were found in the weight of testis, epididymis, and cauda, or sperm motility, count, and morphology or in the estrous cycle length and percentage of time spent in the various estrous stages of treated animals and controls. Inseminated female Sprague-Dawley rats (n = 15 - 20) were exposed to 0, 2000, 3500, or 5000 ppm t-Butyl Alcohol for 7 h/d, via inhalation, in exposure chambers, until day 20 of gestation. Fetotoxicity generally increased with increasing dosage, and fetal weights were slightly depressed at all concentrations of t-Butyl Alcohol. The researchers concluded that exposure to t-Butyl Alcohol evidenced developmental toxicity with effects seen at all concentrations, although these were associated with maternal toxicity.

Details of the inhalation developmental and reproductive toxicity studies summarized below also can be found in Table 3.

Male Sprague-Dawley rats were exposed, whole-body, to vaporized *t*-Butyl Alcohol at 6000 or 12,000 mg/m^3 for 6 wk (7 h/d, 7 d/wk) prior to being mated with non-exposed females.[4] Paternal body weight gain was unrelated to treatment. Differences in behavioral test performance were seen in pups sired by males in both treatment groups, compared to controls. Five pair-wise comparisons in neurotransmitter measurements in pups were statistically significant. Mean concentrations of norepinephrine and β-endorphin were reduced in the cerebellum and met-enkephalin was reduced in the cerebrum of pups sired by 12,000 mg/m^3 males and levels of serotonin in the midbrain and met-enkephalin in the cerebrum were reduced in pups sired by 6000 mg/m^3 males, compared to control pups. The lack of a dose-response relationship or pattern in these effects led the researchers to conclude that these effects likely have little to no biological significance. The lowest-observed-adverse-effect-concentration (LOAEC) and no-observed–effect concentration (NOEC) for paternal body weight and weight gain were determined to be ≥ 12,000 mg/m^3 and 6000 mg/m^3, respectively. The NOAEC for male and female pups was determined to be ≥ 12,000 mg/m^3. Pregnant Sprague-Dawley rats (number/group not specified) were exposed, whole-body, to vaporized 6000 or 12,000 mg/m^3 *t*-Butyl Alcohol from day 1 to day 20 of gestation (upon mating with non-exposed males).[4] A decrease in body weight and food consumption and an increase in water consumption was observed in the 12,000 mg/m^3 dams; no changes were observed in 6000 mg/m^3 dams. Two pair-wise comparisons were statistically significant in behavioral tests of pups born to dams from each treatment group. Five pair-wise comparisons were statistically significant for neurotransmitter measurements of pups delivered by exposed dams; pups born to 12,000 mg/m^3 dams had reduced levels of norepinephrine and β-endorphin in the cerebellum, and reduced met-enkephalin in the cerebrum, while pups born to 6000 mg/m^3 dams had reduced serotonin in the midbrain and met-enkephalin in the cerebrum, compared to controls. The relative severity observed in dams from the high-dose group, in the absence of a dose-response relationship for developmental neurotoxicity in pups, 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.

GENOTOXICITY STUDIES

In Vitro

t-Butyl Alcohol was reported to be non-mutagenic in an Ames test using Salmonella typhimurium "even at high concentrations" in the presence of metabolic activation.[3] t-Butyl Alcohol (100 – 10,000 µg/plate) did not induce mutations in S. typhimurium strains TA98, TA100, TA1535, or TA1537, with or without metabolic activation. In another Ames test, S. typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 were tested with t-Butyl Alcohol at concentrations ranging from 2.9 – 10,000 µg/plate. The results indicated that t-Butyl Alcohol did not cause a significant increase in the number of revertants/plate in any of the strains, with or without metabolic activation; however, there was a slight increase in TA1535 revertants/plate, in the presence and absence of metabolic activation. In a similar test, gasoline-grade t-Butyl Alcohol caused a weak but significant increase in TA1535 revertants/plate, both in the presence and absence of metabolic activation.

No significant increases in mutant frequencies were observed in L5178Y mouse lymphoma cells treated with up to 100 µl/ml t-Butyl Alcohol (99.9%) and gasoline grade t-Butyl Alcohol, in the presence or absence of metabolic activation, with the exception of an increase seen with gasoline grade t-Butyl Alcohol in the absence of metabolic activation. This response was not dose-related and t-Butyl Alcohol was considered non-mutagenic. A small increase in the mutant fraction was seen in an experiment in which L5178Y mouse lymphoma cells were treated with t-Butyl Alcohol without metabolic activation. This result was not reproducible in 3 other experiments in which t-Butyl Alcohol was tested at concentrations of up to 5000 µl/mg.

t-Butyl Alcohol was mutagenic and exhibited a mean lethal concentration of 80 mM in human Chinese hamster ovary cells. An insignificant increase in sister chromatid exchange frequency was observed in Chinese hamster ovary cells treated with up to 20 µl/ml t-Butyl Alcohol, compared to controls. In another study, t-Butyl Alcohol was tested at up to 20 µl/ml for 2 h with metabolic activation and for 24 h without metabolic activation. t-Butyl Alcohol caused a significant increase in sister chromatid exchanges at the high dose without metabolic activation and at the 2 highest doses with metabolic activation.

Details of the in vitro genotoxicity studies summarized below can be found in Table 4.

t-Butyl Alcohol, in dimethyl sulfoxide (DMSO) or water, was not genotoxic in an Ames test performed in accordance with OECD TG 471 when tested at concentrations up to 5000 µg/plate using *S. typhimurium* strain TA102, with or without metabolic activation.[14] In another Ames test, *t*-Butyl Alcohol was tested at 0, 0.75, 1.5, 2.25, 3, or 3.75 mg/plate, using *S. typhimurium* TA102, with metabolic activation.[4] The maximum number of revertants/plate reached approximately 800 at the 2.25 mg/plate concentration compared to 400 revertants/plate for controls. At higher concentrations, the number of revertants/plate decreased in a dose-dependent manner; in the absence of data for negative or solvent controls, the significance of these values is unknown. *t*-Butyl Alcohol was not genotoxic when tested using Chinese hamster ovary cells in a sister chromatid exchange assay (OECD TG 479) or in an in vitro mammalian chromosome aberration test (OECD TG 473), when tested at up to 5000 µg/ml in McCoy's 5A medium, with and without metabolic activation.[4] *t*-Butyl Alcohol was tested at up to 30 mmol/l without metabolic activation in a Comet assay.[4] The article seemed to induce DNA damage in a dose-dependent manner; however, this was partly attributed to cytotoxicity (which had ambiguous results).

CARCINOGENICITY STUDIES

Dermal

The carcinogenic potential of dermally applied t-Butyl Alcohol was evaluated in female ddN mice.[3] 4-nitroquinoline-1-oxide (4NQO) was dermally applied followed by applications of 16.6% t-Butyl Alcohol (actual dosage not specified), in benzene, 6 times/wk for a total of 270 applications. No acute skin damage was observed within 100 d; after 350 d, 2 "erosions" were produced at the application site, which remained for the duration of the observation period. About 150 d after the start of the experiment, and after about 100 applications of t-Butyl Alcohol, 1 neoplasm was observed, which "developed into squamous cell carcinoma rapidly." About 300 d after the start of the experiment, a subcutaneous granuloma was detected. Fifty mice survived after the appearance of the first tumor in the experiment.

Oral

In a 2-yr study, groups of male and female B6C3F$_1$ mice (n = 60/group) were given 540, 1040, or 2070 mg/kg t-Butyl Alcohol and 510, 1020, or 2110 mg/kg t-Butyl Alcohol in drinking water, respectively. The incidence of follicular cell hyperplasia of the thyroid gland was significantly increased in all treated groups of male mice and in the female mice from the 1020 and 2110 mg/kg groups. The incidence of thyroid follicular cell adenoma was significantly increased in high-dose female mice (2110 mg/kg). One thyroid follicular cell carcinoma was observed in a high-dose male (2070 mg/kg). Effects on the urinary bladders included inflammation and hyperplasia of the transitional epithelium for males in the highest dose group and inflammation for females in the highest dose group. There was "equivocal evidence of carcinogenic activity" of t-Butyl Alcohol in male B6C3F$_1$ mice due to marginally increased incidences of follicular cell adenoma or carcinoma of the thyroid gland. Due to the increased incidence of the same in female mice, the researchers concluded that there was "some evidence of carcinogenic activity" of t-Butyl Alcohol in female mice.

Concurrently, groups of male and female F344 rats (n = 60/group) were given 90, 200, or 420 mg/kg t-Butyl Alcohol, and 180, 330, or 650 mg/kg t-Butyl Alcohol, in drinking water, respectively. Proliferative lesions (hyperplasia, adenoma, and carcinoma) in the kidneys of treated male rats, nephropathy in all treated females, and in males given 420 mg/kg t-Butyl Alcohol was observed. Female rats in the 330 and 650 mg/kg groups also exhibited inflammation of the kidneys. Based on the increased incidence of renal tubule adenomas or carcinoma, the researchers concluded that there was "some evidence of carcinogenic activity" of t-Butyl Alcohol in male F344 rats; no evidence of carcinogenic activity in female rats was observed.

OTHER RELEVANT STUDIES

Endocrine Effects

The interactive potential of *t*-Butyl Alcohol was evaluated in an androgen receptor binding assay, performed in accordance with US Environmental Protection Agency (EPA) Office of Prevention, Pesticides, and Toxic Substances (OPPTS) 890.1150.[15] Prostate glands from 90-d-old Sprague-Dawley rats were used to prepare cytosol for the experiments. The reference compound R1881 was used as the positive control; dexamethasone was used as a weak positive control.

t-Butyl Alcohol solutions (up to 10^{-3} M) were tested in tubes containing androgen receptors isolated from the rat prostate tissue in 3 non-concurrent, competitive binding assays. *t*-Butyl Alcohol was classified as a "non-binder" in all 3 assay runs (mean specific binding ≥ 50%); the mean relative binding affinity could not be calculated.

In a steroidogenesis assay, performed in accordance with OECD TG 456, H295R cells were treated with 0.001, 0.01, 0.1, 1, 10, and 100 µM *t*-Butyl Alcohol, in DMSO, for 48 h.[15] Assays were repeated in triplicates. Forskolin, prochloraz, and 22R-hydroxycholesterol were used as positive controls. Testosterone and estradiol levels were measured using high-performance liquid chromatography/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 the 3 assay runs.

The ability of *t*-Butyl Alcohol to inhibit the catalytic activity of aromatase, an enzyme responsible for the conversion of androgen to estrogen, was evaluated in an aromatase assay, using a human recombinant microsome test system.[15] The test followed US EPA guideline OPPTS 890.1200 and was performed in triplicate. Microsomes were tested with final concentrations of 10^{-10} – 10^{-3} M *t*-Butyl Alcohol (at 1% of the total assay volume); 4-hydroxyandrostendione was used as the positive control. *t*-Butyl Alcohol was classified as a non-inhibitor with a mean aromatase activity of 102.3% (± 1.7%), at the highest test concentration.

Cytotoxicity

t-Butyl Alcohol affects the activity of a variety of enzymes and may stabilize or destabilize a variety of biological membranes.[3] 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. t-Butyl Alcohol has no or only a weak effect on rat hepatic mitochondrial respiration and phosphorylation at concentrations of up to 3%. Blood samples from 6 adult female Dorset sheep and 6 adult humans (sexes unspecified) were incubated with 0.1, 0.5, 1, or 5% t-Butyl Alcohol for 1 h, after which methemoglobin and glutathione concentrations were measured. t-Butyl Alcohol caused oxidant stress to erythrocytes as measured by either increased methemoglobin formation and/or decreased glutathione concentrations.

Hepatic Effects

The effect of t-Butyl Alcohol on rat liver function were evaluated in a 3-mo study.[3] Fifteen male Wistar rats were given 15% (v/v) t-Butyl Alcohol in drinking water. Animals were killed after an exposure period ranging from 1 wk up to 3 mo. Exposure to t-Butyl Alcohol induced megamitochondria in the rat hepatocytes after 2- to 3-mo treatment. Proliferation of smooth-surfaced endoplasmic reticulum and an increase in the number of lysosomes and microbodies were also seen. An insignificant decrease in hepatic reduced glutathione concentration and an insignificant increase in diene conjugate formation was observed in 4 male Wistar rats that received a single oral dose of 2540 mg/kg t-Butyl Alcohol, compared to saline controls. Female Wistar rats (unspecified number) had a single 1850 mg/kg dose of t-Butyl Alcohol (25% v/v in water) administered, via gavage. Hepatic triacylglycerols and palmitate uptake into triacylglycerols was increased, but there were no significant changes in hepatic and blood phospholipid concentrations or in the 4-h lactate/pyruvate ratio. The researchers concluded that t-Butyl Alcohol induced a fatty liver, but not by impairing fatty acid oxidation. Twelve female Wistar rats were given a single oral dose of 4 ml/kg t-Butyl Alcohol; 17 h later, the relative liver weight of treated rats was increased, but there was no change in the hepatic nitrogen concentration, or in the fatty acid, triglyceride, cholesterol, or phospholipid concentrations in the blood. Male and female Fischer 344 rats (4/sex/group) received a single dose of either 500 mg/kg t-Butyl Alcohol, 500 mg/kg [^{14}C]t-Butyl Alcohol, or corn oil (controls), via gavage. Renal α2u-globulin levels were significantly higher in the kidney cytosol of treated male rats compared to controls and dialysis of [^{14}C]t-Butyl Alcohol-treated male kidney cytosol with D-limonene supported the hypothesis that t-Butyl Alcohol interacts with α2u-globulin.

Hydroxyl Radical Scavenger

t-Butyl Alcohol has been shown to protect DNA from the effects of radiation.[3] It has been hypothesized that this action may be due to the scavenging of hydroxyl radicals.

Neural Effects

Male Sprague-Dawley rats (unspecified number) received a single oral dose of 3000 mg/kg t-Butyl Alcohol.[3] After approximately 2 h, the rats were decapitated and brain homogenate was incubated with choline for 4 min. Choline uptake was increased in the caudate nucleus and decreased in the hippocampus of treated rats, compared to controls.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Irritation

Animal

In an irritation test, 6 New Zealand rabbits received a single dermal application of a mixture (0.5 ml) containing ethanol and t-Butyl Alcohol (concentrations not specified) to an abraded and an intact site (2.5 cm^2 each).[3] One rabbit exhibited moderate irritation at both the abraded and intact site. Three rabbits exhibited mild irritation at the abraded site, including 1 rabbit which exhibited mild irritation at the intact site. The test article was not considered a primary dermal

irritant to rabbit skin under the conditions of the study. Undiluted t-Butyl Alcohol (99.9% pure) and gasoline-grade t-Butyl Alcohol were evaluated using New Zealand albino rabbits (3/sex) in 2 separate studies. Rabbits received a 0.5 ml application of the test article for 24 h; 2 intact and 2 abraded sites per rabbit were used. Neither test article was considered a primary skin irritant; both test articles were found to be minimally irritating. An unspecified concentration of t-Butyl Alcohol was found to have no irritating effect on the skin of shaved rabbits when observed for 1 wk.

Sensitization

Animal

A guinea pig maximization test was performed using female albino guinea pigs (20 test animals; 10 controls) in accordance with OECD TG 406.[4] Both test and control animals received three pairs of intradermal injections (0.1 ml) on each side of a clipped 4 x 6 cm^2 area of the back during the induction phase. The injections comprised either Freund's complete adjuvant (FCA) and water (1:1), 1% *t*-Butyl Alcohol in water, or 1% *t*-Butyl Alcohol in FCA and water (1:1). One week after receiving the induction injections, an application of 10% sodium lauryl sulfate in petrolatum was made to the injection sites, 24 h prior to a 48-h, occlusive induction application of 100% *t*-Butyl Alcohol. A topical challenge application of 100% *t*-Butyl Alcohol was made for 24 h under an occlusive dressing on day 21 and reactions were scored 24 and 48 h after patch removal. During the induction phase, strong erythema, edema, and necrosis were observed at the intradermal injection sites using FCA, but not at the injection sites that did not involve FCA, in all test and control animals. 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.

Human

An HRIPT was performed on 119 subjects using 60% ethyl alcohol and 0.125% t-Butyl Alcohol.[3] A total of 99 subjects completed the study; subjects who dropped out did so 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.

Phototoxicity

Human

Ultraviolet (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.[6] Based on this lack of absorbance, *t*-Butyl Alcohol does not present a concern for phototoxicity or photoallergenicity.

OCULAR IRRITATION STUDIES

Animal

Nine New Zealand white rabbits each received a 0.1 ml drop of a mixture of ethanol and t-Butyl Alcohol (concentrations unspecified) in 1 eye.[3] The eyes of 6 animals remained unwashed for 24 h, after which time the test article was washed out; the eyes of the other 3 rabbits were washed 30 s after dosing. Ocular effects in the unrinsed treatment group included increased opacity of the cornea, reduced reaction of the iris to light, extreme redness, chemosis, and discharge. Symptoms were less severe in treated eyes that were rinsed. The researchers concluded that the test article was a severe ocular irritant to rabbit eyes. In another ocular irritation study, 0.1 ml of 100% t-Butyl Alcohol was administered to the right eye of 9 New Zealand albino rabbits (5 male, 4 female), in which treated eyes were either washed or unwashed. t-Butyl Alcohol was classified as severely irritating for the unwashed group and moderately irritating to the washed group. In a similar ocular irritation study, 0.1 ml of undiluted gasoline grade t-Butyl Alcohol was classified as a primary eye irritant for both the washed and unwashed groups.

CLINICAL STUDIES

Case Reports

A woman who previously had a positive patch test reaction to ethanol did not react to dermal application of 100% t-Butyl Alcohol.[3] Four female patients did not have any reactions in a 24-h patch test of 10% aqueous t-Butyl Alcohol. A case of allergic contact dermatitis to the t-Butyl Alcohol component of SD-40 alcohol in a commercial sunscreen preparation was described. 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-Butyl Alcohol was applied to the forearms. At 72 h, erythema was observed and at 96 h, vesiculation was observed. No reactions were observed in 2 controls who also had applied t-Butyl Alcohol to their forearms.

Occupational Exposure

The American Conference of Governmental and Industrial Hygienists (ACGIH) has set a threshold limit value (TLV) of 100 ppm that is satisfactory to prevent narcosis with t-Butyl Alcohol.[3] The TLV is the time-weighted average (TWA) concentration for a normal 8-h workday or 40-h workweek and no adverse effects are expected from it. The short-term exposure limit (STEL) 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.

t-Butyl Alcohol is identified as a possible air contaminant in occupational settings (29CFR1910.1000). The National Institute of Occupational Safety and Health (NIOSH) has reported that 1600 ppm *t*-Butyl Alcohol is the concentration immediately dangerous to life or health.[16] The Occupational Safety and Health Administration (OSHA) limits for occupational exposure to *t*-Butyl Alcohol are 100 ppm or 300 mg/m.[17] Additionally, the current STEL for *t*-Butyl Alcohol is 150 ppm (450 mg/m^3).

EXPOSURE ASSESSMENT

The daily exposure to *t*-Butyl Alcohol from various categories/types of cosmetic products was calculated using the Council's survey data[8] on usage concentrations and exposure parameters from different sources (Table 5). The estimated calculations reveal that the daily exposure from aftershave lotions, at the maximum use concentration of 0.91%, represents the highest daily exposure at 14 mg/d across different product categories.

Margin of Safety

A margin of safety (MoS) value of 55,714 was calculated for the highest reported concentration of use, which also results in the highest exposure of *t*-Butyl Alcohol (0.91% in aftershave lotions). Calculations used to obtain this value are detailed below:

Adult human body weight = 60 kg
Skin absorption = 1.5%[6,18]

In a dermal absorption study conducted in male rats, after 72 h, less than 1.5% of ^{14}C-*t*-Butyl Alcohol applied topically was absorbed. According to OECD Guidance Notes on Dermal Absorption, substances generally have a higher permeability through rat skin than through human skin; therefore, a well-conducted in vivo study is unlikely to underestimate dermal absorption in humans.[19]

NOAEL: 195 mg/kg bw/d (oral, in rats; based on reduced bodyweight gain and survival of male rats in a 2-yr, carcinogenicity study, in which the test article was administered via drinking water)[6,20]

$SED_{aftershave\ lotion}$ = (14 mg/d x 1.5%)/60 kg = 0.0035 mg/kg bw/d
$MoS_{aftershave\ lotion}$ = $NOAEL/SED_{dermal}$ = (195 mg/kg bw/d)/(0.0035 mg/kg bw/d) = 55,714

As for the development and reproductive toxicity endpoint, the NOAEL was determined to be 160 mg/kg bw/d,[4,6] which results in an MoS for aftershave lotion of 45,714 accordingly.

The resulting MoS is greater than 100. This threshold is generally considered to be protective, which is derived from multiplying two factors: a 10-fold factor accounts for the extrapolating data from test animals to human being (interspecies extrapolation), and an additional 10-fold for accommodating differences among the human population (intra-species extrapolation).[21]

Additionally, the US EPA has established the human Reference Dose (RfD) of *t*-Butyl Alcohol for oral exposure at 0.4 mg/kg bw/d, which is 114 times greater than the total systemic exposure from the use of aftershave lotion (0.0035 mg/kg bw/d).[5]

SUMMARY

According to the *Dictionary*, *t*-Butyl Alcohol is reported to function in cosmetics as a denaturant, fragrance ingredient, and solvent. The Panel previously reviewed the safety of this ingredient. During its initial review, the Panel considered the available data insufficient to support the safety of *t*-Butyl Alcohol in cosmetics, as described in the safety assessment published in 1989. Upon receiving data which addressed these insufficiencies, and based on the available animal and clinical data, the Panel published an amended final report in 2005 with the conclusion that this ingredient is safe as used in cosmetic products. In accordance with its Procedures, the Panel evaluates the conclusions of previously issued reports approximately every 15 years, and it has been at least 15 years since this assessment has been issued. At the September 2023 meeting, the Panel determined that this safety assessment should be re-opened to evaluate developmental and reproductive toxicity effects of *t*-Butyl Alcohol, to update their evaluation of previously reviewed carcinogenicity studies, and to rectify the test concentration stated in a previously reviewed HRIPT. Additionally, the Panel noted an increase in reported uses and use concentrations, as well as a newly reported use of *t*-Butyl Alcohol in other baby products.

According to 2023 VCRP survey data, *t*-Butyl Alcohol was reported to be used in 136 formulations; in 1998, 32 uses were reported. Results from a 2022 concentration of use survey conducted by the Council indicate that the highest reported maximum use concentration of use for *t*-Butyl Alcohol was at up to 0.91% in aftershave lotions; in 1999, the highest reported frequency of use for *t*-Butyl Alcohol was at up to 0.5% in hair spray aerosol fixatives.

The dermal absorption of *t*-Butyl Alcohol was evaluated in male Sprague-Dawley rats (4/group) in a study performed in accordance with OECD TG 417. Very little of the applied dose was absorbed (less than 1% in tissues and less than 1.5% in excreta) at all time points. The test material showed low potential for dermal absorption and bioaccumulation.

In an oral metabolism study, groups of 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. The major urinary metabolites of *t*-Butyl Alcohol were identified as *t*-butyl alcohol sulfate, 2-hydroxyisobutyrate, and 2-methyl-1,2-propanediol, in rat urine. In an oral metabolism study performed in a human male who received a single dose of 5 mg/kg [^{13}C]*t*-Butyl Alcohol as a gel capsule; major urinary metabolites were identified as 2-methyl-1,2-propanediol and 2-hydroxyisobutyrate, in human urine.

Groups of male and female Fischer 344 rats (4/group/sex) were subjected to a whole-body inhalation exposure of 250, 450, or 1750 ppm *t*-Butyl Alcohol, 6 h/d, for 1 or 8 d. For both sexes, concentrations of *t*-Butyl Alcohol were similar in the blood, liver, and kidneys following a single 6-h exposure; blood and tissue concentrations of *t*-Butyl Alcohol were also lower following repeated exposures. However, concentrations differed between males and females following the repeated, 8-d exposure, possibly corroborating the pharmacokinetic model of *t*-Butyl Alcohol binding to α2u-globulin in male rats.

In a subchronic inhalation toxicity study, rats were exposed, whole body, to up to 6366.13 mg/m^3 *t*-Butyl Alcohol for 13 wk. The NOAEC for local respiratory effects was determined to be 6366.13 mg/m^3.

Groups of male CD-mice (5/group) received a single dose of 0, 400, 1000, or 2000 mg/kg *t*-Butyl Alcohol, in canola oil, via gavage, in a study evaluating acute testicular toxicity. Two non-treatment related deaths occurred in the 400 mg/kg group; no differences were observed in the % change of testosterone or in the serum testosterone of animals treated with *t*-Butyl Alcohol, compared to controls. Testis weights of mice in the 1000 and 2000 mg/kg groups averaged higher than the control and 400 mg/kg groups; the only significant histological difference in the testes of tested mice was a higher percentage of tubules, compared to control animals with sloughing. A developmental and reproductive toxicity study was performed (OECD TG 421) in which male and female albino Sprague-Dawley rats (12/sex/group) received 0, 64, 160, 400, or 1000 mg/kg bw/d *t*-Butyl Alcohol, in water, via gavage. No mortality occurred in the parental generation; besides mild central nervous toxicity observed in the 1000 mg/kg bw/d group (1– 2 h after dosing) and in the 400 mg/kg group (between the second and fourth week of dosing) no other significant effects were observed. There was a significant reduction in the number of live born pups/pregnancy for dams in the 1000 mg/g group. Reduced survival was observed for pups treated with 1000 mg/kg *t*-Butyl Alcohol and offspring born to 1000 mg/kg bw/d dams exhibited lower mean body weights than control offspring. 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. Groups of male Sprague-Dawley rats were exposed, whole-body, to vaporized *t*-Butyl Alcohol at 6000 or 12,000 mg/m^3 for 6 wk (7 h/d, 7 d/wk), prior to being mated with non-exposed females. Paternal body weight gain was unrelated to treatment. A lack of a dose-response relationship or pattern in behavioral performance differences and neurotransmitter levels in pups sired by males in both treatment groups led researchers to conclude that these effects were likely to be of little to no biological significance. The LOAEC and NOEC values for paternal body weight and weight gain were determined to be ≥ 12,000 mg/m^3 and 6000 mg/m^3, respectively. The NOAEC for male and female pups was determined to be ≥ 12,000 mg/m^3. Pregnant Sprague-Dawley rats were exposed to 6000 or 12,000 mg/m^3 vaporized *t*-Butyl Alcohol from day 1 to day 20 of gestation (upon mating with non-exposed males). A decrease in body weight and food consumption and an increase in water consumption was observed in the 12,000 mg/m^3 dams; no changes were observed in 6000 mg/m^3 dams. Only 2-pair wise comparisons were statistically significant in behavioral tests of pups born to dams from each group; reduced levels of norepinephrine, β-endorphin, and met-enkephalin were seen in pups born to 12,000 mg/m^3 dams and reduced serotonin and met-enkephalin levels were observed in pups born to 6000 mg/m^3 dams, compared to controls. The NOAEC for male and female pups was determined to be ≥ 12, 000 mg/m^3.

t-Butyl Alcohol was not genotoxic in 2 separate Ames tests (OECD TG 471), both using *S. typhimurium* strain TA102, when tested at up to 5000 µg/plate, with or without metabolic activation, and at up to 3.75 mg/plate, with metabolic activation, respectively. Chinese hamster ovary cells were exposed to up to 5000 µg/ml *t*-Butyl Alcohol in McCoy's 5A medium in a sister chromatid exchange assay (OECD TG 479) and in an in vitro mammalian chromosome aberration test (OECD TG 473); the test article was non-genotoxic when tested at up to 5000 µg/ml, in both assays. *t*-Butyl Alcohol appeared to induce DNA damage in a dose-dependent manner when tested at up to 30 mmol/l, without metabolic activation, in a Comet assay; these results were partly attributed to cytotoxicity (which had ambiguous results).

Prostate tissue obtained from Sprague-Dawley rats was tested with solutions of up to 10^{-3} M *t*-Butyl Alcohol in an androgen receptor binding assay; the test article was classified as a "non-binder" (mean specific binding ≥ 50% for 3 test runs). The mean relative binding affinity could not be calculated. H295R cells were treated with up to 100 µM *t*-Butyl Alcohol, in DMSO, for 48 h in a steroidogenesis assay, performed in accordance with OECD TG 456. 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 the 3 assay runs. A human recombinant microsome test system was used to evaluate the inhibitive ability of *t*-Butyl Alcohol in an aromatase assay; microsomes were tested with final concentrations of 10^{-10} – 10^{-3} M *t*-Butyl Alcohol. The mean aromatase activity of *t*-Butyl Alcohol was 102.3% (± 1.7%), at the highest test concentration. *t*-Butyl Alcohol was classified as a non-inhibitor of aromatase.

Female albino guinea pigs (20 test animals; 10 controls) were tested with up to 1% *t*-Butyl Alcohol in a guinea pig maximization test, performed in accordance with OECD TG 406. During the induction phase, strong erythema, edema, and necrosis were observed at the intradermal injection sites using FCA, but not at the injection sites that did not involve FCA, in

all test and control animals. 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. Based on UV spectra indicating a lack of absorption between 290 and 400 nm and a molar absorption coefficient below the benchmark of concern *t*-Butyl Alcohol does not present concerns for phototoxicity and photoallergenicity.

The NIOSH reports 1600 ppm *t*-Butyl Alcohol as the concentration immediately dangerous to life or health. The OSHA *t*-Butyl Alcohol occupational exposure limit and STEL are 100 ppm (300 mg/m^3) and 150 ppm (450 mg/m^3), respectively.

An MoS value of 55,714 was calculated for the highest reported concentration and highest exposure of use for *t*-Butyl Alcohol (0.91% in aftershave lotions), based on an NOAEL of 195 mg/kg bw/d from a 2-yr oral toxicity study and 1.5% dermal absorption in rats. Regarding the developmental and reproductive toxicity endpoint, the MoS for aftershave lotions was calculated to be 45,714, using an NOAEL of 160 mg/kg bw/d. The US EPA established the human RfD for *t*-Butyl Alcohol for oral exposure at 0.4 mg/kg bw/d, which is 114 times greater than the total systemic exposure from aftershave lotion (0.0035 mg/kg bw/d).

DISCUSSION

In accordance with its Procedures, the Panel evaluates the conclusions of previously issued reports approximately every 15 yr. The last safety assessment of *t*-Butyl Alcohol was published in 2005, with a conclusion of safe as used in cosmetic products. A re-review was initiated at the September 2023 Panel meeting to evaluate developmental and reproductive toxicity effects, to update the previous discussion of carcinogenicity, and to rectify the erroneous test concentration stated in the 2005 report Abstract of a previously reviewed HRIPT. Upon re-review, the Panel again concluded that *t*-Butyl Alcohol is safe in cosmetics in the present practices of use and concentration.

The Panel noted that *t*-Butyl Alcohol showed low potential for dermal absorption and bioaccumulation. The Panel also determined that a negative guinea pig maximization test mitigated the need for confirmatory sensitization data at maximum concentration of use. The Panel discussed the carcinogenicity studies and determined that the weight-of-evidence does not support a carcinogenic effect. Also, the Panel reiterated that the effects of *t*-Butyl Alcohol on development were likely secondary to maternal toxicity and the effects on learning development were attributed to *t*-Butyl Alcohol in maternal milk and were not an in utero effect. Finally, the Panel noted that because undiluted *t*-Butyl Alcohol was an ocular irritant, ocular irritation data at maximum use concentration would add to the robustness of the safety assessment.

The Panel discussed the issue of possible incidental inhalation exposure resulting from formulations containing *t*-Butyl Alcohol (e.g., at up to 0.11% in perfumes). Inhalation toxicity data present in the report did not raise any concerns. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

The Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Therefore, the Panel has concluded the data are insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that *t*-Butyl Alcohol is safe in cosmetics in the present practices of use and concentration described in this safety assessment.

TABLES

Table 1. Chemical properties of *t*-Butyl Alcohol

Property	Value	Reference
Physical Form	crystals; solid liquid (above 25 °C)	3,17
Color	colorless	17
Odor	camphor	17
Molecular Weight (g/mol)	74.12	3
Specific Gravity (@ 25ºC)	0.78	27CFR21.101
Vapor pressure (mmHg@ 25ºC)	42	17
Vapor Density (mmHg)	2.55	17
Melting Point (ºC)	25.6	3
Boiling Point (ºC)	82.50	3
Solubility	Water, alcohol, ether, and other organic solvents	3
log K_{ow} (@ 25ºC)	0.350 (estimated)	5

Table 2. 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[7]	**1998[3]**	**2022[8]**	**1999[3]**
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

Table 2. 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[7]	**1998**[3]	**2022**[8]	**1999**[3]
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
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.

Table 3 . Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
			ORAL			
t-Butyl Alcohol	canola oil	Male CD-1 mice (5/group)	0, 400, 1000, or 2000 mg/kg	After an initial determination of fecal testosterone levels, animals received a single dose, 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 calcium chloride as positive controls.	Two animals in the 400 mg/kg group died 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 mice in the 1000 and 2000 mg/kg groups averaged 14% higher than the control and 400 mg/kg groups ($p \leq 0.05$). The only significant histological difference was a higher percentage of tubules in the testes, compared to controls, along with sloughing ($7 \pm 2\%$, mean SD, $p \leq 0.05$).	[13]
t-Butyl Alcohol	water	Male and female albino Sprague-Dawley rats F_0:(12/sex/group) F_1:(10/sex/group)	0, 64, 160, 400, or 1000 mg/kg bw/d	OECD TG 421; 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 occurred 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 second and fourth wk of dosing in the 400 mg/kg 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 the other groups. Subsequently, there was a significantly reduced pup survival at the high dose with only 80% survival to postnatal day 4, and 50% survival to postnatal day 21, compared to close to 100% in the other groups. Offspring born to dams treated with *t*-Butyl Alcohol at 1000 mg/kg bw/d exhibited lower mean body weights than 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.	[4]

Table 3 . Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
INHALATION						
t-Butyl Alcohol	air	Male Sprague-Dawley rats (number not specified)	6000 or 12,000 mg/m^3, vaporized	Paternal, whole-body exposure. Both concentrations were administered, in chambers, at different times; approximately 3 mo apart. Daily exposure for 6 wk (7 h/d, 7 d/wk). Controls were used (concurrent vehicle not specified). Due to dosing at different times, comparison between dose groups was not considered appropriate. Exposed males were weighed over the 6-wk study period and mated, post-exposure, with non-exposed females. Upon delivery of pups, the offspring were culled to 4 males and 4 females/ litter and were fostered to untreated controls. Offspring were weighed weekly through 5 wk of age and were observed for behavioral/neurotransmitter (acetylcholine, dopamine, norepinephrine, serotonin, 5-hydroxytryptamine, metenkephalin, β-endorphin, substance P) 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, 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 for pups with paternal exposure to *t*-Butyl Alcohol. In the pups sired by 6000 mg/m^3-exposed males, ascent on the mesh screen was lower than controls (values not provided); no further pair-wise behavioral differences were observed between treated groups and controls. 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. For the neurotransmitter measurements, 5 pair-wise comparisons were statistically significant for pups with paternal exposure to *t*-Butyl Alcohol. In pups sired by 6000 mg/m^3-exposed males, levels of serotonin in the midbrain and met-enkephalin in the cerebrum were reduced, compared to controls. In pups sired by 12,000 mg/m^3-exposed males, the mean concentrations of norepinephrine and β-endorphin were reduced in the cerebellum, and the mean concentration of met-enkephalin was reduced in the cerebrum, compared to control pups. The lack of a pattern of effects or a dose-response relationship led researchers to conclude that the few observed effects were not treatment related 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 NOEC (paternal body weight and weight gain): 6000 mg/m^3 NOAEC (for male and female pups): ≥ 12,000 mg/m^3	[4]

Table 3 . Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
t-Butyl Alcohol	air	Pregnant Sprague-Dawley rats (number not specified)	6000 or 12,000 mg/m^3, vaporized	Maternal, whole-body exposure. Both concentrations were administered, in chambers, 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 weights, behavioral testing and analysis of protein/neurotransmitter levels in the brain were conducted in a similar manner to the previously described study.	No changes in body weight or feed or water consumption were seen in the 6000 mg/m^3 dams. In the 12,000 mg/m^3 dams, a decrease in body weight during the first 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 third wk of gestation, compared to controls. In the offspring, 2 pair-wise comparisons were statistically significant in the behavioral tests. The distance climbed on the mesh screen (ascent) and the mean time held onto wire (10 s vs. 16 s) were both reduced in the pups of 6000 mg/m^3 dams, compared to controls. The mean revolutions per minute of the pups of 12,000 mg/m^3 dams was 26 rpm versus 16 rpm for concurrent controls in the rotorod experiment. Five pair-wise comparisons of neurotransmitter measurements were statistically significant for pups delivered by exposed dams. For pups born to dams in 6000 mg/m^3 group, serotonin was reduced in the midbrain and met-enkephalin was reduced in the cerebrum, compared to controls. For pups born to dams in the 12,000 mg/m^3 group, the mean concentrations 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. A dose-response relationship or discernible pattern of developmental neurotoxicity was not observed in prenatally-exposed pups when examined for up to 60 d postnatally. Furthermore, the relatively severe toxicity observed in maternal animals at the 12,000 mg/m^3 dose 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.	[4]

F_0 – first/parental generation; F_1- second/offspring generation; hCG – human chorionic gonadotropin; LOAEC – lowest-observed-adverse-effect concentration; LOAEL – lowest-observed-adverse-effect level; NOAEC- no-observed-adverse-effect concentration; NOEC- no-observed-effect concentration; NOAEL – no-observed-adverse-effect level; OECD – Organisation for Economic Cooperation and Development; SD – standard deviation; TG – test guideline

Table 4. Genotoxicity studies

Test Article	Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
IN VITRO						
t-Butyl Alcohol	DMSO or water	up to 5000 µg/plate, with or without metabolic activation	*S. typhimurium* TA102	OECD TG 471; Ames test Positive controls: with metabolic activation: 2-Aminoanthracene and 1,8-dihydroxyanthraquinone without metabolic activation: cumene hydroperoxide and mitomycin C	Not genotoxic; No statistical or dose-related increases in the number of mutant colonies was observed.	[14]
t-Butyl Alcohol	water	0, 0.75, 1.5, 2.25, 3, or 3.75 mg/plate, with metabolic activation	*S. typhimurium* TA102	OECD TG 471; Ames test	The maximum number of revertants/plate reached approximately 800 at 2.25 mg/plate compared to 400 for controls. At higher concentrations, the number of revertants/plate decreased in a dose-dependent manner. The significance of these values is unknown since no data were provided for negative or solvent controls.	[4]
t-Butyl Alcohol	McCoy's 5A medium	Up to 5000 µg/ml, with and without metabolic activation	Chinese hamster ovary cells	OECD TG 479; Sister chromatid exchange assay Positive controls: without metabolic activation: mitomycin C (at concentrations of 0.001 and 0.010 µg/ml) with metabolic activation: cyclophosphamide (at concentrations of 0.3 and 2.0 µg/ml	Not genotoxic; 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.	[4]
t-Butyl Alcohol	McCoy's 5A medium	Up to 5000 µg/ml, with and without metabolic activation	Chinese hamster ovary cells	OECD TG 473; in vitro mammalian chromosome aberration test Positive controls: with metabolic activation: cyclophosphamide (at 15 and 50 µg/ml) without metabolic activation: mitomycin C (at 0.25 and 1 µg/ml)	Not genotoxic; The test article did not induce chromosomal aberrations in treated cells.	[4]
t-Butyl Alcohol	not specified	1, 5, 10, or 30 mmol/l, without metabolic activation for 1 h	Human leukemia (HL-60) cells	Comet assay; 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.	The test article seemed to induce DNA damage in a dose-dependent manner; however, this was partly attributed to cytotoxicity (which had ambiguous results).	[4]

DMSO – dimethyl sulfoxide; DNA – deoxyribonucleic acid; OECD – Organisation for Economic Cooperation and Development; TG – test guideline

Table 5. *t*-Butyl Alcohol exposures from daily usage across various categories/types of cosmetic products

Product Category/Type of cosmetics exposure	Daily Exposure by Product Category* (mg/d)	Maximum *t*-Butyl Alcohol Concentration of Use	Daily Exposure Based on the Highest *t*-Butyl Alcohol Use Concentration (mg/d)	Note
Eye lotions	20	0.004 - 0.0042%	0.00084	Exposure amount of eye make-up applied
Mascaras	25	0.01%	0.0025	
Colognes and toilet waters	1500 #	0.097%	1.455	Exposure amount of eau de toilette spray applied
Perfumes	530 γ	0.096 - 0.11%	0.583	
Hair sprays	500 γ			
Aerosol		0.066 - 0.11%	0.55	
Shampoos (non-coloring)	110	0.00014%	0.000154	
Tonics, dressings, and other hair grooming aids	400	0.06%	0.24	Exposure amount of hair styling products applied
Spray				
Lipstick	60	0.0001 - 0.007%	0.0042	
Makeup bases	510	0.006%	0.0306	Exposure amount of liquid foundation applied
Dentifrices	138	0.028%	0.03864	Exposure amount of toothpaste applied
Deodorants	1500			
Not spray		0.89%	13.35	
Other personal cleanliness products	200	0.16%	0.32	Exposure amount of hand wash soap applied
Aftershave lotions	1540 #	0.079 - 0.91%	14	
Beard softeners	154 #	0.029%	0.0447	Exposure amount of shaving cream applied
Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	190	0.0047 - 0.088%	0.1672	Exposure amount of shower gel applied
Face and neck products	1540			Exposure amount of face cream/lotion applied
Not spray		0.016 - 0.044%	0.6776	
Spray		0.094%	1.4476	
Body and hand products	7820			Exposure amount of body lotion applied
Not spray		0.0054 - 0.05%	3.91	
Spray		0.1%	7.82	
Moisturizing products	7820			Exposure amount of body lotion applied
Not spray		0.005 - 0.048%	3.754	
Skin fresheners	308 #	0.003%	0.00924	Exposure amount of face mask applied

***** Exposure parameters are retrieved from the SCCS NoG[21]

Exposure amount is provided by Vermeer Cosmolife[22]

γ Exposure amount is provided by CTFA (currently known as PCPC) habits and practices data, with a retention factor of 0.1 applied[23]

REFERENCES

1. Nikitakis J, Kowcz A. Web-Based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI *Dictionary*). https://incipedia.personalcarecouncil.org/winci/. Washington, D.C.: Personal Care Products Council. Last Updated: 2024. Accessed: 01/25/2024.

2. Elder RL (ed). Final Report on Safety Assessment of *t*-Butyl Alcohol. *J Am Coll Toxicol.* 1989;8(4):627-641.

3. 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.

4. European Chemical Agency (ECHA). ECHA Chem dossier: 2-methylpronan-2-ol (CAS 75-65-0). https://chem.echa.europa.eu/100.000.809/dossier-view/2d926aec-4b25-46d4-a5ce-132a5a833b0f/dbbf255f-6c44-43fb-ada3-128d25780cab_dbbf255f-6c44-43fb-ada3-128d25780cab?searchText=75-65-0. Last Updated: 2018. Accessed: 01/25/2024.

5. U.S. Environmental Protection Agency (EPA). CompTox Chemicals Dashboard v2.3.0: tert-Butyl Alcohol (CAS No. 75-65-0): . https://comptox.epa.gov/dashboard/chemical/properties/DTXSID8020204. Last Updated: 2021. Accessed: 03/02/2024.

6. Api A, Belsito D, Botelho D, et al. RIFM fragrance ingredient safety assessment, 2-methyl-2-propanol, CAS registry number 75-65-0. *Food and Chemical Toxicology.* 2023;173:113512.

7. 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.)

8. 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.)

9. European Union. EUR-Lex: Access to European Union law. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32006D0257&qid=1709340999024. Last Updated: 2024. Accessed: 02/06/2023.

10. Bernauer U, Amberg A, Scheutzow D, Dekant W. Biotransformation of ^{12}C-and 2-^{13}C-labeled methyl *tert*-butyl ether, ethyl *tert*-butyl ether, and *tert*-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(6):651-658.

11. Leavens TL, Borghoff SJ. Physiologically based pharmacokinetic model of methyl tertiary butyl ether and tertiary butyl alcohol dosimetry in male rats based on binding to α2u-globulin. *Toxicol Sci.* 2009;109(2):321-335.

12. Mahler J. 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. *Toxic Rep Ser.* 1997(53):1-56.

13. Billitti J, Faulkner B, Wilson B. Absence of acute testicular toxicity of methyl-tert butyl ether and breakdown products in mice. *Bull Environ Contam Toxicol.* 2005;75:228-235.

14. McGregor D, Cruzan G, Callander R, May K, Banton M. The mutagenicity testing of tertiary-butyl alcohol, tertiary-butyl acetate™ and methyl tertiary-butyl ether in Salmonella typhimurium. *Mutat Res* 2005;565(2):181-189.

15. de Peyster A, Mihaich E, Kim DH, et al. Responses of the steroidogenic pathway from exposure to methyl-tert-butyl ether and tert-butanol. *Toxicology.* 2014;319:23-37.

16. National Institute for Occupational Safety and Health (NIOSH). NIOSH Pocket Guide to Chemical Hazards: *tert*-Butyl Alcohol. https://www.cdc.gov/niosh/npg/npgd0078.html. Last Updated: 2019. Accessed: 03/02/2024.

17. Occupational Safety and Health Administration (OSHA). OSHA Occupational Chemical Database: *tert*-Butyl Alcohol (*tert*- butanol; CAS No. 75-65-0). https://www.osha.gov/chemicaldata/593. Last Updated: Accessed: 03/02/2024.

18. McGregor D. Tertiary-butanol: a toxicological review. *Crit Rev Toxicol.* 2010;40(8):697-727.

19. Organisation for Economic Co-operation and Development. Joint meeting of the chemicals committee and the working party on chemicals, pesticides, and biotechnology. https://www.oecd.org/chemicalsafety/testing/48532204.pdf. Last Updated: Accessed: 03/01/2024.

20. Cirvello JD, Radovsky A, Heath JE, Farnell DR, Lindamood C, 3rd. Toxicity and carcinogenicity of t-butyl alcohol in rats and mice following chronic exposure in drinking water. *Toxicol Ind Health.* 1995;11(2):151-165.

21. Scientific Committee on Consumer Safety (SCCS). The SCCS's notes of guidance for the testing of cosmetic ingredients and their safety evaluation. (12th Revision). SCCS/1647/22. 2023. https://health.ec.europa.eu/document/download/32a999f7-d820-496a-b659-d8c296cc99c1_en?filename=sccs_o_273_final.pdf. Accessed 03/01/2024. Pages1 -203.

22. Selvestrel G, Robino F, Baderna D, et al. SpheraCosmolife: a new tool for the risk assessment of cosmetic products. *ALTEX.* 2021;38(4):565-579.

23. Cosmetic Toiletry and Fragrance Association (CTFA). 2002. (Unpublished data regarding average hairspray and perfume use submitted by CTFA, presently known as Personal Care Products Council.)