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

Status: Draft Amended Report for Panel Review
Release Date: March 4, 2024
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The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This safety assessment was prepared by Preethi Raj, M.Sc, Senior Scientific Analyst/Writer, CIR.

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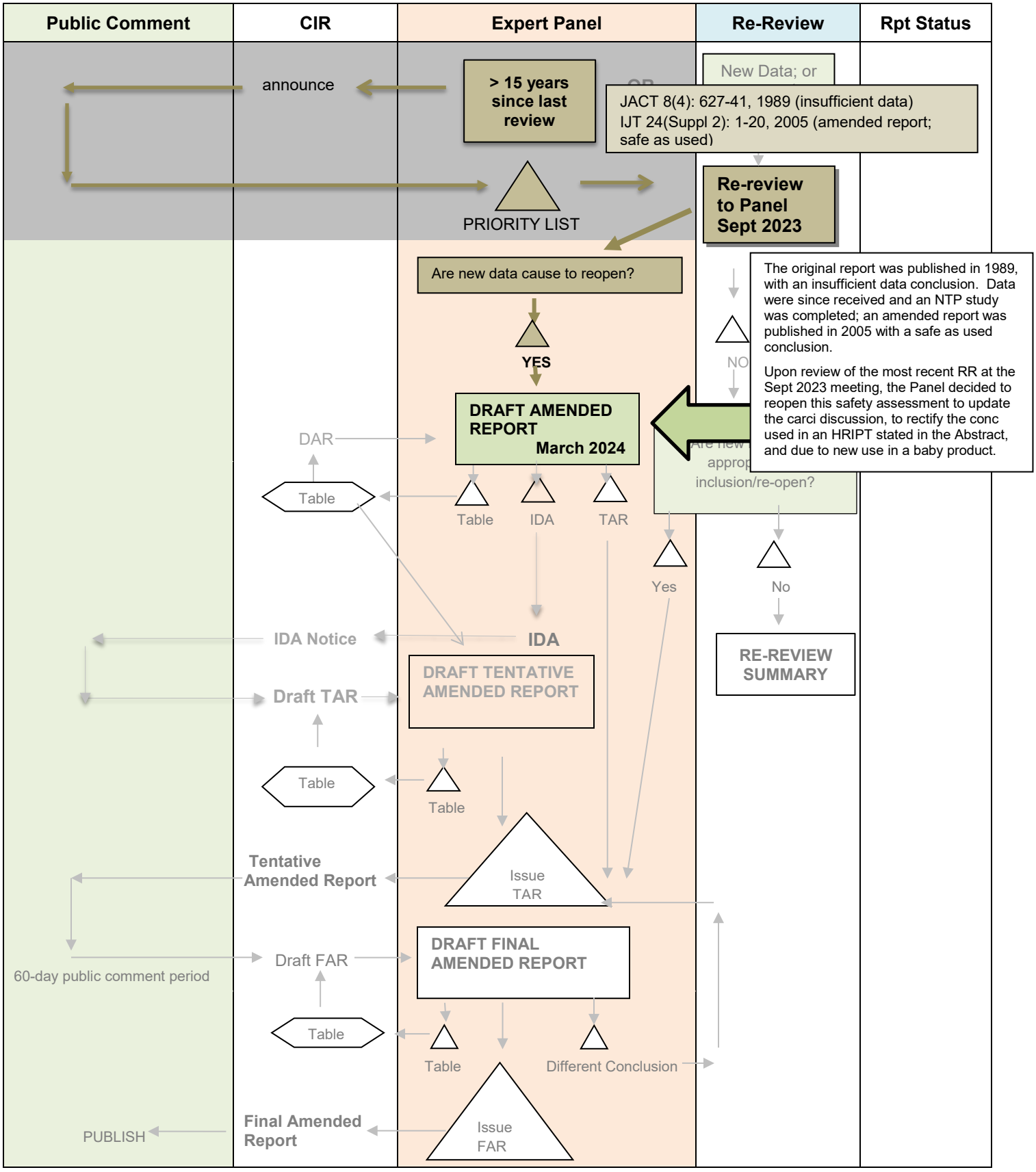
RE-REVIEW FLOW CHART

INGREDIENT/FAMILY *t*-Butyl Alcohol

MEETING March 2024



Cosmetic Ingredient Review

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Memorandum

To: CIR Expert Panel Members and Liaisons
From: Preethi S. Raj, M.Sc.,
Senior Scientific Analyst/Writer, CIR
Date: March 4, 2024
Subject: Amended Safety Assessment of *t*-Butyl Alcohol as Used in Cosmetics

Enclosed is a Draft Amended Report on the Safety Assessment of *t*-Butyl Alcohol as Used in Cosmetics. (It is identified as *report_t-ButylAlcohol_032024* in the pdf document). The Expert Panel for Cosmetic Ingredient Safety (Panel) first published a safety assessment of this ingredient in 1989; the available data were deemed insufficient to make a determination of safety (*originalreport_t-ButylAlcohol_032024*). In response to the needed information, the Panel received human sensitization data for a test substance containing 0.125% *t*-Butyl Alcohol; additionally, the results of the NTP carcinogenicity study became available. Subsequently, the Panel published an Amended Final Report on the Safety Assessment of *t*-Butyl Alcohol as Used in Cosmetics in 2005 (*2005amendedreport_t-ButylAlcohol_032024*). On the basis of the available animal and clinical data in that report, the Panel concluded that *t*-Butyl Alcohol is safe as used in cosmetic products. At its September 2023 meeting, the Panel decided to reopen this safety assessment to evaluate developmental and reproductive effects seen with exposure to 1% *t*-Butyl Alcohol, update the carcinogenicity discussion, and rectify a test concentration stated in a previously reviewed HRIPT; the Panel also noted an increase in reported uses and use concentrations, as well as a newly reported use of *t*-Butyl Alcohol in other baby products.

The reported frequency and concentrations of use of *t*-Butyl Alcohol have increased since the last review. According to 2023 VCRP data, *t*-Butyl Alcohol has 136 reported uses, from 32 reported uses in 1998. In 2023, the maximum reported concentration of use for *t*-Butyl Alcohol was at 0.91% in aftershave lotions; in 1999, *t*-Butyl Alcohol was reported to be used at a maximum concentration of 0.5% in hair spray aerosol fixatives. An exposure assessment of *t*-Butyl Alcohol in twenty different cosmetic product use categories has been prepared by Dr. Jinqiu Zhu and has been included in the report for the Panel's consideration.

Additional supporting documents for this report package include: a flow chart (*flow_t-ButylAlcohol_032024*), report history (*history_t-ButylAlcohol_032024*), search strategy (*search_t-ButylAlcohol_032024*), a data profile (*dataprofile_t-ButylAlcohol_032024*), the minutes from past meetings at which *t*-Butyl Alcohol was discussed (*originalminutes_t-ButylAlcohol_032024*), concentration of use data (*data_t-ButylAlcohol_032024*), and transcripts from the previous meeting at which the current rereview of *t*-Butyl Alcohol was discussed (*transcripts_t-ButylAlcohol_032024*).

If no further data are needed to reach a conclusion of safety, the Panel should formulate a Discussion and issue a Tentative Amended Report. However, if additional data are required, the Panel should be prepared to identify those needs an issue an Insufficient Data Announcement.

t-Butyl Alcohol History

1984

- The Expert Panel for Cosmetic Ingredient Safety (Panel) first reviewed the safety of *t*-Butyl Alcohol in 1989; it was determined that the available data was insufficient to determine safety. The Panel identified the need for several studies, including a 90-d oral toxicity, human sensitization, and UV absorption.

2000 – 2001

- In response to the stated data needs, human sensitization data for a test substance containing 0.125% *t*-Butyl Alcohol as a denaturant were received. Furthermore, upon classification of *t*-Butyl Alcohol as a carcinogen, and receipt of results for NTP carcinogenicity studies, the Panel decided to reopen and re-evaluate the safety of this ingredient.

2005

- Based on the available animal and clinical data following the first review, the Panel issued an Amended Final Report on the Safety of *t*-Butyl Alcohol with the conclusion of safe as used in cosmetics.

September 2023

- An extensive search of the available published literature since 2000 was conducted in accordance with CIR Procedures regarding re-review of these ingredients after ~ 15 years. The Panel determined that this safety assessment should be reopened to evaluate developmental and reproductive effects seen upon exposure to 1% *t*-Butyl Alcohol, update the carcinogenicity discussion, 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.

March 2024

- **A Draft Amended Report is being presented to the Panel for review.**

t-Butyl Alcohol Data Profile* – March 28 – 29, 2024 – Preethi Raj																																	
	Use				Toxico-kinetics			Acute Tox			Repeated Dose Tox			DART			Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	New Rpt	Old Rpt	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
t-Butyl Alcohol	X	O	O	O	X		XO	O	O	O		O	XO		XO	XO	XO		O	O		O	O		X	O	X			O		O

* "X" indicates that new data were available in this category for the ingredient; "O" indicates that data from the original assessment were available

t-Butyl Alcohol

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
t-Butyl Alcohol	75-65-0	✓	✓	✓	✓	✓*	✓*	NR	✓	✓	NR	✓*	NR	✓*	NR	NR	

NR – not reported; ✓*- data available, but is not new, or, relevant

Botanical and/or Fragrance Websites (if applicable)

Ingredient	CAS #	Dr. Duke's	Taxonomy	GRIN	Sigma-Aldrich	AHPA	AGRICOLA	IFRA	RIFM
t-Butyl Alcohol	75-65-0								✓

Search Strategy – from 2000 onwards; last performed 2/07/2024

[*total # of hits / # hits that were useful*]

PubMed

((((((((((((((t-butyl alcohol) OR (75-65-0)) OR (1,1-Dimethylethanol)) OR (2-Methyl-2-Propanol)) OR (2-Methyl-2-propanol)) OR (2-Propanol, 2-MethylTert-Butanol)) OR (tert-Butanol)) OR (Tert-Butyl Alcohol)) OR (Trimethyl Carbinol)) OR (Trimethylmethanol)) OR (tertiary butyl alcohol)) OR (tertiary butanol)) OR (t-butanol)) OR (2-methyl-2-propanol)) OR (trimethyl carbinol)) AND (toxicity) AND (2000:2024[pdat]) – 409, 899 hits/ 10 useful

((((((((((((((t-butyl alcohol) OR (75-65-0)) OR (1,1-Dimethylethanol)) OR (2-Methyl-2-Propanol)) OR (2-Methyl-2-propanol)) OR (2-Propanol, 2-MethylTert-Butanol)) OR (tert-Butanol)) OR (Tert-Butyl Alcohol)) OR (Trimethyl Carbinol)) OR (Trimethylmethanol)) OR (tertiary butyl alcohol)) OR (tertiary butanol)) OR (t-butanol)) OR (2-methyl-2-propanol)) OR (trimethyl carbinol) AND (2000:2024[pdat]) – 10, 114, 017 hits/ 16 useful
AND dermal penetration – 987 hits/0 useful
AND dermal absorption – 1,608 hits/0 useful
AND oral toxicity – 28, 392 hits/2 useful
AND dermal toxicity – 2, 579 hits/0 useful
AND inhalation toxicity – 8,585 hits/0 useful
AND dermal irritation – 786 hits/0 useful
AND dermal sensitization – 659 hits/ 0 useful
AND PPRA – 34 hits/0 useful
AND IL -8 luciferase assay – 460 hits/ 0 useful
AND USENS – 6 hits/0 useful
AND KeratinoSens – 38 hits/0 useful

General search

tert-butanol safety assessment – 1,110,000/3 useful
2-methyl-2-propanol toxicity – 1,140,000 hits/5 useful
tert-butanol dermal toxicity – 896, 000 hits/3 useful

LINKS

Search Engines

- Pubmed - http://www.ncbi.nlm.nih.gov/pubmed
  - appropriate qualifiers are used as necessary
  - search results are reviewed to identify relevant documents
- Connected Papers - https://www.connectedpapers.com/

Pertinent Websites

- wINCI - https://incipedia.personalcarecouncil.org/winci/ingredient-custom-search/
- FDA Cosmetics page - https://www.fda.gov/cosmetics
- eCFR (Code of Federal Regulations) - https://www.ecfr.gov/
- FDA search databases: https://www.fda.gov/industry/fda-basics-industry/search-databases
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras
- SCOGS database: https://www.fda.gov/food/generally-recognized-safe-gras/gras-substances-scogs-database
- Inventory of Food Contact Substances Listed in 21 CFR: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=IndirectAdditives
- Drug Approvals and Database: https://www.fda.gov/drugs/development-approval-process-drugs/drug-approvals-and-databases
- FDA Orange Book: https://www.fda.gov/drugs/drug-approvals-and-databases/approved-drug-products-therapeutic-equivalence-evaluations-orange-book
- OTC Monographs - https://dps.fda.gov/omuf
- Inactive Ingredients Approved For Drugs: https://www.accessdata.fda.gov/scripts/cder/iig/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- EUR-Lex - https://eur-lex.europa.eu/homepage.html
- Scientific Committees (SCCS, etc) opinions: https://health.ec.europa.eu/scientific-committees_en https://health.ec.europa.eu/scientific-committees/scientific-committee-consumer-safety-sccs_en
- ECHA (European Chemicals Agency – REACH dossiers) – https://echa.europa.eu/
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- EFSA (European Food Safety Authority) - https://www.efsa.europa.eu/en
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) IRIS library - https://apps.who.int/iris/
- a general Google and Google Scholar search should be performed for additional background information, to identify references that are available, and for other general information - www.google.com https://scholar.google.com/

Botanical Websites, if applicable

- Dr. Duke's - https://phytochem.nal.usda.gov/
- Taxonomy database - http://www.ncbi.nlm.nih.gov/taxonomy
- GRIN (U.S. National Plant Germplasm System) - https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx
- Sigma Aldrich plant profiler- http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html
- American Herbal Products Association Botanical Safety Handbook (2nd Edition; 2013) - http://abc.herbalgram.org/site/DocServer/AHPABotanicalSafety_FMexcerpt.pdf?docID=4601
- National Agricultural Library NAL Catalog (AGRICOLA) https://agricola.nal.usda.gov/
- The Seasoning and Spice Association List of Culinary Herbs and Spices
- http://www.seasoningandspice.org.uk/ssa/background_culinary-herbs-spices.aspx

Fragrance Websites, if applicable

- IFRA (International Fragrance Association) – https://ifrafragrance.org/
- Research Institute for Fragrance Materials (RIFM) - https://www.rifm.org/#gsc.tab=0 http://fragrancematerialsafetyresource.elsevier.com/
-

SEPTEMBER 2023 PANEL MEETING – INITIAL REVIEW/RE-REVIEW

Belsito Team – September 11, 2023

DR. BELSITO: Okay, we're ready to resume?

DR. SNYDER: t-Butyl Alcohol.

DR. BELSITO: Start your engine. Okay where is this?

DR. SNYDER: Re-review of 1989.

DR. BELSITO: Yeah, I'm just trying to find -- there's also Wave 2 on this.

DR. SNYDER: Yeah.

DR. BELSITO: So, yeah, we published an amended report in 2005 on the basis of the animal and clinical data. It's safe in cosmetic products. It's been 15 years so it's being brought up for a re-review. There's new data, the uses increased, there's a baby product now, use concentrations are up. I think looking at the old data it supports the current conclusion, but I think we should reopen, especially given the baby products.

DR. SNYDER: And also, there's lots of new data, too, so.

DR. BELSITO: Yeah, and lots of new data there.

DR. SNYDER: I agree. It went from 32 to 136 with new.

DR. BELSITO: I think we'll be able to support the safety. I think we just need to reopen it up and take a look at it.

DR. SNYDER: Okay. I agree.

DR. BELSITO: Any other comments?

DR. SNYDER: Well, that Wave 2 about the RIFM safety assessment, 95th percentile, 0.019 percent.

DR. BELSITO: Oh, yeah. Um-hmm.

MS. FIUME: Should you bring that in when we create the draft?

DR. SNYDER: That's what I'm asking him.

DR. BELSITO: Well, I think it's all data.

DR. SNYDER: We have the maximum concentration of use for leave on data. But other than that, it's 0.007.

DR. BELSITO: Right. Okay.

Cohen Team – September 11, 2023

DR. COHEN: And last, but not least, *t*-Butyl Alcohol. So, it is used as a solvent or an alcohol denaturant and as a perfume carrier in cosmetics. In 1989, the panel concluded the data were insufficient to support the safety of its use. Subsequently, data was received to address the insufficiencies and an amended final report was issued in 2005, with the conclusion of safe as used. Since it's been a long time since that report, a search was done, notably we have re-evaluation of the 13-week NTP carcinogenicity toxicity studies, which the author concluded that the tumorigenesis process was rat-specific and not relevant to humans.

In 2022, we have maximum reported concentration of use at 0.91 percent in aftershave, which is up from 0.5 percent in 1999. And we have recent reported use in 136 formulations, which is up considerably.

DR. ROSS: And increased concentration of use, right?

DR. COHEN: Yeah, that's right. All right, I'll open it up because I have some comments for later, but what are the feelings so far? Well, maybe I'll make a comment.

When I went into the original report, in the abstract and in the body of the paper, it says an (HRIPT) test showed no potential for eliciting either dermal irritation or sensitization by 100 percent *t*-Butyl Alcohol. And then, when you go and look at the actual reference in the paper, it says it was performed on 119 individuals using 60 percent ethyl alcohol and 0.125 percent *t*-Butyl Alcohol.

I couldn't find 100 percent study, but maybe you can help me find it because I was a little perplexed by it. I'll try to find it.

So, on Page 15 of the original report, under irritation and sensitization, it talks about 110 individuals being tested at 0.125 *t*-Butyl Alcohol. Ninety-nine finished and the 20 that didn't make it through it seemed to be unrelated. But if you look at the

abstract of that paper, it says at the bottom, "a repeat-insult patch test showed no potential for eliciting either dermal irritation or sensitization by 100 percent *t*-BuOH." Do you see the same thing? You don't have it?

MS. BURNETT: I don't have it. It's not my report. I don't have it in front of me.

DR. COHEN: So, look at towards the end of the abstract and then what's on labeled as Page 15.

DR. HELDRETH: So, you mean in the 2005 amended report?

DR. COHEN: Yeah. On the notable data they talk about apply neat, but it's to guinea pigs. And that's new data so I don't know -- that wouldn't have been in the 2005 report.

DR. ROSS: So, you are in --

DR. COHEN: So, I'm in the last report. The one that --

DR. ROSS: Yeah, yeah. I was just there. Yeah.

DR. COHEN: Right. So, if you look here it says (inaudible).

DR. ROSS: Oh, in the abstract?

DR. COHEN: Look in the abstract and then look at the body of the report which is Page 15. Are you in the old one or the 2000- -- it looks like you're in the old one.

DR. ROSS: I'm in the old one, yeah. It's a hundred percent there and then --

DR. COHEN: I can't reconcile that at all in which case we have to open it, because we don't actually have sensitization data anywhere near, you know, we don't have max use. We don't have it.

DR. HELDRETH: Yeah, just doing a quick scan through here the only test that I see at a hundred percent was out of a case report.

DR. COHEN: It's one case report.

DR. HELDRETH: Yeah.

DR. COHEN: Right, that's not an HRIPT of 119 people.

DR. HELDRETH: No.

DR. COHEN: I think there's some data error here. I don't know where it is, I can't be sure, but if we have max use of 0.91 percent in an aftershave, and the report from 2005 is at roughly the seventh dose, I think we need to reopen it.

DR. BERGFELD: You said if.

DR. COHEN: I did say if. I did say if because if the HRIPT was at a hundred percent on 119 people, I wouldn't reopen the report.

DR. ROSS: I went backwards and forwards. Just my summary. I went backwards and forwards on this one again, I thought it was another interesting one. Increased uses and concentration.

The negatives for not reopening it would be new DART data was similar to previous. The genotox data, as Tom has already pointed out, is negative. The RIFM, non-sens- -- the RIFM had reasonable margin of adequate MOEs on that. It's a non-sensitizer in animals. But I changed it to positive because of two things and to reopen it.

So, I changed my opinion to reopen it. One was if I read the original reports, there were significant DART effects at 1.0 percent and the concentrations of use here have bumped up to 0.9 percent.

DR. COHEN: You see the DART effect at 1.0 percent?

DR. ROSS: Yeah. And then secondly, it would also give you an opportunity to update the carcinogenicity discussion by the mechanism of action not being relevant. I mean, for those two things I'm willing for my opinion to be changed on that. But that's where I came down, to reopen it for those two issues.

DR. BERGFELD: I guess a second would be to clarify what's in there and see what the percentages are before you reopen.

DR. ROSS: Yeah. Well, maybe we do that.

DR. COHEN: I suppose we can table the decision to reopen but I don't know if that's valuable.

DR. HELDRETH: It looks, based on looking at Christina and I, it looks very much like there was an error on what was reported in the abstract. It looks like the hundred percent came from a case report. So, if anything it might be worthwhile to open this and create a new report --

DR. COHEN: And clarify this.

DR. ROSS: Yeah.

DR. HELDRETH: -- so that we can put out a final report that is accurate. Even if nothing changes, I think it's --

DR. COHEN: I think we agree.

DR. HELDRETH: Okay.

DR. COHEN: And you know what, it's an opportunity to deal with the issues that David just brought up as well. It's a solid reopen.

DR. BERGFELD: Either that or clarify.

DR. COHEN: I don't think there's --

DR. SLAGA: I would go with that.

DR. COHEN: I think it doesn't hurt to reopen this.

DR. SLAGA: Yeah.

DR. COHEN: I just make a neat and clean report, which nullifies the old report.

DR. HELDRETH: I think that's ideal.

DR. ROSS: Yeah.

DR. HELDRETH: I think -- you know, we can't reaffirm that report, but we know there's a glaring error right up front.

DR. TILTON: I would support that.

DR. COHEN: Good. Good. Well, everyone's reading their reports so. I want to actually -- any further comments, advice? Warnings for tomorrow?

DR. BERGFELD: Eat your Wheaties tonight.

DR. COHEN: Tom, any thoughts overall? Any concerns?

DR. SLAGA: You mean with this particular re-review? Oh, no.

DR. COHEN: No, I meant tomorrow in our full Panel discussion?

DR. SLAGA: Overall, I thought we had very good discussion for a number of things. Like David I changed my -- I was going for this last one for re-review for do not reopen, but to clarify that hundred percent we should open it.

DR. COHEN: You made a comment about the DART, right? David? On *t*-Butyl Alcohol, the DART at 1 percent?

DR. ROSS: Yeah. The DARTs were reported in the original report at 1 percent, and our concentrations have bumped up to 0.9 percent. So, they're still lower.

DR. COHEN: But not much.

DR. ROSS: But not by much. Yeah. And it gives you an opportunity to update that carcinogenicity discussion as well. Is that enough to reopen? Probably.

DR. COHEN: Okay.

DR. BERGFELD: There's no downside to reopening.

DR. COHEN: No, no. We'll do it. Susan, any other comments for tomorrow?

DR. TILTON: Nope. No other comments.

DR. COHEN: David, anything?

DR. ROSS: Not from me, I think we're good.

DR. COHEN: Okay. We can adjourn?

DR. SLAGA: Adjourned.

MS. RAJ: I realize I was late, and obviously you already went through my ingredients, but here are some documents for Pentapeptides that may be useful.

DR. COHEN: Yeah, this is the Don one. Okay. Yeah, I read through that one.

DR. ROSS: I went through the definition of wrinkles.

DR. COHEN: Yeah. Well, that has good definitions.

DR. ROSS: Yeah, there were good pictures, yeah.

DR. COHEN: Good photography. Anyway, so we can be officially -- can I have this? We're officially adjourned and off the record now?

Full Panel – September 12, 2023

DR. BELSITO: Yeah. We published an amended final report on the safety of t-Butyl Alcohol as used in cosmetics in 2005. We found it safe as used. It's been 15 years. We're being asked to re-review it. We have new data that showed use increase, use in one baby product, use concentrations up. I think looking at the data in the old report, it will support the safety, but we thought given the increases in the use in a baby product, we should reopen it.

DR. BERGFELD: So, that's a motion? Dr. Cohen?

DR. COHEN: Second.

DR. BERGFELD: Second.

DR. COHEN: Just a quick discussion. So we'll second it with a quick discussion. In the original report -- well not the original report, the second -- you know, the re-review. This is in 2005. The abstract talks about an HRIPT at 100 percent. And when you read the rest of the report, I don't think that's correct. The HRIPT is at 0.125 percent. It's quite a bit off, so we need to re-review that data. So, if we're re-opening, we're going to re-litigate the entire thing.

DR. BELSITO: Right. But it also reinforces my opinion that you shouldn't always believe what was previously written in the literature.

DR. COHEN: Well it was within the report, right.

DR. BELSITO: I understand.

DR. COHEN: One part of the report has it one way, and so we'll make that correction.

DR. BELSITO: I understand.

DR. BERGFELD: So, any other comments regarding the t-Butyl Alcohol?

DR. ROSS: There was a couple of things that we felt that there were DART effects at 1 percent and the concentrations of use have increased to 0.9 percent. And we wanted to update the carcinogenicity discussion in the dossier. So, there were two other points.

DR. BERGFELD: Okay. Any other comments to be added to the document? All right, moving on then to the re-review summaries, Benzaldehyde. Dr. Cohen?

SEPTEMBER 1986 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT

Dr. Bergfeld stated that her team was recommending an insufficient data conclusion for this ingredient. The data lacking included: a) 90-day study, b) data on human sensitization, c) and a UV absorbance spectrum (if absorbance is observed at greater than 290 nm, then photosensitization data will be required). She noted that data were lacking on ocular irritation but were not being requested because *t*-Butyl Alcohol was expected to be a severe ocular irritant. This would also be noted in the discussion of the report.

Dr. McEwen inquired as to whether the voluminous literature on alcohol dependency and withdrawal may have contained any of the requested data. Ms. Brandt stated that all of the literature was contained in the original document and had been reviewed by the team. Dr. Carlton requested an opportunity to review this literature again.

The Panel then unanimously approved and accepted this report with an insufficient data conclusion, with the inadequacies as listed above. The insufficient data announcement will shortly be announced for a 90-day comment period.

JUNE 1987 PANEL MEETING – SECOND REVIEW/DRAFT TENTATIVE REPORT

Dr. Elder noted that a Tentative Final Report had been released on this ingredient in September 1986 outlining the need for a 90-day oral study, human sensitization data, and an ultraviolet spectrum. Data from an NTP 90-day oral study were received from CTFA and an ultraviolet spectrum was received from Dr. Shank. No clinical data were received. Therefore, the recommendation was for this report to go insufficient due to the lack of human sensitization data.

Dr. Bergfeld commented that *t*-Butyl Alcohol was used in many fragrances and as this was an area of high sensitization in her clinic, the human sensitization data were critical to the evaluation of this ingredient. Dr. Schroeter concurred. It was also noted that Drs. Shank and Hoffmann had both done a UV spectrum and had obtained the same results: no absorption above 290 nm. Dr. Bergfeld requested that the discussion note that as no absorption was seen above 290 nm, photosensitization data were not required.

Dr. Bergfeld requested that the minutes reflect the fact that Drs. Shank and Hoffmann had run UV spectra purely out of interest and that this should not set a precedent for further such activity by the Panel. Dr. Schroeter commented that this showed the simplicity of obtaining these data and therefore industry should always be willing to supply them. Dr. McEwen noted that what is easy in an academic setting is not always easy in a commercial setting.

Dr. McEwen requested that the discussion note that the Panel had recently approved the use of *n*-butyl alcohol for cosmetic use and explain why the clinical data for *n*-butyl alcohol were not relevant to *t*-Butyl Alcohol. It was noted that the report had originally been on both alcohols but had been split because there were no dermal data on *t*-Butyl Alcohol. Dr. Bergfeld commented that the lack of animal and human sensitization data on *t*-Butyl Alcohol did not allow extrapolation from *n*-Butyl Alcohol to *t*-Butyl Alcohol.

The Panel then unanimously approved an "insufficient data" conclusion for *t*-Butyl Alcohol based on the lack of human sensitization data. The final report will shortly be announced for a 90-day comment period.

MAY 2000 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT

Dr. Belsito noted that *t*-Butyl Alcohol is used primarily as a denaturant in cosmetics, but can also be used as a solvent. Use concentration data indicate that this ingredient is used at a concentration of 0.5% in a hairspray and at a concentration of 0.3% in a skin freshener. Dr. Belsito recalled that a Final Report with an insufficient data conclusion on *t*-Butyl Alcohol was issued in 1987, and noted that the Panel determined that human sensitization data were needed for completion of the safety assessment. He also noted that this report is being re-evaluated by the Panel because a human skin sensitization study on 0.125% *t*-Butyl Alcohol was received in response to the insufficient data conclusion that was issued.

Dr. Belsito stated that his Team determined that *t*-Butyl Alcohol is safe as used as a denaturant, but that the available data are insufficient for evaluating the safety of this ingredient for use as a solvent in cosmetic products. He said that inhalation toxicity data (because of ingredient use in a hairspray) and a review of the NTP study findings are needed in order for the Panel to complete its safety assessment of *t*-Butyl Alcohol as a solvent in cosmetic products.

In response to Dr. Bergfeld's concerns, Dr. Belsito said that the issue of dermal absorption will be addressed in the NTP study and that use concentration data have been received from CTFA.

Dr. Schroeter favored tabling the Panel's review of *t*-Butyl Alcohol until the September 11-12, 2000 Panel meeting, pending the results of the NTP study and the incorporation of all new data that have been received to date into the safety assessment.

Dr. McEwen noted that the human skin sensitization study provided was done on an alcohol with *t*-Butyl Alcohol used as a denaturant, and that the negative results for 0.125% *t*-Butyl Alcohol in the study indicate that *t*-Butyl Alcohol is safe for use as a denaturant in cosmetics at that concentration. He added that it appears that the use of *t*-Butyl Alcohol as a solvent at or above that concentration would be regarded as safe as well.

Dr. Belsito said that there is a concern relating to the concentration in the finished cosmetic product at which t-Butyl Alcohol is used as a denaturant versus its use as a solvent. Dr. Belsito noted that he is aware of information suggesting that *t*-Butyl Alcohol is a carcinogen, and that the NTP study results would be needed in order for the Panel to conclude that *t*-Butyl Alcohol can be used safely at concentrations higher than those associated with its use as a denaturant.

Dr. Andersen said that based on today's discussion, it is clear that the Panel intends to do the following: (1) re-evaluate its previous safety assessment of *t*-Butyl Alcohol, (2) review a draft report at a subsequent Panel meeting that would include the NTP study results, a complete review of the scientific literature on *t*-Butyl Alcohol published since 1987, and the new human skin sensitization data on 0.125% *t*-Butyl Alcohol, and (3) consider the issuance of a new conclusion based on the new data that have been accrued.

Dr. Andersen added that the Panel expressed concern over inhalation toxicity relative to other cosmetic uses of *t*-Butyl Alcohol, which may be considered an informal request for data from the cosmetics industry.

Dr. Bergfeld confirmed that the Final Report on *t*-Butyl Alcohol has been published and noted that, possibly, an amended Final Report will be issued to reflect the Panel's current deliberations.

DECEMBER 2000 MEETING – SECOND REVIEW/DRAFT TENTATIVE REPORT

In July of 1987, the Panel issued a Final Report on *t*-Butyl Alcohol with an insufficient data conclusion. It was determined that only human skin sensitization data were needed for completion of this safety assessment. In response to the Panel's data request, negative human skin sensitization data on an alcohol containing *t*-Butyl Alcohol (0.125%) as a denaturant were received and these data were reviewed at the May 18-19, 2000 Panel Meeting. The fact that *t*-Butyl Alcohol has been classified as a carcinogen and the need for the results of the NTP carcinogenicity study for inclusion in this safety assessment were also discussed.

The Panel's action plan for further review of *t*-Butyl Alcohol was summarized as follows at the May Panel meeting: (1) re-evaluate the published CIR safety assessment of *t*-Butyl Alcohol, (2) review a draft report at a subsequent Panel meeting that would include the NTP study results, a complete review of the scientific literature on *t*-Butyl Alcohol published since 1987, and the new human skin sensitization data on 0.125% *t*-Butyl Alcohol, and (3) consider the issuance of a new conclusion based on the new data that have been accrued.

Dr. Schroeter noted that since the May 18-19, 2000 Panel meeting, carcinogenicity studies by the NTP as well as Cirvello et al. (1985) were received and need to be reviewed in detail by the Panel. He then proposed that the report be tabled, pending the Panel's review of these studies.

FEBRUARY 2001 MEETING – THIRD REVIEW/DRAFT TENTATIVE REPORT

Dr. Schroeter recalled that the Panel tabled the review of this ingredient at the December 4-5, 2000 Panel meeting, pending the Panel's detailed review of the NTP carcinogenicity data that were received.

After reviewing the carcinogenicity data, Dr. Schroeter said that his Team determined that the test concentrations that were of concern relative to carcinogenicity are well above cosmetic use concentrations of *t*-Butyl Alcohol. Dr. Schroeter noted that carcinogenicity was observed at concentrations greater than 2.5%, and that the dose-response generally was not good at higher doses in rats or mice. After considering these data along with the negative Ames test and mammalian genotoxicity data, Dr. Schroeter's Team concluded that concern over the carcinogenicity of *t*-Butyl Alcohol in cosmetic products is not warranted.

The Panel voted unanimously in favor of issuing a Tentative Amended Final Report with a safe as used conclusion on *t*-Butyl Alcohol.

Dr. Bergfeld asked Dr. Schroeter to elaborate on the content of the report discussion.

Dr. Schroeter said that the NTP study would have to be mentioned, addressing the concentrations at which carcinogenicity was observed and cosmetic use concentrations of *t*-Butyl Alcohol.

Dr. Belsito said that there was no definite evidence of carcinogenicity in the NTP study. He noted that the NTP has a system for grading carcinogenicity, and that some evidence suggesting that *t*-Butyl Alcohol was carcinogenic was found in this study.

Concerning some of the findings in the NTP study, Dr. Belsito noted that his Team suggested that the renal tumors observed in male F344/N rats may have been due to an effect of the α-2-microglobulin, which is known to cause an increase in the incidence of renal adenomas in that species. Dr. Belsito added that tumors of the thyroid gland were observed only in female $B6C3F_1$ mice. He also noted that tumor formation (mice and rats) occurred at high doses and that responses were variable. Dr. Belsito said that these findings should be mentioned in the report discussion.

Dr. Slaga noted that the concern over some evidence of carcinogenicity in male rats, but not in female rats, and follicular thyroid hyperplasia, adenomas, and carcinomas in male and female mice needs to be addressed in the report discussion.

Dr. Shank said that the negative genotoxicity data on *t*-Butyl Alcohol should also be mentioned.Dr. Belsito added that reproductive effects of *t*-Butyl Alcohol should also be included. He noted that an increased number of stillbirths at high doses and an effect on the development of learning in newborns were noted in reproductive and developmental toxicity studies. Concerning postnatal development, Dr. Belsito recalled that when the pups were fostered by a mother that was not being fed *t*-Butyl Alcohol, the pups performed much better in behavioral tests. Thus, the presence of *t*-Butyl Alcohol in maternal milk was probably responsible for the behavioral changes.

Dr. Belsito noted that Dr. Snyder had indicated his desire to review the reproductive and developmental toxicity data on *t*-Butyl Alcohol in detail.

Dr. Klaassen requested that the carcinogenicity data be included in a table indicating the doses administered and the responses that were observed.

The Panel voted unanimously in favor of tabling the Final Report on *t*-Butyl Alcohol, pending the Panel's detailed review of the carcinogenicity data that were received.

SEPTEMBER 2001 PANEL MEETING - FOURTH REVIEW/DRAFT AMENDED FINAL REPORT

In July of 1987, the Panel issued a Final Report on *t*-Butyl Alcohol with an insufficient data conclusion. It was determined that only human skin sensitization data were needed for completion of this safety assessment. In response to the Panel's data request, negative human skin sensitization data on an alcohol containing *t*-Butyl Alcohol (0.125%) as a denaturant were received, and these data were reviewed at the May 18-19, 2000 Panel meeting. The fact that *t*-Butyl Alcohol has been classified as a carcinogen and the need for NTP carcinogenicity study results for inclusion in this safety assessment were also discussed.

The Panel's action plan for further review of *t*-Butyl Alcohol was summarized as follows at the May 18-19, 2000 Panel meeting: (1) re-evaluate the published CIR safety assessment of *t*-Butyl Alcohol, (2) at a subsequent Panel meeting, review a draft report that would include the NTP study results, a complete review of the scientific literature on *t*-Butyl Alcohol published since 1987, and the new human skin sensitization data on 0.125% *t*-Butyl Alcohol, and (3) consider the issuance of a new conclusion based on the new data that have been accrued.

At the February 12-13, 2001 Panel meeting, the Panel issued a Tentative Amended Final Report with a safe as used conclusion, and the issuance of an Amended Final Report with this conclusion is being considered at today's meeting.

Dr. Belsito noted that his Team revised the last sentence in the third paragraph of the report discussion to read as follows: Overall, the Panel decided that the studies on *t*-Butyl Alcohol showed that it was unlikely to have significant carcinogenic potential as currently used in cosmetic formulations. The first sentence in the last paragraph of the discussion was also revised as follows: In its consideration of the reproductive and developmental toxicity data, the Panel noted that the increased incidence of still births occurred at high exposure levels and was likely secondary to maternal toxicity.

Dr. Belsito also noted that the chronic inhalation toxicity study (Prescott-Matthews et al., 1988) summarized in the report text is from a meeting abstract, and that this study is now published as Borghoff et al. (2001). The Prescott-Matthews et al. (1988) reference will be deleted.

The Panel voted unanimously in favor of issuing an Amended Final Report with the following conclusion: Based on the available animal and clinical data in this report, the CIR Expert Panel concludes that *t*-Butyl Alcohol is safe as used in cosmetic products. The preceding changes as well as other minor editorial changes in the report text were also approved by the Panel.

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

Status: Draft Amended Report for Panel Review
Release Date: March 4, 2024
Earliest Possible Review: March 28 – 29, 2024

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

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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
TG	test guideline
US	United States
UV	ultraviolet
VCRP	Voluntary Cosmetic Registration Program

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% (which is comparable to the highest reported concentration of use), 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]

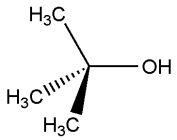

Figure 1. *t*-Butyl Alcohol

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. It has been further noted that volume quantities of t-Butyl Alcohol are prepared using the Oxirane process for the manufacture of propylene oxide which produces t-Butyl Alcohol as a by-product.

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 are submitted by the cosmetic industry via 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 are provided by cosmetic product categories, based 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 is reported to be used in 136 formulations, 115 of which are 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 is 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 has 1 reported use in other baby products (concentration of use 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. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

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

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 and has been used as an example of a nonmetabolizable alcohol.

Animal

Dermal

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 h, 24 h , 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.

Oral

In experimental data, the liquid/air 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.[3] The elimination of t-Butyl Alcohol from rat blood has been shown to be slow. 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 in urine. As a percentage of dose, the average extra glucuronic acid excreted over 24 h was 24.4%. No aldehydes or ketones were detected in the expired air of a rabbit given 6 ml t-Butyl Alcohol (route unspecified).

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 metabolites identified in [^{13}C]*t*-Butyl Alcohol-dosed rats, were *t*-butyl alcohol glucuronide, *t*-butyl alcohol sulfate, 2-hydroxyisobutyrate, and 2-methyl-1,2-propanediol. [^{13}C]acetone, *t*-Butyl Alcohol, and its glucuronide represented minor metabolites.

Inhalation

t-Butyl Alcohol was shown to be slowly eliminated from the blood of mice.[3] Nine male Swiss-Webster mice 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. Upon inhalation of 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 with mice (unspecified number). 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. 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. 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 genders following the repeated, 8-d exposure. The researchers stated this finding possibly corroborated the pharmacokinetic model of *t*-Butyl Alcohol binding to α2μ-globulin (a protein found in the kidney) in male rats.

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

The acute dermal toxicity of 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.[3] 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. In another 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. 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 whole body, inhalation of 0, 450, 900, 1750, 3500, or 7000 ppm t-Butyl Alcohol for 6 h plus 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. Eighty 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 $\alpha 2\mu$-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 $\alpha 2\mu$-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 (methods and numbers 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 B6C3F1 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 wk 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, 6 male and 4 female mice from the 4% group, 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 B6C3F1 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 B6C3F1 mice (60/sex/group) were given t-Butyl Alcohol, in drinking water, at concentrations 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, maternal animals 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. No significant differences were found in the weight of the testis, epididymis, and cauda or sperm motility, count, and morphology of male B6C3F1 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 B6C3F1 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 highest 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 6000 or 12,000 mg/m^3 *t*-Butyl Alcohol for 6 wk (7 h/d, 7 d/wk) and were 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 to Salmonella typhimurium in an Ames test "even at a high concentration," and when the bacterial suspension was preincubated with the test material 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, 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.[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 B6C3F1 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 EPA 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. The test article 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 from 1 wk, up to 3 mo. Exposure to t-Butyl Alcohol induced megamitochondria in the rat hepatocytes after 2 – 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 α2µ-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 α2µ-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 about 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. 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 for test and control animals. Since some sites in each animal used FCA, all 30 animals displayed this reaction. All non-FCA injection sites resulted in no reaction. No reactions were observed either 24 or 48 h after dermal challenge with the undiluted test article. *t*-Butyl Alcohol was considered a non-sensitizer.

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.[16] 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 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, t-Butyl Alcohol was classified as a primary eye irritant for both the washed and unwashed groups.

CLINICAL STUDIES

Case Reports

A woman who had 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% t-Butyl Alcohol in water.

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 of 100 ppm that is satisfactory to prevent narcosis with t-Butyl Alcohol.[3] The threshold limit value is the time-weighted average concentration for a normal 8-h workday or 40-h workweek and no adverse effects are expected from it. The short-term exposure limit is that concentration to which workers can be exposed for 15 min without suffering ill effects. Four 15-min periods are permitted per day with at least 60 min between exposure periods. In addition, the daily threshold limit value must not be exceeded. National Institute of Occupational Safety and Health (NIOSH) has reported that 8000 ppm t-Butyl Alcohol is the concentration immediately dangerous to life or health.

t-Butyl Alcohol is identified as a possible air contaminant in occupational settings (29CFR1910.1000). The present NIOSH and Occupational Safety and Health Administration (OSHA) limits for occupational exposure to *t*-Butyl Alcohol are 100 ppm or 300 mg/m^3.[17,18]

EXPOSURE ASSESSMENT

CIR staff calculated the daily exposure to *t*-Butyl Alcohol from various categories/types of cosmetic products, 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.

A margin of safety (MoS) value of 55,714 was calculated for the highest reported concentration of use for *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%[16,19]

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.[20]

NOAEL: 195 mg/kg bw/d (oral, rats, 2 yr)[21]

$SED_{aftershave\ lotion}$ = (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

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).[22]

RISK ASSESSMENT

Based on the Crème RIFM aggregate exposure model v1.0, the total systemic exposure to *t*-Butyl Alcohol as a fragrance ingredient is 0.000062 mg/kg/d, the majority of which is attributed to inhalation exposure (0.000061 mg/kg/d).[16] This systemic exposure value was used in conjunction with NOAEL values from toxicity studies to calculate margin of exposure (MoE) values, namely:

MoE for repeated dose toxicity: 195 mg/kg/d ÷ 0.000062 mg/kg/d = 3,145,161

MoE for reproductive toxicity: 160 mg/kg/d ÷ 0.000062 mg/kg/d = 2,580,645

According to RIFM, the 95th percentile concentration of *t*-Butyl Alcohol in fine fragrances is 0.019%.[16] In a carcinogenic risk assessment conducted on thyroid tumors, according to US Environmental Protection Agency (EPA) guidelines, the human Reference Dose (RfD) was estimated to be 220 µg/kg/d.[16] This RfD value is 3500 times greater than the total systemic exposure from fragrances (0.000062 mg/kg/d).

SUMMARY

According to the *Dictionary*, *t*-Butyl Alcohol is reported to function in cosmetics as a denaturant, fragrance ingredient, and solvent. 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 seen at 1%, 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 is 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 is 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 glucuronide, *t*-butyl alcohol sulfate, 2-hydroxyisobutyrate, and 2-methyl-1,2-propanediol. In another oral metabolism study performed in a human male who received a single dose of 5 mg/kg [^{13}C]*t*-Butyl Alcohol orally as a gel capsule, the major urinary metabolites were identified as 2-methyl-1,2-propanediol and 2-hydroxyisobutyrate, while unconjugated *t*-Butyl Alcohol, *t*-butyl alcohol glucuronide, and traces of *t*-butyl alcohol sulfate were identified as the minor urinary metabolites.

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 genders following the repeated, 8-d exposure, possibly corroborating the pharmacokinetic model of *t*-Butyl Alcohol binding to α2μ-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 wk 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 6000 or 12,000 mg/m^3 *t*-Butyl Alcohol for 6 wk (7 h/d, 7 d/wk), and were 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/m3 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 tested with 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 in either assay. *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 for test and control animals. Since some sites in each animal used FCA, all 30 animals displayed this reaction. All non-FCA injection sites resulted in no reaction. No reactions were observed either 24 or 48 h after dermal challenge with the undiluted test article. *t*-Butyl Alcohol was considered a non-sensitizer. 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.

An MoS value of 55,714 was calculated for the highest reported concentration of use for *t*-Butyl Alcohol (0.91% in aftershave lotions), based on an NOAEL from a 2-yr oral toxicity study (195 mg/kg/d) and 1.5% dermal absorption in rats. Based on the Crème RIFM aggregate exposure model v1.0 and derived from the systemic exposure value in conjunction with toxicity studies, the MOE for repeated dose toxicity of *t*-Butyl Alcohol was 3,145,161 and the MoE for reproductive toxicity of *t*-Butyl Alcohol was 2,580,645. According to RIFM, the 95th percentile concentration of *t*-Butyl Alcohol in fine fragrances is 0.019%. In a carcinogenic risk assessment conducted on thyroid tumors, according to US EPA guidelines, the human RfD was estimated to be 220 µg/kg/d, which is 3500 greater than the total systemic exposure from fragrances (0.000062 mg/kg/d).

PREVIOUS (2005) DISCUSSION

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

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

In the NTP study there was some evidence of carcinogenicity in male rats and female mice. Specifically, NTP found a small increase in renal carcinomas in male rats, but not female rats, and a small increase in thyroid carcinomas in female mice, but not male mice. The CIR Expert Panel considered that this pattern of findings was not consistent between different sexes in different species and was not likely indicative of a carcinogenic effect of t-Butyl Alcohol. Perhaps more importantly, the Panel found an absence of a true dose response in the NTP study, further suggesting the absence of a carcinogenic effect. In addition, the Panel concluded that the renal tubule effects found in male rats was likely an effect of α2µ-globulin. Overall, the Panel decided that the studies on t-Butyl Alcohol showed that it was a weak carcinogen (at most) and unlikely to have significant carcinogenic potential as currently used in cosmetic formulations.

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

DISCUSSION

To be developed.

CONCLUSION

To be determined.

TABLES

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

Property	Value	Reference
Physical Form	crystals; solid liquid (above 25 °C)	3,18
Color	colorless	18
Odor	camphor	18
Molecular Weight (g/mol)	74.12	3
Specific Gravity (@ 25ºC)	0.78	27CFR21.101
Vapor pressure (mmHg@ 25ºC)	42	18
Vapor Density (mmHg)	2.55	18
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 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 Concentration of Use	Daily Exposure Based on the Highest 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	5000 γ			
Aerosol		0.066 - 0.11%	5.5	
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	1540			Exposure amount of face cream applied
Not spray		0.005 - 0.048%	0.7392	
Skin fresheners	308 #	0.003%	0.00924	Exposure amount of face mask applied
Other skin care preparations	2160	0.01%	0.216	Exposure amount of hand cream used

***** Exposure parameters are retrieved from the SCCS NoG[22]

Exposure amount is provided by Vermeer Cosmolife[23]

γ Exposure amount is provided by CTFA (currently known as PCPC) habits and practices data[24]

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3

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

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

INTRODUCTION

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

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

CHEMICAL AND PHYSICAL PROPERTIES

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

Other names for *t*-BuOH include tertiary butyl alcohol, tert-butyl alcohol, tertiary butanol, tert-butanol, *t*-butanol, 2-methyl-2-propanol, and trimethyl carbinol.[1-3]

t-BuOH is available in the form of colorless, hygroscopic crystals with a camphoraceous odor. The crystals become a clear liquid above 25.5°C. *t*-BuOH is soluble in water, alcohol, ether, and other organic solvents.[3-7] Chemical and physical properties of *t*-BuOH are presented in Table 1.

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

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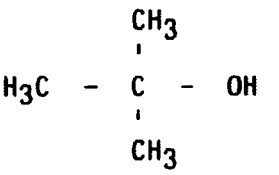

FIG. 1. t-Butyl Alcohol.

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

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

t-BuOH used in cosmetics typically contains 99.5% *t*-BuOH, a maximum of 0.002% acidity (as acetic acid), a maximum of 0.1% water, and a maximum of 0.001% nonvolatile matter.[4]

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

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

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

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

USE

Cosmetic Use

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

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

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

Product formulations containing *t*-BuOH may be applied as many as several times a day and may remain in contact with the skin for variable periods following application. Daily or occasional use may extend over many years.[17]

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

Noncosmetic Use

t-BuOH is permitted as an indirect food additive. *t*-BuOH may be used in formulating defoaming agents used in the preparation and application of coatings for paper and paperboard; these coatings may be safely used as components of articles intended for use in producing, manufacturing, packing, processing, preparing, treating, packaging, transporting, or holding food.[9] *t*-BuOH may be safely used in surface lubricants employed in the manufacture of metallic articles that contact food; it may be used in surface lubricants used in the rolling of metallic foil or sheet stock, provided that the total residual lubricant remaining on the metallic article in the form in which it contacts food does not exceed 0.015 mg/square inch of metallic food–contact surface.[9]

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

t-BuOH has been used as an alcohol denaturant, a flotation agent, a dehydration agent, a solvent, and an octane booster in gasoline. It has been used in paint removers, as a chemical intermediate, and in chemical analyses.[3,5]

BIOLOGY

Effects on Enzymes and Membranes

t-BuOH affects the activity of a variety of enzymes and may stabilize or destabilize a variety of biological membranes. These effects vary with concentration and with temperature and may be due to perturbation of protein conformation, structural changes in membrane lipids, or disturbance of lipid–protein interactions.[19–22]

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

Action as a Hydroxyl Radical Scavenger

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

Environmental Occurrence

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

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

t-BuOH is not a substrate for alcohol dehydrogenase or for catalase and has been used as an example of a nonmetabolizable alcohol. However, the results of recent investigations have indicated that *t*-BuOH is not as inert metabolically as previously assumed.[29,30] *t*-BuOH is a hydroxyl radical scavenger; in rat liver microsomes, it can be oxidatively demethylated by hydroxyl radicals generated from NADPH-dependent microsomal electron transfer to yield formaldehyde.[29,31] Baker et al.[32] investigated the in vivo metabolism of *t*-BuOH to acetone in Long-Evans rats and inbred Sprague-Dawley rats after intraperitoneal doses of 1 g/kg *t*-BuOH. *t*-BuOH concentration in the blood was measured over a 24 h period; the half-life of *t*-BuOH was 9.1 h. Acetone, produced by the metabolism of *t*-BuOH, was also detected in the blood. Acetone was slowly eliminated from the blood by excretion in the urine and

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expired air, but the quantity excreted was highly variable. Baker et al.[32] injected two rats with 1.75 g/kg β-[^{14}C]*t*-BuOH. Over a 24 h period, 68.7% of the total dose was recovered from one rat and 93.2% was recovered from the other rat as CO_2 and acetone. When the animals were injected with 1.5 g/kg of a 1:1 mixture of α-[^{13}C]*t*-BuOH and *t*-BuOH, more acetone than expected was recovered. *t*-BuOH was a source of acetone, but also may have stimulated acetone production from other sources. Treatment of rats with U-[^{14}C]hexadecanoic acid and *t*-BuOH followed by collection of respiratory gases indicated that *t*-BuOH did not affect fatty acid synthesis.

t-BuOH is eliminated slowly from the blood of rats. *t*-BuOH was dissolved in water and a dose of 25 mmol/kg was administered by gastric intubation to female Wistar rats (number unspecified).[33] The *t*-BuOH blood concentration at 2 h was 13.24 mM, at 5 h it was 12.57 mM, and at 20 h it was 11.35 mM.

A 5.7 (w/v) solution of *t*-BuOH in saline was administered by gastric intubation to four to six female Sprague-Dawley rats every 8 h for 1 or 2.5 days; *t*-BuOH was administered in an amount inversely proportional to the degree of intoxication in order to maintain a uniform blood *t*-BuOH concentration of 60–100 mg percent.[34] The rats were then given *t*-BuOH to elevate their blood concentrations to between 125 and 150 mg percent, and blood was taken from the tails and sampled for *t*-BuOH. Eighteen hours were required to eliminate *t*-BuOH completely from the blood when the rats were treated for 2.5 days, and 26 h were required when the rats were treated for 1 day; the rate of elimination of 1.2 g/kg *t*-BuOH was 0.7 mmol/kg rat/h. Acetaldehyde was not detected in the blood or brain of rats treated for 3 days with *t*-BuOH. *t*-BuOH did not affect the oxygen uptake or pyridine nucleotide redox state of perfused rat liver.

t-BuOH is also slowly eliminated from the blood of mice. McComb and Goldstein[35] administered a single intraperitoneal dose of 8.1 mmol/kg *t*-BuOH to nine male Swiss-Webster mice; *t*-BuOH was eliminated from the blood in 8–9 h. The same mice then inhaled *t*-BuOH vapor for 3 days; the concentration of *t*-BuOH vapor administered was that which maintained a mean blood concentration of 8 mM *t*-BuOH. The researchers found it necessary to raise the *t*-BuOH vapor concentration progressively to maintain a given concentration of *t*-BuOH in the blood. *t*-BuOH was not detected in the blood 3 h after the mice were removed from the vapor chamber. A single intraperitoneal dose of 8.1 mmol/kg *t*-BuOH was administered (to an unspecified number of mice) 4 h after the end of a 3 day inhalation period; no *t*-BuOH was detected in the blood 3 h later. The increased elimination rate of *t*-BuOH may have been due to metabolic tolerance; more *t*-BuOH may have been conjugated and eliminated in animals previously exposed to *t*-BuOH.

The intragastric administration of *t*-BuOH to rats increased the rate of elimination of subsequently administered ethanol in comparison with the rate of elimination of ethanol by rats not given *t*-BuOH.[36]

Kamil et al.[37] administered 12 mmol of *t*-BuOH by stomach tube to three chinchilla rabbits. *t*-BuOH was conjugated to a large extent with glucuronic acid, and glucuronides were readily isolated from the rabbit urine; as a percentage of dose, the average extra glucuronic acid excreted over 24 h was 24.4%. The researchers suggested that volatile alcohols might also be elimi-

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nated to some extent in an unchanged state by the lungs. No aldehydes or ketones were detected in the expired air of a rabbit given 6 ml *t*-BuOH (route unspecified).

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

ANIMAL TOXICOLOGY

Oral Studies

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

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

A dose of 25 mmol/kg (1.85 g/kg) *t*-BuOH as a 25% by volume solution in water was administered by gastric intubation to female Wistar rats (unspecified number).[33] Control rats received water. *t*-BuOH concentration in blood dropped only a small amount between 2 and 20 h after dosing. Blood free fatty acid concentration was unchanged at 2 h and increased at 5 h, and triacylglycerol concentration was decreased at 20 h. Hepatic triacylglycerols were increased at 2 and at 5 h. There were no significant changes in hepatic and blood phospholipid concentrations or in the 4 h lactate/pyruvate ratio. Hepatic palmitate uptake into triacylglycerols was increased at 2, 5, and 20 h, and palmitate incorporation into serum triacylglycerols was about 50% of control values at 5 and 20 h. The researchers concluded that *t*-BuOH induced a fatty liver, but not by impairing fatty acid oxidation.

A group of 12 female Wistar rats was given 4 ml/kg *t*-BuOH in a single oral dose.[42,43] Seventeen hours later, in comparison with a control group of rats, the relative weight of the liver was significantly increased, but there was no change in the hepatic nitrogen concentration, or in the fatty acid, triglyceride, cholesterol, or phospholipid concentrations in the blood.

A 3 g/kg dose of *t*-BuOH was administered orally to male Sprague-Dawley rats (unspecified number).[44] Later (unspecified time but 2 h later is likely), the rats were decapitated and brain homogenate was incubated with choline for 4 min at 37°C. Choline uptake was increased in the caudate nucleus and decreased in the hippocampus in comparison with control rats.

Four male Wistar rats were given a single oral dose of 2.54 g/kg *t*-BuOH.[30] Control rats received saline. Six hours after administration, the hepatic reduced glutathione concentration was decreased, although not significantly, and diene conjugate formation was increased, although not significantly, in comparison with the control rats.

An indwelling gastric fistula was surgically implanted 4 days after birth into eight Long-Evans rats from each of six litters to implement an artificial feeding method.[45] Four rats from each litter received milk formula containing a mean daily dose of *t*-BuOH that ranged from 0.60 to 2.69 g/kg on postnatal

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days 4 through 7 and then received only milk formula for the next 11 days. The other 4 rats from each litter received only milk formula. At postnatal day 18, all the rats were decapitated, various organs were weighed, and biochemical analyses were performed. Only 26 of 48 animals survived the experiment; the major cause of death was a poor fistulation procedure or gastric bloating. Blood concentrations of *t*-BuOH ranged from 33.0 to 66.0 mg/100 ml of blood during alcohol administration. No differences between groups were observed in emergence of teeth, eye opening, or unfolding of the ears. No significant differences were observed between treated and control rats in body, liver, and heart weights, but the brains weighed significantly less in the treated rats; treated rats had decreased protein in the forebrains and decreased DNA in the hindbrains.

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

Groups of 10 male and 10 female B6C3F$_1$ mice were given drinking water containing 0, 0.25, 0.5, 1.0, 2.0, and 4.0% *t*-BuOH for 90 days.[47] Average dosages for males were 0, 319.3, 726.3, 1565.8, 2838.8, and 6247.2 mg/kg/day, and average dosages for females were 0, 568.3, 941.7, 1731.8, 4362.9, and 7475.8 mg/kg/day. Six of 10 male mice and 4 of 10 female mice died receiving the highest dosage. There was a dosage-related depression in weight gain in the males that received water containing 1, 2, and 4% *t*-BuOH and in the females that received water containing 2 and 4% *t*-BuOH. Hyperplasia of the transitional epithelium of the urinary bladder and inflammation of the urinary bladder were observed. Other pathologic effects were considered secondary to inanition. The no-effect concentration for direct chemical effects was 1% for male mice and 2% for female mice.

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Dermal Studies

Renkonen and Tier[48] conducted an experiment to investigate the intradermal irritation of *t*-BuOH to rabbits. There were no vehicle controls. Eight rabbits were injected intradermally with *t*-BuOH (vehicle unspecified). The size of the local skin reaction after injection of 35 mg *t*-BuOH was 14 mm^2, and after 10 mg *t*-BuOH was 43 mm^2. No explanation of the significance of these results was provided.

SPECIAL STUDIES

Animal Reproduction and Teratology

Groups of 15 pregnant Swiss-Webster mice were fed liquid diets containing *t*-BuOH at concentrations of 0.5, 0.75, and 1.0% (w/v) from days 6 to 20 of gestation.[49] Control mice were fed only the liquid diet. The 1.0% *t*-BuOH group was fed ad libitum. The other groups were pair-fed based on the consumption of the 1.0% *t*-BuOH group. The average maternal weight gain over the 20 days was 64% for the controls and 62, 52, and 51% for the 0.50, 0.75, and 1.0% *t*-BuOH-fed groups, respectively. Approximately one-half of the maternal animals in each group were replaced with untreated surrogate mothers within 24 h of delivery of litters to determine the role of maternal nutritional and behavioral factors on the young. Length of gestation, gross structural abnormalities, and number of deaths were recorded. Weight measurements, pinna detachment, eye opening, and behavioral test scores for the young were determined various times during days 2–22 postparturition. The total number of litters from 15 animals was 11 (77%) in the control group, 12 (80%) in the 0.5% *t*-BuOH group, 8 (53%) in the 0.75% group, and 7 (47%) in the 1.0% group. The average number of neonates per litter was 10.4 in the control group, 10.3 in the 0.5% *t*-BuOH group, 7.4 in the 0.75% group, and 5.3 in the 1.0% group. The average "fetal" weight at day 2 was 1.78 g in the control group, 1.66 g in the 0.5% *t*-BuOH group, 1.45 g in the 0.75% group, and 1.10 g in the 1.0% group. There was a dosage–response relationship between *t*-BuOH concentration in the diet and total number of stillborns (number of stillborns per litter size not given); there were 3 stillborns in the control group, 6 in the 0.5% *t*-BuOH group, 14 in the 0.75% group, and 20 in the 1.0% group. Pinna detachment occurred between days 6 and 8 in all the groups. Eyes opened in the 1.0% *t*-BuOH group at around day 16; this was 2–4 days later than in the other groups. Postnatal weight gain was decreased over the first 10 days in the nonfostered 0.75 and 1.0% groups in comparison to the other groups. There was a general dosage–response relationship between higher *t*-BuOH exposure in utero and poorer behavioral performance of pups. Fostered pups performed significantly better than nonfostered pups in three of four behavioral tests. All the treated groups did eventually recover and acquire the same level of performance on the behavioral tests.

Anderson et al.[50] determined the effect of *t*-BuOH on in vitro fertilization of Swiss-Webster mice gametes. Capacitated epididymal mouse spermatozoa were added to mouse oocytes with cumulus masses and, after a 24 h

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incubation, the eggs were examined for fertilization. *t*-BuOH, at a concentration of 87 mM, was added to both the capacitation and the culture media. It did not affect the in vitro fertilization capacity of spermatozoa.

Mutagenicity

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

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

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

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

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

Carcinogenicity

Hair was clipped from the backs close to the base of the tail of female ddN mice, chemicals were applied to this bared skin, and the mice were observed for 450 days.[59] Moribund animals were killed and tissues were examined. In the first experiment, 0.05 mg 4-nitroquinoline-1-oxide (4NQO) in benzene was applied to the mice 3 times a week for a total of 20 applications. No acute skin damage was observed. In 50 surviving mice, there was 1 small papilloma and no "skin tumors." In a second experiment, 4NQO was applied as in the first experiment and was followed by applications of 16.6% *t*-BuOH (actual dosage unspecified) in benzene 6 times a week for a total of 270 applications. No acute skin damage was observed within about 100 days. After 350 days, two "erosions" were produced at the application site and these remained for the duration of the observation period. About 150 days after the start of the experiment and after about 100 applications of *t*-BuOH, one neoplasm was observed and "it developed into squamous cell carcinoma rapidly." About 300 days after the start of the experiment, a subcutaneous granuloma was detected. Fifty mice survived after the appearance of the first tumor in the experiment.

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

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CLINICAL ASSESSMENT OF SAFETY

A woman who had a positive patch test reaction to ethanol was tested with 100% *t*-BuOH.[61] The alcohol was applied for 48 h and the site was scored at 3, 24, and 48 h after removal of the test material. The woman had a negative reaction to *t*-BuOH.

Four female patients were tested on the upper back with 1 and 10% *t*-BuOH in water.[62] The patches were applied for 24 h and reactions were read 24 and 48 h after removal. None of the women had any reaction to *t*-BuOH.

Edwards and Edwards[18] described a case of allergic contact dermatitis to the *t*-BuOH component of SD-40 alcohol in a commercial sunscreen preparation. A man who had a widespread, pruritic, red, vesicular eruption of his face, neck, arms, and chest and who had used a variety of sunscreens was patch-tested with sunscreens and with the individual components of the product to which he reacted. A 70% concentration of *t*-BuOH was applied to the forearms. At 72 h, erythema was observed and at 96 h, vesiculation was observed. No reactions were observed in two controls who also had applied *t*-BuOH to their forearms.

Dermatitis has also been observed when *t*-BuOH is applied to the skin; it caused slight pain, moderate hyperemia and erythema, dryness, and vesiculation.[63-65]

The ACGIH has set a threshold limit value of 100 ppm and a short-term exposure limit of 150 ppm that is satisfactory to prevent narcosis with *t*-BuOH.[63] The threshold limit value is the time-weighted average concentration for a normal 8 h workday or 40 h workweek and no adverse effects are expected from it. The short-term exposure limit is that concentration to which workers can be exposed for 15 min without suffering ill effects. Four 15 min periods are permitted per day with at least 60 min between exposure periods. In addition, the daily threshold limit value must not be exceeded.[2] NIOSH has reported that 8000 ppm *t*-BuOH is the concentration immediately dangerous to life or health.[11]

SUMMARY

t-BuOH is a tertiary aliphatic alcohol that is used as a solvent or an alcohol denaturant and as a perfume carrier in cosmetics. *t*-BuOH absorbs ultraviolet light at 275 nm and does not absorb at any longer wavelength. In 1986, *t*-BuOH was reported as an ingredient in 10 hair and facial skin care preparations at concentrations ranging from $\leq 0.1\%$ to between 0.1 and 1%.

t-BuOH is not a substrate for alcohol dehydrogenase. In rat liver microsomes, *t*-BuOH can be oxidatively demethylated by hydroxyl radicals to yield formaldehyde. Acetone was found in the blood, urine, and expired air of rats following the intraperitoneal administration of *t*-BuOH. *t*-BuOH was slowly eliminated from the blood of rats and mice; elimination is more rapid in animals previously exposed to *t*-BuOH.

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

The oral administration of *t*-BuOH to mice during pregnancy resulted in poorer initial behavioral performance of pups. The pups did eventually recover. *t*-BuOH did not affect the in vitro fertilization capacity of mouse spermatozoa.

t-BuOH was not mutagenic in the *Salmonella*/mammalian-microsome mutagenicity test, did not increase the incidences of penicillin or streptomycin resistance in *Micrococcus aureus*, and did not induce adenine independence in adenine-dependent *Neurospora crassa*. *t*-BuOH was mutagenic to cultured human–Chinese hamster ovary hybrid cells at a cytotoxic dose.

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

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

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

DISCUSSION

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

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

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

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

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

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

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

CONCLUSION

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

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

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

INTRODUCTION

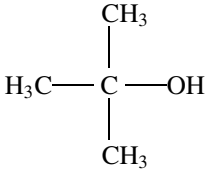
In its initial safety assessment of t-Butyl alcohol (t-BuOH), the Cosmetic Ingredient Review (CIR) Expert Panel concluded that the available data were insufficient to support the safety of this ingredient in cosmetics (CIR 1989). The studies that were needed in order to complete a safety assessment were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

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

CHEMISTRY

Definition and Structure

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



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

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[1]Reviewed by the Cosmetic Ingredient Review Expert Panel. This report was prepared by Melody Chen, CIR Scientific Analyst and Writer.

Physical and Chemical Properties

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

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

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

Methods of Production

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

In the 4th edition of the *Encyclopedia of Chemical Technology*, the butyl alcohol entry states that t-BuOH is produced as a by-product from the isobutane oxidation process for producing propylene oxide (Kirk-Othmer 1992a). In the butylenes entry, it is further noted that volume quantities of t-BuOH are prepared using the Oxirane process for the manufacture of propylene oxide which produces t-BuOH as a by-product (Kirk-Othmer 1992b).

TABLE 1
Chemical and physical properties of t-BuOH

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

Analytical Methods

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

Impurities

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

USE

Cosmetic

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

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

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

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

Noncosmetic

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

TABLE 2
Product formulation data on t-BuOH

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

*These concentrations are not alcohol denaturant uses.

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

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

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

Use as an Alcohol Denaturant

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

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

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

GENERAL BIOLOGY

Absorption, Distribution, Metabolism, and Excretion

Absorption

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

Distribution

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

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

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

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

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

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

A 5.7 (*w/v*) solution of t-BuOH in saline was administered by gastric intubation to 4 to 6 female Sprague-Dawley rats every 8 h for 1 or 2.5 days; t-BuOH was administered in an amount inversely proportional to the degree of intoxication in order to maintain a uniform blood t-BuOH concentration of 60 to 100 mg % (Thurman et al. 1980). The rats were then given t-BuOH to elevate their blood concentrations to between 125 and 150 mg %, and blood was taken from the tails and sampled for t-BuOH. Eighteen hours were required to eliminate t-BuOH completely from the blood when the rats were treated for 2.5 days, and 26 h were required when the rats were treated for 1 day; the rate of elimination of 1.2 g/kg t-BuOH was 0.7 mmol/kg rat/h. Acetaldehyde was not detected in the blood or brain of rats treated for 3 days with t-BuOH. t-BuOH did not affect the oxygen uptake or pyridine nucleotide redox state of perfused rat liver.

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

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

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

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

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

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

Metabolism

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

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

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

These authors also injected two rats with 1.75 g/kg β-[^{14}C]t-BuOH. Over a 24-h period, 68.7% of the total dose was recovered from one rat and 93.2% was recovered from the other rat as CO_2 and acetone. When the animals were injected with 1.5 g/kg of a 1:1 mixture of α-[^{13}C] t-BuOH and t-BuOH, more acetone than expected was recovered. t-BuOH was a source of acetone, but also may have stimulated acetone production from other sources. Treatment of rats with U-[^{14}C] hexadecanoic acid and t-BuOH followed by collection of respiratory gases indicated that t-BuOH did not affect fatty acid synthesis (Baker et al. 1982).

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

Excretion

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

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

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

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

Cytotoxicity

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

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

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

Hydroxyl Radical Scavenger

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

ANIMAL TOXICOLOGY

Oral Toxicity

Acute

The LD_{50} of t-BuOH for white rats (unspecified strain) was 3.5 g/kg (details of experiment unspecified) (Schaffarzick and Brown 1952).

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

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

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

A 3 g/kg dose of t-BuOH was administered orally to male Sprague-Dawley rats (unspecified number) (Hunt et al. 1979). Later (unspecified time but 2 h later is likely), the rats were decapitated and brain homogenate was incubated with choline for 4 min at 37°C. Choline uptake was increased in the caudate nucleus and decreased in the hippocampus in comparison with control rats.

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

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

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

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

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

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

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

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

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

Short-Term

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

Elf Atochem North American Incorporated (1994a) conducted a study in which five groups of five male and five female B6C3F$_1$ mice received 0.125%, 0.25%, 0.50%, 1%, and 2% (*w/v*) t-BuOH in their drinking water for 14 days. A control group was given tap water. Changes in body weight were difficult to interpret; in both males and females the two high-dose groups outgained the three lower-dose groups. No scientific rationale was provided for these results. Males given 2% t-BuOH consumed 34% less water than the controls in the first week and 28% less than the controls in the second week, whereas the males given 1% t-BuOH consumed 29% less in the first week and 7% less than the controls in the second week. The females given 1% and 2% t-BuOH drank less water in the first week than the controls, but drank 12% and 18% more in the second week.

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

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

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

Subchronic

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

increased but insignificant were testicular interstitial adenomas and thymomas in males; and lung adenomas and pituitary adenomas/carcinomas in females. The results for $B6C3F_1$ mice in the same study showed that t-BuOH affected livers in males (fatty changes); urinary bladders (chronic inflammation and hyperplasia of transitional cell epithelium) in both males and females; and thyroids (proliferative changes including hyperplasia and neoplasia) in both males and females (Arco Chemical Company 1992).

As reported in Lindamood et al. (1992) and Takahashi et al. (1993), groups of F344 rats and $B6C3F_1$ mice (10 male and 10 female) were given 0%, 0.25%, 0.5%, 1%, 2%, and 4% (*w/v*) t-BuOH in drinking water for 94 to 95 days). All high-dose rats, six male and four female mice given 4%, t-BuOH, died before the end of the study. A significant decrease in body weight occurred in all dose levels of male rats; female rats given 4% t-BuOH; male mice given 1%, 2%, and 4% t-BuOH; and female mice given 2% and 4% t-BuOH. The 0.25% to 2% dose groups showed a statistically significant decrease in body weight gain. Water consumption decreased in the high-dose groups of both sexes and species whereas it increased in the low-dose groups in male rats. Clinical signs for both sexes of rats included emaciation, ataxia, blood in the urine, and hypoactivity. Males also exhibited paraphimosis while females also exhibited urine staining of the fur. In mice, clinical signs included emaciation, ataxia, abnormal posture, and hypoactivity.

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

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

In a study reported both by Elf Atochem North American Incorporated (1994b) and Amoco Corporation (1994), groups of $B6C3F_1$ mice and F344 rats (10 male, 10 female) received 0.25%, 0.5%, 1%, 2% or 4% t-BuOH (*w/v*) in their drinking water for 13 weeks. The control groups received plain tap water. In both species, t-BuOH was found to be more toxic to males than to females.

Among the rats, nine males and two females in the 4% group died between the 4th and 13th weeks. A reduction in growth rate was seen among males in the 1% and higher dose levels with the controls outgaining them by 16% to 104%. The control females outgained the 2% and 4% groups by 11% and 46%, respectively. Histopathology revealed the presence of papillary hyperplasia of the transitional epithelium of the urinary bladder in five males and two females in the 4% group. This group also had a decrease in the cell population of bone marrow in nine males and three females.

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

The NTP (1995) reported a study in which groups of F344 rats and $B6C3F_1$ mice (10 male, 10 female) were given 0, 2.5, 5, 10, 20, or 40 mg/ml t-BuOH in their drinking water for 13 weeks. Treatment-related mortalities occurred at the highest concentration in male and female rats and mice. In addition, mean body weight gains of these groups were significantly lower than those of the controls. There was decreased water consumption by treated rats and by the 20 and 40 mg/ml groups of mice during the first week indicating decreased palatability of the dosed water. Some liver toxicity was suggested by a slight increase in serum alanine aminotransferase activity in all exposed groups of female rats.

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

Chronic

In a 2-year NTP study (1995), groups of 60 male F344 rats were given 1.25, 2.5, or 5 mg/ml (90, 200, 420 mg/kg) t-BuOH; groups of 60 female rats were given 2.5, 5, or 10 mg/ml (180, 330, 650 mg/kg) t-BuOH; and groups of 60 male and 60 female $B6C3F_1$ mice were given 5, 10, or 20 mg/ml (540, 1040, 2070 mg/kg for males; 510, 1020, 2110 mg/kg for females) t-BuOH in their drinking water. Controls were given untreated water. Survival of the male rats given 5 mg/ml t-BuOH was significantly lower than that of the controls. Survival among exposed female rats was lower than that of controls, especially in

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

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

Acute Dermal Toxicity

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

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

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

Inhalation Toxicity

Acute

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

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

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

Short-Term

The NTP (1997) reported a study in which groups of five male and five female F344 rats and $B6C3F_1$ mice were exposed to t-BuOH by whole-body inhalation to target concentrations of 0, 450, 900, 1750, 3500, and 7000 ppm for 6 h plus T_{90} per day, 5 days per week, over an 18-day period.

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

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

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

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

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

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

Subchronic

NTP (1997) reported results of a study in which groups of F344 rats and $B6C3F_1$ mice (10 male, 10 female) were exposed to t-BuOH for 6 h plus T_{90} per day, 5 days per week, for 13 weeks. Actual concentrations used were 135, 270, 540, 1080, and 2100 ppm. The only t-BuOH–related death that occurred was that of a 2100 ppm male mouse. Body weight gains were similar to those of the controls for all exposed rats, but were significantly less for the 135 and 270 ppm male and the 1080 and 2100 ppm female mice. Female rats (2100 ppm) were emaciated and hypoactive.

Other effects of t-BuOHs exposure included slight anemia and decreased serum alkaline phosphatase activity in exposed male rats and a marked increase in the number of segmented neutrophils in the 2100 ppm male mice. In rats, kidney weights of 1080 ppm males and 2100 ppm males and females were significantly greater than those of the controls. Likewise, liver weights of the 1080 and 2100 ppm female rats and mice were greater than those of the controls (NTP 1997).

Acute Intravenous Toxicity

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

Ocular Irritation

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

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

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

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

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

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

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

Dermal Irritation

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

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

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

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

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Groups of F344 rats and $B6C3F_1$ mice (10 male, 10 female) were exposed to t-BuOH for 6 h plus T_{90} per day, 5 days per week, for 13 weeks. Actual concentrations used were 135, 270, 540, 1080, and 2100 ppm. No significant differences were found in the weight of the testis, epididymis, and cauda; and sperm motility, count, and morphology for males. For females, no significant differences were found in the estrous cycle length or percentage of time spent in the various stages (NTP 1997).

Groups of 60 male F344 rats were given 1.25, 2.5, or 5 mg/ml (90, 200, 420 mg/kg) t-BuOH; groups of 60 female rats were given 2.5, 5, or 10 mg/ml (180, 330, 650 mg/kg) t-BuOH; and groups of 60 male and 60 female $B6C3F_1$ mice were given 5, 10, or 20 mg/ml (540, 1040, 2070 mg/kg for males; 510, 1020, 2110 mg/kg for females) t-BuOH in their drinking water for 2 years. Controls were given untreated water (NTP 1995). No significant differences in sperm morphology or motility were found for male rats or mice; and for female rats, no significant differences in the length of the estrous cycle or percentage of time spent in the various estrous stages occurred. However, for female mice, estrous cycle length was significantly increased in the highest-dose group whereas percentage of time spent in the various estrous stages did not differ from controls.

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

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

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

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

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

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

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

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

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

The teratogenic effects of t-BuOH were studied using cells from chicken embryo wing buds (Kulyk and Hoffman 1996). Cultures were fed with medium containing 0.1% to 4% (*v/v*) t-BuOH. t-BuOH was effective at enhancing cartilage differentiation. The authors postulated that this could interfere with proper skeletal morphogenesis.

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

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

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

GENOTOXICITY

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

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

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

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

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

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

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

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

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

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

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

CARCINOGENICITY

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

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

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

In a 2-year NTP study (1995), groups of 60 male F344 rats were given 1.25, 2.5, or 5 mg/ml (90, 200, 420 mg/kg) t-BuOH; groups of 60 female rats were given 2.5, 5, or 10 mg/ml (180, 330, 650 mg/kg) t-BuOH; and groups of 60 male and 60 female $B6C3F_1$ mice were given 5, 10, or 20 mg/ml (540, 1040, 2070 mg/kg for males; 510, 1020, 2110 mg/kg for females) t-BuOH in their drinking water. Controls were given untreated water. Because an interim evaluation of 10 rats/sex/dose was performed at 15 months, 50 rats/group completed the study.

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

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

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

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

*Average severity of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
**$p \leq .05$.
***$p \leq .01$.

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

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

*Average severity of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
**$p \leq .05$.
***$p \leq .01$.

$B6C3F_1$ mice due to marginally increased incidences of follicular cell adenoma or carcinoma of the thyroid gland. Due to the increased incidences found in female mice, it was concluded that there was "some evidence of carcinogenic activity" of t-BuOH in female mice (NTP 1995).

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

CLINICAL ASSESSMENT OF SAFETY

Irritation and Sensitization

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

TABLE 6
Incidence of thyroid and bladder lesions in $B6C3F_1$ mice (NTP 1995; Cirvello et al. 1995)

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

Case Reports

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

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

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

Occupational Health and Safety

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

SUMMARY

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

An RIPT test showed no potential for eliciting either dermal irritation or sensitization by100% t-BuOH. Dermatitis can result from dermal exposure of humans to t-BuOH.

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

DISCUSSION

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

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

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

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

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

CONCLUSION

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

REFERENCES

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Concentration of Use by FDA Product Category – t-Butyl Alcohol

Product Category	Maximum Concentration of Use
Eye lotions	0.004-0.0042%
Mascaras	0.01%
Colognes and toilet waters	0.097%
Perfumes	0.096-0.11%
Hair sprays	
Aerosol	0.066-0.11%
Shampoos (noncoloring)	0.00014%
Tonics, dressings, and other hair grooming aids	
Spray	0.06%
Lipstick	0.0001-0.007%
Makeup bases	0.006%
Dentifrices	0.028%
Deodorants	
Not spray	0.89%
Other personal cleanliness products	0.16%
Aftershave lotions	0.079-0.91%
Beard softeners	0.029%
Skin cleansing (cold creams, cleansing lotions, liquids, and pads)	0.0047-0.088%
Face and neck products	
Not spray	0.016-0.044%
Spray	0.094%
Body and hand products	
Not spray	0.0054-0.05%
Spray	0.1%
Moisturizing products	
Not spray	0.005-0.048%
Skin fresheners	0.003%
Other skin care preparations	0.01%

Information collected in 2022

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