Amended Safety Assessment of Butoxyethanol as Used in Cosmetics

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Release Date:	February 14, 2025
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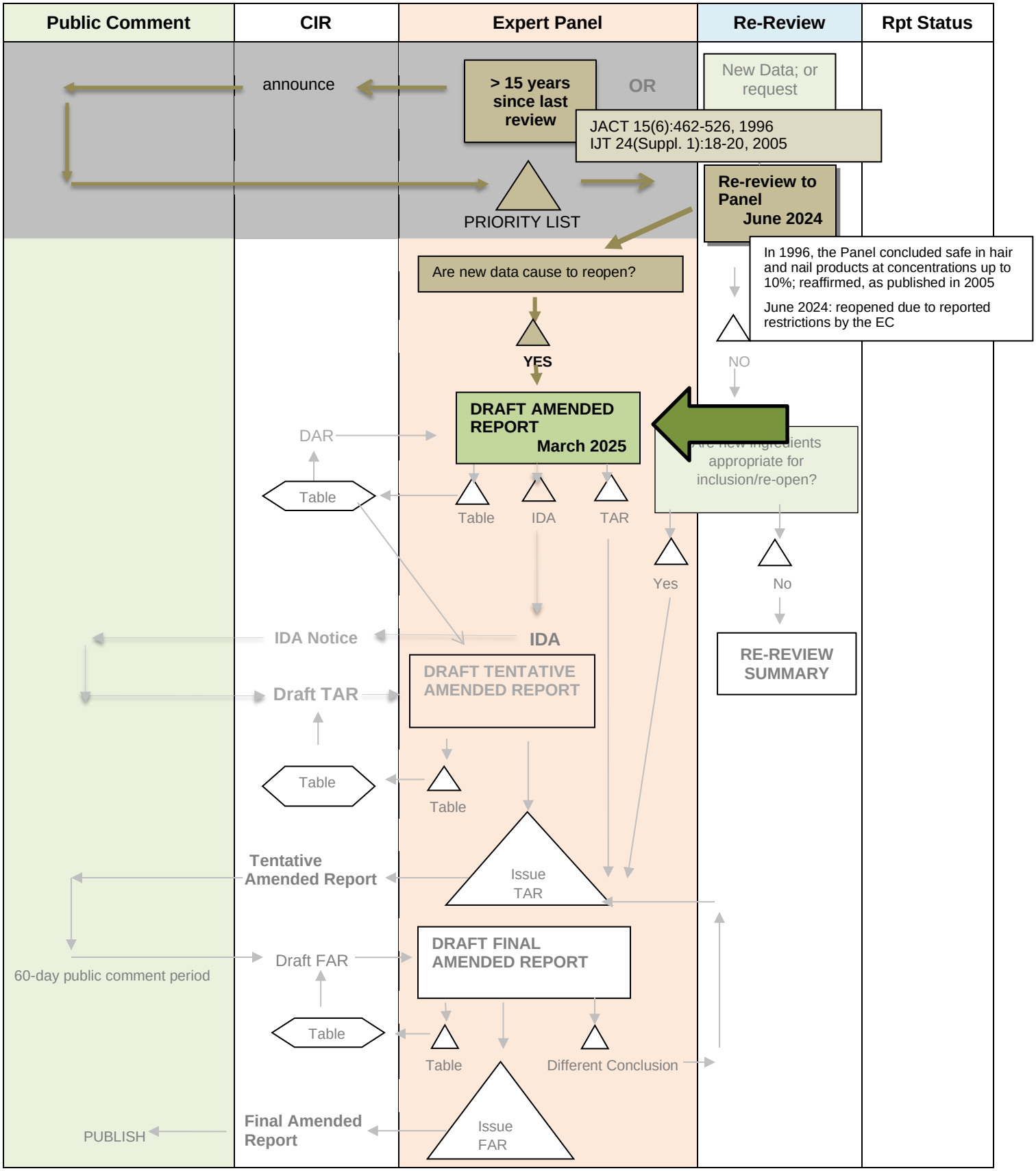
The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Samuel Cohen, M.D., Ph.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume, M.B.A. This safety assessment was prepared by Christina Burnett, M.S., Senior Scientific Analyst/Writer, CIR.

555 13th Street, NW, Suite 300W, Washington, DC 20004
cirinfo@cir-safety.org

RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Butoxyethanol

MEETING March 2025



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Christina L. Burnett, M.S., Senior Scientific Analyst/Writer, CIR
Date: February 14, 2025
Subject: Amended Safety Assessment of Butoxyethanol as Used in Cosmetics

Enclosed is the Draft Amended Report on the Amended Safety of Butoxyethanol as Used in Cosmetics. (It is identified as *report_Butoxyethanol_032025* in the pdf document). The original review of Butoxyethanol was published in 1996 with the conclusion that "Butoxyethanol is safe in hair and nail products at concentrations up to 10.0%" (*originalreport1996_Butoxyethanol_032025*). The Panel reassessed the safety of Butoxyethanol in cosmetics because of questionable evidence of carcinogenicity (in mice and rats) in a two-year NTP inhalation carcinogenicity study on Butoxyethanol that was published in 2000. The Panel determined that the results were not relevant to humans, and reaffirmed the original conclusion in a re-review that was published in 2005 (*rereview2005_Butoxyethanol_032025*).

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. In June 2024, the Panel determined that this safety assessment should be re-opened for re-evaluation due to reported restrictions by the European Commission on the use of Butoxyethanol. Excerpts from the 1996 report are disseminated throughout the text of this document, as appropriate, and are identified by *italicized* text.

According to RLD that CIR received in 2024, Butoxyethanol is used in 81 formulations, with 79 uses reported in hair dyes and colors. A single use each was reported in perfumes and makeup fixatives. VCRP survey data received in 2023 reported Butoxyethanol was used in 3 hair dyes and colors. When comparing the VCRP data received in 2023 to that received in 2001, the frequencies of use for Butoxyethanol have greatly decreased since the re-review was performed; in 2001, Butoxyethanol was reported to have 110 uses, with the majority in hair coloring formulations. No uses were reported in response to the concentration of use survey conducted by the Council in 2020 (*data_Butoxyethanol_032025*). In 2001, the maximum concentration of use range for Butoxyethanol was 3% in leave-on products (i.e., eye shadow, blushers, and nail polish and enamel formulations) and 50% in rinse-off products (i.e., nail polish and removers). Concentrations of use were not reported for hair coloring preparations in 2001.

Additional supporting documents for this report package include a flow chart (*flow_Butoxyethanol_032025*), report history (*history_ Butoxyethanol_032025*), search strategy (*search_Butoxyethanol_032025*), data profile (*dataprofile_Butoxyethanol_032025*), transcripts from the June 2024 meeting (*transcripts_Butoxyethanol_032025*), and the minutes from all the meetings at which Butoxyethanol were discussed during the original reviews (*originalminutes_Butoxyethanol_032025*).

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

Butoxyethanol History

1996 – The CIR Final Report on the Safety Assessment of Butoxyethanol was published in the *Journal of the American College of Toxicology*. The Panel concluded that Butoxyethanol is safe in hair and nail products at concentrations up to 10.0%

2005 – The reaffirmed conclusion in a re-review of Butoxyethanol was published in 2005 in the *International Journal of Toxicology*.

June 2024 – The Panel determined that this safety assessment should be re-opened for re-evaluation due to reported restrictions by the European Commission on the use of Butoxyethanol.

Butoxyethanol Data Profile* – March 2025 – Christina Burnett																															
	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	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Butoxyethanol CAS No. 111-76-2	**X**	**O**	**O**	XO	X	XO	X	XO	XO	XO	O	XO	XO	O	XO	XO	X	O	X		XO			X	O	O	X	XO		X

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

Butoxyethanol

Ingredient	CAS #	PubMed	FDA	CompTox	ChemPort	NIOSH	NTIS	NTP	FEMA	EU	ECHA	SIDS	SCCS	AICIS	FAO	WHO	Web
Butoxyethanol	111-76-2	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

Search Strategy

PubMed

Search performed from 2000 to present
(butoxyethanol) OR (111-76-2[EC/RN Number]) – 202 results, 60 relevant

ECHA

Entry for CAS # 111-76-2 resulted in finding a dossier for Butoxyethanol. Pertinent data not found in the original report is summarized in the above table.

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
- CompTox: https://comptox.epa.gov/dashboard/chemical/pubmed-abstract-sifter/DTXSID3039242; https://www.epa.gov/comptox-tools/downloadable-computational-toxicology-data#LM
- eChemPortal: https://www.echemportal.org/echemportal/
- DeepDyve: https://www.deepdyve.com/
- 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
- 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/

JUNE 2024 PANEL MEETING –RE-REVIEW

Belsito Team – June 3, 2024

DR. BELSITO: Okay. So, butoxyethanol, this is a component for re-review. So, it's been 15 years since the previous review, but we initially reviewed it in 1996.

Kevin, I need your help with the screen here. Hold on. Okay, I got it. Sorry about that, I'm using a new computer.

So, the Expert Panel first published a review of the safety of butoxyethanol in '96. We concluded it's safe in hair and nail products at concentrations of up to ten percent. The Panel reconsidered the re-review and reaffirmed the conclusion in 2005. And because it's been 15 years, we're doing another re-review in accordance with the procedures. In April of 2024, an extensive search of the literature was performed for studies from 2000 onward, and there were a lot of new studies identified.

And so, I looked through all of those studies. In terms of what I found is there's been a marked decrease in use in this ingredient, with no concentrations reported to the PCPC. At the last review, we allowed it at ten percent in hair dyes and 50 percent in nail products. But we have no information now as to whether it's used in nail products at all; it appears to be just semi-permanent dyes. The SCCS has issued an opinion on this, limiting the concentration to hair dyes only. So, I actually thought we might need to reopen this to look at the new data and assess why the SCCS has given a restriction, and also in terms of currently we're saying it's okay in nail products up to 15 percent. Also, we have no concentrations of use. So, that was my conclusion from reading all of this.

DR. KLAASSEN: I have to mention -- oops.

DR. RETTIE: Go ahead.

DR. KLAASSEN: I have to --

DR. BELSITO: Is it because my mic is open?

DR. KLAASSEN: Okay, I'll continue. I have to mention on this one, for the first time since I've been this committee, that I found an error in the structure of a chemical. What you have here, this is supposed to be butoxyethanol, and the structure on Page 16 is butoxymethanol. That was quite surprising. I usually don't look at the chemistry quite that close, but it hit me between the eyeballs. Unless I don’t know how to read, even if my chemistry is even worse than that.

DR. RETTIE: It's just missing carbon. But, yeah, we should probably correct that. It looks like a holdover from the last report.

DR. HELDRETH: Yeah, that's actually a copy of the old report. Before my time, so I can't be blamed.

MS. BURNETT: Not us.

DR. KLAASSEN: Not blaming you guys. Just want to make sure it doesn't continue.

DR. RETTIE: Is there a likelihood we'll get MOCRA data for this at any point to update concentrations, or is it just up in the air?

DR. HELDRETH: Well, Cosmetics Direct is supposed to be -- the door closed here at the beginning of July. So, yeah, by the time we get this, it's hopeful that we would have --

DR. EISENMANN: It won't get concentrations from Cosmetics Direct.

DR. HELDRETH: Right.

DR. EISENMANN: When this came out, I gave everybody a chance to give me a concentration again, and nobody has come back. So, I've been trying, but nobody's telling -- I only get people saying they're not using it. So, there's really not much use with it.

DR. BELSITO: But we will at least get an idea if what you're getting back from your company is somewhat correct. Because if we're getting a high number of reports from FDA and we're not seeing that reflected, we'll also get from FDA categories of use, right. And so, we can see if, in fact, it's being used in nail products and you're not getting reports on that. So, there will be a little bit more of a check and balance with that data. Bottom line is FDA is not going to have concentrations of use. They're just going to have use categories.

DR. RETTIE: We probably also want to look at updating boilerplates. It seemed like we need an ethoxylation ethylene oxide boilerplate to go in and perhaps update the metabolism since we didn't have any in the original report of note. So, yeah, reopen I think.

DR. SNYDER: Inaudible.

DR. BELSITO: Paul. Hold on, Paul. We're hardly hearing you again. Try it now.

DR. KLAASSEN: Okay, go ahead.

DR. SNYDER: Okay.

DR. BELSITO: Yeah.

DR. KLAASSEN: Perfect.

DR. SNYDER: My observation was the same as your, Don, there's several of these that have had a lot of new data. And so, I think that when we get that much new data I think we need to look at it carefully. And so, I was also wanting to reopen to look at the new data.

DR. RETTIE: In my read, there was not really any new tox data of particular note, other than ocular irritation and some cytotox.

DR. BELSITO: And, Allan, you wanted a new ethoxylation boilerplate? Or a look at it?

DR. RETTIE: A look at it at least. I felt in terms of making the compound they use ethylene oxide, so typically that's a flag for us to look at our boilerplates.

DR. BELSITO: If we reopen it, were there any glaring data needs that you saw at this point for any endpoints? I mean, I didn't. The purpose to reopen it was to reassess the data that we currently have.

DR. RETTIE: I didn't see anything glaringly missing, just updates.

DR. BELSITO: Paul?

DR. SNYDER: Same for me.

DR. KLAASSEN: Same here.

DR. BELSITO: Okay. So, we want to reopen, correct the chemical structure, look at the ethoxylation boilerplate, assess why the SCCS gave restriction, and look at all the new data that we have, and also hopefully get some clarification on concentrations of use and products used with the new FDA reporting system. Anything else that I'm missing?

DR. SNYDER: One thing, Don, was that EU restriction to only as a solvent, so I wanted to see what that was based on too.

DR. BELSITO: Yeah, I have that, Paul. Look at why the SCCS restricted it.

DR. SNYDER: Okay. Thank you.

DR. EISENMANN: Usually, their restrictions are placed on what the industry has requested they look at.

DR. BELSITO: Okay. Anything else before we move on? Okay.

Cohen Team – June 3, 2024

DR. COHEN: Okay. Butoxyethanol. First published the review in 1996 with a conclusion that butoxyethanol is safe in hair and nail products at concentrations up to ten percent. In 2002 the panel considered re-review of this report for the first time and reaffirmed the 1996 conclusion which was published in 2005. So, it should be noted that at the time of the original report there were no restrictions for butoxyethanol use in cosmetic products. However, European regulators regarding cosmetic ingredients now categorize butoxyethanol an Annex III.

A list of substances which cosmetic products must not contain except subject to restrictions laid down. Butoxyethanol may only be used as a solvent in oxidative hair dye products up to four percent and a solvent in non-oxidative hair dye products at up to two percent and must not be in aerosol dispensers.

DR. BERGFELD: And that was the ban from Europe?

DR. COHEN: That’s the European restrictions laid down. Use of butoxyethanol since the initial re-review was performed -- the survey and it’s decreased. In 2001, butoxyethanol was in 110 cosmetic products with the majority in hair dyes and colors. In 2021 the survey reported a maximum concentration of use of three to 50 percent with the highest of 50 percent in nail polish and enamel removers.

No concentrations of use were reported for hair dye formulation and the 2023 VCRP, it was reported in three hair dye formulations with no concentration of use reported to the counsel in 2020. There was a lot of new data in this dossier. Quite a bit. I’m wondering whether we ought to reopen this because of the volume of new information.

DR. ROSS: I thought it was mainly if -- I was on the fence here but to argue for the reopening I guess you would say the E.U. ranks are in conflict with our previous conclusion. So, our original document says up to ten percent. The E.U. Annex III, again, very specific on concentrations. Four percent and two percent in oxidative and non-oxidative hair products respectively. So, you would say, yeah, you should reopen and there’s a lot of new data. We should reopen. But it’s a lot of work for three uses and --

DR. TILTON: Well, we don't know.

DR. COHEN: We really don't know, and the other thing is the question about aerosols concerned me a little bit. I don't know if it's being used in any aerosols, and we may want to address that.

DR. TILTON: I mean, I don't know if we can reaffirm the past conclusion without reevaluating all the new data. And it was previously found safe up to ten percent including the nail products and, so I feel like we need to reevaluate the current data and rationale for why restrictions have been put in place.

DR. ROSS: I agree with that. In fact, I just found a final note saying that we should reopen based on the E.U. Annex III.

DR. COHEN: Yeah. I had a reopen on this one. There was just too much information to put in a re-review summary and we couldn't.

DR. BERGFELD: No, we've had other summaries have a lot of stuff in it. I think the biggest thing for me is the fact that it's been banned, and we have discordance in concentration, so it's considered sound. I think that has to be resolved.

DR. COHEN: And by the way, we have a clear concentration in the conclusion that's five times higher in products that have been surveyed.

MS. BURNETT: Yeah. We did see the explanation in the re-review summary for when that was reported, right? It was, so when we did the first re-review that was noted that it was used up to 50 percent in nail products and the panel at the time explained that it doesn't absorb through the nail. There was something else, but that was the rationale for allowing that concentration to be that high for nail polish.

DR. COHEN: But why would the conclusion have it for ten per- --

MS. BURNETT: I don't know why they didn't --

DR. COHEN: -- but it says hair and nail products at up to ten percent --

MS. BURNETT: I know.

DR. COHEN: -- and then they re- -- it sounds like they were just justifying this digression, but the conclusion was resolute.

MS. BURNETT: Right.

DR. COHEN: All right.

MS. FIUME: So, what it really comes down to is that companies are using it higher than the conclusion concentration that was stated then they either have the data to support it or they're just not following the conclusion. So, for the panel the conclusion concentration would take precedence over what the reported concentration is.

DR. COHEN: Right. So, when we open this report, or presumably open this report, we don't have consensus on it yet. The max use, if there's concentrations of 50 percent, that becomes the concentration in this report unless we're specific in our conclusion.

MS. FIUME: Which you can based on the safety (inaudible) --

DR. COHEN: Yeah.

DR. BERGFELD: I want to draw your attention to the discussion on this particular ingredient. It talks about the nail. Of course, we just discussed this, but in the ten percent, it is in wash off products because it highlights colors, bleaches, shampoos. And then there were specific contact dermatitis sensitization seen on two or more patients. That was safe in rinse off or leave on. That says severe ocular irritation on the undiluted with moderate and no corneal injuries were observed in concentrations of 15 and five percent on (inaudible). I'm not sure; we've got to check that.

But anyhow, there's the discussion about the concentrations and why we went to the concentration that we had here which was ten percent.

DR. COHEN: But then why in the re-review is the 50 percent discussed? You said it's in the re-review.

MS. BURNETT: Mm-hmm. PDF page 81.

DR. BERGFELD: Yeah. I think I read that, too. It was in the nail product, the 50 percent, and the adjustment wouldn't penetrate the nail.

MS. BURNETT: PDF page 81. It's, like, the bottom of the page. Just after --

DR. COHEN: The panel also considered current data indicating that butoxyethanols used in concentrations up to 50 percent in nail polish and enamel removers. In light of its previous limitation of ten percent in hair and nail products, however it was concluded that the increased use concentration was of little concern because of the nail plate is made up of dead tissue and thus the amount of absorption through the nail would be negligible.

DR. BERGFELD: May or not be true. Penetrate pretty easily.

DR. ROSS: I see some more quantitative risk assessments on here.

DR. COHEN: Yes, because this can be acquired or absorbed through other sources.

DR. BERGFELD: Cuticle?

DR. COHEN: You can go through inhalation.

DR. ROSS: Inhalation. I mean, bottom line is we're going to have to reopen.

DR. BERGFELD: Reopen it.

DR. COHEN: We'll see if we get unanimity tomorrow.

Full Panel – June 4, 2024

DR. BELSITO: The Expert Panel first published the review of safety of Butoxyethanol in 1996. We concluded that it is safe in hair and nail products at concentrations of up to 10 percent. Because it's been at least 15 years since we've done that -- oh, I'm sorry, in 2002 -- we considered a rereview for the report and reaffirmed the '96 conclusion in 2005. Because it's been 15 years, we're looking at this again. And the literature was scoured in April of 2024.

Since the time that we've last looked at it, it appears not to be used in nail anymore but continues to be used in hair. Concentrations are lower, but we did receive data that the SCCS has restricted this as a solvent and, also, reduced the concentration. And we thought that we needed to reopen this to look at that data, also to look at the ethoxylation boilerplate, determine why the SCCS has restricted it to solvent use. And hopefully at that point we'll have some FDA data that tell us whether there are uses other than hair use.

DR. COHEN: Second.

DR. BERGFELD: Any other discussion regarding reopening this review?

DR. COHEN: Look, Don, it went through, everything.

DR. BERGFELD: Seeing none, call the question, all those in favor? Paul?

DR. SNYDER: I agree.

DR. BERGFELD: Unanimous, thank you.

FEBRUARY 10-11, 1992 PANEL MEETING – INTIAL REVIEW

Full Panel

Dr. Elder began the discussion with a short description of the history of Butoxyethanol. He commented that CIR has received a final commitment for human irritation and sensitization patch test from CMA. This commitment was then passed out to the Panel members. Dr. Elder asked if there was any other data the Panel wanted on this compound.

Dr. Bergfeld remarked that the Panel expected the data, or an update, within 3 months. She then moved to postpone discussion on this ingredient until the next Panel meeting. The motion was seconded.

Dr. McEwen asked if the Panel would want any more data in the future.

Dr. Bergfeld said that the Panel would have to wait until they received the data to see if they would need any other information for a final decision. She recognized that it was important for the Panel to request all of the information necessary all at once, rather than a little at a time.

Dr. Belsito said that he was interested in Butoxyethanol's effects on calcium metabolism in the scalp and its long-term effects on adhesion and proliferation of keratinocytes. He asked if this was a compound strictly for wash-off products.

Dr. Shank pointed out that there was a subchronic dermal study in the review.

Dr. Belsito replied that there were no human studies, and there was no good animal model for conditions such as psoriasis. He stated that it would be possible to see effects in humans that wouldn't be seen in animals.

Dr. Elder pointed out that in some previous reviews, there were ingredients whose use was limited to wash-off products in the conclusion. He suggested that it would probably be sufficient in this case to say "safe as used", because the products Butoxyethanol is used in are wash-off products.

Dr. Bergfeld requested that the discussion reflect these concerns about leave-on versus wash-off products. She asked Dr. Belsito if this restriction would be enough, instead of requesting the calcium metabolism data.

Dr. Belsito said that it would be sufficient.

Dr. Elder said that he would make inquiries of the Food and Drug Administration as to the products, wash-off and/or leave-on, that Butoxyethanol is used in.

The Panel decided to postpone further discussion of Butoxyethanol until the next Panel meeting, when the requested data, or an update on that data, should be presented.

NOVEMBER 22-23, 1993 MEETING – SECOND REVIEW/DRAFT REPORT

Full Panel

Dr. Belsito noted that his Team had made editorial changes in the draft report discussion that had been distributed. Particularly, the statement indicating that Butoxyethanol is nephrotoxic in rats was modified to include the routes of administration. Dr. Belsito's Team also concluded that Butoxyethanol is safe as used in cosmetics.

Dr. Schroeter stated that his Team agrees that Butoxyethanol is safe as used; however, nephrotoxic effects should not be mentioned in the discussion, because the respective studies are not relevant to use of Butoxyethanol as a cosmetic ingredient. Furthermore, the statement on dermal carcinogenicity of a product containing 2.5% Butoxyethanol and 90.0% Stoddard solvent should also be deleted from the discussion because the following statement from EPA is included in the test of the CIR report: "In general, it is not necessarily surprising that a product containing approximately 90.0% Stoddard solvent (a refined petroleum distillate with a typical boiling range of approximately 300 to 400 °F) is carcinogenic considering the increasing body of evidence which indicates that most (if not all) petroleum derivatives may possess some degree of oncogenic or co-oncogenic potential."

Dr. Slaga noted that a negative dermal carcinogenicity study on a hair dye formulation containing 10% Butoxyethanol is included in the text; thus, the carcinogenicity of Butoxyethanol really doesn't need to be addressed in the report discussion.

The Panel agreed to delete the second paragraph of the draft discussion, and the Panel's revised discussion now reads as follows: Undiluted Butoxyethanol was severely irritating to the eyes of rabbits. However, moderate corneal injury was observed in rabbits after the instillation of 15% aqueous Butoxyethanol and no corneal injury was observed after 5% aqueous Butoxyethanol was instilled into the eyes of rabbits. The Expert Panel is aware of these effects and, also, that Butoxyethanol

has reportedly been used in hair dyes at concentrations ranging from ≤ 0.1% to 10%. Use of Butoxyethanol within this concentration range, in effect, attenuates the ocular irritation potential of this ingredient in hair dyes. The Panel also deleted any other information that was deemed not additive to the report.

The Panel approved the conclusion that Butoxyethanol is safe as used in hair dyes at concentrations up to 10% and voted in favor of issuing a Tentative Final Report on this ingredient.

FEBRUARY 28-MARCH 1, 1994 MEETING – FINAL REPORT

Full Panel

The Panel voted in favor of issuing a Final Report on Butoxyethanol. [No further discussion recorded.]

MAY 23-24, 1994 MEETING – NARROW USE CONCLUSION DISCUSSION

Full Panel

Dr. Andersen noted that at the last Panel meeting, it was concluded (final conclusion) that Butoxyethanol is safe for use in hair dyes at concentrations up to 10%. However, after issuance of the Final Report, current data indicating that Butoxyethanol is used in other product types were received from FDA: tonics, dressings, and other hair grooming aids (1 product), hair dyes and colors (117 products), hair bleaches (2 products), and nail products and enamels (1 product). Based on the new information, Dr. Andersen wanted to know whether or not the current conclusion should be changed.

Dr. Shank said that the 10% concentration limit should not be based on ocular irritation studies, as stated in the report discussion. He recommended that the limitation should be based on negative results at the only concentration (10% Butoxyethanol) tested in the human repeated insult patch test. It was noted that Butoxyethanol was not tested at a concentration of 10% in ocular irritation studies, and that the available ocular irritation data indicated that 5% Butoxyethanol was safe and that 15% Butoxyethanol induced corneal injury.

Dr. Andersen said that the other reason for establishing the 10% concentration limit was that this represented the highest use concentration reported to FDA in 1989. He also noted that the record does not support that the Panel's decision was based on dermal irritation and sensitization data.

Dr. Bergfeld said that the 10% concentration limit should have been based on human skin irritation and sensitization data, and that the Final Report discussion must now be changed to reflect this.

Based on the current FDA frequency of use data, the Panel agreed that the Final Report discussion should be revised to reflect the Panels basis (human skin irritation and sensitization data) for establishing the 10% concentration limit and that the conclusion should be revised to read as follows: On the basis of the animal and clinical data included in this report, the Expert Panel concludes that Butoxyethanol is safe in hair and nail products at concentrations up to 10%.

The Panel voted in favor of reissuing the Final Report on Butoxyethanol with a revised discussion and conclusion, and noted that the announcement of this document would be followed by a 90-day comment period.

SEPTEMBER 12-13, 1994 – REVISED FINAL REPORT

Full Panel

Dr. Bergfeld noted that comments on this ingredient were not received during the 90-day comment period for the Tentative Report. The Panel unanimously approved the issuance of a Final Report on Butoxyethanol.

FEBRUARY 2002 MEETING – FIRST RE-REVIEW

Team Minutes (minutes not broken out by Team Leaders) – February 11, 2002

On behalf of the Ethylene and Propylene Glycol Ethers Panel of the American Chemistry Council, Dr. Tipton Tyler presented a review of studies sponsored by the Council and an analysis of the National Toxicology Program lifetime inhalation exposures of mice and rats to Butoxyethanol. He noted that the finding of pheochromocytomas of the female rat adrenal gland was not statistically significant and that these cytomas were within the historical control frequency. He argued that the liver neoplasms observed in male mice were suggestive of oxidative stress subsequent to red blood cell hemolysis and iron deposition in the liver. He stated that, compared to rats, humans have a higher hepatic antioxidant capacity and would not experience the same cascade of events leading to liver tumors. He also addressed the forestomach neoplasms found in female mice, relating them to the prolonged exposure and the irritation produced by Butoxyethanol. Since humans have no comparable anatomic structure, he stated that the relevance to humans was questionable. Both Dr. Tyler and Dr. Sue Lewis participated with the teams in discussing the NTP data.

Full Panel – February 12, 2002

A CIR Final Report on the safety of Butoxyethanol in cosmetics was issued in 1994 with the following conclusion: On the basis of the animal and clinical data included in this report, the Expert Panel concludes that Butoxyethanol is safe in hair and nail products at concentrations up to 10.0%. The Final Report was published in the Journal of the American College of Toxicology in 1996.

Dr. Belsito stated that re-review of the safety of Butoxyethanol in cosmetics is being considered because of questionable evidence of carcinogenicity (in mice and rats) in a two-year NTP inhalation carcinogenicity study on Butoxyethanol that was published in 2000. He noted that after review of this study, his Team determined that the results are not relevant to man for the following reasons: (1) Pheochromocytomas observed in one rodent species were within the historical control range. (2) Liver cancer observed in a male rat was thought to have been due to hemolysis. [It has been shown that rodent red blood cells are very sensitive to hemolysis by Butoxyethanol, and that this effect was due to iron overload.] (3) An increased incidence of forestomach cancers was observed in female mice. [The forestomach is not a relevant organ to look at, and this effect was thought to have been due to irritation, but not genotoxicity.]

Dr. Belsito added that the CIR Final Safety Assessment on Butoxyethanol should not be reopened for the reasons stated in the preceding paragraph.

Dr. Slaga said that it has to be emphasized that the NTP study results on Butoxyethanol are not relevant to humans. He added that it is not the intent of the Panel to imply that, in general, animal models are not relevant to humans.

Dr. Slaga also noted that the finding of forestomach tumors after inhalation exposure is an unusual finding. He said that, in the past, these tumors have been observed after large amounts of a chemical (e.g., BHA) accumulate and cause irritation of the forestomach.

Dr. Bergfeld wanted to know if a report discussion should be developed to address the current 50% use concentration for Butoxyethanol in nail polish and enamel removers, considering that use concentrations up to 10% are stated in the published CIR Final Report.

Dr. Marks said that no safety data are available that would allow the Panel to comment on the new use concentration. However, he noted that there would be significantly less absorption through the nail plate.

Dr. Belsito said that a discussion, if needed, could be developed to address the new use concentration, noting that the nail is made up of dead tissue and that the amount of absorption through the nail is going to be negligible.

Dr. Marks noted that the in vitro hematotoxicity study by Ruchaud et al., (1992), cited in the published CIR Final Report on Butoxyethanol, has been retracted. This information was provided by Dr. Tipton Tyler at yesterday's Team meetings.

Dr. Belsito said that the deletion of this reference would have no substantive effect on the safety assessment.

Dr. Belsito recalled that it was suggested in Teams that the NTP terminology for characterizing the carcinogenic potential of a chemical (e.g., terminology used in CIR Final Report on t-Butyl Alcohol) should be used in the Butoxyethanol re-review document when referring to the carcinogenic potential of this chemical in the NTP inhalation carcinogenicity study.

The Panel unanimously concluded that the CIR Final Safety Assessment on Butoxyethanol should not be reopened.

Dr. Bergfeld noted that the Panel will have an opportunity to review the points recommended for inclusion in the discussion at a subsequent Panel meeting.

Dr. Andersen said that the ingredients considered for re-review at today's meeting represent the first group that will be included in the 2002 Annual Review, and more ingredients will be added as the year progresses. The 2002 Annual Review, including any comments received during the 90-day comment period, will be reviewed by the Panel prior to publication next year.

Amended Safety Assessment of Butoxyethanol as Used in Cosmetics

Status: Draft Amended Report for Panel Review
Release Date: February 14, 2025
Panel Meeting: March 13 - 14, 2025

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.; Samuel Cohen, M.D., Ph.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, 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, M.B.A. This safety assessment was prepared by Christina Burnett, M.S., Senior Scientific Analyst/Writer, CIR.

555 13th Street, NW, Suite 300W, Washington, DC 20004
cirinfo@cir-safety.org

ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
ATP	adenosine trisphosphate
BEI	Biological Exposure Index
CFR	Code of Federal Regulations
CHO	Chinese hamster ovary
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
Dictionary	web-based *International Cosmetic Ingredient Dictionary and Handbook*
EC	European Commission
ECHA	European Chemicals Agency
EPA	Environmental Protection Agency
EU	European Union
FDA	Food and Drug Administration
G-6-PDH	glucose-6-phophate dehydrogenase
HET-CAM	hen's eye test-chorioallantoic membrane
HRIPT	human repeated insult patch test
IARC	International Agency for Research on Cancer
IRIS	integrated risk information system
LOAEL	lowest-observed-adverse-effect level
MDA	malondialdehyde
MOE	margin of exposure
MOS	margin of safety
MoCRA	Modernization of Cosmetics Regulation Act
MRI	magnetic resonance imaging
MTT	3-[4,5-dimethylthiazol-2-yl]2,5-diphenyl tetrazolium bromide
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NA	not applicable
NR	not reported
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
OH8dG	8-hydroxy-2'-deoxyguanosine
Panel	Expert Panel for Cosmetic Ingredient Safety
RLD	Registration and Listing Data
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SCCP	Scientific Committee on Consumer Products
SED	systemic exposure dose
SHE	Syrian hamster embryo
TG	test guideline
US	United States
VCRP	Voluntary Cosmetic Registration Program

INTRODUCTION

This assessment reviews the safety of Butoxyethanol as used in cosmetic formulations. According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (*Dictionary*), Butoxyethanol functions as a fragrance ingredient, solvent, and viscosity decreasing agent in cosmetic products.[1]

In the Cosmetic Ingredient Review (CIR) report published in 1996, on the basis of the animal and clinical data included in the report, the Expert Panel for Cosmetic Ingredient Safety (Panel) concluded that "Butoxyethanol is safe in hair and nail products at concentrations up to 10.0%."[2] Subsequently, at the February 2002 meeting, the Panel discussed the need to reassess safety in cosmetics because of questionable evidence of carcinogenicity (in mice and rats) in a two-year National Toxicology Program (NTP) inhalation carcinogenicity study on Butoxyethanol that was published in 2000. However, the Panel determined that the results were not relevant to humans, and thus reaffirmed this conclusion, as published in 2005.[3]

In June 2024, the Panel determined that this safety assessment should be re-opened for re-evaluation due to reported restrictions by the European Commission (EC) on the use of Butoxyethanol. Excerpts from the summaries of the 1996 report are disseminated throughout the text of this re-review document, as appropriate, and are identified by *italicized* text.

This report 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 January 2025. 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 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 chemical and toxicological data on Butoxyethanol included in this safety assessment were obtained from robust summaries of data submitted to the European Chemicals Agency (ECHA) by companies as part of the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) chemical registration process.[4] Additionally, data were obtained from opinions produced by the Scientific Committee on Consumer Products (SCCP) of the EC.[5] These data summaries are available on the databases for ECHA and the EC, respectively, and when deemed appropriate, information from the summaries has been included in this report.

CHEMISTRY

Definition and Structure

According to the *Dictionary*, Butoxyethanol (CAS No. 111-76-2) is the ether alcohol that conforms to the structure depicted in Figure 1.[1]

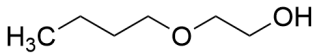

Figure 1. Butoxyethanol

Chemical Properties

Chemical properties for Butoxyethanol are summarized in Table 1. Butoxyethanol is a colorless liquid with a molecular weight of 118.18 g/mol.[2] The log P_{ow} at 25ºC is 0.81.[4]

Method of Manufacture

Butoxyethanol is usually prepared by the reaction of ethylene oxide with butyl alcohol and may also be prepared by the direct alkylation of ethylene glycol with an agent such as dibutyl sulfate. [2] *Another method for the production of Butoxyethanol is the reaction of butyl alcohol with ethylene carbonate or 2-chloroethanol. Butoxyethanol may also be produced through reacting ethylene glycol with butyl bromide.*

Composition and Impurities

Water accounts for not more than 0.2% of the composition of Butoxyethanol.[2] *The purity of technical-grade Butoxyethanol ranges from 98 - 99.5%. Commercial samples of glycol ethers invariably contain small quantities of peroxides. A supplier reported impurities as follows: acidity (0.001 mEq/g), carbonyl (210 - 276 ppm), peroxide (66 - 169 ppm), dioxane (0.35 ppm), ethylene oxide (0.015 ppm), ethylene glycol (0.32%), butanol (420 - 450 ppm), butyraldehyde (210 - 330 ppm), diethylene glycol (100 ppm), butyl carbitol (< 100 ppm), and "heavies" (0.1%).*

The SCCP reported that Butoxyethanol is ≥ 99% pure.[5] Impurities are as follows: 2-butoxyethoxyethanol (≤ 0.3% w/w), 1,2-ethanediol (≤ 0.5% w/w), 1-butanol (≤ 0.2% w/w), and water (< 0.2% w/w). Butylated hydroxytoluene (0.008 - 0.012% w/w) may be added to prevent the formation of peroxides.

USE

Cosmetic

The safety of the cosmetic ingredient addressed 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 Butoxyethanol in cosmetics. Data included herein were obtained from the FDA and in response to a survey of maximum use concentrations conducted by the Personal Care Products Council (Council), and it is these values that define the present practices of use and concentration. Frequencies of use obtained from the FDA include data from the Voluntary Cosmetic Registration Program (VCRP) database as well as Registration and Listing Data (RLD). As a result of the Modernization of Cosmetics Regulation Act (MoCRA) of 2022, the VCRP was terminated in 2023 and, as of 2024, manufacturers and processors have been mandated to register and list their products (and ingredients therein) with the FDA (i.e., RLD). An exception is made for small businesses, which are exempt from MoCRA reporting for most cosmetic product categories. However, to utilize the exemption, the small business must not sell eye area products, injected products, internal use products, or products that alter appearance for more than 24 h.[6] Please note, at this time, it is not appropriate to contrast data from the VCRP and RLD to determine a trend in frequency of use because there are numerous differences in the ways the data for the VCRP and the RLD were collected and processed, and because reporting frequency of use is now mandatory (as opposed to the past practice of voluntary reporting). Although the VCRP program is now defunct, trends in frequency of use from the RLD alone are not yet possible in that a baseline is currently not available.

According to RLD that CIR received in 2024, Butoxyethanol is used in 81 formulations, with 79 uses reported in hair dyes and colors (Table 2).[7] A single use each was reported in perfumes and makeup fixatives. VCRP survey data received in 2023 reported Butoxyethanol was used in 3 hair dyes and colors.[8] When comparing the VCRP data received in 2023 to that received in 2001, the frequencies of use for Butoxyethanol have greatly decreased since the first re-review was considered; in 2001, Butoxyethanol was reported to have 110 uses, with the majority in hair coloring formulations.[3,8] No uses were reported in of the concentration of use survey conducted by the Council in 2020.[9] In 2001, the maximum concentration of use range for Butoxyethanol was 3% in leave-on products (that resulted in dermal contact) and 50% in rinse-off products (nail polish and enamel removers).[3] Of note, concentrations of use were not reported for hair coloring products at the time of the first re-review (2001 data).

The RLD reported that this ingredient may be used in cosmetic sprays and powders, and could possibly be inhaled; for example, Butoxyethanol is reported to be used in a perfume (concentration not reported).[7,9] 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.

Some products containing Butoxyethanol may be marketed for use with airbrush delivery systems. With the advent of MoCRA and the current product categories outlined by the FDA, it is now mandatory that cosmetic products used in airbrush delivery systems be reported as such for some, but not all, product categories in the RLD. In other words, a reliable source of frequency of use data regarding the use of cosmetic ingredients in conjunction with airbrush delivery systems is now available in some instances. None of the reported product categories for Butoxyethanol as listed in the RLD include a designation using airbrush application, so it is possible that this ingredient is used with airbrush delivery systems, but not reported as such. Additionally, the Council currently surveys the cosmetic industry for maximum reported use concentrations of ingredients in products which may be used in conjunction with an airbrush delivery system; thus, this type of data may also be available when submitted. Nevertheless, no concentration of use data were provided indicating airbrush application. Nevertheless, 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. Without information regarding the consumer habits and practices data or product particle size data (or other relevant particle data, e.g., diameter) related to this use technology, the data profile is incomplete, and the Panel is not able to determine safety for use in airbrush formulations. Accordingly, the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

In the European Union (EU), Butoxyethanol is categorized in Annex III, the list of substances which cosmetic products must not contain except subject to the restrictions laid down.[10] For this ingredient, the regulation states that Butoxyethanol may only be used as a solvent in oxidative hair dye products at up to 4%, a solvent in non-oxidative hair dye products at up to 2%, and must not be used in aerosol dispensers (sprays). The SCCP opinion gives these same restrictions on use in hair dye formulations, as related to direct application to the hair/scalp.[5] The opinion does not include any other cosmetic exposure, such as exposure to other types of cosmetics or possible aerosol/spray products.

Non-Cosmetic

Butoxyethanol is used as an industrial solvent for resins and varnishes, in hydraulic fluids, and in the formulation of floor polishes, floor waxes, and cleaning compositions for leather, upholstery, and glass.[2] *Food-related uses of Butoxyethanol described in the Code of Federal Regulations (CFR) are as follows: component of adhesives (21 CFR 175.105), defoaming agent used in the manufacture of paper and paperboard (21 CFR 173.315), solvent in polysulfide polymer/polyepoxy resins for contact with dry food (21 CFR 176.210), use in sanitizing solutions (21 CFR 177.1650), and use in flume water (concentration not to exceed 1 ppm) for washing sugar beets prior to slicing (21 CFR 178.1010).*

In addition to the regulations described above, residue of Butoxyethanol is exempted from the requirement of a tolerance when used in accordance with good manufacturing practices as an ingredient in an antimicrobial pesticide formulation, provided it is applied on a semi-permanent or permanent food-contact surface (other than being applied on food packaging) with adequate draining before contact with food (40 CFR 180.940).

TOXICOKINETIC STUDIES

The results of rat and human studies indicated that ^{14}C-Butoxyethanol was percutaneously absorbed and metabolized to butoxyacetic acid, with excretion in urine, after oral, dermal, or inhalation exposure.[2] *The in vitro percutaneous absorption of Butoxyethanol was demonstrated using rat, guinea pig, and human skin samples. When ^{14}C-Butoxyethanol was administered orally to rats, the major route of excretion was via the urine, and butoxyacetic acid was the major urinary metabolite. In one study, an analysis of tissues excised subsequent to oral administration indicated that the greatest specific activity was detected in the thymus, followed by the spleen and liver.*

Dermal Penetration

Dermal penetration studies for Butoxyethanol are summarized in Table 3. In human skin samples, Butoxyethanol absorbed more rapidly through damaged skin than undamaged skin (3.39 mg/cm^2/h vs 1.19 mg/cm^2/h).[5] In solution, a steady state flux of 544 ± 64 nmol·cm^{-2}/h (0.064 mg/cm^2/h) was found with human dermatomed skin.[11] In a study with oxidative hair dye formulations, the dermal absorbance for formulations with 5 and 10% Butoxyethanol was 61 ± 29 µg/cm^2 (12.1 ± 5.9%) and 125 ± 73 µg/cm^2 (12.5 ± 7.3%), respectively.[5] Butoxyethanol penetrated human skin up to 6-fold more rapidly from aqueous solution (50%, 450 mg/ml) than from the neat solvent (900 mg/ml); a corresponding increase in apparent permeability coefficient was observed as Butoxyethanol concentration in water decreased.[12] Permeation rates of Butoxyethanol through unoccluded rat dermatomed skin (16%) were greater than rat whole skin (8%), while absorption through human dermatomed skin (4%) was lower than the rat.[13] Absorption of undiluted Butoxyethanol through occluded rat dermatomed skin in vitro was 18%. This same study found that Butoxyethanol absorption was enhanced by application in methanol to 23%. In a study with full-thickness human skin with undiluted Butoxyethanol, approximately 0.16% of the absorbed Butoxyethanol was metabolized to butoxyacetic acid.[14]

Following topical application of undiluted Butoxyethanol in rats, 28% of the dose was absorbed after 24 h.[13] The major routes of excretion included the urine (19%), expiration as carbon dioxide (6%) and feces (0.4%): little of the dose remained in the carcass (1.3%). In a study with human volunteers exposed to Butoxyethanol vapors, urinalysis results showed that baseline dermal absorption of Butoxyethanol vapor was 11% of the total absorbed dose. [15] Higher temperatures and greater humidity increased dermal absorption. The rate of percutaneous absorption of Butoxyethanol is greater in aqueous solutions than when it is applied neat or diluted in ethanol.[16,17]

Absorption, Distribution, Metabolism, and Excretion

Human

Dermal

Six male volunteers were dermally exposed for 4 h to 50% aq. Butoxyethanol on the arm for 30 min.[18] Butoxyethanol in blood and total and free 2-butoxyacetic acid in the urine were measured. After dermal exposure, 147.1 and 346 mg of free and total 2-butoxyacetic acid, respectively, were excreted in the urine at up to 48 h after exposure. The proportion of conjugated 2-butoxyacetic acid in single urine samples increased after dermal exposure from 45% in the first collection period to 92% after 48 h. The elimination half-life of total 2-butoxyacetic acid following dermal exposure was longer than that of free butoxyacetic acid (5.1 h and 3.8 h, respectively).

Inhalation

Six male volunteers were exposed via inhalation by mouth to 93 mg/m^3 Butoxyethanol for 30 min.[18] Butoxyethanol in blood and total and free 2-butoxyacetic acid in the urine were measured. After exposure, the 24-h cumulative excretion of free and total 2-butoxyacetic acid in urine was 5.5 and 12.8 mg, respectively. The interindividual variation in the cumulative excreted amount after inhalation exposure was higher (49%) for free 2-butoxyacetic acid than for total 2-butoxyacetic acid (31%).

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Butoxyethanol was slightly toxic (mean LD_{50} = 0.58 g/kg) when administered dermally to rabbits.[2] *In dermal toxicity studies with rats and guinea pigs, mean LD_{50} values of 2.52 and 0.23 ml/kg, respectively, have been reported. Butoxyethanol was slightly toxic (mean LD_{50} = 2.8 g/kg) in an acute oral toxicity study involving rats. In oral toxicity studies with guinea pigs and rabbits, LD_{50} values of 1.20 and 0.35 g/kg, respectively, have been reported. In acute inhalation toxicity studies, animal mortality rates were related to both the concentration of Butoxyethanol and the duration of exposure.*[2] *In mice, 7- and 32-h exposure to 770 ppm Butoxyethanol caused 12.5 and 81.25% mortality, respectively. Exposures of 7 and 32 h to 1220 ppm Butoxyethanol caused 68.75 and 100% mortality, respectively. At a concentration of 800 ppm Butoxyethanol, none of 6 rats exposed died at 4 h, but 3 animals died at 8 h.*

Additional acute toxicity studies are summarized in Table 4. In dermal studies, the LD_{50} for Butoxyethanol was greater than 2000 mg/kg in rats and guinea pigs.[4,5] The dermal LD_{50} in rabbits ranged from 307 to greater than 2000 mg/kg. The oral LD_{50} for mice was 1519 mg/kg in fasted animals and 2005 mg/kg in fed animals; LD_{50} in rats ranged from 880 to 2100 mg/kg. All rabbits that received 695 or 1500 mg/kg orally died, and the oral LD_{50} for Beagle dogs was greater than 695. In inhalation studies, no deaths were observed in rats that were exposed up to 2.25 mg/l for 3 h, but all rats died after being exposed to 4.25 mg/l for 8 h.[5] In guinea pigs, the LC_{50} was greater than 2.25 mg/l Butoxyethanol following a 4-h exposure.[19] The LC_{50} was less than 2.0 mg/l in rabbits that were exposed to Butoxyethanol for 7 h.[5]

Repeated Dose Toxicity Studies

Dermal necrosis and hemoglobinuria were observed in rabbits that received repeated topical applications of Butoxyethanol.[2] Effects noted in rats that received repeated oral doses of Butoxyethanol (dose range 222 - 1000 mg/kg) included the following: decreased erythrocytes count, packed cell volume, and hemoglobin concentration; increased weight of spleen, liver, and kidneys; and decreased weight of the thymus. Observations made in animals subjected to repeated inhalations of Butoxyethanol (concentration range 77 - 500 ppm) included increased erythrocyte fragility, decreased hemoglobin concentration and erythrocyte count, and pathological changes in the kidneys, liver, and lungs. Increased kidney weight was observed in guinea pigs subjected to repeated inhalation of Butoxyethanol concentrations ranging from 203 to 495 ppm. Effects were not noted in animals exposed to 20 and 62.5 ppm Butoxyethanol.

Additional repeated-dose toxicity studies are summarized in Table 5. In an oral 6-wk toxicity study in rats that received up to 885 mg/kg/d Butoxyethanol, the NOAEL was less than 222 mg/kg/d.[4,5] Significant toxicity was observed at 885 mg/kg/d. Adverse effects observed included changes to red blood cells, splenic congestion, and liver anisokaryosis starting at 222 mg/kg/d. In inhalation studies, no adverse effects were observed in studies in male guinea pigs and male Beagle dogs that were exposed for 7 h/d for 2 wk to approximately 400 - 411 ppm (2.02 mg/l) Butoxyethanol.[5] A no-observed-adverse-effect concentration (NOAEC) could not be established in studies with mice, rats, rabbits, and cats exposed to 537 ppm (2.63 mg/l) Butoxyethanol for 6 h/d for 15 d; a NOAEC was determined to be 537 ppm for guinea pigs following this exposure.[4] In a 90-d inhalation study in rats, the NOAEC was less than 50 ppm for rats exposed to Butoxyethanol 7 h/d for 5 d/wk. Adverse effects included an increase in erythrocyte osmotic fragility and an increase in relative kidney to bodyweight ratio.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

In an in vitro study, 100% embryo lethality was induced at Butoxyethanol concentrations greater than 12.5 mM.[2] Dermal applications of Butoxyethanol did not cause embryotoxicity or fetotoxicity, and there were no significant differences in visceral or skeletal defects. In oral teratogenicity studies, the only significant finding in mice dosed with Butoxyethanol was a significant difference in the number of resorptions between experimental and control groups. In rats, oral administration of Butoxyethanol did not result in any increase in the incidence of fetal malformations over that noted in the control group; embryo/fetal effects included increased resorptions and non-live implants. In an inhalation teratogenicity study involving rats, ventricular septal defects and absent or severely shortened innominate arteries were noted in fetuses; results of fetal skeletal examinations and statistical analyses were not available. The results of other inhalation teratogenicity studies involving rats included mixed results regarding significant differences in the number of resorptions. In one of these studies, there were no statistically significant increases in the incidence of external, visceral, skeletal, or total malformations. The inhalation of Butoxyethanol in rabbits caused a significant reduction in unossified sternebra 6, rudimentary rib (first lumbar vertebra), and the number of viable implants per litter. However, there were no statistically significant increases in the number of litters with one or more fetuses with external, visceral, skeletal, or total malformations.

Neither oral nor inhaled doses of Butoxyethanol caused testicular atrophy in rats or mice, respectively. However, oral administration of Butoxyethanol to mice resulted in a significant decrease in the number of viable litters.

Oral

In a two-generation reproductive toxicity study, male and female CD-1 mice were exposed for a period of 14 wk to 720, 1340 or 2050 mg/kg Butoxyethanol in drinking water.[4] Significant adverse reproductive effects were observed in the females at very high dose levels which also caused severe general toxicity, including death (6/20 deaths in the 1340 mg/kg group; 13/20 deaths in the 2050 mg/kg group). An insufficient number of pups from 1340 mg/kg group were available to mate in the F_1 generation, so only pups from the 720 mg/kg dose group were used for the next mating cycle. At 720 mg/kg, the only adverse finding was a marginal statistically significant reduction in pup weight in the F_1 generation. This reduction was only 3% compared to controls and was not repeated in the F_2 generation, so it was not considered a significant adverse finding. Thus, the conservative lowest-observed-adverse-effect level (LOAEL) was determined to be 720 mg/kg/d. The reproductive no-observed-adverse-effect level (NOAEL) for Butoxyethanol was determined to be 720 mg/kg/d. A NOAEL for developmental toxicity could not be derived.

The SCCP noted that the lowest fetal NOAEL was 100 mg/kg bw/d in a rat study that was reported on in the original CIR safety assessment.[2,5] It was based on effects observed at 200 mg/kg bw/d: increased fetal lethality without malformations. These effects were observed with maternal toxicity (hemolytic anemia) and reduced body weight gain observed at 100 mg/kg bw/d. The maternal NOAEL was 30 mg/kg bw/d in this study. This NOAEL based on 3-d exposure to 100 mg/kg bw/d is considerably lower than the NOAEL based on 90-d repeated toxicity studies.

GENOTOXICITY STUDIES

Butoxyethanol was not mutagenic in the Ames test or in point mutation, forward mutation, and chromosomal aberration assays involving Chinese hamster ovary (CHO) cells.[2] *Butoxyethanol also did not induce sister chromatid exchanges in metabolically activated cultures of CHO cells. However, positive and negative results were noted without metabolic activation. Butoxyethanol was weakly mutagenic in an unscheduled DNA synthesis assay.*

Additional genotoxicity data are summarized in Table 6. In Syrian hamster embryo (SHE) cells, Butoxyethanol (0.5 - 20 mM) did not induce cellular transformation.[20] No increase in DNA damage was observed in a comet assay using SVEC4-10 mouse endothelial cells following exposure of up to 10 mM Butoxyethanol or 10 mM butoxyacetic acid (a metabolite).[21]Genotoxicity was not observed in an in vivo micronucleus induction test in mice at up to 1100 mg/kg Butoxyethanol or in a *Pig-a* assay in rats at up to 450 mg/kg Butoxyethanol.[4,22]

CARCINOGENICITY STUDIES

In dermal carcinogenicity studies with rats, a hair dye containing Butoxyethanol (10%) was not carcinogenic, but a rust-preventive product containing Butoxyethanol (2.5%) was carcinogenic.[2] *It should be noted that the latter product contained 90.9% Stoddard solvent (a refined petroleum distillate).*

In 2000, the NTP published the results of 2-yr inhalation studies of Butoxyethanol in male and female B6C3F1 mice and male and female F344/N rats.[23] *The mice were exposed to 0, 62.5, 125, or 250 ppm for 6 h/d, 5 d/wk, for 104 wk, and the rats were exposed to 0, 31.2, 62.5, or 125 ppm for the same time period. In male mice of the 250 ppm group, increased incidences of hemagiosarcoma of the liver, a marginal increase in the incidences of forestomach squamous cell papilloma, and an increase in the incidences of hepatocellular carcinoma were observed. In female mice of the 250 ppm, increased incidences of forestomach squamous cell papilloma or carcinoma (mainly papilloma) were observed. Increased incidences of forestomach neoplasms in the mice occurred in groups in which ulceration and hyperplasia were also present. No evidence of carcinogenic activity from exposure to Butoxyethanol was observed in male rats; however, there was equivocal evidence of carcinogenic activity in female rats exposed to 125 ppm Butoxyethanol based on the increased combined incidences of benign or malignant pheochromocytoma (mainly benign) of the adrenal medulla. In both species of animals, exposure to Butoxyethanol caused a mild regenerative anemia and effects secondary to the anemia.*

Overall, the International Agency for Research on Cancer (IARC) determined that Butoxyethanol is not classifiable as to its carcinogenicity to humans (Group 3).[24] In the evaluation, there was inadequate evidence in and limited evidence in experimental animals for carcinogenicity of Butoxyethanol. The US Environmental Protection Agency (EPA) integrated risk information system (IRIS) assessment of Butoxyethanol determined that the human carcinogenicity of this ingredient could not be determined at the time the assessment was performed, but "suggestive evidence" for cancer exists from laboratory animal studies performed on mice and rats by the NTP.[25]

Mechanism

In Vitro

In a study examining the mode of action for Butoxyethanol induced hemangiosarcomas,.[21] hemolyzed red blood cells activated macrophages, as evidenced by increased tumor necrosis factor (TNF)-α, while neither Butoxyethanol or butoxyacetic acid (a metabolite) increased TNF-α from macrophages. The effect of activated macrophages on endothelial cell DNA damage and DNA synthesis was also studied. Co-culture of endothelial cells with activated macrophages increased endothelial cell DNA damage after 4 or 24 h and increased endothelial cell DNA synthesis after 24 h. These data demonstrate that Butoxyethanol and related metabolites do not directly cause DNA damage. Supportive evidence also demonstrated that damaged red blood cells, iron, and/or products from macrophage activation (possibly reactive oxygen species) produce DNA damage in endothelial cells and that activated macrophages stimulate endothelial cell proliferation.

Oral

Gene expression data from the bone marrow, liver, and spleen of B6C3F1 male mice (10 animals per group) exposed via gavage to a single dose (4 h) or 7 daily doses of 900 mg/kg Butoxyethanol in deionized water were used to develop a mechanistic model of hemangiosarcoma.[26] The resulting mechanistic model confirmed previous work proposing that Butoxyethanol induced macrophage activation and inflammation in the liver. In addition, the model supported local tissue hypoxia in the liver and spleen, coupled with increased erythropoietin signaling and erythropoiesis in the spleen and bone marrow, and suppression of mechanisms that contribute to genomic stability, events that could be contributing factors to hemangiosarcoma formation. Finally, an immunohistochemistry method demonstrated that tissue hypoxia was present in the spleen and bone marrow.

OTHER RELEVANT STUDIES

Cytotoxicity

In a study that investigated the effects of Butoxyethanol (0.1 - 25 mM) on cell viability and on the hydrogen peroxide-induced damage in the human neuroblastoma (SH-SY5Y) cells, Butoxyethanol alone did not affect lactate dehydrogenase

release and 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyl tetrazolium bromide (MTT) reduction.[27] A concentration-dependently enhanced cytotoxic effect of hydrogen peroxide (0.5 mM) was observed with Butoxyethanol.

Immunotoxicity

Butoxyethanol did not induce immunotoxicity in rats.

Nephrotoxicity

In studies with rats, Butoxyethanol was nephrotoxic when administered intravenously, but was not nephrotoxic when administered intraperitoneally.

Hematotoxicity

Hemolytic effects of Butoxyethanol were observed in a number of in vitro and in vivo hematotoxicity studies.[2]

Groups of 4 male and 4 female F344 rats were exposed orally to single daily doses of Butoxyethanol at 250 mg/kg bw for 2, 3, or 4 d.[28] The rats were examined for hemolysis and histopathological evidence of disseminated thrombosis on days 2, 3, 4, and 29 (the latter in an additional group that was dosed for 4 d). Time-course Butoxyethanol-related erythrocytic changes were statistically significant in both sexes. Evidence of thrombosis and infarction was seen mainly in females dosed more than once with widespread thrombotic crisis after 2 or 3 dosing, likely explicable by the more significant morphological changes in erythrocytes and hemolysis observed in this gender. Thrombosis and infarction in the heart, brain, lungs, eyes, and bones were observed.

In another study employing similar protocol as above, groups of male and female F344 rats were exposed orally to single daily doses of Butoxyethanol at 250 mg/kg bw for 2, 3, or 4 d.[29] Blood was taken before the rats were killed on days 2, 3, 4, and 29 (the latter in an additional group that was dosed for 4 d). The administration of Butoxyethanol did not affect red blood cells aggregability but markedly enhanced their adherence to extracellular matrix; such enhancement was correlated with adherence to cultured endothelial cells. Red blood cell/endothelial cell interaction has been shown to be a potent catalyst of vascular occlusion in hemolytic hemoglobinopathies; thus, the enhanced red blood cell adherence to endothelial cell is a likely mechanism by which thrombosis and organ infarct are induced in Butoxyethanol-treated rats.

Age and dose-related differences in sensitivity to Butoxyethanol were investigated in groups of five 6- and 12-week-old female F344 rats at 62.5, 125, or 250 mg/kg/d of Butoxyethanol via oral gavage for up to 4 d.[30] The maximum hemolytic response, resulting in decreased erythrocyte count and higher mean cell volume, occurred in the 12-wk-old rats treated with the 250 mg/kg/d dose of Butoxyethanol. The highest increase in intracellular adhesion molecule-1 levels occurred in the 12-wk-old rats treated with 125 and 250 mg/kg Butoxyethanol. No intravascular thrombi were noted in the 6-wk-old Butoxyethanol-treated animals. The majority of intravascular thrombi occurred in the 12-wk-old rats treated with 250 mg/kg Butoxyethanol.

The nature of hemolytic effects induced by Butoxyethanol was analyzed for glucose-6-phosphate dehydrogenase (G-6-PDH) activity, adenosine triphosphate (ATP), pyruvate and thiols levels in peripheral blood of male Wistar rats.[31] In addition, the susceptibility to autoxidation of rat erythrocyte lipids was evaluated. Groups of 5 rats were treated with single doses of 0.625, 1.25, or 5.0 mM/kg Butoxyethanol. Analyses were performed 24 h after dosing. A decrease of ATP level in a dose-dependent manner and an increase in protein- and nonprotein-bound sulfhydryl groups were observed. These results indicate that an acute hemolytic effect of Butoxyethanol is not associated with alterations in G-6-PDH activity and the susceptibility of erythrocyte lipids to autoxidation. Increases in protein- and nonprotein-bound sulfhydryl groups seem to indicate the selective hemolysis of the aged erythrocytes. The increase in pyruvate and thiol levels may protect erythrocytes against the appearance of oxidative stress.

Hepatotoxicity

The hepatic effects of Butoxyethanol were studied in male B6C3F1 mice and male F344 rats.[32] Via daily gavage, the mice received 0, 225, 450, and 900 mg/kg/d and rats received 0, 225, and 450 mg/kg/d, 5 times per wk. Following treatment for up to 90 d, DNA synthesis, oxidative damage, hematocrit, and iron deposition were measured in the livers. An increase in hemolysis was observed in Butoxyethanol-treated rats and mice in a dose-dependent manner. An increase in the percentage of iron-stained Kupffer cells was observed following treatment with 450 and 900 mg/kg in mice and 225 and 450 mg/kg in rats. A biphasic increase in oxidative damage was seen in mouse liver after 7 and 90 d of treatment with Butoxyethanol, whereas no increases were observed in treated rat liver. Vitamin E levels were reduced by Butoxyethanol treatment in both mouse and rat livers; however, the basal level of vitamin E was approximately 2.5-fold higher in rat than in mouse liver. A similar biphasic induction of DNA synthesis was seen following in the mouse. In the mouse liver, increased DNA synthesis was observed in hepatocytes at 90 d and in endothelial cells at 7 and 14 d at all doses. No change in DNA synthesis was seen in Butoxyethanol-treated rat liver. No apparent differences in apoptosis and mitosis in the liver were observed in mouse and rat liver between Butoxyethanol treatment groups and untreated controls.

Butoxyethanol induced oxidative stress in the liver of male B6C3F1 mice (groups of 5) following 7-d treatment by gavage at 0, 450, or 900 mg/kg.[33] The study also examined whether Butoxyethanol (at 1 and 5 mM), 2-butoxyacetic acid, or iron ($FeSO_4$) produced oxidative stress in B6C3F1 mouse and F344 rat hepatocytes. Oxidative stress was examined by measuring oxidative DNA damage (8-hydroxy-2'-deoxyguanosine; OH8dG), lipid peroxidation (malondialdehyde (MDA)

formation), and cellular vitamin E concentrations. Neither Butoxyethanol or 2-butoxyacetic acid induced changes in the oxidative stress parameters examined in either rat or mouse hepatocytes. In contrast, iron produced a dose-related increase in OH8dG and MDA and a decrease in vitamin E levels following 24 h treatment. Mouse hepatocytes were more sensitive than rat hepatocytes to the oxidative damage induced by the iron. Iron-induced oxidative stress was not increased by co-treatment of iron with either Butoxyethanol or 2-butoxyacetic acid. These results support the proposal that the induction of hepatic oxidative stress by Butoxyethanol in vivo occurs secondary to induction of hemolysis and iron deposition in the liver rather than as a direct action of Butoxyethanol or 2-butoxyacetic acid.

Cardiovascular Effects

Atrial thrombosis was evaluated in 2-yr bioassays of more than 500 chemicals reported on by the NTP.[34] Incidences of atrial thrombosis were increased in high-dose groups involving 13 compounds, including Butoxyethanol. One of 5 female F344 rats that inhaled 500 ppm Butoxyethanol for 14 wk experienced left atrium thrombosis, hemolytic anemia, thrombocytosis, and systemic thrombosis.

Osteotoxic Effects

In a study of bone injury associated with thrombosis from hemolytic disorders, groups of 4 male and 4 female F344 rats were given 4 daily doses of 250 mg Butoxyethanol/5 ml water/kg bw via gavage.[35] Tail vertebrae were studied by histopathology and magnetic resonance imaging (MRI). Thrombosis and infarction were seen in both sexes, but females were more severely affected. Lesions were characterized by extensive medullary fat necrosis, granulomatous inflammation, fibroplasia, growth plate degeneration, and new woven bone formation adjacent to necrotic bone trabeculae. MRI mean and standard deviation tissue-density data for both sexes indicated a significant ($p \leq 0.05$) decrease following 4-d treatment and a significant increase ($p \leq 0.05$) following an additional 24 d without treatment.

A second study with a similar protocol as above utilized only female F344 rats.[36] The rats were given 0, 250, or 300 mg of Butoxyethanol/kg bw/d via gavage for 4 consecutive days. The rats were then killed 2 h or 26 d after the final treatment. The treated animals displayed a darkened purple-red discoloration on the distal tail. Histopathological evaluation, including phosphotungstic acid-hematoxylin staining of animals killed 2 h after the final treatment, revealed disseminated thrombosis and infarction in multiple organs, including bones. X-ray analysis found premature thinning of the growth plate occurred in the calcaneus, lumbar and coccygeal vertebrae, femur, and ilium of the treated animals. Areas of decreased radiographic densities were seen in the diaphysis of the femur of all treated animals. The bones were then examined histologically and showed a range of changes, including loss or damage to growth plates and necrosis of cortical bone. No thrombi were seen in the animals sacrificed at 30 d, but bone and growth plate changes consistent with prior ischemia were noted.

DERMAL IRRITATION AND SENSITIZATION STUDIES

Butoxyethanol was, at most, moderately irritating to rabbit skin.[2] In a human repeated insult patch test (HRIPT) involving 201 healthy subjects, 10% Butoxyethanol was not a sensitizer. Definite erythema and barely perceptible erythema were observed in 1 and 13 subjects, respectively, during the challenge phase. Reactions ranging from barely perceptible to definite erythema were observed in 63 subjects during induction.

Irritation

Animal

In a study using 5 New Zealand White rabbits, undiluted Butoxyethanol (0.5 ml) was applied under occlusion to an area of 6 cm^2 on shaved skin for 4 h.[4] The application site was then rinsed. Slight to moderate erythema and very slight edema were observed. The effects were persistent and still very slightly visible at the end of the 14-d observation period. The mean erythema score was 1.7 out of 4 and the mean edema score was 0.

In another irritation study, 6 albino rabbits received undiluted Butoxyethanol (0.5 ml) under occlusive conditions to an area of 2.54 cm^2 for 24 h.[4,5] Test sites were not rinsed. Very slight edema and erythema were observed at the 72-h observation period. The mean primary dermal irritation index was 1.5 out of 8.

Sensitization

Animal

The sensitization potential of Butoxyethanol was assessed in a guinea pig maximization study in accordance with Organisation for Economic Co-operation and Development (OECD) test guideline (TG) 406.[4,5] Ten Dunkin-Hartley guinea pigs were used in the study. During the induction phase, 0.5% Butoxyethanol in 0.9% was injected intradermally and 25% Butoxyethanol in saline was applied topically under occlusion (2 x 4 cm^2 area) for 48 h. After an ~2-wk rest, the challenge phase was performed topically under occlusion (8 mm diameter patch) for 24 h with 10% Butoxyethanol in saline. A rechallenge application was performed 1 wk after the initial challenge. Skin irritancy was observed, but it was determined the Butoxyethanol was not a skin sensitizer in this study.

OCULAR IRRITATION STUDIES

Undiluted butoxyethanol was an ocular irritant in rabbits.[2] *Additional testing in rabbits found Butoxyethanol at 5% caused no corneal injury, while 15% Butoxyethanol caused moderate corneal injury.*

Additional ocular irritation data are summarized in Table 7. Butoxyethanol tested undiluted and in 10% solution was irritating in a hen's eye test-chorioallantoic membrane (HET-CAM) assay and a corneal swelling test.[4] In several in vivo rabbit studies, undiluted Butoxyethanol was irritating to eyes.[4,5]

CLINICAL STUDIES

Case Reports

A 53-yr-old male patient accidentally co-ingested ethanol and 150 - 250 ml of pure Butoxyethanol.[37] The patient developed rapid obtundation, severe airway edema, hypotension, and prolonged acidosis. The patient recovered without sequelae.

Occupational Exposure

The application of an ink wash solvent, diluted to a concentration of 0.5 - 2.5% Butoxyethanol, did not induce sensitization or photosensitization reactions in 5 workers who had been exposed to Butoxyethanol in the workplace.[2]

Screening was performed in 34 screen-printing workers exposed to Butoxyethanol and in 37 non-exposed clerical workers.[38] The exposed group of workers usually worked 8 h/d for 5 d/wk. A Chi-square test showed the reticulocyte percentages and corrected reticulocyte counts to be significantly higher in the exposed group. T-tests showed a significant increase in white blood cell counts, reticulocyte percentages, and corrected reticulocyte count (i.e., reticulocyte index) in the exposed group, with *p*-values of 0.002, 0.004, and 0.002, respectively. Multivariate analysis showed the odds ratio for the corrected reticulocyte counts to be 16.30 for the exposed group, when compared with that of the control group.

Firefighter exposure to ingredients in firefighting foam, including Butoxyethanol, was assessed by biomonitoring methods during 3 consecutive firefighting training sessions.[39] The firefighters' short-term exposure to Butoxyethanol was analyzed by urinalysis of 2-butoxyacetic acid. In 2 of the training sessions, the average urinary excretions of 2-butoxyacetic acid (1.4 mmol/mol creatinine) exceeded the reference limit of the occupationally unexposed population (0.5 mmol/mol creatinine). In the third session, the average urinary excretion of 2-butoxyacetic acid was 0.8 mmol/mol creatinine.

In a 5-d work week, 31 decal transfer workers with direct contact (hands) with a 10% dilute aqueous solution of Butoxyethanol were exposed to an average of 1.7 ppm of Butoxyethanol in the air.[40] Correlation of Butoxyethanol in the air and post-shift urinary 2-butoxyacetic acid (a Butoxyethanol metabolite) levels was poor. Post-shift total 2-butoxyacetic acid levels in urine on Monday and Friday (446.8 and 619.4 mg/g creatinine, respectively) were around 223 and 310% of the American Conference of Governmental Industrial Hygienists (ACGIH) proposed Biological Exposure Index (BEI; 200 mg/g creatinine). Higher levels of total 2-butoxyacetic acid were observed in the urine of subjects exposed to low-level 2-butoxy-acetic acid in air, likely because of direct dermal contact.

RISK EXPOSURE

MOE is a quantitative factor calculated for cosmetic ingredients by dividing the NOAEL obtained for an ingredient in an animal experiment by the estimated systemic exposure dose (SED) for the ingredient in humans, generally according to US EPA and EC SCCS guidelines. The MOE is sometimes referred to as margin of safety (MOS), despite the parameters being definitionally different.

The SCCP calculated an MOE value for Butoxyethanol as a solvent in hair dye preparations to be 27.[5] This calculation is based on the NOAEL of 30 mg/kg bw/d (from a rat maternal toxicity study) and a systemic exposure dose (SED) of 1.11 mg/kg bw (skin area surface of 700 cm^2 x absorption through skin of 95 $\mu g/cm^2$ x 0.001 (unit conversion)/typical human bw of 60 kg). The SCCP determined that the inter-species dynamics uncertainty factor should be 0.01, rather than the default value of 2.5, due to the significantly higher resistance of humans to hemolysis compared to rodents. To ensure a cautious and conservative assessment, the SCCP further adjusted this factor to 0.1. Taking toxicokinetics and toxicodynamics into account, the minimal protective MOE was set at 4, calculated as follows: 4 (inter-species kinetics) × 0.1 (inter-species dynamics) × 3.2 (intra-species kinetics) × 3.2 (intra-species dynamics) = 4. Since the derived MOE is 27, which exceeds the minimum requirement of 4, it is considered to provide sufficient protection for the use of Butoxyethanol as a solvent in hair dye formulations.

SUMMARY

Butoxyethanol is reported to function as a fragrance ingredient, solvent, and viscosity decreasing agent in cosmetics, according to the *Dictionary*. The Panel first reviewed the safety of Butoxyethanol in a report published in 1996 with the conclusion that "Butoxyethanol is safe in hair and nail products at concentrations up to 10.0%." The Panel reaffirmed this conclusion after determining that the questionable evidence of carcinogenicity (in mice and rats) observed in a two-year NTP inhalation carcinogenicity study was not relevant to humans, as published in 2005. 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. In June 2024, the Panel determined that this safety assessment should be re-opened for re-evaluation due to reported restrictions by the EC on the use of Butoxyethanol.

According to RLD that CIR receive in 2024, Butoxyethanol is used in 81 formulations, with 79 uses reported in hair dyes and colors. A single use each was reported in perfumes and makeup fixatives. VCRP survey data received in 2023 reported Butoxyethanol was used in 3 hair dyes and colors. When comparing the VCRP data received in 2023 to that received in 2001, the frequencies of use for Butoxyethanol greatly decreased since the original re-review was considered; in 2001, Butoxyethanol was reported to have 110 uses, with the majority in hair coloring formulations. No uses were reported in the concentration of use survey conducted by the Council in 2020. In 2001, the maximum concentration of use range for Butoxyethanol was 3% in leave-on products and 50% in rinse-off products; concentrations of use were not reported for hair coloring products at the time of the first re-review.

In the EU, Butoxyethanol is categorized in Annex III, the list of substances which cosmetic products must not contain except subject to the restrictions laid down. For this ingredient, the regulation states that Butoxyethanol may only be used as a solvent in oxidative hair dye products at up to 4%, a solvent in non-oxidative hair dye products at up to 2%, and must not be used in aerosol dispensers (sprays). The SCCP opinion gives these same restrictions on use in hair dye formulations, as related to direct application to the hair/scalp. The opinion does not include any other cosmetic exposure, such as exposure to other types of cosmetics or possible aerosol/spray products.

In human skin samples, Butoxyethanol absorbed more rapidly through damaged skin than undamaged skin (3.39 $mg/cm^2/h$ vs 1.19 $mg/cm^2/h$). In solution, a steady state flux of 544 ± 64 $nmol \cdot cm^{-2}/h$ (0.064 $mg/cm^2/h$) was found with human dermatomed skin. In a study with oxidative hair dye formulations, the dermal absorbance for formulations with 5 and 10% Butoxyethanol was 61 ± 29 $\mu g/cm^2$ (12.1 ± 5.9%) and 125 ± 73 $\mu g/cm^2$ (12.5 ± 7.3%), respectively. Butoxyethanol penetrated human skin up to 6-fold more rapidly from aqueous solution (50%, 450 mg/ml) than from the neat solvent (900 mg/ml); a corresponding increase in apparent permeability coefficient was observed as Butoxyethanol concentration in water decreased. Permeation rates of Butoxyethanol through unoccluded rat dermatomed skin (16%) were greater than rat whole skin (8%), while absorption through human dermatomed skin (4%) was lower than the rat. Absorption of undiluted Butoxyethanol through occluded rat dermatomed skin in vitro was 18%. This same study found that Butoxyethanol absorption was enhanced by application in methanol to 23%. In a study with full-thickness human skin with undiluted Butoxyethanol, approximately 0.16% of the absorbed Butoxyethanol was metabolized to butoxyacetic acid.

Following topical application of undiluted Butoxyethanol in rats in vivo, 28% of the dose was absorbed after 24 h. The major routes of excretion included the urine (19%), expiration as carbon dioxide (6%) and feces (0.4%): little of the dose remained in the carcass (1.3%). In a study with human volunteers exposed to Butoxyethanol vapors, urinalysis results showed that baseline dermal absorption of Butoxyethanol vapor was 11% of the total absorbed dose. Higher temperatures and greater humidity increased dermal absorption. The rate of percutaneous absorption of Butoxyethanol is greater in aqueous solutions than when it is applied neat or diluted in ethanol.

Volunteers were dermally exposed for 4 h to 50% aq. Butoxyethanol or exposed via inhalation to 93 mg/m^3 Butoxyethanol for 30 min. After dermal exposure, 147.1 and 346 mg of free and total 2-butoxyacetic acid, respectively, were excreted in the urine at up to 48 h after exposure. After inhalation exposure, the 24-h cumulative excretion of free and total 2-butoxyacetic acid in urine was 5.5 and 12.8 mg, respectively.

In acute dermal studies, the LD_{50} for Butoxyethanol was greater than 2000 mg/kg in rats and guinea pigs. The dermal LD_{50} in rabbits ranged from 307 to greater than 2000 mg/kg. The oral LD_{50} for mice was 1519 mg/kg in fasted animals and 2005 mg/kg in fed animals; the LD_{50} in rats ranged from 880 to 2100 mg/kg. All rabbits that received 695 or 1500 mg/kg orally died, and the oral LD_{50} for Beagle dogs was greater than 695. In acute inhalation studies, no deaths were observed in rats that were exposed up to 2.25 mg/l for 3 h, but all rats died after being exposed to 4.25 mg/l for 8 h. In guinea pigs, the LC_{50} was greater than 2.25 mg/l Butoxyethanol following a 4 h exposure. The LC_{50} was less than 2.0 mg/l in rabbits that were exposed to Butoxyethanol for 7 h.

In an oral 6-wk toxicity study in rats that received up to 885 mg/kg/d Butoxyethanol, the NOAEL was less than 222 mg/kg/d. Significant toxicity was observed at 885 mg/kg/d. Adverse effects observed included changes to red blood cells, splenic congestion, and liver anisokaryosis starting at 222 mg/kg/d. In inhalation studies, no adverse effects were observed in studies in male guinea pigs and male Beagle dogs that were exposed for 7 h/d for 2 wk to approximately 400-411 ppm (2.02 mg/l) Butoxyethanol. A NOAEC could not be established in studies with mice, rats, rabbits, and cats exposed to 537 ppm (2.63 mg/l) Butoxyethanol for 6 h/d for 15 d; a NOAEC was determined to be 537 ppm for guinea pigs. In a 90-d inhalation study in rats, the NOAEC was less than 50 ppm exposed to Butoxyethanol 7 h/d for 5 d/wk. Adverse effects included an increase in erythrocyte osmotic fragility and an increase in relative kidney to bodyweight ratio.

In an oral two-generation reproductive toxicity study, the conservative LOAEL for Butoxyethanol was determined to be 720 mg/kg/d based on a statistically significant reduction in pup weights in the F_1 generation. The reproductive NOAEL for Butoxyethanol was determined to be 720 mg/kg/d. An NOAEL for developmental toxicity could not be derived. The SCCP noted that the lowest fetal NOAEL was 100 mg/kg bw/d in a rat study that was reported on in the original CIR safety

assessment. It was based on effects observed at 200 mg/kg bw/d: increased fetal lethality without malformations. These effects were observed with maternal toxicity (hemolytic anemia) and reduced body weight gain observed at 100 mg/kg bw/d. The maternal NOAEL was 30 mg/kg bw/d in this study.

In SHE cells, Butoxyethanol (0.5 - 20 mM) did not induce cellular transformation. No increase in DNA damage was observed in a comet assay using SVEC4-10 mouse endothelial cells following exposure of up to 10 mM Butoxyethanol. Genotoxicity was not observed in an in vivo micronucleus induction test in mice at up to 1100 mg/kg Butoxyethanol or in a *Pig-a* assay in rats at up to 450 mg/kg Butoxyethanol.

Overall, IARC determined that Butoxyethanol is not classifiable as to its carcinogenicity to humans (Group 3). In the evaluation, there was inadequate evidence in and limited evidence in experimental animals for carcinogenicity of Butoxyethanol. The US EPA IRIS assessment of Butoxyethanol determined that the human carcinogenicity of this ingredient could not be determined at the time the assessment was performed, but "suggestive evidence" for cancer exists from laboratory animal studies performed on mice and rats by the NTP. In a study of the mechanism of carcinogenicity using mice exposed to a single dose or 7 daily doses of 900 mg/kg Butoxyethanol in water, Butoxyethanol induced macrophage activation and inflammation in the liver. In addition, the model supported local tissue hypoxia in the liver and spleen, coupled with increased erythropoietin signaling and erythropoiesis in the spleen and bone marrow, and suppression of mechanisms that contribute to genomic stability, events that could be contributing factors to hemangiosarcoma formation.

Butoxyethanol (0.1 - 25 mM) enhanced the cytotoxic effect of hydrogen peroxide in a concentration-dependent manner. In a study examining the involvement of hemolysis and macrophage activation in Butoxyethanol carcinogenesis, DNA damage was produced by hemolyzed red blood cells, ferrous sulfate, and hydrogen peroxide in mouse endothelial cells. Butoxyethanol and related metabolites did not directly cause the DNA damage. Supportive evidence demonstrated that damaged red blood cells, iron, and/or products from macrophage activation (possibly reactive oxygen species) produce DNA damage in endothelial cells and that activated macrophages stimulate endothelial cell proliferation. In studies with rats that received up to 250 mg/kg/d Butoxyethanol, thrombosis and infarction of the organs were observed. A decrease of ATP level in a dose-dependent manner and an increase in protein- and nonprotein-bound sulfhydryl groups were observed following treatment with singled dose of up to 5.0 mM/kg Butoxyethanol.

Following treatment for up to 90 d with Butoxyethanol in mice (up to 900 mg/kg/d) and rats (up to 450 mg/kg/d, an increase in hemolysis was observed in Butoxyethanol-treated rats and mice in a dose-dependent manner. A biphasic increase in oxidative damage was seen in mouse liver after 7 and 90 d of treatment with Butoxyethanol, whereas no increases were observed in treated rat liver. In the mouse liver, increased DNA synthesis was observed in hepatocytes at 90 d and in endothelial cells at 7 and 14 d at all doses. No change in DNA synthesis was seen in Butoxyethanol-treated rat liver. No apparent differences in apoptosis and mitosis in the liver were observed in mouse and rat liver between Butoxyethanol treatment groups and untreated controls. Butoxyethanol induced oxidative stress in the liver of male mice following 7-d treatment by gavage of up to 900 mg/kg. Neither Butoxyethanol or 2-butoxyacetic acid induced changes in the oxidative stress parameters examined in either rat or mouse hepatocytes. Mouse hepatocytes were more sensitive than rat hepatocytes to the oxidative damage induced by the iron. Iron-induced oxidative stress was not increased by co-treatment of iron with either Butoxyethanol or 2-butoxyacetic acid.

In studies of bone injury associated with thrombosis from hemolytic disorders in rats that received up to 300 mg/kg/d Butoxyethanol via gavage, lesions were characterized by extensive medullary fat necrosis, granulomatous inflammation, fibroplasia, growth plate degeneration, and new woven bone formation adjacent to necrotic bone trabeculae. Bones examined histologically showed a range of changes, including loss or damage to growth plates and necrosis of cortical bone.

In dermal irritation studies in rabbits with undiluted Butoxyethanol under occlusive conditions, slight erythema and edema were observed. Butoxyethanol was determined not to be a skin sensitizer, but produced irritation, in a guinea pig maximization study that used induction concentrations of 0.5% (intradermally) and 25% (topically) and a challenge concentration of 10%.

Butoxyethanol tested undiluted and in 10% solution was irritating in a HET-CAM assay and a corneal swelling test. In several in vivo rabbit studies, undiluted Butoxyethanol was irritating to eyes.

A 53-yr-old male patient developed rapid obtundation, severe airway edema, hypotension, and prolonged acidosis following accidental ingestion of pure Butoxyethanol. Occupational exposure studies have been performed on workers exposed to Butoxyethanol in screen-printing, decal transfer, and firefighting foam.

The SCCP calculated a MOE value for Butoxyethanol as a solvent in hair dye preparations to be 27. This calculation is based on the NOAEL of 30 mg/kg bw/d (from a rat maternal toxicity study) and a systemic exposure dose (SED) of 1.11 mg/kg bw (skin area surface of 700 cm^2 x absorption through skin of 95 $\mu g/cm^2$ x 0.001 (unit conversion)/typical human bw of 60 kg). The SCCP determined that the inter-species dynamics uncertainty factor should be 0.01, rather than the default value of 2.5, due to the significantly higher resistance of humans to hemolysis compared to rodents. To ensure a cautious and conservative assessment, the SCCP further adjusted this factor to 0.1. Taking toxicokinetics and toxicodynamics into account, the minimal protective MOE was set at 4. Since the derived MOE is 27, which exceeds the minimum requirement of 4, it is considered to provide sufficient protection for the use of Butoxyethanol as a solvent in hair dye formulations.

PREVIOUS DISCUSSION – ORIGINAL REPORT (1998)

Current cosmetic product formulation data (1994) submitted to FDA indicate that Butoxyethanol is used in hair and nail products.[2] *Data submitted to FDA in 1989 indicate that Butoxyethanol was used in hair colors, bleaches and shampoos at concentrations up to 10%. At the expected maximum use concentration of 10%, there was no evidence of skin sensitization reactions to aqueous Butoxyethanol in a repeated insult patch test involving 201 subjects. Thus, 10% Butoxyethanol is a safe concentration for product formulations intended for rinse-off or leave-on application to human skin.*

The Panel recognizes the severe ocular irritation potential of undiluted Butoxyethanol, but acknowledges that moderate and no corneal injuries were observed at concentrations of 15 and 5% aqueous, respectively. In consideration of these results, the ocular irritation potential of Butoxyethanol is attenuated at concentrations expected in cosmetic product formulations.[2]

PREVIOUS DISCUSSION –RE-REVIEW (2005)

The Panel noted that the in vitro hematotoxicity study by Ruchaud et al. (1992), cited in the published Final Report, has been retracted and agreed that the deletion of this reference would have no substantive effect on this safety assessment.[3]

The CIR Expert Panel discussion focused on the questionable evidence of carcinogenicity (in rats and mice) in a 2-year inhalation carcinogenicity study on Butoxyethanol that was published by the NTP in 2000. The conclusions for rats and mice were as follows: no evidence of carcinogenic activity in male rats; equivocal evidence of carcinogenic activity in female rats, based on increased combined incidences of benign or malignant pheochromocytoma (mainly benign) of the adrenal medulla; some evidence of carcinogenic activity in male mice, based on increased incidences of hemangiosarcoma of the liver; marginal increase in incidences of forestomach squamous cell papilloma and increase in incidences of hepatocellular carcinoma may have been exposure related; and some evidence of carcinogenic activity in female mice, based on increased incidences of forestomach squamous cell papilloma or carcinoma (mainly papilloma).

After reviewing the NTP study, the Panel noted that the results are not relevant to man for the following reasons: (1) Pheochromocytomas observed in one rodent species were within the historical control range. (2) Liver cancer observed in a male rat was thought to have been due to hemolysis [it has been shown that rodent red blood cells are very sensitive to hemolysis by Butoxyethanol, and that this effect was due to iron overload]. (3) An increased incidence of forestomach cancers was observed in female mice after inhalation exposure. The forestomach is not a relevant organ for evaluation, and this effect was thought to have been due to irritation, but not genotoxicity.

The Panel also considered current data indicating that Butoxyethanol is used at concentrations up to 50% in nail polish and enamel removers, in light of its previous limitation of 10.0% in hair and nail products. However, it was concluded that the increased use concentration was of little concern because the nail plate is made up of dead tissue, and, thus, the amount of absorption through the nail would be negligible.

DISCUSSION

To be developed.

CONCLUSION

To be determined.

TABLES

Table 1. Chemical properties

Property	Value	Reference
Physical Form	Clear, thin liquid	2
Color	Colorless	5
Odor	Mild, sweet ester/ether	4
Molecular Weight (g/mol)	118.18	2
Specific Gravity	0.890 - 0.906	2
Viscosity (kg/(s x m @ 20 ºC)	336.5	4
Vapor pressure (mmHg @ 140 ºC) (mmHg @ 25 ºC)	300 0.88	2
Melting Point (ºC)	< -40 -77 -74.8	2 5
Boiling Point (ºC)	171 - 172	2
Water Solubility	Miscible with water	2
Other Solubility	Miscible with methanol and ether in all proportions	2
log P_{ow} (@ 25 ºC & pH 7)	0.81	4
Disassociation constant (pKa @ 20 ºC)	15 (QSAR estimation)	4

Table 2. Frequency (RLD/VCRP) and concentration of use of Butoxyethanol according to likely duration and exposure and by product category

	# of Uses			*Max Conc of Use*	
	RLD (2024)[7]	**VCRP (2023)[8]**	**VCRP (2001)[3]**	**% (2020)[9]**	**% (2001)[3]**
Totals*	**81**	**3**	**110**	**NR**	**3-50**
summarized by likely duration and exposure**					
Duration of Use					
Leave-On	***	*NR*	*3*	*NR*	*3*
Rinse-Off	***	*3*	*107*	*NR*	*50*
Diluted for (Bath) Use	***	*NR*	*NR*	*NR*	*NR*
Exposure Type					
Eye Area	***	NR	NR	NR	3
Incidental Ingestion	***	NR	NR	NR	NR
Incidental Inhalation-Spray	***	NR	2[a]	NR	NR
Incidental Inhalation-Powder	***	NR	NR	NR	NR
Dermal Contact	***	NR	NR	NR	3
Deodorant (underarm)	***	NR	NR	NR	NR
Hair - Non-Coloring	***	NR	2	NR	NR
Hair-Coloring	***	3	107	NR	NR
Nail	***	NR	1	NR	3-50
Mucous Membrane	***	NR	NR	NR	NR
Baby Products	***	NR	NR	NR	NR
as reported by product category					
Eye Makeup Preparations (not children's)					
Eye Shadow	NR	NR	NR	NR	3
Fragrance Preparations	***1***				
Perfumes	1	NR	NR	NR	NR
Hair Preparations (non-coloring)					
Tonics, Dressings, and Other Hair Grooming Aids	NR	NR	2	NR	NR
Hair Coloring Preparations	***79***				
Hair Dyes and Colors (all types requiring caution statements and patch tests)	79	3	94	NR	NR
Hair Tints	NR	NR	3	NR	NR
Hair Shampoos (coloring)	NR	NR	8	NR	NR
Hair Bleaches	NR	NR	2	NR	NR
Makeup Preparations (not eye; not children's)	***1***				
Blushers and Rouges (all types)	NR	NR	NR	NR	3
Makeup Fixatives	1	NR	NR	NR	NR
Manicuring Preparations					
Nail Polish and Enamel	NR	NR	1	NR	3
Nail Polish and Enamel Removers	NR	NR	NR	NR	50

NR – not reported

*The total FOU provided for RLD refers to the ingredient count supplied by FDA, and is not a summation of the number of uses per category because each product may be categorized under multiple product categories. For data supplied via the VCRP or by the Council survey, the sum of all exposure types may not equal the sum of total uses because each ingredient may be used in cosmetics with multiple exposure types.

**Likely duration and exposure are derived from VCRP and survey data based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

***In the RLD, each ingredient may be reported under several product categories, making a summation of RLD misleading in comparison to VCRP data. Accordingly, RLD are presented below by product category (as supplied by FDA), but are not summarized by likely duration and exposure.

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

Table 3. Dermal penetration studies on Butoxyethanol

Vehicle	Test System	Concentration/Dose	Protocol	Results	Reference
			IN VITRO		
not reported	human abdominal skin; damaged (n = 4) and undamaged (n = 8)	not reported	Dermal penetration study performed as 2 experiments using liquid Butoxyethanol; skin was exposed to the test material in Franz-type diffusion cells; absorption results were measured twice due to high variability in the first experiment	Mean absorption rates were: 0.857 ± 0.282 mg/cm^2/h in the first experiment and 1.52 ± 0.37 mg/cm^2/h in the second experiment. The damage ratio (permeability constant after contact with Butoxyethanol/permeability constant before contact with Butoxyethanol) was 3.25 ± 3.33 in the first experiment and 5.14 ± 4.99 in the second experiment. Due to the high variability in each experiment, mean absorption rates were calculated separately for the undamaged and damaged skin. The results from the damaged skin specimens were about 3 times higher than the undamaged skin (3.39 mg/cm^2/h vs 1.19 mg/cm^2/h). When the results from the four cells showing the high damage ratio are excluded from the calculation of the overall mean result, the mean damage ratio is 1.66 ± 1.31.	5
tested neat and in water	full thickness or dermatomed human breast skin	100 or 200 µl in aq. solution; 10 µl neat	Dermal absorption study of liquid Butoxyethanol using flow-through diffusion cells; measured for 24 h	In solution, a steady state flux of 544 ± 64 nmol·cm^{-2}/h (0.064 mg/cm^2/h) was found with dermatomed skin. Reducing the dose to 100 µl decreased the steady state flux by about 55%. With full thickness skin, the time to steady state increased and the steady state flux decreased. Absorption rates of undiluted Butoxyethanol in finite dose exceeded those measured with aqueous solutions, though the apparent permeability coefficient was higher with the aqueous doses.	11
oxidative hair dye formulations, mixed 50/50 w/w with developer containing hydrogen peroxide	human skin	20, 500, or 1000 µg/cm^2; formulation contained 5 or 10% butoxyethanol (final on-skin concentrations were 2.5 and 5% butoxyethanol, respectively)	Dermal absorption study performed in accordance with OECD TG 28; 2 different formulations containing 5 or 10% ^{14}C-Butoxyethanol were mixed with equal volume of developer and applied to skin for 30 min; skin surface was 1.76 cm^2; at end of exposure, skin was washed; diffusion was monitored during the 24 h following application and receptor fluid was collected 2, 4, 6, 10, 21, and 24 after the beginning of exposure.	Total recovery for the formulation containing 5% Butoxyethanol was 84 ± 4%; for the 10% formulation, total recovery was 81 ± 4%. Dermal absorbance for formulation with 5% Butoxyethanol was 61 ± 29 µg/cm^2 (12.1 ± 5.9%); for the 10% formulation, dermal absorbance was 125 ± 73 µg/cm^2 (12.5 ± 7.3%).	5
tested neat and in water	synthetic polydimethylsiloxane membrane, rat skin, and human skin	900 mg/ml neat and 0.9 -810 mg/ml in aq. solution	Dermal absorption study using in vitro diffusion system that assessed the influence of water mixtures on the absorption of ^{14}C-Butoxyethanol in a synthetic membrane (400 µm thickness), dermatomed rat skin (280 µm thickness), and dermatomed human skin (320 µm thickness); Scoot Dick-Newcastle cells and Eagles minimum essential medium receptor fluid were utilized in this study; study performed under partially occluded conditions	Butoxyethanol penetrated human skin up to 6-fold more rapidly from aqueous solution (50%, 450 mg/ml) than from the neat solvent; a corresponding increase in apparent permeability coefficient was observed as Butoxyethanol concentration in water decreased. The maximum penetration rate of water also increased in the presence of Butoxyethanol. Absorption through a synthetic membrane obeyed Fick's Law and absorption through rat skin showed a similar profile to human skin but with a lesser effect.	12

Table 3. Dermal penetration studies on Butoxyethanol

Vehicle	Test System	Concentration/Dose	Protocol	Results	Reference
undiluted or prepared in methanol	rat whole and dermatomed skin (280 µm); human dermatomed skin (330 µm)	aliquots of 10 µl	Percutaneous absorption test of ^{14}C-Butoxyethanol using in vitro flow through diffusion cell technique, results compared with the in vivo absorption in rat skin (described below); some samples of skin were occluded with parafilm; absorption was measured for up to 24 h after application	Permeation rates of Butoxyethanol through unoccluded rat dermatomed skin (16%) were greater than rat whole skin (8%), while absorption through human dermatomed skin (4%) was lower than the rat. Absorption of undiluted Butoxyethanol through occluded rat dermatomed skin was 18%. Butoxyethanol absorption (23%) was enhanced by application in methanol. Distribution analysis and microautoradiography demonstrated the lack of Butoxyethanol accumulation within the skin in vitro. This was reflected in the absence of first-pass metabolism of Butoxyethanol during percutaneous penetration through viable human or rat skin in vitro, despite rat skin cytosol having the potential to metabolize Butoxyethanol.	13
undiluted	full-thickness excised human skin	200 µl/cm^2; 115.2 mg	Dermal absorption and metabolism study of ^{14}C-Butoxyethanol; skin samples were placed on transwell supports and placed with the underside of the skin in contact with receptor fluid; absorption and metabolism of Butoxyethanol to butoxyacetic acid was monitored for 24 h	In total 64.94 ± 0.04 mg of Butoxyethanol or its metabolites were removed from the surface of the skin at 24 h, representing the equivalent of 56% of the applied dose, the equivalent of 17.5% of the applied dose was recovered from the receiver fluid, 3% from within the skin and the remaining 23.5% of the dose was lost to the atmosphere through evaporation. After 24 h, a total of 31.5 µg of butoxyacetic acid had been produced representing approximately 0.03% of the applied dose. Approximately 0.16% (31.5 µg as a percentage of the total amount of Butoxyethanol reaching the receiver fluid (20.17 mg)) of the absorbed Butoxyethanol was metabolized to butoxyacetic acid.	14
			ANIMAL		
undiluted	male Wistar rats; number not reported	100 µl	Percutaneous absorption test of ^{14}C-Butoxyethanol in rats; results compared with the in vitro absorption in rat skin (described above); animals received test material on clipped skin, test sites were occluded; blood was drawn at 1, 4, 7, and 24 h post dosing; animals were placed in sealed metabolism cages to message radioactivity in expired air; urine was collected; animals were killed for tissue distribution studies	Following topical application, 28% of the dose was absorbed after 24 h. The major routes of excretion included the urine (19%), expiration as carbon dioxide (6%) and feces (0.4%): little of the dose remained in the carcass (1.3%). Free Butoxyethanol (0.5%), butoxyacetic acid (8%), glucuronide conjugate (3%), sulfate conjugates (0.7%) and ethylene glycol (0.6%) were detected in urine. Distribution analysis and microautoradiography demonstrated the lack of Butoxyethanol accumulation within the skin in vivo. This was reflected in the absence of first pass metabolism of Butoxyethanol during percutaneous penetration through rat skin in vivo, despite rat skin cytosol having the potential to metabolize Butoxyethanol.	13
			HUMAN		
none	4 volunteers, 2 males and 2 females	50 ppm for 2 h	Study to investigate the influence of temperature, humidity, and clothing on dermal absorption of Butoxyethanol vapors; volunteers were exposed to Butoxyethanol vapors on 9 occasions; for 8/9 exposures, volunteers wore air-fed half masks to supply clean air; absorption was measured through urinalysis for 2-butoxyacetic acid; urine was collected before and after each exposure at 0, 4, 6, 8, 10, 12, 22, 26, 30 and 34 h; baseline conditions were 25°C, 40% relative humidity, shorts and T-shirt	Urinalysis results showed that baseline dermal absorption of Butoxyethanol vapor was 11% of the total absorbed dose. Higher temperature (30°C) and greater humidity (65%) increased dermal absorption. Wearing whole-body overalls did not attenuate absorption. By combining several factors together in the occupation scenario, dermal absorption of vapors was significantly increased with a mean of 39% of the total absorbed dose.	15

Table 3. Dermal penetration studies on Butoxyethanol

Vehicle	Test System	Concentration/Dose	Protocol	Results	Reference
diluted in water (17%) or diluted in ethanol (17%)	3 volunteers, 2 males and 1 female	10 µl	Butoxyethanol (≥ 99.4% pure) was applied diluted in water or diluted in ethanol on the skin of healthy volunteers and occluded with Finn Chambers to prevent evaporation. Confocal Raman micro-spectroscopy measurements were done following application after 15 min and 3 h. The concentration of Butoxyethanol as a function of distance to the skin surface was calculated and further analyzed with regard to mass transport into the stratum corneum and the flux through the stratum corneum.	Butoxyethanol penetrated markedly faster when dissolved in water as compared to ethanol (after 15 min: 104 µg/cm^2 compared to 22.3 µg/cm^2; after 3 h: 321 µg/cm^2 compared to 23.3 µg/cm^2).	16
aqueous solution	6 male volunteers	50%, 90%, or 100% w/w	Volunteers were dermally exposed (8 ml) to Butoxyethanol for 4 h on the arm over an area of 40 cm^2. Each volunteer was exposed twice to the 50% solution, and once each to the 90% solution and to Butoxyethanol neat. Dermal absorption parameters were calculated from 24-h excretion of total butoxyacetic acid in urine and Butoxyethanol in blood. The time-weighted average dermal fluxes were calculated from the urine and blood data.	The dermal absorption of Butoxyethanol from aqueous solutions was markedly higher than that of neat Butoxyethanol. The dermal fluxes obtained from cumulative 24-h excretion of 2-butoxyacetic acid amounted to 1.34, 0.92, and 0.26 mg/cm^2/h for 50, 90 and 100% Butoxyethanol, respectively. The dermal fluxes calculated from the Butoxyethanol blood data amounted to 0.92 and 0.74 mg/cm^2/h for 50 and 90% Butoxyethanol, respectively. The permeation rates into the blood reached a plateau between 60 and 120 min after the start of exposure, indicating achievement of steady-state permeation. The apparent permeability coefficient K(p), was 1.75 x 10^{-3} and 0.88 x 10^{-3} cm/h for 50 and 90% Butoxyethanol, respectively.	17

Table 4. Acute toxicity studies on Butoxyethanol

Vehicle	Animals/Group	Concentration/Dose	Protocol	LD_{50}/LC_{50}/Results	Reference
			DERMAL		
none	groups of 5 male and 5 female Sprague-Dawley rats	2000 mg/kg	Acute dermal toxicity study in accordance with OECD TG 402; rats received test material under semi-occlusive patch; test site wiped clean with cotton wool moisten with water after 24 h and animals were observed for 14 d after administration	LD_{50} > 2000 mg/kg; no clinical signs of toxicity during observation period and no deaths were observed	4,5
none	groups of 5 male and 5 female Sprague-Dawley rats	2000 mg/kg	Acute dermal toxicity study in accordance with OECD TG 402; rats received test material under occlusive patch for 24 h; animals were observed for 14 d after administration	LD_{50} > 2000 mg/kg; 1 female found dead 2 d after dosing; clinical signs of toxicity in 2 females included ataxia, pallor of the extremities, emaciation, lethargy, decreased respiratory rate, labored respiration, and tiptoe gait with incidents of ptosis and red/brown staining around the eyes; hunched posture was observed in most animals at end of exposure period; no abnormalities observed at necropsy in surviving animals; in animal that died during the study, hemorrhagic lungs, dark liver, dark kidneys, sloughing of the non-glandular epithelium of the stomach, and hemorrhage of the small and large intestines were observed	4
none	Groups of 5 male and 5 female Hartley guinea pigs	2000 mg/kg	Acute dermal toxicity study; guinea pigs received test material under occlusive patch; test sites were washed after 24 h and animals were observed for 14 d after administration	LD_{50} > 2000 mg/kg; no clinical signs of toxicity during observation period and no deaths were observed; all animals gained weight and no signs of organ toxicity at necropsy	4,5
none	groups for 4 male and female rabbits, strain not specified	3 doses, not specified	Acute dermal toxicity study; rabbits received test material under occlusive patch for 24 h; no further details provided	LD_{50} = 612 mg/kg (680 ml/kg); no further details provided	4

Table 4. Acute toxicity studies on Butoxyethanol

Vehicle	Animals/Group	Concentration/Dose	Protocol	LD_{50}/LC_{50}/Results	Reference
none	groups of 5 male New Zealand White rabbits	153, 307, 614, or 1239 mg/kg	Acute dermal toxicity study in accordance with OECD TG 402; rabbits received test material under occlusive patch for 24 h; test site wiped with cotton wool; animals observed for 14 d after administration	LD_{50} = 307 mg/kg; at lower doses, clinical effects included anorexia, slight depression, cyanosis, ataxia, and soft feces; at higher doses, clinical effects included salivation, nasal discharge, iritis, significant depression, labored breathing and prostration	4
none	groups of 5 male and 5 female New Zealand White rabbits	1000 and 2000 mg/kg, tested sequentially	Acute dermal toxicity study in accordance with OECD TG 402; rabbits received test material under semi-occlusive patch; test site wiped clean with cotton wool moisten with water after 24 h; animals were observed for 14 d after administration	LD_{50} > 2000 mg/kg; no deaths at 1000 mg/kg; at 2000 m/kg, 1 female died on day 2 and 1 male and 1 female were killed *in extremis* on day 1; no signs of systemic toxicity noted in 1000 mg/kg dose group, very slight to well-defined erythema/slight edema at test stie; in 2000 mg/kg dose group, lethargy, stained urine, decreased respiratory rate, hunched posture, yellow skin and eyes observed along with isolated incidents of righting reflex, hypothermia, ataxia, and diarrhea, test site had very slight to well defined erythema/slight edema; no abnormalities observed at necropsy in the 1000 mg/kg dose group, animals that died in the 2000 mg/kg dose group before study end had pale kidneys, dark liver, hemorrhage of the gastric mucosa, and red fluid in the bladder, no abnormalities noted in surviving animals	4,5
none	groups of 5 male and 5 female New Zealand White rabbits	500, 707, or 1000 mg/kg	Acute dermal toxicity study in accordance with OECD TG 402; rabbits received test material under occlusive patch; test site wiped clean with cotton wool moisten with water after 24 h; animals were observed for 14 d after administration	LD_{50} = 841 mg/kg for all animals (LD_{50} males = 1060 mg/kg and LD_{50} females = 667 mg/kg); clinical signs of toxicity in all groups were lethargy, ataxia, red stained urine, diuresis, decreased respiratory rate, hunched posture, and yellow skin and eyes; no adverse effects were observed at necropsy in animals that survived until study end; in animals that died during the study, hemorrhagic lungs, dark kidneys, dark or pale liver or patchy liver pallor, and red fluid in the bladder were observed	4,5
			ORAL		
none	groups of 5 male CD-1 mice	not specified, but within 2.6 - 168 mM/kg	Acute oral toxicity study in accordance with OECD TG 401; mice received a single oral dose via gavage; study conducted with fed and fasted groups	LD_{50} = 1519 mg/kg in fasted mice and 2005 mg/kg in fed mice; clinical signs in both fed and fasted mice were inactivity, labored breathing, rapid respiration, anorexia, slight to moderate weakness, tremors, proration, and death; animals that died exhibited bloody urine, and/or blood in the stomach and intestines (fasted animals); these conditions were not noted in animals that survived until study termination; hematuria was noted in the intermediate dose fed group at necropsy and was blood noted in the stomach of some fed mice which died before study end	4,5
none	groups of 5 male CD/BR rats	not specified, but within 2.6 - 168 mM/kg	Acute oral toxicity study in accordance with OECD TG 401; rats received a single oral dose via gavage; study conducted with fed and fasted groups	LD_{50} = 1746 mg/kg in both fed and fasted rats; clinical signs in both fed and fasted animals were inactivity, labored breathing, rapid respiration, anorexia, slight-to-moderate weakness, tremors, prostration, and death; animals that died had bloody urine and/or blood in the stomach and intestines (fasted animals), these conditions were not noted in animals that survived until study termination; hematuria was noted in high dose fasted and fed animals at necropsy	4,5
water	groups of 10 male and 10 female rats, strain not specified	200 - 1600 mg/kg	Acute oral toxicity study in accordance with OECD TG 401; rats received a single oral dose via gavage	LD_{50}= 880 mg/kg in males and 615 mg/kg in females; signs of toxicity in the 1000 – 1600 mg/kg dose groups included staggering, reduced general state, atony, abdominal and lateral position, irregular respiration, dyspnea, and hemolytic urine; surviving animals had squatting posture and scrubby fur 4 d after administration, but these animals were without findings after 6 d. Signs of toxicity in the 200 - 800 mg/kg dose groups included slight staggering, accelerated respiration, and hemolytic urine; 1 d after administration, squatting posture, straight fur, and reddened eyes occurred; surviving animals rebounded with no abnormalities at study end; animals that died during the study had anemic musculature, hemoglobinuric nephrosis, blood-colored urine, adipose, livers, and blurred snouts at necropsy; no remarkable abnormalities were observed at necropsy in animals that survived until study end	4
none	groups of 3 female CDF rats	130 - 2000 mg/kg	Acute oral toxicity study; rats received a single oral dose via gavage	LD_{50} = 1900 mg/kg; clinical signs included staining of perineal region (130 - 1000 mg/kg), rough hair coats (1000, 2000 mg/kg), lethargy, rapid shallow breathing, palpebral closure (2000 mg/kg), and necrosis of tails in all surviving animals in the 1000 and 2000 mg/kg dose groups	4

Table 4. Acute toxicity studies on Butoxyethanol

Vehicle	Animals/Group	Concentration/Dose	Protocol	LD_{50}/LC_{50}/Results	Reference
water	groups of male and female Wistar rats, number not reported	test material given as a 5 or 10% (vol/wt) solution, or in one case, undiluted	Acute oral toxicity study; route not specified	LD_{50} = 2100 mg/kg in males and 1850 mg/kg in females; clinical signs included sluggishness, ruffling of coats, prostration, and narcosis at doses at or above the LD_{50}; necropsy on rats that died revealed congestion of hemorrhaged lungs, mottled livers, severely congested kidneys, and hemoglobinuria	4
water	groups of 3 male and 3 female rabbits	695 and 1500 mg/kg	Acute oral toxicity study; rabbits received a single oral dose via gavage	All animals died during the observation period (up to 48 h) in both dose groups; clinical signs in the high-dose group included atonia, lateral position, hyperpnea, staggering gait, and hyperemia; bloody red coloration of the eye occurred in 2 animals in the lower dose group; at necropsy, the high-dose animals had hyperemia and edema of the lung, hematuria and tubular necrosis of the kidney, hemorrhagia in the interstitial tissues of the adrenal gland, and follicular hemorrhagia in the spleen; lower dose animals had hyperemia, heart muscle adiposis, acute hemolytic nephrosis, pulmonary edema, and acute lymphocytopenia	4
water	1 male and 1 female Beagle dog	695 mg/kg	Acute oral toxicity study; dogs received a single oral dose via gavage	LD_{50} > 695; no remarkable clinical signs or abnormalities at necropsy were observed	4
INHALATION					
none	groups of 3 male 3 and female rats, strain not specified	1.44 mg/l for 3 h or 4.25 mg/l for 8 h	Acute inhalation toxicity study; rats were exposed to test material via whole body exposure for 3 or 8 h followed by a 7-d observation period	No deaths were observed to 1.44 mg/l exposure for 3 h, but all animals died after an 8 h exposure to 4.25 mg/l; clinical signs of toxicity included acute bloody urine, apathy, scrubby fur, intermittent respiration, mucous membrane irritation, and slight anemia; at necropsy, bloody nether regions, hematuria, liver anemia, and blood clotting the bladder were observed in the 8 h exposure group and chronic bronchitis was observed in the 3 h exposure group	4
none	groups of 3 male and 3 female Sprague-Dawley rats	2.25 mg/l for 3 h or 4.26 mg/lg for 7 h	Acute inhalation toxicity study; rats were exposed to test material via whole body exposure for 3 or 7 h followed by a 14-d observation period	LC_{50} > 4.26 mg/ml; no deaths were observed after 3 h exposure, 2/6 animals died after 7-h exposure; clinical signs of toxicity included eyelid closure, slight salivation, accelerated respiration, hemorrhagic urine, apathy, crouch position, unstable gait, scrubby and contaminated fur, and anemic ears; at necropsy, animals that died during the observation period had acute dilation of the right side of the heart and sallow left ventricle, moderate acute exhalation of the lungs, clay-gray toned liver, bloody ulcerations of the glandular stomach, and hematinic intestinal contents	4
not reported	groups of 6 male and female Dunkin-Hartley guinea pigs	not fully described, at minimum 2.25 mg/l	Acute inhalation toxicity study in accordance with OECD TG 433; guinea pigs were exposed to test material via snout-only, single exposure for 4 h followed by a 14-d observation period	LC_{50} > 2.25 mg/l; no deaths occurred attributable to exposure to test material; no clinical signs of toxicity or adverse gross pathology observed	19
air	groups of 4 male albino rabbits	~2.02 mg/l (400 - 411 ppm)	Acute inhalation toxicity study; rabbits were exposed to test material via whole body exposure for 7 h followed by a 7-d observation period; study was repeated with fresh animals 2 more times	LC_{50} < 2.0 mg/l; mortality rate was 75%; clinical signs included poor coordination of extremities and loss of equilibrium; at necropsy, rabbits that died during the study had reddish ocular and nasal discharges, yellow discoloration of the sclera, congested kidneys, hematuria, hemorrhagic ulcers in the gastric mucosa, mottled or yellow discoloration of the liver, slight congestion of the lungs and nasal turbinates	4

Table 5. Repeated-dose toxicity studies on Butoxyethanol

Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
			ORAL			
water	male CR, COBS, CD, BR albino rats	6 wk	0, 222, 443, or 885 mg/kg/d	Repeated dose oral toxicity study performed in accordance with OECD TG 407; rats received test material 5 d/wk via gavage	NOAEL $\leq$ 222 mg/kg/d; significant toxicity was seen in the 885 mg/kg/d dose group, most significant adverse effects were changes to the red blood cells (reduced count, decreased hemoglobin and increased mean corpuscular hemoglobin) from the 222 mg/kg/d dose group upwards; other effects in the low dose group were equivocal changes in the kidney (proteinaceous casts and hemosiderin) and changes attributed to the dosing method (stomach hyperkeratosis); splenic congestion and stomach hyperkeratosis observed in virtually all animals at all doses, the latter is likely to be at least partly related to the dosing method, so the findings are uncertain in terms of relevance and extramedullary hematopoiesis was evident in the spleens of treated animals; liver anisokaryosis was also seen at 222 mg/kg/d.	4
			INHALATION			
air	male and female mice (20 total, strain not specified),	15 d	2.63 mg/l (537 ppm)	Animals were exposed to test material via whole-body exposure for 6 h/d	NOAEC could not be established; adverse effects aside from death included general anemia, deep blue concrement in urinary bladder, adipose liver, and discolored liver and lung	4
air	male and female rats (10 total, strain not specified) (strain not specified)	15 d	2.63 mg/l (537 ppm)	Animals were exposed to test material via whole-body exposure for 6 h/d	NOAEC could not be established; adverse effects aside from death included hematuria, anemic and adipose liver, and edematous soft tissue of the lung.	4
air	32 Carworth E strain male rats	90 d	0 or 50 ppm	Rats were exposed to test material via whole-body exposure for 7 h/d, 5 d/wk	NOAEC < 50 ppm; adverse effects included an increase in erythrocyte osmotic fragility and an increase in relative kidney-to-bw ratio, but not the absolute weight ratio	4
air	groups of 8 male guinea pigs, strain not specified	~2 wk	~2.02 mg/l (400 - 411 ppm)	Guinea pigs were exposed to test material via whole body exposure for 7 h followed by a 7-d observation period; 7-h was then repeated, and then for half the animals, the exposure was repeated for 5 consecutive days	LC_{50} > 2.0 mg/l; no adverse effects observed	5
air	male guinea pigs (10 total, strain not specified)	15 d	2.63 mg/l (537 ppm)	Animals were exposed to test material via whole-body exposure for 6 h/d	NOAEC was determined to be 537 ppm	4
air	1 male and 1 female Himalayan rabbit	15 d	2.63 mg/l (537 ppm)	Animals were exposed to test material via whole-body exposure for 6 h/d	NOAEC could not be established; adverse effects aside from death included lung edema, anemic liver, and deep blue-purple kidneys.	4
air	1 male and 1 female cat (strain not specified)	15 d	2.63 mg/l (537 ppm)	Animals were exposed to test material via whole-body exposure for 6 h/d	NOAEC could not be established; adverse effects aside from death included liver adiposis	4
air	2 male Beagle dogs	~ 2 wk	~2.02 mg/l (400 - 411 ppm)	Dogs were exposed to test material via whole-body exposure for 7 h followed by a 7-d observation period; 7 h exposure was then repeated, and for half the animals, the exposure was repeated for 5 consecutive days	LC_{50} > 2.0 mg/l; salivation occurred, no other adverse effects observed	5

Table 6. Genotoxicity studies on Butoxyethanol

Vehicle	Concentration/Dose	Test System	Protocol	Results	Reference
			IN VITRO		
DMSO	0.5 - 20 mM	Syrian hamster embryo (SHE) cells	SHE cell transformation study; cells were treated with test material for 7 d	Butoxyethanol did not induce cellular transformation.	20
not reported	1, 5 or 10 mM	SVEC4-10 mouse endothelial cells	Comet assay; Butoxyethanol and its metabolites, 2-butoxyacetaldehyde (0.1 - 1.0 mM) and 2-butoxyacetic acid (1 - 10 mM) were tested; exposure duration was 2, 4, or 24 h	No increase in DNA damage was observed following Butoxyethanol exposure	21
			IN VIVO		
phosphate-buffered saline	0,17.19, 34.38, 68.78, 137.5, 550, or 1100 mg/kg	groups of 5 male B6C3F1 mice	Micronucleus induction test; mice injected intraperitoneally 3 times at 24 h intervals	Butoxyethanol did not cause an increase in polychromatic erythrocytes; a statistically significant increase in the number of micronucleated polychromatic erythrocytes observed in the 138 mg/mg dose group when compared to control but not observed in any other dose group; all mice died in the 1100 mg/kg dose group	4
not reported	0, 10, 35, 100, 250, or 450 mg/kg	groups of 6 male Wistan-Han IGS rats	*Pig-a* assay; rats received test material via gavage using both single administration and 28-d treatment regimens; mutant frequencies were assessed on days 15 and ~30 for both treatment protocols and also on days 43 and 57 for the 28-d protocol	Not mutagenic; in single dose, a statistically significant increase in the percentage of reticulocytes on day 15 at doses of 100 - 450 mg/kg was observed, but there was no effect on either mutant reticulocytes or erythrocyte frequences at any doses test. In 28-d dosing, a statistically significant increase in percentage of reticulocytes on day 15 and 29 at doses of 100 - 450 mg/kg was observed; on day 43 there was slight but statistically significant decreasing trend in percentage of reticulocytes compared with controls at 250 and 450 mg/kg.; on day 57, reticulocyte values were comparable with controls; no increase in mutant reticulocyte or erythrocyte frequency was observed at any doses on days 15, 29, or 43 or on mutant erythrocyte frequency on day 57	22

Table 7. Ocular irritation studies on Butoxyethanol

Vehicle	Concentration/Dose	Test Population	Protocol	Results	Reference
			IN VITRO/EX VIVO		
doubly distilled water	undiluted and in 10% solution	3 eggs from White Leghorn hens	HET-CAM assay; observed for 3.5 min after application	Irritating; with undiluted test material, slight hemorrhagia was observed in all eggs after 8 - 15 sec, moderate intra- and extravascular coagulation in all eggs after 24 - 47 sec; with 10% solution, slight hemorrhagia in all eggs after 26 - 36 sec, moderate intra- and extravascular coagulation observed in all eggs after 40 51 sec	4
none	undiluted	enucleated eyes from New Zealand White rabbits	Corneal swelling test; treatment duration was 5 h	Irritating to eyes; corneal swelling of over 100% with no sign of recovery observed	4
			ANIMAL		
none	undiluted; 0.1 ml applied	3 New Zealand White rabbits, sex not specified	Ocular irritation study performed in accordance with OECD TG 405; eyes were instilled with test material and washed 24 h after treatment, prior to 24-h reading; observations made at 1, 24, 48, and 72 h and 7, 14, and 21 d	Irritating to eyes; 1 rabbit had slight corneal opacity that was reversible in 21 d; signs of a slight iris injury observed that was reversible in 7 d; a medium to severe irritation of the conjunctivae also observed and was reversible in 21 d	4,5

Table 7. Ocular irritation studies on Butoxyethanol

Vehicle	Concentration/Dose	Test Population	Protocol	Results	Reference
none	undiluted; 0.1 ml applied	6 albino rabbits, sex not specified	Ocular irritation study; eyes instilled with test material and were not rinsed; observations made at 24, 48, and 72 h and 7 d	Irritating to eyes; mild effects to the cornea and severe effects to the conjunctivae were observed in all animals at all time points; mild iritis was observed in the majority of the animals at most time points and was not reversed after the 72-h observation period	4,5
none	undiluted; 0.1 ml applied	6 New Zealand White rabbits, sex not specified	Ocular irritation study performed in accordance with OECD TG 405; eyes instilled with test material and were not rinsed; observations made at 24, 48, and 72 h	Irritating to eyes; negligible iritis and corneal effects observed; very mild chemosis and moderate to significant redness/conjunctivitis observed, which persisted until the end of observation but showed signs of recovery	4,5
none	undiluted; 0.1 ml applied	3 New Zealand White rabbits, sex not specified	Ocular irritation study performed in accordance with OECD TG 405; not specified if eyes were rinsed after instillation of the test material; observations made at 24, 48, and 72 h	Irritating to eyes; conjunctival effects were persistent, lasting until within 21 d in 2 animals; corneal and iridial effects were fully reversible within 14 d	4
none	undiluted; “one drop” applied	2 Vienna White rabbits, sex not specified	Ocular irritation study; one drop of test material applied to conjunctival sac of the right eye, left eye received physiological solution of sodium chloride and served as control; animal observed several times on treatment day and 14 d afterward; not reported if eyes were rinsed	Irritating to eyes; conjunctival and corneal effects were marked; corneal effects disappeared within 8 d	4

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Final Report on the Safety Assessment of Butoxyethanol[1]

Abstract: Butoxyethanol is an ether alcohol used as a solvent in hair and nail products at concentrations up to 10%. This ingredient is absorbed through the skin, metabolized to butoxyacetic acid, and excreted in urine. Acute inhalation toxicity was related to concentration and duration of exposure; pathological changes occur in the kidneys, liver, and lungs. Butoxyethanol was only slightly toxic in an acute oral study in rats and in a dermal study in rabbits. Butoxyethanol was nephrotoxic in an intravenous study in rats, but not when administered intraperitoneally. No evidence of genotoxicity was seen in a battery of tests with metabolic activation, but positive and negative effects were seen in the absence of metabolic activation. A dermal study of a cosmetic product containing 10% Butoxyethanol was not carcinogenic in rats, whereas a rust-preventive product containing 2.5% Butoxyethanol was carcinogenic (90.9% of the rust preventive was a petroleum distillate). There is some evidence for reproductive and developmental toxicity in oral and inhalation studies involving rats, rabbits, and mice, but no such effects in dermal studies in rats. Clinical tests and reports from occupational exposures indicate Butoxyethanol to be an irritant when inhaled. Butoxyethanol was not a sensitizer or photosensitizer in clinical tests. Undiluted Butoxyethanol is recognized to be a severe ocular irritant, but aqueous concentrations of 15 and 5% produced only moderate and no corneal injury, respectively. In consideration of these data, the Cosmetic Ingredient Review Expert Panel concluded that this ingredient may be used safely in hair and nail cosmetic products at concentrations up to 10%. **Key Words:** Butoxyethanol—Dermal—Toxicity—Carcinogenicity.

CHEMISTRY

Butoxyethanol (CAS no. 111-76-2) is the ether alcohol that conforms to the formula shown in Fig. 1 (Wenninger and McEwen, 1995*a*). It is a transparent liquid that is soluble in water and in most organic solvents (Budvari, 1989). Water accounts for not more than 0.20% of its composition (Nikitakis and McEwen, 1990). The purity of technical-grade Butoxyethanol ranges from 98 to 99.5% (Nelson et al., 1984). Other names for this chemical are 2-Butoxyethanol, Butyl Cellosolve, Ethylene Glycol Monobutyl Ether, Monobutyl Ethylene Glycol Ether, Butyl Oxitol, and Ethanol, 2-Butoxy (Wenninger and McEwen, 1995*a*). The chemical and physical properties of Butoxyethanol are summarized in Table 1.

Methods of Production

Butoxyethanol is usually prepared by the reaction of ethylene oxide with butyl alcohol and may also be prepared by the direct alkylation of ethylene glycol with an agent such

[1] Reviewed by the Cosmetic Ingredient Review Expert Panel.

Address correspondence and reprint requests to Dr. F. A. Andersen at Cosmetic Ingredient Review, 1101 17th St. N.W., Suite 310, Washington, D.C. 20036, U.S.A.

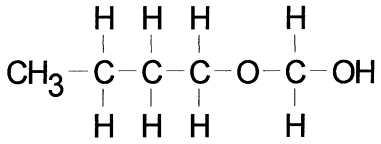

FIG. 1. Chemical formula for Butoxyethanol.

as dibutyl sulfate (Rowe and Wolf, 1982). Other methods for the production of Butoxyethanol are the reaction of butyl alcohol with ethylene carbonate or 2-chloroethanol and also via the reaction of ethylene glycol with butyl bromide (Budavari, 1989).

Analytical Methods

Butoxyethanol has been analyzed via the following methods: infrared spectroscopy (Nikitakis and McEwen, 1990; Krasavage, 1986), gas chromatography (Grant et al., 1985; Krasavage, 1986), gas chromatography employing flame ionization detection (Dugard et al., 1984), and HPLC (Ghanayem et al., 1990*a*).

Maximum ultraviolet absorbance of Butoxyethanol (1.0% vol/vol in methanol) was observed at 229 nm. A peak was also observed at 214 nm (Midwest Research Institute, 1984).

Impurities

Commercial samples of glycol ethers invariably contain small quantities of peroxides (McGregor, 1984).

The results of analyses of Butoxyethanol obtained from Union Carbide Co. are as follows: acidity (0.001 mEq/g), carbonyl (210–276 ppm), peroxide (66–169 ppm), dioxane (0.35 ppm), ethylene oxide (0.015 ppm), ethylene glycol (0.32%), butanol (420–450

TABLE 1. *Properties of Butoxyethanol*

Property	Description	Ref.
Molecular weight	118.18	Lide (1993)
Form	Clear, thin liquid	Nikitakis and McEwen (1990)
Odor	Mild	Mackison et al. (1978)
Solubility	Miscible with water, methanol, and ether in all proportions	Nikitakis and McEwen (1990)
Specific gravity	0.890–0.906	Nikitakis and McEwen (1990)
Refractive index	1.4156–1.4204	Nikitakis and McEwen (1990)
Boiling point (°C)	171–172	Budavari (1989)
Freezing point (°C)	<–40	Krasavage (1986)
	–77	NIOSH (1990)
Vapor pressure (mm Hg)	300 at 140°C	Sax (1979)
	0.88 at 25°C	NIOSH (1990)
Flash point (closed cup; °C)	60	Budavari (1989)
Autoignition temperature (°C)	238	Rowe and Wolf (1982)
Flammability limits (vol. % in air)	1.10–12.7	NIOSH (1990)
Evaporation rate (butyl acetate = 1)	0.1	NIOSH (1990)

ppm), butyraldehyde (210–330 ppm), diethylene glycol (100 ppm), butyl carbitol (<100 ppm), and heavies (0.1%) (Bushy Run Research Center, 1981*a*).

USE

Purpose in Cosmetics

Butoxyethanol is used as a solvent and viscosity-decreasing agent in hair dyes and colors and in tonics, dressings, and other hair-grooming aids (Wenninger and McEwen, 1995*b*).

Scope and Extent of Use in Cosmetics

The product formulation data submitted to the Food and Drug Administration (FDA) in 1994 indicate that Butoxyethanol is used in a total of 121 cosmetic products (see Table 2). Hair dyes and colors containing Butoxyethanol may be marketed with patch test instructions and a caution statement.

Concentration-of-use values are no longer reported to FDA by the cosmetics industry (*Federal Register,* 1992). However, product formulation data submitted to FDA in 1989 indicated that Butoxyethanol was used in 111 cosmetic products at the following concentrations: >0.1–1.0% (1 product), >1.0–5.0% (28 products), and >5.0–10.0% (82 products) (FDA, 1989).

International Use

Butoxyethanol is included in the list of cosmetic ingredients that have been approved for use in cosmetic formulations marketed in Japan (Nikko Chemicals Co. Ltd., 1992). This ingredient is not included in the list of ingredients prohibited from use in cosmetic products marketed in the European Economic Community (1993).

Surfaces to Which Applied

Cosmetic products containing Butoxyethanol are applied to the hair and may come in contact with the skin and ocular and nasal mucosae.

TABLE 2. *Product formulation data on Butoxyethanol (FDA, 1994)*

Product category	Total no. of formulations in category	Total no. containing Butoxyethanol
Hair dyes and colors (all types requiring caution statement and patch test)	995	117
Hair bleaches	115	2
Tonics, dressings, and other hair-grooming aids	563	1
Nail polish and enamel	142	1
1994 totals	—	121

Frequency and Duration of Application

Product formulations containing Butoxyethanol may be used as often as daily and monthly. Many of the products may be expected to remain in contact with body surfaces as briefly as a few minutes and as long as a few months. Each product has the potential for being applied many times over a period of several years.

Noncosmetic Use

Butoxyethanol is used as an industrial solvent for resins and varnishes, in hydraulic fluids, and in the formulation of floor polishes, floor waxes, and cleaning compositions for leather, upholstery, and glass (Rambourg-Schepens et al., 1988). Food-related uses of Butoxyethanol described in the Code of Federal Regulations (CFR) are as follows: component of adhesives (21 CFR 175.105), defoaming agent used in the manufacture of paper and paperboard (21 CFR 173.315), solvent in polysulfide polymer/polyepoxy resins for contact with dry food (21 CFR 176.210), use in sanitizing solutions (21 CFR 177.1650), and use in flume water (concentration not to exceed 1 ppm) for washing sugar beets prior to slicing (21 CFR 178.1010).

BIOLOGICAL PROPERTIES

Effect on Skeletal Muscle Relaxation

In vesicles of sarcoplasmic reticulum prepared from rabbit fast skeletal muscle and crayfish tail muscle, Butoxyethanol (0.8%) decreased the maximum Ca^{2+} uptake from 162 nmol Ca^{2+}/mg protein (control) to 152 nmol Ca^{2+}/mg protein. Butoxyethanol (0.8%) also increased the subsequent Ca^{2+} efflux rate to three times that of the control rate. The Ca^{2+} uptake rate by vesicles of sarcoplasmic reticulum was almost completely inhibited by 2% Butoxyethanol. Butoxyethanol did not significantly inhibit the activity of basal ATPase, but markedly increased Ca^{2+}-dependent ATPase activity. In the isolated phrenic nerve/diaphragm preparation from the rat, Butoxyethanol (1.0%) markedly increased muscle tone; muscle contractions were not produced because of the tetanus induced by 2% Butoxyethanol (Mayahara et al., 1982).

Inhibition of Metabolic Cooperation

Chinese hamster V79 cells that display a specific form of cell-to-cell communication called metabolic cooperation were cultured with Butoxyethanol (added to culture medium). The phenomenon of metabolic cooperation between V79 cells depends on cell-to-cell contact and the resulting formation of permeable gap junctions. In the metabolic cooperation assay, mutant V79 cells that lack the enzyme hypoxanthineguanine phosphoribosyl-transferase (HGPRT) are cultured with wild-type cells that possess the enzyme. The enzyme-deficient cells (HGPRT–) cannot phosphoribosylate 6-thioguanine (6-TG) or 8-azaguanine. The wild-type population (HGPRT+) has the HGPRT enzyme and phosphoribosylates 6-TG to a toxic metabolite that kills the cells. When HGPRT– (6-TGr) cells are cocultured with HGPRT+ (6-TGs) cells, the toxic metabolite is transferred from the 6-TGs to the 6-TGr cells and either kills some or all of the HGPRT– mutants. Four hours after the two cell types were seeded in culture dishes, Butoxyethanol was added (10

plates/concentration tested), followed by 6-TG. Butoxyethanol significantly increased the recovery of 6-TGr cells in coculture with 6-TG cells. At concentrations of 0.005 and 0.035 *M* Butoxyethanol, there was ~5 and 45% recovery of 6-TGr cells, respectively. Cytotoxic effects became significant at concentrations of ≥0.031 *M*. Cytotoxicity was defined as interference with the colony-forming ability of the V79 cells (Welsch and Stedman, 1984). Butoxyethanol-induced inhibition of metabolic cooperation in Chinese hamster V79 cells has also been demonstrated in another study [Loch-Caruso et al., 1984; National Institute for Occupational Safety and Health (NIOSH), 1990].

Pharmacological Activity

Butoxyethanol (3.0% in saline) had the following pharmacological actions at the doses indicated: antispasmodic in dogs (0.03–0.66 g/kg i.v. dose), sympatholytic in dogs (0.2–2.0 g/kg i.v. dose), parasympathomimetic in dogs (0.2–2.0 g/kg i.v. dose), vasodilator in dogs (0.2–2.0 g/kg i.v. dose), decrease in respiratory rate and volume in dogs (0.2–2.0 g/kg i.v. dose), diuretic in dogs (0.2–2.0 g/kg i.v. dose), CNS depressant in dogs (0.2–2.0 g/kg i.v. dose), cardiotoxic in dogs (0.2–2.0 g/kg i.v. dose), diuretic in guinea pigs (1.0 g/kg i.p. dose), diuretic in rats (0.2–0.8 g/kg i.p. dose), CNS depressant in rats (0.1–1.0 g/kg i.p. dose), and sedative and/or narcotic in rats (0.2–0.8 g/kg i.p. dose) (Mellon Institute of Industrial Research, 1954).

Butoxyethanol (3.0% in saline) did not have any of the following pharmacological activities at the doses indicated: analgesic in rats (0.1–0.5 g/kg i.p. dose), anti-cholinesterase in rats (0.2–1.0 g/kg i.p. dose), parasympatholytic in dogs (0.2–2.0 g/kg i.v. dose), sympathomimetic in dogs (0.2–2.0 g/kg i.v. dose), ganglionic blocking in dogs (0.2–2.0 g/kg i.v. dose), CNS stimulant in dogs (0.2–2.0 g/kg i.v. dose), antihistaminic in guinea pigs (1.0 g/kg i.p. dose), curare-like activity in mice (0.01–0.4 g/kg i.p. dose), and CNS stimulant in rats (0.01–1.0 g/kg i.p. dose) (Mellon Institute of Industrial Research, 1954).

Behavioral Effects

The effect of repeated inhalation of Butoxyethanol vapor on animal behavior was evaluated using female Carworth rats (weight 160 ± 20 g). Four groups of rats (8–10 per group) were exposed to 50, 100, 200, and 400 ppm Butoxyethanol, respectively, 5 days/week (4 h/day) for 10 days. Conditioned avoidance–escape behavior was studied according to a modification of the pole climb method of Cook and Weidley (1957). Behavioral criteria included the abolishment or significant deferment of avoidance response (conditioned or buzzer response) and escape response (unconditioned or buzzer-shock response). The animals were trained to respond to both stimuli within 2 s. Deferment of avoidance or escape responses of >6 s was considered significant. Repeated inhalation of all concentrations of Butoxyethanol had no effect on growth or behavioral performance. Transient hematuria was observed in groups exposed to 200 and 400 ppm Butoxyethanol (Goldberg et al., 1964).

Percutaneous Absorption

The results of eight percutaneous absorption studies are summarized in Table 3. Overall the results suggest that absorption increases as a function of time and that a semiocclusive covering increases absorption.

TABLE 3. *Percutaneous absorption of Butoxyethanol*

Animals	Dose	Results	Ref.
6 rats (172–290 g)	200 mg/kg applied dermally	20–23% of applied radioactivity detected in urine within 48 h after application	Bartnik et al. (1987)
24 rats (160–192 g)	200 mg/kg applied dermally	Major portion of ingredient metabolized to butoxyacetic acid	Bartnik et al. (1987)
9 guinea pigs (517–760 g)	91.5 μmol/kg i.v. (avg. of 10 experiments)	Avg. blood clearance value of 128 ml/min/kg body wt	Johanson and Fernstrom (1986)
9 guinea pigs (517–760 g)	Undiluted ingredient (avg. of 10 experiments) applied dermally	Avg. skin uptake rate of 0.25 μmol/min/cm^2	Johanson and Fernstrom (1986)
14 guinea pigs (497–930 g)	Undiluted ingredient and 5, 10, 20, 40, and 80% vol/vol dilutions applied dermally	Avg. relative skin uptake rate approximately the same after application of 5, 10, 20, and 100% and twice as great after application of 40 and 80%	Johanson and Fernstrom (1988)
Dorsal skin from rats and guinea pigs	^{14}C-ingredient: 5,409 μg/cm^2	Under semiocclusive conditions, 66.7% (rat) and 11.2% (guinea pigs) of applied dose absorbed after 6 h; under nonocclusive conditions, 5.6% of applied dose absorbed after 1 h	Bartnik et al. (1987)
Dorsal skin from rats and guinea pigs; human skin obtained at autopsy	^{14}C-ingredient, 10% concentration: 100 μg/cm^2 applied, semiocclusive and nonocclusive conditions	For all skin samples, absorption was greater under semiocclusive conditions	Bartnik et al. (1987)
Abdominal skin obtained from humans	Undiluted ingredient	Rate of absorption = 0.198 mg/cm^2	Dugard et al. (1984)

The percutaneous absorption of Butoxyethanol was evaluated using six male (269–290 g) and six female (172–196 g) rats. Prior to administration, [^{14}C]Butoxyethanol (^{14}C-carbons 1 and 2) was diluted with Butoxyethanol and distilled water to final specific activities of 130.3 μCi/ml (applied to male rats) and 90.8 μCi/ml (applied to female rats). After each animal was anesthetized, the test substance was applied (dose 200 mg/kg) to shaved skin of the back. Urine was collected at 0- to 8-, 8- to 24-, and 24- to 48-h intervals. At the end of the experiment, the animals were killed with ether. Within 48 h after application, ~20–23% of the applied radioactivity was detected in the urine; no notable differences in excretion between males and females were found. At the end of the experiment, 4.3 and 8.2% of the applied radioactivity was detected on the skins of males and females, respectively (Bartnik et al., 1987).

In another experiment, the percutaneous absorption of [^{14}C]Butoxyethanol (^{14}C-carbons 1 and 2) was evaluated using 24 female Wistar rats (weights between 160 and 192 g). Both the preparation of the test solution and its cutaneous application were carried out in a manner similar to that stated in the preceding paragraph. Some animals were killed with ether at 0.5, 1, 2, 4, 6, 8, 16, and 24 h postapplication. Blood was collected via heart puncture and centrifuged. Radioactivity in either the blood or the plasma was determined via scintillation spectrometry. To determine the amount of butoxyacetic acid (metabolite of Butoxyethanol) that was not bound to protein, pooled plasma (groups of three animals) was centrifuged and the ultrafiltrate was analyzed via HPLC. Measurements indicated that the greatest counts of radioactivity ([^{14}C]Butoxyethanol) in both blood and plasma occurred at 2 h postapplication. The same observation was made following measurements of butoxyacetic acid in plasma ultrafiltrate. Based upon concentrations of butoxyacetic acid in the plasma ultrafiltrate, it appeared that the major portion of Butoxyethanol absorbed was metabolized to butoxyacetic acid (Bartnik et al., 1987).

Both the rate of percutaneous absorption and the elimination kinetics of Butoxyethanol were evaluated in 10 individual experiments using nine female outbred guinea pigs (weights 517–760 g) per experiment. The animals were kept under pentobarbital anesthesia throughout the experiment. The test substance (10.7 mg/ml Butoxyethanol in 0.3 *M* sodium chloride) was administered intravenously (dose range 42–94 μmol/kg body wt) to each animal, and blood samples were obtained at 5, 10, 20, 30, 40, 50, 60, 75, 90, 105, and 120 min postadministration. One or two glass rings (exposure area 3.14 cm^2) were then glued to skin on the back of each animal that had been clipped free of hair. At 150 min postadministration, each ring was filled with undiluted Butoxyethanol (1 ml) and sealed with a coverglass. Arterial blood samples were obtained at the time intervals previously mentioned. Following intravenous administration, the concentration of Butoxyethanol in the blood declined rapidly. The average blood clearance value (10 experiments, average dose 91.5 μmol/kg) for Butoxyethanol was 128 ml/min/kg body wt. Following epicutaneous administration, the concentration of Butoxyethanol in the blood increased rapidly and reached a plateau during the latter half of the 2-h exposure period. The average uptake rate for skin (10 experiments) was 0.25 (range 0.05–0.46) μmol/min/cm^2 (Johanson and Fernstrom, 1986).

Using essentially the same procedure described above, Johanson and Fernstrom (1988) evaluated the percutaneous absorption of Butoxyethanol solutions (5, 10, 20, 40, and 80% vol/vol in water) and undiluted Butoxyethanol using female outbred guinea pigs (weights 497–930 g). The animals remained under pentobarbital anesthesia throughout the 6-h experiment, after which all animals were killed. Blood concentrations of both diluted and undiluted Butoxyethanol increased as a function of time. During the last hour of exposure to 100% Butoxyethanol, the average concentration in the blood was 4.6 μmol/L (range 1.8–14.2 μmol/L) and the cutaneous uptake rate was 132 nmol/min/cm^2 (range 64–414 nmol/min/cm^2). The average relative uptake rate of Butoxyethanol was approximately the same following the administration of 5, 10, 20, and 100% Butoxyethanol. However, the uptake rate was twice as great after the administration of 40 and 80% Butoxyethanol.

Bartnik et al. (1987) also determined the cutaneous absorption of [^{14}C]Butoxyethanol (in vitro). They used the method of Zesch and Schaefer (1973) to study absorption by fresh dorsal skin from hairless rats, frozen dorsal guinea pig skin, and human skin from the flexor aspect of the arm (obtained at autopsy). Connective and fatty tissues were removed

from each skin sample. The penetration chamber consisted of a donor chamber and a water-jacketed receptor chamber equipped with a magnetic stirrer for circulation and filled with physiological saline. Excised skin was positioned such that the visceral side was bathed by the fluid in the receptor chamber. Each solution of [^{14}C]Butoxyethanol (specific activity 58.6–85.6 μCi/ml) was placed on the outer surface of the skin, and fluid in the cell was sampled at different intervals and assayed for radioactivity. Cutaneous absorption was measured either without the upper penetration chamber (nonocclusive) or with the two penetration chambers connected (semiocclusive). The time course of absorption (percent of applied radioactivity) by hairless rat skin under semiocclusive conditions was as follows: 19.4% (after 1 h), 66.7% (after 6 h), and 94.3% (after 16 h). For comparison purposes between species, the authors noted that 11.2% of [^{14}C]Butoxyethanol applied under semiocclusive conditions to guinea pig skin was absorbed after 6 h. Comparing experimental conditions, the authors noted that hairless rat skin, under nonocclusive conditions, absorbed 5.6% of the [^{14}C]Butoxyethanol applied after 1 h. Absorption of diluted [^{14}C]Butoxyethanol (10% in water, 100-μg/cm^2 dose) applied to skin under semiocclusive conditions after 1 h was as follows: 43.3% (hairless rat skin), 17.7% (guinea pig skin), and 17.3% (human skin). Under nonocclusive conditions, comparative values were 11% (hairless rat skin), 8.6% (guinea pig skin), and 6.9% (human skin). The authors concluded that absorption increases with time of exposure and that absorption is greater under semiocclusive conditions.

In another study, the absorption of undiluted Butoxyethanol across isolated human abdominal epidermis in vitro was evaluated. Epidermal membranes were placed in glass diffusion cells. After an initial determination of permeability to tritiated water was made, Butoxyethanol was applied to the outer epidermal membrane surface. The appearance of Butoxyethanol in an aqueous ''receptor'' phase bathing the underside of the epidermis was quantified via a gas chromatographic technique employing flame ionization detection. A final determination of tritiated water permeability was compared with initial values, such that any irreversible alterations in epidermal barrier function induced by Butoxyethanol were detected. Butoxyethanol had a slow rate of absorption (0.198 mg/cm^2/h) (Dugard et al., 1984). Although not listed in Table 3, similar results were reported for Butoxyethanol in an earlier in vitro percutaneous absorption study (similar procedure) involving human abdominal epidermal membranes (Imperial Chemical Industries, 1982).

Metabolism, Distribution, and Excretion

The results of eight studies on the metabolism, distribution, and excretion of Butoxyethanol are shown in Table 4. These data suggest that butoxyacetic acid is the major metabolite, that metabolism is mainly by alcohol dehydrogenase in the liver, and that metabolism is required for toxicity; most excretion is in the urine.

The metabolism and excretion of Butoxyethanol were evaluated using male albino rats (weights ~250 g). The rats were exposed to Butoxyethanol (2,000 mg/m^3 air) for 1 h in a closed glass box. Urine was collected 20 h before and after exposure. After exposure and transmethylation, two peaks were observed on gas chromatograms of the acidic fraction of the urine. One of the peaks was reportedly an artifact, but the mass spectrum of the other peak was consistent with that of the trimethylsilyl derivative of *n*-butoxyacetic acid (Jonsson and Steen, 1977).

TABLE 4. *Metabolism, distribution, and excretion of Butoxyethanol*

Animals	Dose	Results	Ref.
Rats (250 g)	2,000 mg/m^3 air	Trimethylsilyl derivative of *n*-butoxyacetic acid detected in urine	Jonsson and Steen (1977)
Rats (11–12 wks old)	4.3, 49, and 438 ppm in air	Majority of ^{14}C eliminated in urine; butoxyacetic acid was major urinary metabolite; quantity of ^{14}C metabolized proportional to exposure concentration	Sabourin et al. (1992)
Rats (9–13 wks old)	^{14}C-ingredient: 125 and 500 mg/kg (oral doses)	At 48 h postadministration, urine was primary route of excretion; butoxyacetic acid was major urinary metabolite; nonglandular stomach had highest tissue counts of radioactivity	Ghanayem et al. (1987*c*)
Rats (4–5 and 9–13 wks old)	^{14}C-ingredient: 500 mg/kg (oral dose)	Compared with older rats, greater portion of dose was excreted as CO_2 and in urine in young rats	Ghanayem et al. (1987*a*)
Rats (11–12 wks old)	^{14}C-ingredient: 290, 860, and 2,590 ppm in water	Most of ^{14}C either excreted in urine (50–60% of dose) or exhaled as CO_2 (8–10%); butoxyacetic acid was major urinary metabolite	Medinsky et al. (1990)
Rats (210–300 and 425–480 g)	^{14}C-ingredient: 31.25, 62.5, and 125 mg/kg (i.v. dose)	Mean concentration–time profiles of total radioactivity in whole blood and plasma directly proportional to dose	Ghanayem et al. (1990*a*)
Rats (200–214 g)	^{14}C-ingredient: 118 mg/kg (s.c. dose)	Within 72 h postinjection, 78% of administered radioactivity detected in urine; <1% in feces; greatest specific activity detected in thymus at end of experiment	Bartnik et al. (1987)
Perfused rat livers	Concentrations of 0.057–2.7 m*M*	Extraction ratio decreased with increasing concentrations of Butoxyethanol; assuming venous equilibrium, estimate of apparent maximum elimination rate ranged from 0.59 to 1.3 μmol/min/g	Johanson et al. (1986*b*)

The metabolism and distribution of Butoxyethanol were evaluated using three groups of male F344/N rats (11–12 weeks old). The rats were to have been exposed to 5, 50, or 450 ppm [^{14}C]Butoxyethanol for 6 h using plastic, nose-only exposure tubes. The actual exposure concentrations were 438 ± 12, 49 ± 2, and 4.3 ± 2.3 ppm (n = 72 at each concentration). In the first group of rats (n = 5), fractional uptake of Butoxyethanol and body burden were determined at the end of exposure. The body burden is a measure of total ^{14}C in rats at the end of exposure. The excretion of Butoxyethanol equivalents and major metabolites was determined in the second group of rats (n = 4). In the third group of rats (n = 30), blood ^{14}C and metabolite concentrations were determined after exposure to 5 ppm [^{14}C]Butoxyethanol only. The amount of Butoxyethanol inhaled was proportional to the exposure concentration, up to 49 ppm. The difference between the body burden and the total excreta and residual collected was not statistically significant, indicating complete recovery of ^{14}C during the collection of excreta. The quantity of [^{14}C]Butoxyethanol equivalents metabolized was measured as total ^{14}C in the urine, feces, and exhaled $^{14}CO_2$ and remaining in the carcass at 66 h postexposure. This quantity was the same as the total excreta and residual in the body; the parent compound contributed <6% to the total excreta. The ''total excreta and residual in body'' is defined as total ^{14}C recovered in excreta, gas traps, and ^{14}C remaining in the carcass of rats placed in metabolism cages for excreta collection. The quantity of ^{14}C metabolized was proportional to the exposure concentration (Sabourin et al., 1992).

In the same study, hemoglobinuria was noted in four rats in the 450-ppm group that were to have been used for excreta collection. However, because two of the animals died within 24 h, only excretion data from the two survivors were presented. At all exposure concentrations, the majority of ^{14}C was eliminated in the urine, and <7.0% of the parent compound was exhaled. Ten to 20.0% of the [^{14}C]Butoxyethanol equivalents remained in the carcass up to 66 h postexposure. Approximately 7.0% of the excreted ^{14}C was in the form of $^{14}CO_2$, indicating complete metabolism of the ethanol moiety of Butoxyethanol. Butoxyacetic acid was the major urinary metabolite at all exposure concentrations, and smaller amounts of ethylene glycol and butoxyethanol glucuronide were also detected. An analysis of whole-blood samples obtained from three rats exposed to 4.3 ppm [^{14}C]Butoxyethanol indicated that the majority of the Butoxyethanol equivalents were present in the plasma. Butoxyacetic acid was the major plasma metabolite (Sabourin et al., 1992).

The distribution and excretion of [^{14}C]Butoxyethanol and its metabolite were evaluated using male F344 rats (9–13 weeks old). After [^{14}C]Butoxyethanol was diluted with Butoxyethanol, a single 125- or 500-mg/kg dose (50–60 μCi/kg) was administered to each animal via gavage at a dose volume of 5 ml/kg in water. The animals were killed with ether at 48 h postadministration and tissues excised. Regarding the following results, each value (%) reported represents the average of values from three rats. Forty-eight hours after the administration of 125- and 500-mg/kg doses of [^{14}C]Butoxyethanol, ~18 and 10% of the dose was exhaled as $^{14}CO_2$, respectively. Between 2 and 3% of administered [^{14}C]Butoxyethanol was excreted in the feces. The percentage of the 125-mg/kg dose that was excreted in the urine (70%) at 48 h was significantly greater than that for the 500-mg/kg dose (40%). Metabolites of Butoxyethanol detected in the urine were expressed as the percentage of total radioactivity in the urine fraction. Butoxyacetic acid was the only urinary metabolite detected 48 h after administration of the 125-mg/kg dose. The glucuronide conjugates of Butoxyethanol (23.8%) and butoxyacetic acid (75.3%) were de-

tected in the urine 48 h after administration of the 500-mg/kg dose. At 8 h postadministration, the following urinary metabolites of Butoxyethanol (125-mg/kg dose) were detected: butoxyacetic acid (74.4%), glucuronide conjugate of Butoxyethanol (21.3%), and sulfate conjugate of Butoxyethanol (2.7%). Less than 2% Butoxyethanol was also present. Butoxyacetic acid (76.4%) and the glucuronide conjugate of Butoxyethanol (23.0%) were detected in the urine 8 h after the administration of the 500-mg/kg dose; however, Butoxyethanol was not detected. Compared with the 125-mg/kg dose group, tissue concentrations of [^{14}C]Butoxyethanol at 48 h postadministration were significantly greater in specific organs of rats that received 500-mg/kg doses. In 125- and 500-mg/kg dose groups, the greatest concentration of radioactivity was detected in the stomach, followed by the liver and kidneys. After [^{14}C]Butoxyethanol was incubated with whole-blood samples from rats for 1, 2, and 3 h, Butoxyethanol was the only species detected (Ghanayem et al., 1987*a*).

The metabolism and excretion of Butoxyethanol (>99% purity) were evaluated using young (4–5 weeks old) and adult (9–13 weeks old) male F344 rats according to the procedure in the preceding paragraph. [^{14}C]Butoxyethanol (specific activity 6.32 mCi/mmol) was administered in 500-mg/kg doses. There was a significantly higher portion of the administered dose that was eliminated as CO_2 in young rats as compared with that eliminated by older rats. Similarly, a significantly higher portion of the administered dose was excreted in the urine of young rats. In the analysis of urinary metabolites by HPLC, the butoxyacetic acid/Butoxyethanol-glucuronide + Butoxyethanol-sulfate ratio was significantly greater in older rats (Ghanayem et al., 1987*b*).

Butoxyethanol was administered to male F344/N rats (11–12 weeks old) at concentrations of 290, 860, and 2,590 ppm in drinking water over a period of 24 h. Butoxyethanol was administered as 2-butoxy[U-^{14}C]ethanol, and each rat received ~15 μCi of radioactivity. Exhaled air was removed from each cage via a vacuum pump and collected in a series of gas traps. Both urine and feces were collected over a period of 72 h. Most of the ^{14}C was either excreted in the urine or exhaled as CO_2. Of the dose administered, 50–60% was eliminated in the urine as butoxyacetic acid and 8–10% was eliminated as CO_2. The results of HPLC chromatograms of urine samples collected during the 12- to 24-h period after dosing indicated that the majority of the radioactivity had a retention time that was similar to that of butoxyacetic acid, the major Butoxyethanol metabolite. A small but significant amount of the radioactivity cochromatographed with the ethylene glycol standard. Radioactive peaks identified as the glucuronide conjugate of Butoxyethanol and unmetabolized Butoxyethanol were also reported (Medinsky et al., 1990).

The kinetics of [^{14}C]Butoxyethanol metabolism and clearance was evaluated using male Fischer 344 rats that were either 3–4 months old (210–300 g) or 12–13 months old (425–480 g). The animals were divided into groups of three to four animals, and a single bolus dose (31.25, 62.5, or 125.0 mg/kg; dose volume 1 ml/kg) of Butoxyethanol was administered intravenously. [^{14}C]Butoxyethanol was diluted, as needed, with Butoxyethanol such that 45–55 μCi/kg was administered. Blood samples (0.35 ml) were collected at 5, 15, and 30 min and at 1, 2, 4, 6, and 8 h postadministration and then combusted to $^{14}CO_2$; plasma samples did not undergo combustion. Total radioactivity in whole blood and in plasma was calculated as micrograms of Butoxyethanol equivalent per milliliter. In both age groups (3–4 and 12–13 months), the mean concentration–time profiles of total Butoxyethanol-derived radioactivity in whole blood and in plasma were directly propor-

tional to the dose administered. Consistently greater concentrations were attained in older rats. Additionally, the mean concentration of total Butoxyethanol-derived radioactivity in the plasma was consistently greater than that in whole blood in both age groups. As determined by HPLC analysis, Butoxyethanol and its metabolite, butoxyacetic acid, were the only two identifiable chemical species detected in plasma from rats of both age groups. There was no significant effect of dose on the half-life or the volume of distribution of Butoxyethanol. An increase in the maximum plasma concentration of Butoxyethanol and a decrease in systemic clearance of Butoxyethanol were noted in response to an increase in dose. No relationship was found between the ages of the animals tested and either the half-life, volume of distribution, or systemic clearance of Butoxyethanol. However, the maximum plasma concentration of Butoxyethanol was age dependent. Specifically, a significant increase in the maximum plasma concentration of Butoxyethanol was observed in older rats, but not in younger rats. For butoxyacetic acid, a metabolite of Butoxyethanol, a significant increase in its half-life was found only in older rats dosed with 31.25 and 125.0 mg/kg Butoxyethanol, but not 62.5 mg/kg. Both age and dose had significant effects on the maximum plasma concentration and half-life of butoxyacetic acid. These parameters were greater in the older rats (Ghanayem et al., 1990*a*).

The inhibition of Butoxyethanol metabolism by pretreatment of rats with pyrazole or cyanamide caused a significant increase in the half-life of intravenously administered Butoxyethanol and a significant decrease in its systemic clearance. Additionally, cyanamide caused a decrease in the volume of distribution of Butoxyethanol, whereas pyrazole had no effect. Concerning butoxyacetic acid kinetics, pyrazole significantly decreased the maximum plasma concentration and half-life of butoxyacetic acid. Cyanamide increased the half-life of butoxyacetic acid and decreased the maximum concentration in the plasma (Ghanayem et al., 1990*a*). Pyrazole and cyanamide inhibited the metabolism of Butoxyethanol to butoxyacetic acid via the inhibition of alcohol dehydrogenases and aldehyde dehydrogenases, respectively, and both have protected rats against Butoxyethanol-induced hemolytic anemia (Ghanayem et al., 1987*c*). Results of the present study indicated that pyrazole and cyanamide were associated with significant changes in butoxyacetic acid kinetics (Ghanayem et al., 1990*a*). These results are supportive of earlier findings indicating that the metabolism of Butoxyethanol to butoxyacetic acid was required for hematotoxicity (Carpenter et al., 1956).

When rats were treated with probenecid (inhibitor of the renal transport of organic acids) prior to intravenous administration of Butoxyethanol, no effects on the maximum plasma concentration, volume of distribution, or systemic clearance of Butoxyethanol were found. However, the half-life of butoxyacetic acid was significantly increased. These findings support the hypothesis that butoxyacetic acid is eliminated via the renal organic acid transport system and that this pathway may play a critical role in the detoxification of butoxyacetic acid (Ghanayem et al., 1990*a*).

The distribution and excretion of Butoxyethanol were evaluated using three adult male Wistar rats (weights 200–214 g). Prior to administration, [^{14}C]Butoxyethanol was diluted with Butoxyethanol and distilled water to a specific activity of 37.5 μCi/ml. Each animal was injected subcutaneously (scapular region) with a single 118-mg/kg dose (not exceeding 99 μCi/kg) of Butoxyethanol. During the 72-h period after administration, expired air, feces, and urine were collected. At the end of the experiment, the animals were killed via CO_2 asphyxiation and the following organs and tissues removed: liver, kidneys, spleen,

adipose tissue, testes, thymus, sternum (including bone marrow), and blood. Within 72 h after injection, 78% of the administered radioactivity was detected in the urine. Less than 1% of the radioactivity was detected in the feces and 10% was eliminated as $^{14}CO_2$ in the exhaled air. At the end of the experiment, the greatest specific activity was detected in the thymus, followed by the spleen and liver (Bartnik et al., 1987).

The elimination kinetics of Butoxyethanol (six experiments) was evaluated using the isolated perfused rat liver. The liver was isolated, placed in a perfusion chamber, and allowed to equilibrate for 15 min. Approximately 82 m*M* Butoxyethanol in Krebs–Henseleit buffer was infused into the mixing chamber via a syringe pump. Concentrations of Butoxyethanol (0.057–2.7 m*M*) were achieved by varying the pump rate. Each concentration was maintained for 10 min. Two samples from the perfusion medium and four from the perfusate were collected during the last 4 min. Perfusion flow was held constant at ~20 ml/min and the temperature of the liver at 37°C. Elimination of Butoxyethanol from the rat liver was concentration dependent. The extraction ratio decreased with increasing concentrations of Butoxyethanol. The extraction ratio was defined as the quotient between the amount of Butoxyethanol extracted and the amount presented to the liver. Assuming venous equilibrium, estimates of the apparent maximum elimination rate of Butoxyethanol ranged between 0.59 and 1.3 μmol/min/g. In the presence of 17.1 m*M* ethanol, the extraction ratio of Butoxyethanol decreased from 0.44 ± 0.03 (n = 10) to 0.11 ± 0.03 (n = 18). When ethanol was withdrawn, the liver almost returned to its previous elimination capacity in ~10 min. The drastic effect of ethanol on the elimination of Butoxyethanol supports the hypothesis that Butoxyethanol is metabolized mainly via oxidation by alcohol dehydrogenase in the rat liver (Johanson et al., 1986*b*).

TOXICOLOGY

Acute Inhalation Toxicity

Acute inhalation toxicity studies on Butoxyethanol have been conducted using rats, mice, and guinea pigs. The results of these studies are summarized in Table 5. The LC_{50} of Butoxyethanol in rats is in the range of 450–500 ppm.

Short-Term Inhalation Toxicity

The results of several short-term inhalation toxicity studies are shown in Table 6 and described in detail herein. In addition to hematologic effects, liver and kidney weights were increased with these exposures in a wide range of experimental animals.

Fifteen male and 15 female rats (weights not stated) were selected for exposure to 500 ppm Butoxyethanol 5 days/week (7 h/day) for a total of 30 exposures. A total of 13 female rats died either during or shortly after the first exposure. At necropsy, pulmonary congestion and hemorrhage, along with generalized congestion of the other organs, were noted. The remaining two rats died after the second and third exposures, respectively. A total of seven male rats died either during or after the first exposure, and only three survived the 30-day exposure period. Pathological changes were observed in two of the three survivors. One rat had moderate swelling of the convoluted and loop tubules of the kidneys. The other rat had moderate swelling of the liver and the convoluted and loop tubules of the kidneys. Anemia and increased fragility of erythrocytes were also noted

TABLE 5. *Acute inhalation toxicity of Butoxyethanol*

Animals	Dose	Results	Ref.
6 rats (174–336 g)	7-, 3-, and 1-h exposures to 902, 908, and 910 ppm; 1-h exposure to 910 ppm	Deaths: 4 (902 ppm), 1 (908 ppm), 0 (910 ppm)	Shell Research Limited (1982)
3 groups of 12 rats (4–5 wks old)	3 groups: 4-h exposure to 202, 523, and 867 ppm	Deaths: 12 (867 ppm), 5 (523 ppm), 0 (202 ppm)	Dodd et al. (1983)
3 groups of 6 male rats (mean wts 128.4–146.7 g)	3 groups: 4-h exposure to 202, 523, and 867 ppm	LC_{50} = 486 ppm	Bushy Run Research Center (1980*a*)
3 groups of 6 female rats (mean wts 100.6–115.6 g)	3 groups: 4-h exposure to 202, 523, and 867 ppm	LC_{50} = 450 ppm	Bushy Run Research Center (1980*a*)
6 rats (120–130 g)	8-h exposure to 800 ppm	3 deaths at 8 h; no deaths at 4 h	Carpenter et al. (1956)
3 groups of 6 rats (125–150 g)	3 groups: 4 h (500 ppm), 8 h (500 ppm), 4 h (800 ppm)	Deaths: 1 (500 ppm, 4 h)	Mellon Institute of Industrial Research (1952)
2 groups of 6 rats	2 groups: 4 h (500 ppm) and 8 h (500 ppm)	Deaths: 1 (500 ppm, 4 h)	Carpenter et al. (1956)
6 rats	8-h exposure to 475 ppm	No deaths	Mellon Institute of Industrial Research (1952)
13 male rats (380–500 g); 23 female rats	7-h exposure to 375 ppm	Deaths: 11 males, 23 females	Carpenter et al. (1956)
10 rats (275–298 g)	1-h exposure to 200 mg/L	No deaths	MB Research Laboratories (1977)
Mice (no. not stated; 250–330 g)	7- and 32-h exposures to 1,210 ppm	Deaths: 68.75% (7 h), 100% (32 h)	Werner et al. (1943*a*)
Mice (no. not stated)	7- and 32-h exposures to 770 ppm	Deaths: 12.5% (7 h), 81.25% (32 h)	Werner et al. (1943*a*)
16 mice (wts ~20 g)	7-h exposures to various concentrations	Minimum LC_{50} = 700 ppm	Werner et al. (1943*a*)
Guinea pigs (no. not stated)	7- and 9-h exposures to 1,300 ppm	Deaths: 50% (7 h and 9 h)	Tyler (1984)

(Mellon Institute of Industrial Research, 1952). In that the concentration tested in the preceding experiment induced 90% mortality, a total of 15 female rats (weights not stated) were exposed to an average concentration of 314 ppm Butoxyethanol according to the same procedure. All of the rats died within 3 days. Pathological changes in the kidneys, liver, and lungs were comparable with those observed in female rats exposed to 500 ppm Butoxyethanol. Blood in the urine, increased fragility of erythrocytes, and hemoglobin in the serum due to hemolysis of blood drawn by cardiac puncture were also noted (Mellon Institute of Industrial Research, 1952).

Rats (strain not stated; weights 125–150 g) were exposed repeatedly to various concentrations of Butoxyethanol. The results were as follows: None of six female rats died (six 8-h exposures to 125 ppm), one of five female rats died (four 8-h exposures to 250 ppm), none of six female rats died (six 8-h exposures to 250 ppm), and three of six male rats died (two 8-h exposures to 500 ppm) (Mellon Institute of Industrial Research, 1952).

TABLE 6. *Short-term inhalation toxicity of Butoxyethanol*

Animals	Dose	Results	Ref.
30 rats (weights not stated)	30 exposures to 500 ppm (5 days/wk, 7 h/day)	20 rats died during or after first exposure; 3 rats survived to day 30	Mellon Institute of Industrial Research (1952)
6 rats (125–150 g)	Two 8-h exposures to 500 ppm	3 rats died	Mellon Institute of Industrial Research (1952)
2 groups of rats (5 and 6 rats, 125–150 g)	2 groups: four 8-h and six 8-h exposures to 250 ppm	1/5 rats (four 8-h exposures) died	Mellon Institute of Industrial Research (1952)
6 rats (125–150 g)	Six 8-h exposures to 125 ppm	No deaths	Mellon Institute of Industrial Research (1952)
2 groups of 23 rats (wts ~205 g)	Exposure to 135 and 320 ppm 5 days/wk (7 h/day) for 5 wks	No. of deaths not significantly different from that in untreated (23 rats) control group; transient hematological effects	Werner et al. (1943*c*)
Groups of 15 male (av. wt 190 g) and female (av. wt 140 g)	30 exposures to 62.5, 125, and 250 ppm 5 days/wk (7 h/day)	Combined results: significant increases in liver and kidney wts (125 and 250 ppm)	Mellon Institute of Industrial Research (1952)
10 guinea pigs (334–499 g)	30 exposures to 62.5, 125, and 250 ppm 5 days/wk (7 h/day)	Significant increase in kidney wt (250 ppm)	Mellon Institute of Industrial Research (1952)
4 groups of 16 rats (4–5 wks old)	4 groups: exposures to 0, 20, 86, and 245 ppm for 5 consecutive days (6 h/day)	No deaths; significantly depressed numbers of erythrocytes and increased liver wt (245 ppm)	Dodd et al. (1983)
4 groups of 16 rats (30–34 days old)	4 groups: exposures to 0, 20, 86, and 245 ppm for 9 days (6 h/day)	Significant depression of erythrocyte count and increased liver wt (86 and 245 ppm)	Bushy Run Research Center (1981*b*)
2 groups of 10 guinea pigs	2 groups: exposures to 375 and 495 ppm for 30 days	2 deaths within 12 exposures (495 ppm); 1 death after 7 exposures (375 ppm)	Mellon Institute of Industrial Research (1955)
5 groups of 10 guinea pigs (435–500 g)	5 groups: exposures to 54, 107, 203, 376, and 494 ppm 5 days/wk (7 h/day) for 30 days	Deaths: 1 (376 ppm), 2 (494 ppm)	Carpenter et al. (1956)
1 dog	2 days of exposure to 617 ppm	Death at 13.5 h	Mellon Institute of Industrial Research (1955)
2 dogs	Exposure to 415 ppm 5 days/wk (7 h/day) for 12 wks	Decreased erythrocyte count and hemoglobin concentration; increased blood urea concentrations	Werner et al. (1943*b*)
2 dogs (1 male, 1 female, 14.15 and 13 kg)	Repeated exposures to 385 ppm (7 h/day)	Male and female dogs died after 28 and 8 days	Mellon Institute of Industrial Research (1955)
2 dogs (1 male, 1 female, 11.9 and 9.95 kg)	Exposure to mean concentration of 200 ppm for 31 days	Values for erythrocyte fragility increased slightly (male and female); slight decrease in erythrocyte count and hemoglobin concentration (female)	Mellon Institute of Industrial Research (1955)
2 *Rhesus* monkeys	Exposure to 104 ppm for 10 days and 210 ppm for remaining 30 days	1 monkey died of multiple splenic tumors after 5 exposures to 104 ppm; decreased erythrocyte count (104 and 210 ppm)	Mellon Institute of Industrial Research (1955)

In another study, two groups of 23 adult Wistar rats (weights ~205 g) were exposed to 135 and 320 ppm Butoxyethanol, respectively, 5 days/week (7 h/day) for a total of 5 weeks. Additional groups of rats were exposed to other glycol ethers at concentrations ranging from 310 to 390 ppm. An untreated group of 23 rats served as the control. Gross and microscopic examinations were performed on four pairs of rats per group. In each group, four pairs of rats were killed 1, 3, and 5 weeks after the initiation of exposure and 1 week after the termination of exposure, respectively. The remaining 15 rats in each group, used for growth and hematological studies, were killed 3 weeks after the termination of exposure; microscopic examination was then performed. Compared with controls, experimental animals did not have significant alterations in body weight. The experimental groups also did not experience any increase in mortality over that noted in the control group. After 1 week of exposure, an increase in the reticulocyte count and significant decreases in hemoglobin concentration and erythrocyte count were noted in the experimental group exposed to 320 ppm Butoxyethanol; values for these parameters subsequently returned to normal. Upon histological examination, hemosiderosis was not significant in the spleens of rats from experimental groups exposed to Butoxyethanol; differences in the number of myeloid cells between experimental groups and the control were very slight. The only conspicuous histopathologic finding in livers from experimental rats was a decrease in cytoplasmic density. The specific glycol ether exposure groups in which this finding was noted were not identified. However, based on these data, the authors stated that, seemingly, any of the glycol ether derivatives may cause a decrease in cytoplasmic density in hepatic cells (Werner et al., 1943*c*).

Groups of 15 male (average weight 190 g) and 15 female (average weight 140 g) Sherman rats and also 10 male guinea pigs (weights 334–499 g) were exposed to Butoxyethanol concentrations of 62.5, 125, and 250 ppm 5 days/week (7 h/day) for a total of 30 exposures. Butoxyethanol exposure did not have any effect on the mean weight gain of rats or guinea pigs that completed the study. The combined results for male and female rats indicated that both 125 and 250 ppm Butoxyethanol caused a significant increase in liver weight (as a percentage of body weight) over that of the control group. A similar increase in kidney weight was also apparent at these two concentrations. When the results for male and female rats were evaluated separately, 250 ppm Butoxyethanol did not cause a significant increase in weight of kidneys. The only statistically significant effect observed in guinea pigs, increase in weight of kidneys, was observed after exposure to 250 ppm Butoxyethanol. These findings prompted the authors to infer that, compared to the rat, the guinea pig was less susceptible to toxic effects of Butoxyethanol. Blood was found in the urine at 7 h only in the 250-ppm group as follows: 10 of 15 female rats, 1 of 15 male rats, but 0 of 10 guinea pigs. No blood was seen in any animal at this exposure level at later times. At a concentration of 62.5 ppm, Butoxyethanol did not produce any effects in guinea pigs or rats (Mellon Institute of Industrial Research, 1952).

The toxicity of Butoxyethanol (>99%) was evaluated using six groups composed of 16 Fischer 344 rats each (4–5 weeks old; 8 males, 8 females/group). Four groups of animals were exposed, 6 h/day for 5 consecutive days, to the following concentrations of Butoxyethanol, respectively: 0 ppm, 20 ppm (97 mg/m^3), 86 ppm (415 mg/m^3), and 245 ppm (1,183 mg/m^3). These values represent mean chamber concentrations of Butoxyethanol. The control group was exposed to air. After a 2-day nonexposure period, exposures resumed for 4 consecutive days. On the day prior to termination, blood samples for

hematologic measurements were obtained via the orbital bleeding of rats that had been anesthetized with methoxyfurane. Necropsy was performed on the morning after the last day of Butoxyethanol exposure. Two additional groups of rats served as experimental (245-ppm exposure) and control groups, respectively. Unlike the other four groups, each was allowed a 14-day recovery period after the ninth exposure. None of the animals died in any of the treatment groups either during or after exposure. Significantly depressed numbers of erythrocytes, hemoglobin, and mean corpuscular hemoglobin concentration (~20% below the control) were observed in males and females of the 245-ppm exposure group. No effect on hematologic parameters was present in rats exposed to 20 ppm Butoxyethanol. Male and female rats of the 245-ppm exposure group and female rats of the 86-ppm exposure group had significantly larger liver weights. Liver weights were expressed as percentages of body weight. The absolute mean liver weight for 245-ppm-exposed females was significantly greater than the control mean, while absolute liver weights for 245-ppm-exposed males and 86-ppm-exposed females were greater, but not statistically significant when compared with respective control values. After the 14-day recovery period, mean liver weights for control and 245-ppm exposure groups were not significantly different (Dodd et al., 1983).

Groups of rats (8 males, 8 females/group; 30–34 days old) were exposed to Butoxyethanol vapor at concentrations of 0 ppm, 20 ppm (97 mg/m^3), 86 ppm (415 mg/m^3), and 245 ppm (1,183 mg/m^3), respectively, 6 h/day for 9 days. The animals were killed and necropsied on the day after the last exposure. An additional 15 rats (8 males, 7 females) were also exposed to 245 ppm, and an additional 14 rats (7 males, 7 females) served as controls. These extra groups of animals were used for determining, if necessary, the reversibility of any alterations in the parameters assessed during the 9-day exposure period. Unlike the other groups, these rats were observed for 14 days after the last exposure and killed on day 14. In the 245-ppm exposure group, statistically significant smaller body weights were reported for male and female rats. This effect gradually disappeared during the 14-day observation period. Depression of body weight gain was also observed in female rats exposed to 86 ppm Butoxyethanol and continued throughout the exposure period. No significant changes in body weight were found in rats exposed to 20 ppm Butoxyethanol. Hematological findings noted in male and female rats exposed to 245 ppm Butoxyethanol included significant reduction of the erythrocyte count, decreased hemoglobin content, and decreased mean corpuscular hemoglobin concentrations. Significant increases in mean corpuscular volume, nucleated erythrocytes, and reticulocytes were also observed in rats of the 245-ppm exposure group; a significant increase in lymphocytes was noted only in males. Substantial recovery of these hematological changes was observed after the 14-day observation period; however, the values were still significantly different from those of control rats. The leukocyte counts for rats of the 245-ppm group returned to control values during the observation period. Male and female rats exposed to 86 ppm Butoxyethanol had significant, but less profound, changes in hematological parameters when compared with rats of the 245-ppm exposure group. No effects on hematological parameters were observed in rats of the 20-ppm exposure group. Liver weights (expressed as a percentage of body weight) were significantly greater in male and female rats exposed to 245 ppm Butoxyethanol and in female rats exposed to 86 ppm Butoxyethanol, but not in rats exposed to 20 ppm Butoxyethanol when compared with control values. Gross lesions in all exposure groups were not more fre-

quent than or different from those observed in the control group (Bushy Run Research Center, 1981*b*).

Groups of 10 male particolor guinea pigs (weights not stated) were exposed to metered concentrations of 375 and 495 ppm Butoxyethanol for 30 days. Two male guinea pigs died within 12 exposures to 495 ppm Butoxyethanol, and one animal died after 7 exposures to 375 ppm Butoxyethanol. The mean weights in both exposure groups decreased slightly by the third day of exposure. Only the weights of guinea pigs exposed to 375 ppm Butoxyethanol were significantly below those of the control group. A significant increase in kidney weight in both exposure groups was also observed. Only three guinea pigs that died during exposure had major tissue damage, which was mostly pulmonary hemorrhage and congestion (Mellon Institute of Industrial Research, 1955).

A total of five groups of guinea pigs (10/group; weights 435–580 g) inhaled 54-, 107-, 203-, 376-, and 494-ppm concentrations of Butoxyethanol, respectively, 5 days/week (7 h/day) for a total of 30 days. Exposures took place in 200- to 7,900-L exposure chambers. None of the animals exposed to 54, 107, or 203 ppm Butoxyethanol died. Mortality in the 376- and 494-ppm exposure groups was 1 of 10 and 2 of 10, respectively. Significant pulmonary congestion and cloudy swelling of the convoluted and loop tubules of the kidneys were observed in animals that died. Kidney weights in 203-, 376-, and 494-ppm exposure groups were significantly greater than those reported for the control group. No significant differences in liver weight between experimental and control groups were observed (Carpenter et al., 1956).

The toxicity of Butoxyethanol (617 ppm) was evaluated using a female mongrel dog (weight not stated). The dog died 13.5 h into the 2-day exposure period. Both vomiting and extreme weakness were noted during exposure. The alterations found at necropsy included moderate congestion of the kidneys and lungs (Mellon Institute of Industrial Research, 1955).

Two dogs were exposed to 415 ppm Butoxyethanol 5 days/week (7 h/day) for 12 weeks. Increased ocular and nasal secretions, indicative of mild irritation, were observed during the exposure period. A maximal decrease in erythrocyte count and hemoglobin concentration was observed after 4–6 weeks of exposure and remained at approximately the same concentration until exposures were discontinued. Increased blood urea concentrations were initially observed within 1 week after the initiation of exposure. Urea concentrations remained elevated throughout the course of exposure and declined after exposure had been terminated (Werner et al., 1943*b*).

In another study, a male Basenji yearling dog and a female mixed breed Basenji (Springer × Kerry Blue) yearling dog were exposed repeatedly, 7 h/day, to ~385 ppm Butoxyethanol. Male and female dogs weighed 14.15 and 13.0 kg, respectively. The male dog died after 28 days of exposure and the female dog after 8 days. The following observations were made in both animals: loss of weight, transitory increase in erythrocyte fragility, incidental ocular and nasal infection, generalized weakness, apathy, anorexia, and increased leukocyte count. Additionally, the test substance induced vomiting in the female dog several times during the first 4 days of exposure, and the male dog had a significant decrease in erythrocyte count and hemoglobin concentration. Erythrocyte fragility in the male dog decreased to initial hemolysis in 0.32% saline and complete hemolysis in 0.20% saline 27 days after maximum values of initial hemolysis in 0.54% saline and complete hemolysis in 0.42% saline had been reached within 7 days. Elevated

plasma fibrinogen levels of 1.75 and 2.26 g/100 ml in the male dog after 26 and 27 exposures, respectively, were the only positive results from blood chemistry tests. These values were several times greater than the generally accepted control value, 0.52 g/100 ml, for dogs. An analysis of a 16-h urine sample from the male dog, taken after day 25 of exposure, indicated the presence of 55 mg of butoxyacetic acid. The more remarkable observations at necropsy included moderate to severe congestion and hemorrhages of the lungs, congestion of the liver in both animals, and congestion of both kidneys in the female dog. Gross observations were confirmed by microscopic examination. A large subcapsular hematoma in one of the adrenal glands of the female dog was also noted (Mellon Institute of Industrial Research, 1955).

Both a male and a female Basenji dog (both aged 6 months) were exposed to a mean concentration of 200 ppm Butoxyethanol for a total of 31 days. Male and female dogs weighed 11.9 and 9.95 kg, respectively. Blood chemistry tests were performed immediately after days 15, 22, and 29 of exposure and immediately before day 28 of exposure. Weekly hematological tests were also performed. An erythrocyte permeability test was also performed 1 day before the animals were killed. The values for erythrocyte fragility in the male and female dogs increased slightly. Additionally, a slight decrease was found in both the erythrocyte count and the hemoglobin concentration of the female dog. The erythrocyte permeability of both dogs was greater than that for control dogs, but the difference was not statistically significant. The results of blood chemistry tests indicated no significant alterations. Necropsy findings included slight capillary enlargement or breakdown in the lungs of both dogs; microscopic examinations did not confirm the findings observed grossly. An analysis of 16-h urine samples from the male and female dog, taken after day 14 of exposure, indicated the presence of 100 and 42.5 mg of butoxyacetic acid, respectively. Ocular damage was not observed (Mellon Institute of Industrial Research, 1955).

Two female *Rhesus* monkeys were exposed to 104 ppm Butoxyethanol for 10 days. One monkey (weight not stated) died of multiple splenic neoplasms after five exposures. In the other animal (weight 4.2 kg), no significant alteration in the hematological profile was observed during the exposure period. Additionally, the erythrocyte fragility values, usually determined after a 7-h exposure, remained equal to those determined prior to treatment (initial hemolysis in 0.52% saline and complete hemolysis in 0.44% saline). A decrease in the erythrocyte count was also observed. Because no significant hematological alterations were observed, the concentration of Butoxyethanol was increased to 210 ppm for the remaining 30 days of exposure. After four exposures to 210 ppm, the values increased to initial hemolysis in 0.60% saline and complete hemolysis in 0.52% saline; these values usually persisted for the remainder of the 30-day exposure period. After a 17-h to 2.5-day nontreatment period, erythrocyte fragility tests were performed. The fragility values were usually smaller than values that had been determined immediately after exposure. At the conclusion of the 30 exposures to 210 ppm Butoxyethanol, the erythrocyte count had decreased to a value that was less than half of the original erythrocyte count. A similar pattern, with respect to the hemoglobin concentration, was also observed. Additionally, after 14 exposures to 210 ppm Butoxyethanol, the plasma fibrinogen concentration was 1.22 g/100 ml. This value was four times greater than what is generally accepted as a control value. Vomiting was the clinical sign most commonly reported during 30 days of exposure to 210 ppm Butoxyethanol. Necropsy results included

caseous nodules in the lungs that were congested and hemorrhagic. All of the remaining tissues examined appeared normal. Microscopic findings included patchy areas of congestion with inflammatory cells and a small amount of peribronchial fibrosis. Congestion was also observed in the liver and kidneys (Mellon Institute of Industrial Research, 1955).

Subchronic Inhalation Toxicity

This section presents the results of six studies, including two from the National Toxicology Program (NTP). As in the previous section, hematologic findings predominate and are seen across a number of species.

Groups of 70 mature male C3H mice (weights not stated) were exposed to metered concentrations of 100, 200, and 400 ppm Butoxyethanol, respectively, 7 h/day (Mellon Institute of Industrial Research, 1955). The animals were killed at the following intervals: 15 mice from each group (after 30 exposures), 15 mice from each group (after 60 exposures), 10 mice from each group (after 90 exposures), and 11–18 mice from each group (after 90 days of exposure followed by a 42-day nontreatment period). Ten mice from each group were also selected for erythrocyte fragility tests after various periods of exposure. Statistically significant alterations in weight of the liver were observed in mice subjected to 30-, 60-, and 90-day exposures to 400 ppm Butoxyethanol and in mice exposed to 200 ppm Butoxyethanol for 60 days. No significant deviations from the controls were noted in mice killed 42 days after the end of the 90-day exposure period. Neither the mortality rate nor gross lesions were significantly different. At microscopic examination of 80 kidneys from mice subjected to various durations of exposure, no significant tissue changes were found at any of the three concentrations of exposure. The number of mice with blood in the urine immediately after exposure was proportional to the concentration at the time of exposure. Increased erythrocyte fragility was noted in mice of all three exposure groups. The increase in erythrocyte fragility noted after day 89 of exposure was no greater than that observed after day 1 of exposure. Additionally, the results of an analysis of blood samples obtained after a 17-h nontreatment period indicated that values for erythrocyte fragility had essentially returned to normal.

In an NTP study, the subchronic inhalation toxicity of Butoxyethanol was evaluated using groups of 10 B6C3F1 mice/sex/group. The groups were exposed, whole-body inhalation, to Butoxyethanol at concentrations of 0, 31, 62.5, 125, 250, and 500 ppm, respectively, for 13 weeks. Four males and four females exposed to 500 ppm Butoxyethanol either died or were found moribund and killed. Reductions in terminal body weight of 4.5, 6.4, and 11.4% were reported for male mice exposed to 125, 250, and 500 ppm, respectively. Terminal body weights were reduced by 7.7% in female mice exposed to 500 ppm Butoxyethanol. Clinical signs that are consistent with the known hemolytic effect of Butoxyethanol in rodents were most prevalent in male and female mice at concentrations of 250 and 500 ppm. The results of hematological evaluations indicated that the primary alteration in the hematopoietic system was moderate regenerative anemia, with increased platelet counts at higher concentrations of exposure. For males and females, the no-observed-effect level for hematopoietic effects was 62.5 ppm. At necropsy, splenomegaly was observed in two female mice from the 500-ppm exposure group. Significant increases in liver-to-body weight ratios ($p < 0.01$) were noted in males from 250- and 500-ppm exposure groups and in females exposed to 500 ppm Butoxyethanol.

The following histopathological findings were associated with Butoxyethanol exposure: testicular degeneration, excessive splenic extramedullary hematopoiesis and hemosiderosis, hemosiderin accumulation in Kupffer cells, renal tubular degeneration, and hemosiderin deposition. Gastric necrosis, ulceration, inflammation, and epithelial hyperplasia with a presumed secondary pleuritis were also noted. The no-observed-effect levels for histopathological findings were 31 ppm (females) and 62.5 ppm (males) (NTP, 1993*a*).

In another NTP study, the subchronic inhalation toxicity of Butoxyethanol was evaluated using groups of 10 F344/N rats/sex/group. The groups were exposed, whole-body inhalation, to Butoxyethanol concentrations of 0, 31, 62.5, 125, 250, and 500 ppm for 13 weeks. Five females from the 500-ppm exposure group and one male exposed to 250 ppm were found moribund. Compared with controls, there was a 9.5% reduction in terminal body weight in rats exposed to 500 ppm Butoxyethanol. Clinical signs that are consistent with the known hemolytic effect of Butoxyethanol were most prevalent in rats (both sexes) dosed with 125, 250, and 500 ppm Butoxyethanol, respectively. The results of hematological evaluations indicated that the primary effect on the hematopoietic system was a mild to moderate regenerative anemia. For males, the no-observed-effect level for Butoxyethanol-induced hematologic effects was 62.5 ppm. However, hematologic effects were observed in female mice from all exposure groups. At necropsy, gross lesions that were related to Butoxyethanol exposure were observed only in females from the 500-ppm group. Missing distal portions of the tail were reported for all females that survived and two moribund females that were killed. Multiple dark foci in the kidneys and diffusely pale livers were also observed in moribund female rats that were exposed to 500 ppm Butoxyethanol. Significant increases in liver-to-body weight ratios were reported for rats (both sexes) in 250- and 500-ppm exposure groups. Additionally, liver weights, right kidney weights, and their respective organ/body weight ratios were increased in mid- and high-dose groups. The following histopathological findings were associated with Butoxyethanol exposure: excessive splenic extramedullary hematopoiesis, hemosiderin accumulation in Kupffer cells, hepatocellular necrosis, centrilobular hepatocellular degeneration, renal tubular degeneration and hemosiderin deposition, and bone marrow hyperplasia. In male and female rats, the no-effect levels for hepatic lesions were 62.5 and 31 ppm, respectively (NTP, 1993*a*).

The subchronic (90-day) inhalation toxicity of Butoxyethanol (>99%) was evaluated using four groups of 32 Fischer 344 rats (4–5 weeks old; 16 males, 16 females/group). The four groups of animals were exposed 5 days/week (6 h/day) for 13 weeks to the following concentrations of Butoxyethanol, respectively: 0 ppm (air exposed), 5 ppm (24 mg/m^3), 25 ppm (121 mg/m^3), and 77 ppm (372 mg/m^3). These values represented mean chamber concentrations of Butoxyethanol; air flow through the chambers was maintained at ~2,000 L/min. On the day prior to termination of exposure, blood samples for hematologic measurements were obtained via the orbital bleeding of rats that had been anesthetized with methoxyfurane. Individual urine samples were collected over a 3-h period on the morning prior to termination of the experiment. No deaths occurred at any time during the study. With the exception of a transient decrease in body weight gain (weeks 2–4 of exposure) in female rats of the 77-ppm exposure group, mean body weights were similar to controls. After the sixth week (31 exposures), female rats of the 77-ppm exposure group had significant decreases in their erythrocyte counts (13% below control value) and hemoglobin, accompanied by an 11% increase in mean corpuscular hemoglobin above

that of controls. At the conclusion of the 90-day study (66 exposures), the hematologic effects seen in female rats had either lessened or returned to control values. In male rats of the 77-ppm exposure group, a 5% decrease in the erythrocyte count was observed after 90 days (66 exposures). This was the only significant hematologic finding reported for male rats. No significant differences in serum chemistry and urinalysis determinations were observed at any level of exposure. No gross or microscopic lesions were found in rats treated with Butoxyethanol (Dodd et al., 1983).

The subchronic inhalation toxicity of Butoxyethanol was also evaluated using a total of 128 male and female inbred Fischer 344 rats (30–34 days old). Four groups of 32 rats (16 males, 16 females/group) were exposed to 0 ppm, 5 ppm (24 mg/m^3), 25 ppm (121 mg/m^3), and 75 ppm (372 mg/m^3) Butoxyethanol vapor, respectively, 5 days/week (6 h/day) for ~90 days. The mean weights for the groups tested were as follows: 157.3 g (0-ppm exposure), 154.7 g (5 ppm), 163.3 g (24.6 ppm), and 161.0 g (77 ppm). Twelve rats (six males, six females) per group were killed at ~1.5 months prior to the end of exposure; blood samples were obtained, but necropsy was not performed. The remaining animals were killed and necropsied after the last exposure; histopathological evaluations were performed. Blood samples were collected prior to each scheduled necropsy and urine was collected only prior to the second series of necropsies. Clinical pathologic examinations, including urinalysis, hematology, and serum clinical chemistry, were performed on all animals. Butoxyethanol exposure did not cause death in any of the test groups. During the early weeks of exposure, slightly decreased body weight gain and feed consumption (compared with female controls) were observed in female rats exposed to 77 ppm Butoxyethanol. However, by the middle of the exposure period, these values were similar to those of the control group. Minimal reductions in erythrocyte counts, hemoglobin, and packed cell volume, with a slight increase in mean corpuscular hemoglobin, were also observed only in female rats exposed to 77 ppm Butoxyethanol. Organ weights were not different among groups and no gross or microscopic lesions resulted from treatment (Bushy Run Research Center, 1981*a*).

Both a male (weight 9.40 kg, 8 months old) and a female (6.10 kg, 8 months old) wire-haired terrier were exposed to Butoxyethanol vapor (100 ppm) 7 h/day for a total of 90 days. The dogs were not appreciably affected by exposure to 100 ppm Butoxyethanol. However, at the halfway point of the exposure period, a transient doubling of the leukocyte count was observed in both animals. The female dog's leukocyte count returned essentially to preexposure values, but the male's leukocyte count was ~50% greater at the conclusion of the exposure period. Additionally, an appreciable decrease in the male dog's packed cell volume (43.0% packed cell volume before the first exposure to 34.5% after 90 exposures) was observed. The results of a urinalysis indicated the presence of butoxyacetic acid in 24-h urine samples from the male (100 mg butoxyacetic acid detected) and female (94 mg butoxyacetic acid detected) dogs (Mellon Institute of Industrial Research, 1955).

Acute Oral Toxicity

In addition to the expected studies in rats, this section includes findings in a wide variety of experimental animals.

In an acute oral toxicity study involving female rats (weights 90–120 g; number not stated), the mean LD_{50} for undiluted Butoxyethanol was 2,800 mg/kg (Carpenter et al., 1956).

The oral toxicity of Butoxyethanol (>99% purity) was evaluated using male F344 rats of the following age groups: 4–5 weeks, 9–13 weeks, 5–6 months, and 16 months. For comparative purposes, young and adult rats refer to 4- to 5-week-old and 9- to 13-week-old rats, respectively. Single oral doses of Butoxyethanol (32, 63, 125, 250, and 500 mg/kg) were administered to groups of 6–12 animals, respectively, corresponding to each age bracket, with the exception of rats of 5- to 6-month-old and 16-month-old age groups that did not receive 250- or 500-mg/kg doses. Each dose was dissolved in water to allow gavage administration of 5 ml/kg; the control group received water. Rats were killed with ether at 2, 4, 8, 24, and 48 h postadministration. A significant, dose-dependent increase in the splenic weight/body weight ratio was observed in young and adult rats that received 125- or 500-mg/kg doses. Values approached controls within 48 h after administration. A significant decline in numbers of erythrocytes, hemoglobin concentration, and packed cell volume was detected in adult rats, but not in young rats, that received 125 mg/kg Butoxyethanol. In rats dosed with 500 mg/kg Butoxyethanol (young and adult rats) and adult rats dosed with 125 mg/kg Butoxyethanol, a significant increase in the concentration of free hemoglobin in the plasma was found. By 24 h postadministration, values were comparable with those of the control group. For the entire range of doses tested in groups of young and adult rats, a dose-dependent decline in erythrocytes, hemoglobin, and packed cell volume at 24 h postadministration was found. Focal necrosis of hepatocytes was observed in 9- to 13-week-old rats that received 250- and 500-mg/kg doses of Butoxyethanol. Evidence of regression of these lesions was noted at 48 h postadministration. In the kidneys, small and dark pink protein droplets, compatible with hemoglobin, were seen in the proximal tubular epithelium of rats that received 125-, 250-, and 500-mg/kg doses. Evidence of regression of these renal changes was noted at 48 h postadministration (Ghanayem et al., 1987*a*). Subsequent to this publication, dose- and time-dependent increases in packed cell volume, followed by a decline as hemolysis occurred, were identified using an impedance-based analyzer (Ghanayem et al., 1990*b*). In this publication, the authors concluded that earlier results obtained using the laser hematology analyzer were inaccurate, because with this technique, erythrocytic swelling was not detected. With the laser hematology analyzer, cell size is characterized by diameter and not volume; the opposite is true with the impedance-based analyzer (Ghanayem et al., 1990*b*).

The acute oral toxicity of Butoxyethanol was evaluated by Bushy Run Research Center (1980*b*) using nonfasted male Wistar rats (3–4 weeks old, 90–120 g). The test substance was administered via stomach tube to four groups of rats (5/group) in doses of 1.25, 2.5, 5.0, and 10.0 ml/kg, respectively. The LD_{50} was 2.68 ml/kg (95.0% confidence limits = 1.85–3.88 ml/kg). Necropsy findings included dark livers, distended stomachs, red-colored kidneys and adrenal glands, and blood in the intestines. There were no remarkable observations in animals that survived.

In acute oral toxicity studies involving rats, mean LD_{50} values of 530 mg/kg (weights 260–320 g, females) and 3,000 mg/kg (weights 30–60 g, males) for aqueous Butoxyethanol have been reported (Carpenter et al., 1956). The following mean LD_{50} values for aqueous Butoxyethanol have been reported in other animal species: 320 mg/kg in male rabbits (weights 2,700–3,200 g) and 370 mg/kg in male rabbits (weights 1,500–3,000 g), 1,200 mg/kg in male and female guinea pigs (weights 200–300 g) (Carpenter et al., 1956), and 1,000–1,600 mg/kg in mice (weights not stated) (Tyler, 1984).

Two further acute oral toxicity studies were conducted by MB Research Laboratories, Inc.

(1976). In the first study, four groups of 10 male Wistar albino rats (weights 200–300 g) were intubated with 0.67, 1.31, 2.56, and 5.0 g/kg of Butoxyethanol, respectively. Mortality rates were as follows: 0/10 (0.67-g/kg dose), 3/10 (1.31-g/kg dose), 9/10 (2.56-g/kg dose), and 10/10 (5.0-g/kg dose). The LD_{50} was 1.59 g/kg (95% confidence limits = 1.11–2.27 g/kg).

In the second study (MB Research Laboratories, Inc. 1977), the acute oral toxicity of Butoxyethanol was evaluated as before, but the groups received doses of 5.0, 7.12, 10.14, and 14.43 g/kg. After dosing, the animals were observed for 14 days. Mortality rates were as follows: 0/10 (5.0-g/kg dose), 5/10 (7.12-g/kg dose), 9/10 (10.14-g/kg dose), and 10/10 (14.43-g/kg dose). At necropsy, observations reported for all treatment groups included dark livers and kidneys and dark areas on the lungs. An LD_{50} of 7.5 g/kg (95% confidence limits = 5.9–9.5 g/kg) was calculated. The Mellon Institute of Industrial Research (1952) also conducted acute toxicity studies in rats, guinea pigs, and rabbits. The acute oral toxicity of 10.0% Butoxyethanol in water was evaluated using male (three groups of five) and female (four groups of five) nonfasted albino rats of the Sherman strain. The weights of both male and female rats ranged from 90 to 114 g. The three groups of male rats received doses of 2.0, 2.52, and 3.16 g/kg, respectively, and the four groups of female rats doses of 1.58, 2.0, 2.52, and 3.16 g/kg, respectively. All doses were administered via stomach tube, and the animals were observed during a 14-day period after dosing. The mean LD_{50} values for male and female rats were 2.6 g/kg (range 2.3–2.9 g/kg) and 2.3 g/kg (range 1.9–2.8 g/kg), respectively. Some males of the 2.52- and 3.16-g/kg dose groups died, and deaths occurred in all dose groups of females. The administration of doses at or above the LD_{50} resulted in such clinical signs as sluggishness, ruffled coats, prostration, and narcosis. Necropsy findings in animals that died included congested or hemorrhagic lungs, mottled livers, congested kidneys, and blood in the urine. The hematuria was found in males dosed with 3.16 g/kg and females that received doses as low as 1.58 g/kg. In a second acute oral toxicity study involving rats, an LD_{50} of 1.5 g/kg (range 1.2–1.9 g/kg) was reported, but no details were given. In guinea pigs, the LD_{50} for Butoxyethanol was 1.20 g/kg (range 0.96–1.50 g/kg), while in rabbits, the LD_{50} was 0.35 g/kg (range 0.33–0.38 g/kg). The weights, strain, and number of guinea pigs or rabbits tested were not stated (Mellon Institute of Industrial Research, 1952).

Short-Term Oral Toxicity

Four studies are included here, including one NTP study in mice and rats. Overall, little short-term oral toxicity is shown.

The oral toxicity of Butoxyethanol was evaluated in three groups of five male mice (weights and strain not stated) gavaged with doses of 500, 1,000, and 2,000 mg/kg, respectively, for 5 weeks (5 days/week). Reduced erythrocyte counts were observed in animals of the 500-mg/kg group. Reduced erythrocyte counts and testicular atrophy (one mouse) were observed in mice of the 1,000-mg/kg group. All animals of the 2,000-mg/kg group died (Tyler, 1984).

In another study, the oral toxicity of Butoxyethanol (99.9% purity) was evaluated using male F344 rats (~4 weeks old). Two groups of 24 animals received oral doses of 500 and 1,000 mg/kg, respectively, for 4 consecutive days. Doses of Butoxyethanol (diluted with water) were administered in 5-ml/kg volumes. After administration of the final dose, six

animals from each group were bled and then killed. Control animals (n = 24) were dosed with water. Necropsy was performed on rats killed on days 1, 4, 8, and 22 postadministration. Treatment-related reductions in the rate of body weight gain were seen only in animals that received 1,000-mg/kg doses. Dose-related increases in relative weights of spleen, liver, and kidneys and reduced thymic weights were observed on day 1 of recovery; except for slight elevations in spleen and liver weights, the weight changes had returned to normal by day 22. Marrow hyperplasia, with no evidence of hemorrhage, was also observed in treatment groups. Animals in the 1,000-mg/kg dose group had reduced numbers of erythrocytes, packed cell volume, and hemoglobin and markedly elevated mean corpuscular volume, reticulocyte counts, and mean corpuscular hemoglobin at the end of treatment. Most of these changes returned to normal during the recovery period, although mean corpuscular volume and mean corpuscular hemoglobin remained slightly elevated on day 22 postadministration. Leukocyte counts were reduced at day 1 in animals of the 1,000-mg/kg dose group, but gradually increased with time; however, control values were not reached by the end of the recovery period. The effects on erythrocytes and leukocytes were also present, but less severe in rats of the 500-mg/kg dose group (Grant et al., 1985).

Krasavage (1986) evaluated the oral toxicity of Butoxyethanol (99.5% pure) using three groups of 10 adult male albino rats of the COBS CD (SD) BR strain. The test substance (undiluted) was administered via gavage to the three groups at doses of 222, 443, and 885 mg/kg, respectively, over a period of 6 weeks (5 days/week). Control animals were dosed with distilled water. At the end of the 6-week period, blood samples were collected from the inferior vena cava. Animals were then killed and necropsy was performed. A dose-dependent decrease in body weight gain was observed, which, along with decreased feed consumption, in the 885-mg/kg dose group, was significantly different from that observed in the control group. Significant dose-dependent decreases in hemoglobin concentration, erythrocyte count, and mean corpuscular hemoglobin concentration were also reported. These effects on erythrocytes, as well as increased mean corpuscular hemoglobin and mean corpuscular volume, were the most significant toxic effects induced by Butoxyethanol. Effects that were secondary to those observed in erythrocytes included increased splenic weights, splenic congestion, and hemosiderin accumulation in the liver and kidneys. Relative liver weights and serum alkaline phosphatase (443-and 885-mg/kg doses) and serum alanine aminotransferase (885-mg/kg doses) activities were also increased. A significant reduction in blood glucose concentration occurred in animals that received 885-mg/kg doses. There was no evidence of toxicity in the testes, bone marrow, thymus, or leukocytes.

The short-term oral toxicity of Butoxyethanol (99.0% pure) was investigated by the NTP in F344/N rats and B6C3F_1 mice (5–7 weeks old; 5 males, 5 females/dose). The rats received oral doses (in drinking water) of 100, 150, 250, 400, or 650 mg/kg daily for 14 days; the control group received water. None of the rats died or was killed prior to the end of the study. In all of the treatment groups, the final mean body weights and body weight gains of male rats were similar to those noted in controls. However, for female rats dosed with 650 mg/kg Butoxyethanol, the final mean body weight was lower than that for the control group. Clinical signs of toxicity were not observed, and there were no test substance-induced gross lesions. Changes in organ weight were considered minimal. Compared with controls, changes (increases and decreases) in relative and absolute thymus

weights were noted in groups of female rats; however, these changes were not significantly different. Decreases in relative and absolute thymus weights, not statistically significant when compared with controls, were observed in male rats at all but one of the doses tested; mean values for absolute thymic weight were the same in control and 400-mg/kg dose groups. In the mice, the same doses as in rats produced no deaths prior to the end of the study. Compared with the control group, absolute and relative thymus weights were significantly lower ($p \leq 0.05$) in male mice. However, significant changes in absolute or relative thymic weight were not observed in groups of female mice (NTP, 1993*b*).

Subchronic Oral Toxicity

Three studies are included, including one NTP study in mice and rats. Among the positive effects, hematologic findings predominate in the NTP study in rats, but were not found in mice.

Subchronic studies were conducted by the Mellon Institute of Industrial Research and by the NTP. In the first of two Mellon Institute studies, four groups of Sherman stock rats (5 males, 5 females/group) were fed daily diets containing Butoxyethanol concentrations of 0.03% (0.018 g/kg/day), 0.125% (0.076 g/kg/day), 0.5% (0.31 g/kg/day), and 2.0% (1.54 g/kg/day) for 90 days. Untreated rats (five males, five females) served as controls. The mean weight gain of the surviving rats in the 2.0% group was significantly less than that of the control group. Liver weight, expressed as percentage of body weight, was significantly increased in rats of the 0.5% and 2.0% groups. Kidney weight, expressed as a percentage of body weight, was also increased in the 2.0% group. On days 3 and 6, tests for the presence of blood in pooled urine samples from 0.5%, 2.0%, and control groups were negative. In each animal that died, pneumonia was accompanied by renal and hepatic lesions that are usually present in pneumonia-related deaths common in rats. At necropsy of uninfected rats, no lesions were found. The authors concluded that there were no deaths that were directly attributable to toxic effects of Butoxyethanol. The ''no-effect'' level of Butoxyethanol for 90-day repeated oral doses was between doses of 0.076 and 0.31 g/kg/day (Mellon Institute of Industrial Research, 1952).

In the second subchronic oral toxicity study, Butoxyethanol was evaluated using four groups of 20 DW rats (45 days old, 94–172 g). The four groups (10 males, 10 females/group) were fed Butoxyethanol concentrations of 0.01, 0.05, 0.25, and 1.25% in the diet, respectively, for a maximum of 93 days. Untreated rats (10 males, 10 females) served as controls. At the conclusion of dosing, all survivors were killed. Of the 100 rats tested, one male animal dosed with 1.25% Butoxyethanol died; lesions associated with pneumonia were observed at necropsy. Significant depression of feed consumption was noted only in both male and female rats that received 1.25% Butoxyethanol. Reduced feed consumption was also observed in male rats dosed with 0.25% Butoxyethanol and, to a lesser extent, in those dosed with 0.01% Butoxyethanol. The body weight gain in male and female rats dosed with 1.25% Butoxyethanol was reduced to approximately one-half that of the control group. Statistically significant reduction of weight gain was also noted in males that received 0.25% Butoxyethanol. Only at the first weighing (after 2 days of dosing) was body weight reduction significant in rats that received 0.05% Butoxyethanol (Mellon Institute of Industrial Research, 1963).

Butoxyethanol (99.0% pure) was evaluated in a 13-week NTP (1993*b*) study involving F344/N rats and $B6C3F_1$ mice (5–7 weeks old; 10 males, 10 females/dose). The test substance, in drinking water, was administered daily to groups of rats in doses of 750, 1,500, 3,000, 4,500, or 6,000 ppm, respectively; the control group received distilled water. None of the experimental animals died or was killed prior to the end of the study. The following hematological alterations were observed in male rats: Mild anemia, indicated by a decrease in erythrocyte counts, was noted at doses of 3,000, 4,500, and 6,000 ppm Butoxyethanol, respectively; thrombocytopenia was noted at doses of 4,500 and 6,000 ppm. At weeks 1 and 13, mild decreases in hemoglobin concentration were noted and reticulocyte counts were moderately increased; sporadic decreases in hemoglobin concentration were noted at week 3. Also, at week 1, mildly to moderately increased lymphocyte counts (lymphocytosis and neutrophilia) were noted at doses of 3,000, 4,500, and 6,000 ppm. Alkaline phosphatase activity was also increased, at week 1, in multiple groups of male rats and, at week 13, in the 6,000-ppm dose group. Increased alkaline phosphatase activity is consistent with mild cholestasis. No consistent changes in hematocrit were noted in male rats. Hematological alterations were also noted in female rats: Decreases in erythrocyte counts and, less consistently, hematocrit and hemoglobin concentrations were noted in most groups. These observations were indicative of mild to moderate anemia. Mild increases in platelet counts were noted at week 1 in female rats from higher dose groups; decreased platelet counts were noted at weeks 3 and 13. Marked leukocytosis (neutrophilia and lymphocytosis) was also noted at week 1. Additionally, at week 1, alkaline phosphatase activity was mildly increased in female rats from the high-dose group. Mild increases in alkaline phosphatase activity were noted in the two highest dose groups at week 13. Organ weight and gross pathology changes were limited to the thymus and uterus. Absolute thymus weights of male rats in the 4,500-ppm dose group ($p < 0.01$) and male and female rats in the 6,000-ppm dose group ($p < 0.05$) were significantly lower than in controls. A reduction in size of the uterus in rats from 4,500- and 6,000-ppm dose groups was the only gross lesion that was considered related to test substance administration.

Histopathologic lesions that were related to test substance administration were observed in the liver, spleen, and bone marrow of male and female rats. Hepatic lesions, which included cytoplasmic alteration, hepatocellular degeneration, and pigmentation, were more prominent in the three highest dose groups (3,000, 4,500, and 6,000 ppm), but were observed also in most of the male and female rats that were dosed with Butoxyethanol. Hyperplasia of bone marrow in dosed rats consisted of increased cellularity of hematopoietic cells in the midshaft of the femur, with a decrease in the amount of marrow fat cells relative to that seen in controls. A corresponding increase in hematopoiesis and hemosiderin pigment was also present in the spleen (NTP, 1993*b*).

In the 13-week mouse study, the same doses as in rats were administered. None of the mice died or were killed prior to the end of the study. There were no significant clinical observations in male or female mice or gross or microscopic lesions that were related to the administration of Butoxyethanol (NTP, 1993*b*).

Acute Dermal Toxicity

A variety of skin penetration and acute dermal toxicity studies were performed with Butoxyethanol.

A skin penetration test for Butoxyethanol was conducted using rats (strain and weights not stated). The test substance was applied via a catheter to clipped skin of the entire trunk, and the area was covered with either vinyl, Saran, or polyethylene sheeting secured with rubber bands. Each animal was immobilized during the 4-h exposure period. At the end of exposure, the covering was removed and excess test substance blotted from the skin. The animals were observed for a period of 14 days. The LD_{50} was 2.52 ml/kg (range 1.86–3.41 ml/kg) (Mellon Institute of Industrial Research, 1961).

The acute dermal toxicity of Butoxyethanol was evaluated using eight male New Zealand white rabbits (3–5 months old). Two groups of four rabbits received doses of 0.5 and 1.0 ml/kg Butoxyethanol, respectively. The test substance was applied under impervious sheeting that had been wrapped around clipped, intact skin of the trunk. The rabbits were immobilized during the 24-h application period, and excess fluid was removed to prevent ingestion. The LD_{50} was 0.630 ml/kg (95% confidence limits = 0.386–1.03 ml/kg). At necropsy, the lesions in rabbits that died included orange-red-colored lungs, dark spleens, very dark red kidneys, orange-colored peritoneum and intestines, and blood in the urine. No remarkable observations were made in animals that survived (Bushy Run Research Center, 1980*b*).

The acute dermal toxicity of undiluted Butoxyethanol was evaluated using four male and four female New Zealand white rabbits (4–6 months old). The test substance was applied to clipped skin of the trunk, and the site was covered with an impervious occlusive covering for 6 h. The dermal LD_{50} values in male and female rabbits were 0.707 and 0.630 ml/kg, respectively. Observations included necrosis at the application site, marked discoloration of the kidneys, and either hematuria or hemoglobinuria (Bushy Run Research Center, 1980*b*).

In a skin penetration study, undiluted Butoxyethanol was applied to the skin of male New Zealand albino rabbits (average weight 2.5 kg, number of animals not stated). The animals were 3–5 months old. The test substance was maintained in contact with skin of the trunk, clipped free of hair, for 24 h with Vinylite sheeting. The animals were observed for a period of 14 days after application. The mean LD_{50} was 0.56 ml/kg (range 0.48–0.64 ml/kg). Extreme congestion of the kidneys, blood in the urine, paleness of the liver, and engorgement of the spleen were noted in animals that died. In earlier studies of undiluted Butoxyethanol (dose 0.56 ml/kg), mortality rates were 100.0% (10 rabbits tested, average weight 3.0 kg) and 50.0% (6 rabbits tested, weights not stated). Blood in the urine was a common finding in both of these studies (Mellon Institute of Industrial Research, 1952).

In another study, the acute dermal toxicity of Butoxyethanol (>99.5% pure) was evaluated using six groups of six female New Zealand rabbits. The six groups of animals received doses of 0.08, 0.10, 0.12, 0.15, 0.20, and 0.25 ml/kg, respectively. Each dose was epicutaneously applied to the skin for a period of 8 h. Untreated animals served as controls. Mortalities were recorded daily during a 15-day period after exposure. Gross and microscopic examinations were performed on animals that died and on those that survived to day 15. The following deaths were reported: 0.08 ml/kg dose (two rabbits), 0.10 ml/kg (two rabbits), 0.12 ml/kg (four rabbits), 0.15 ml/kg (five rabbits), 0.20 ml/kg (five rabbits), and 0.25 ml/kg (six rabbits). Additionally, the results of microscopic examination of tissues from animals that died during the observation period were as follows: congestion in the liver with small necrotic foci, passive congestion in the spleen with atrophic white pulp, pulmonary inflammation with congestion and thickening of alveolar walls, enlarged

kidneys, and necrosis of the epidermis and dermis. Animals that survived the observation period had no lesions that were different from the control group and 0.08- and 0.10-ml/kg dose groups. Animals in the remaining groups had cutaneous lesions and persistent renal lesions. All cutaneous lesions had healed by the end of the observation period (Duprat and Gradiski, 1979).

The acute dermal toxicity of Butoxyethanol was evaluated using 10 New Zealand white rabbits (weights 2.3–3.0 kg). A single dose (2.0 g/kg) of the test substance was applied to abraded skin of the abdomen that had been clipped free of hair. The test site was then covered with gauze, and the trunk was wrapped with an impervious material. At the end of the 24-h application period, the animals were observed daily for 14 days. The following signs of toxicity were observed at 24 h postapplication: lacrimation, blood in the urine, flaccid muscle tone, and anorexia. Additionally, spontaneous movement was not observed. All of the animals died on day 2 of the observation period (MB Research Laboratories, Inc. 1976).

In another study involving New Zealand white rabbits (four groups of four), the acute dermal toxicity of Butoxyethanol was evaluated. The four groups received doses of 0.25, 0.5, 1.0, and 2.0 g/kg Butoxyethanol, respectively. Applications were made to abraded abdominal skin that had been clipped free of hair. The test site was then covered with gauze, and the trunk was wrapped with an impervious material. At the end of the 24-h application period, the animals were observed daily for 14 days. One of the animals dosed with 0.5 g/kg Butoxyethanol and all of the animals that received larger doses died. There were no deaths in the 0.25-g/kg dose group. The LD_{50} was 0.58 g/kg (confidence limits = 0.31–0.85 g/kg) (MB Research Laboratories, Inc. 1976). No deaths were reported in another acute dermal toxicity study in which 10 New Zealand white rabbits received a single dose of Butoxyethanol (2.0 g/kg) according to the same procedure. Reactions observed at 24 h postapplication included erythema (five rabbits; Draize score = 1) and erythema and edema (five rabbits; Draize scores = 1) (MB Research Laboratories, Inc. 1977). Dermal LD_{50} values of 404–502 mg/kg (male rabbits) have been reported in other studies (NIOSH, 1990).

In an acute dermal toxicity test involving guinea pigs (Mellon Institute of Industrial Research, 1952), the number of animals and weights were not stated, but the LD_{50} for Butoxyethanol was recorded as 7.1 ml/kg (range 6.0–8.6 ml/kg).

Roudabush et al. (1965) evaluated the acute dermal toxicity of Butoxyethanol (undiluted) in male Hartley guinea pigs (weights 400–900 g). A minimum of three doses was applied, usually to four animals per dose. In one group of animals, the test substance was applied via a cellulose pad to clipped and depilated abdominal skin. In the other group, the test substance was applied (cellulose pad) to clipped and depilated abraded skin of the back. The test substance was held in place via an impervious cuff in both groups of animals; edges of the cuff were taped to the skin. LD_{50} values for abraded and intact skin, calculated according to the method of Finney (1952), were 0.30 and 0.23 ml/kg, respectively.

Short-Term Dermal Toxicity

The dermal toxicity of Butoxyethanol was evaluated using 24 male and 24 female New Zealand white rabbits (12–13 weeks old, 1.71–2.75 kg). Three groups of rabbits (five per

sex per group) were tested with Butoxyethanol concentrations of 5.0, 25, and 50%, respectively, and one group of four rabbits was tested with 100% Butoxyethanol. Two negative control groups (five males, five females) were treated with water. The test substance was introduced, via syringe or pipette, under a vinyl sheet that had been secured around the clipped dorsal surface of each animal. During the first week of treatment, the animals were subjected to 5 consecutive days of exposure (6 h/day). After a 2-day nontreatment period, the animals were subjected to an additional 4 consecutive days of exposure. At the conclusion of exposure, the animals were observed for a period of 14 days and then killed. None of the male rabbits died during the study. At necropsy, the lesions observed in one moribund female were not treatment related. By the sixth day of treatment, necrosis was severe and edema was moderate in animals that received applications of 100% Butoxyethanol. Butoxyethanol (50%) caused necrosis of the skin in one of five males and four of five females, and only erythema was observed in animals treated with 5 and 25% Butoxyethanol. In male rabbits, no statistically significant changes in body weight or organ weight (as percentage of body weight) were observed at any of the concentrations tested. In female rabbits, no statistically significant changes in organ weight were observed. However, body weights of female rabbits that received 100% Butoxyethanol were significantly less than those of the control group on days 3, 5, 8, 10, 11, and 18. Body weights of female rabbits that received 25% Butoxyethanol were significantly greater than those of the control group. Systemic effects observed in rabbits treated with 50 and 100% Butoxyethanol included hemoglobinuria (dose related in severity and time to onset) and equivocal proteinuria. Both effects were reversible at the conclusion of the application period. Concentrations of 5 and 25% Butoxyethanol did not cause any systemic effects. Gross findings included some thickening of the skin in male rabbits treated with 100% Butoxyethanol. Neither gross nor microscopic lesions observed were treatment related (Bushy Run Research Center, 1980*c*).

Subchronic Dermal Toxicity

The subchronic dermal toxicity of Butoxyethanol (in distilled water) was evaluated using 40 male and 40 female New Zealand white rabbits (weights 2.2–3.1 kg). The animals were divided into four groups of 20 (10 males, 10 females/group). Three groups of animals were dosed with 2.8% (10-mg/kg doses), 14.3% (50-mg/kg doses), and 42.8% (150-mg/kg doses) concentrations of Butoxyethanol, respectively. Twenty rabbits (control group) were dosed with distilled water. In each group, doses (0.35-ml/kg volumes) were applied to clipped skin of the back for 13 weeks (5 days/week). For each exposure, the test substance was applied to the skin for 6 h. Each site was covered with an occlusive dressing that was secured with hypoallergenic tape. The occlusive dressing was covered with three layers of polyvinylidene chloride wrap. Three animals died and one moribund animal was killed. These deaths were not related to administration of the test substance. Also, lesions observed in animals killed at the end of the study were not due to administration of the test substance. Such changes were related to subclinical parasitic or bacterial changes. No test substance-induced changes were noted in the other parameters measured: clinical observations, skin irritation, body weight changes, feed consumption, hematology parameters, erythrocyte fragility, differential leukocyte count, serum chemistry, organ weights, and final body weight or organ/final body weight ratios (Wil Research Laboratories, Inc. 1983).

Acute Intravenous Toxicity

Undiluted Butoxyethanol was injected intravenously into the ear vein of male albino rabbits (numbers and weights not stated). Increased respiration was observed in rabbits dosed with 0.05 ml/kg and transient prostration in rabbits dosed with 0.1 ml/kg. A dose of 0.2 ml/kg resulted in sudden death. The LD_{50} of undiluted Butoxyethanol was 0.14 ml/kg (Mellon Institute of Industrial Research, 1952).

The mean LD_{50} for female rats (numbers and weights not stated) injected intravenously with 3.0% Butoxyethanol in saline was 0.38 g/kg (range 0.29–0.50 g/kg). All of the rats died within 24 h after the injection of 0.3 g/kg; blood in the urine was noted at 3 h postinjection. The cause of death was pulmonary hemorrhage (Mellon Institute of Industrial Research, 1952).

In another study involving rabbits (numbers and weights not stated), the acute intravenous LD_{50} for 3.0% Butoxyethanol in saline was 0.50 g/kg (range 0.38–0.65 g/kg) (Mellon Institute of Industrial Research, 1952).

Acute Subcutaneous Toxicity

Single doses of Butoxyethanol were administered subcutaneously to 13 rabbits (weights and strain not stated). A dose of 180 mg/kg Butoxyethanol temporarily induced slight renal inflammation, and doses of 360–1,800 mg/kg caused death, associated with inflammation of the kidneys, within 20–72 h. Within 2 h after the administration of 2,700 mg/kg Butoxyethanol, death resulting from respiratory paralysis was noted (Gross, 1943).

In another study, no signs of illness were noted in a cat (weight not stated) injected subcutaneously with 900 mg/kg Butoxyethanol. However, a second cat injected subcutaneously with 1,800 mg/kg died at day 3 postinjection; signs of renal injury were noted (Gross, 1943).

Skin Irritation

The skin irritation potential of undiluted Butoxyethanol was evaluated in four studies in rabbits. In the first study, five rabbits (strain not stated) were exposed. The test substance (0.1 ml) was applied to intact skin of the abdomen; sites were not covered. Reactions were graded (scale of 1–10; Grade 1 = no signs of injury) based on the appearance of either moderate or marked capillary injection, erythema, edema, or necrosis within 24 h. Moderate capillary injection (Grade 2) was observed in two rabbits, and no signs of irritation were observed in the remaining three rabbits (Bushy Run Research Center, 1980*b*).

In the second study, male and female New Zealand white/white or Dendermont albino rabbits (weights 2.75–5.2 kg) were exposed to Butoxyethanol (in sweet almond oil) at concentrations of 5, 10, 25, and 50%. Applications were made to shaved skin adjacent to the spinal column (dorsolumbar zone) via a Teflon exposure chamber containing a patch immersed with 0.5 ml of the test substance. Each concentration remained in contact with the skin for 4 h. An exposure chamber containing 0.5 ml of sweet almond oil (vehicle control) was applied at a site on the opposite side of the spinal column. Untreated skin also served as a control. At the end of exposure, patches were removed and sites were washed with a detergent solution and water. Sites were examined for signs of erythema and edema

formation 1, 24, 48, and 72 h after patch removal and scored according to the Draize scale. Butoxyethanol was classified as a nonirritant (Jacobs et al., 1987).

In the third study, in six New Zealand white rabbits, Butoxyethanol was applied for 24 h to abraded and intact skin sites (clipped free of hair) on the left and right sides of the spinal column, respectively, via patches made of surgical gauze. Each patch, containing 0.5 ml of the test substance, was secured with adhesive tape, and the trunk of each animal was then wrapped with an impervious material. Reactions were scored 24 and 72 h after patch removal, and a primary irritation score was calculated. A primary irritant is defined as a substance that is not corrosive and has an empirical score of ≥ 5 [16 CFR 1500.3 (c)(4) and 1500.41]. Butoxyethanol was classified as a nonirritant (primary irritation score = 0.09) (MB Research Laboratories, Inc. 1977).

In the final study, Butoxyethanol was evaluated using six male New Zealand white rabbits. A patch containing 0.5 ml of the test substance was applied for 4 h to intact skin (clipped free of hair) on each animal. The authors concluded that Butoxyethanol was moderately irritating to the skin of rabbits (72-h mean irritation score = 2.7). The 72-h mean irritation score was defined as the sum of the mean erythema and edema scores at 72 h postdosing (Rohm and Haas Co., 1983).

Ocular Irritation

The ocular irritation potential of Butoxyethanol was evaluated using six healthy rabbits (strain not stated). The test procedure was divided into two phases, separated by a 6-month period. In the first phase, undiluted Butoxyethanol (100 μl) was instilled into the conjunctival sac of each rabbit; eyes were not rinsed. Mean scores for erythema, chemosis, and corneal opacity were determined at 24, 48, and 72 h postinstillation according to the Draize scale. In the second phase, Butoxyethanol (100 μl) was instilled into the conjunctival sac of each of three rabbits from the original group of six. In addition to the mean scores determined in the first phase of testing, a mean score for percentage of corneal swelling was also determined during the second phase. Corneal thickness was measured (in vivo) with an ultrasonic pachometer at 24, 48, and 72 h postinstillation. Ocular lesions were considered significant or to have the potential for causing serious ocular damage if mean scores of ≥ 2 (corneal opacity), 2.5 (erythema), and 2 (chemosis) were reported. Based upon a plot of mean scores (34 different substances, rabbit eyes in vivo) for corneal opacity versus mean percentage of corneal swelling, it was deduced that a mean value of 80% for corneal swelling corresponds to a mean corneal opacity score of 2. The mean scores reported were as follows: 2.51 (erythema), 0.89 (chemosis), 1.73 (corneal opacity), and 70% (corneal swelling) (Jacobs and Martens, 1989).

In another study, in nine male and female New Zealand white rabbits, the test substance (0.1 ml) was instilled onto the corneal surface, and the animal was then allowed to blink freely. The cornea and surrounding area were covered with the test substance, even though ~25% of the volume instilled was lost from the treated eye. The eyes of three animals were rinsed after instillation of Butoxyethanol. Reactions were scored at 24, 48, and 72 h and days 7, 14, and 21 postinstillation according to the Draize scale (0–110). Ocular irritation reactions persisted beyond day 21, and the authors concluded that Butoxyethanol was severely irritating to the eyes of rabbits (Rohm and Haas Co., 1983).

Butoxyethanol (0.1 ml) was instilled into the conjunctival sac of one eye of six New

Zealand white rabbits. Contralateral eyes served as controls. Reactions were graded in accordance with the Consumer Product Safety Act (16 CFR 1500.42) and the Draize scale (0–110). Total Draize ocular irritation scores ranged from 11 to 17, and the test substance was classified as an ocular irritant (MB Research Laboratories, Inc. 1977).

Undiluted Butoxyethanol (0.005 ml) and 5.0% (0.5 ml) and 15.0% (0.5 ml) Butoxyethanol in distilled water were instilled into the conjunctival sac of five rabbits (strain not stated). Reactions were scored immediately after instillation and again at 24 h postinstillation after staining with fluorescein. Undiluted Butoxyethanol caused severe corneal injury and iritis. Moderate corneal injury and no corneal injury were observed after the instillation of 15.0 and 5.0% Butoxyethanol, respectively (Bushy Run Research Center, 1980*b*).

Nephrotoxicity

The nephrotoxicity of Butoxyethanol was evaluated using five female Sprague–Dawley rats (2–4 months old, 150–250 g). The animals were injected intraperitoneally with Butoxyethanol (0.055 g/kg in olive oil or water) five times per week for 2 weeks, and the urinary excretion of *N*-acetyl-β-D-glucosaminidase, β_2-microglobulin, and albumin was determined. The dose of Butoxyethanol injected (0.055 g/kg) represented 10.0% of the intraperitoneal LD_{50}. Animals in the control group were injected with olive oil (2.5 ml/kg). Increased urinary excretion of albumin and β_2-microglobulin is indicative of glomerular damage and tubular impairment, respectively. The baseline excretion of *N*-acetyl-β-D-glucosaminidase represented 20.0% of the total activity in the kidneys (Lockwood and Bosmans, 1979). Butoxyethanol did not affect the urinary excretion of albumin, *N*-acetyl-β-D-glucosaminidase, or β_2-microglobulin (Bernard et al., 1989).

In another study, the nephrotoxicity of Butoxyethanol was evaluated using 10 female SPF Sprague–Dawley rats (weights 200 ± 20 g). Such an evaluation was accomplished via the use of urine samples for the analysis of various parameters of renal function. The test substance, 0.034 ml/kg body wt in physiological saline (2.5 ml/kg), was injected intravenously into each animal. During the next 4 days, 24-h urine samples were collected. Control 24-h urine samples were collected prior to intravenous injection. In urine collected on the second day, the albumin content and lactate dehydrogenase activity were increased. Additionally, β-galactosidase activity was decreased in urine collected on the fourth day. Urinary activity of leucine arylamidase and the volume and osmotic pressure of the urine remained unchanged. Butoxyethanol caused a microhematuria (~50 erythrocytes/μl) in two of the rats on day 1 postinjection. The authors considered the results consistent with disturbances in the whole nephron (Freundt and Helm, 1986).

Cytotoxicity

The in vitro cytotoxicity of Butoxyethanol was evaluated using Chinese hamster ovary (CHO) cells. The EC_{50} (concentration allowing ~50% of the seeded cells to form colonies) served as the index of cytotoxicity. Initially, the cells were maintained in the logarithmic phase of growth and subcultured twice weekly. Karyotype and growth rate, checked monthly, were stable. For the cytotoxicity determination, ~200 cells were seeded into 25-cm^2 culture flasks. Butoxyethanol was added to the medium 4–5 h later. At 16 h, the medium was renewed and cells were allowed to grow in colonies for 6–7 days. The

resulting colonies were dried, fixed with methanol, stained, and counted. Butoxyethanol was classified as a cytotoxic compound (EC_{50} = 6.9 mg/ml) (Jackh et al., 1985).

Hematotoxicity

This section presents details of the hematotoxicity findings in 20 separate studies of Butoxyethanol in a wide range of experimental animals and humans. Tests were both in vivo and in vitro. The results of each of these studies are summarized in Table 7.

In Vivo Exposure

A series of inhalation studies were reported by the Mellon Institute of Industrial Research in rats, rabbits, and dogs. A group of six female rats (weights not stated) inhaled Butoxyethanol (200 ppm) 5 days/week (7 h/day) followed by a 17-h nontreatment period. The fragility of erythrocytes was determined before and after each exposure daily for a period of 9 days. Each day, erythrocyte fragility increased from initial hemolysis in 0.48% saline (before exposure) to initial hemolysis in 0.52–0.68% saline after exposure. By the end of the nontreatment period, the values for erythrocyte fragility had returned to normal. In another group of six female rats concurrently exposed to 200 ppm Butoxyethanol, the rats became anemic after 26 h of exposure over a period of 4 days. The erythrocyte count was approximately half that of the preexposure value, and hemoglobin had decreased from ~90.0 to 65.0%. After five exposures (6 or 7 h) to 200 ppm Butoxyethanol, followed by a 7-day nontreatment period, a marked improvement in both the erythrocyte count and the hemoglobin values was observed. However, in most cases, these values remained below those determined prior to exposure. When the rats were subjected to three additional exposures, the erythrocyte count was reduced and hemoglobin values were smaller than those noted after the first five exposures. Subsequently, after an 8-day nontreatment period, a measurable increase in both erythrocyte count and hemoglobin values was observed. However, erythrocyte counts remained below normal values. Both leukocyte counts and values for erythrocyte fragility were within control ranges (Mellon Institute of Industrial Research, 1952).

In a second study, rats (numbers and weights not stated) inhaled Butoxyethanol (25 ppm) for 7 h. While increased erythrocyte fragility was seen in the 25-ppm group, it was observed in only one of four rats exposed to 10 ppm (Mellon Institute of Industrial Research, 1952).

One rabbit (weight not stated) inhaled 200 ppm Butoxyethanol for 10 h. Erythrocyte fragility was determined periodically until the cells had returned to normal. The saline concentrations resulting in initial and complete hemolysis of blood samples, respectively, were as follows: 0.60–0.44% (3–180 min postexposure), 0.52–0.40% (240–420 min postexposure), 0.48–0.40% (480 min postexposure), and 0.48–0.36% (25 h postexposure) (Mellon Institute of Industrial Research, 1952).

In a study involving two dogs (weights not stated), inhalation (3 days, 7 h/day) of 665 ppm Butoxyethanol did not cause any increase in erythrocyte fragility. Dogs were said to be unlike rats in their response to Butoxyethanol vapor (Mellon Institute of Industrial Research, 1952). In a second inhalation study involving two dogs (weights not stated), single 8-h exposures to 419 and 624 ppm Butoxyethanol also did not increase erythrocyte fragility (Mellon Institute of Industrial Research, 1952).

After each of four rats was fed 10.0% aqueous Butoxyethanol (dose 1.48 g/kg), erythrocyte fragility was increased markedly. In one rat, the saline concentrations resulting in initial and complete hemolysis of blood samples were 0.48 and 0.40%, respectively, 15 min after dosing. The respective saline concentrations were 0.72% (initial hemolysis) and 0.60% (complete hemolysis) in the remaining three rats at 50 min, 130 min, and 4.5 h after dosing (Mellon Institute of Industrial Research, 1952).

The hemolytic activity of Butoxyethanol was evaluated using 15 female Wistar rats (average body weight 161 ± 9 g). The test substance was applied (single dose) to three groups of three rats at doses of 260, 375, and 500 mg/kg, respectively. A dose of 200 mg/kg was applied to each of six rats. Applications were made to shaved skin of the back. After test substance application, all animals were given 1.5 ml of tap water via intubation, followed by an additional 1.5 ml after 5 h. At 6 h postapplication, blood was sampled retroorbitally and animals were killed via CO_2 asphyxiation. At a dose of 200 mg/kg, Butoxyethanol did not induce hemolysis or hemoglobinuria. Doses of 260, 320, 375, and 500 mg/kg caused hemolytic effects, indicated by such findings as an increase in mean cell volume, a lowered erythrocyte count and hemoglobin value, and hemoglobinuria in some of the test animals in each dose group. There was no discernible dose–response relationship between 260- and 500-mg/kg doses (Bartnik et al., 1987).

In another study, the hemolytic activity of Butoxyethanol was evaluated using 20 male Wistar rats (weights 200 ± 7 g). The test substance was administered (injection into tail vein) to five groups of rats (four rats per group) in doses of 25, 37.5, 50, 62.5, and 75 mg/kg, respectively. Doses were adjusted to a constant volume of 5 ml/kg and infused at a rate of 1 ml/min. At 3 and 6 h postinjection, blood was collected retroorbitally. The animals were killed with ether, and additional blood samples were obtained via cardiac puncture. The sera were analyzed for hemoglobin via spectrophotometry; measurements were made at 541 nm. Hemoglobin was not detected in the sera of rats injected with a dose of 62.5 mg/kg. However, in half of the animals receiving 75-mg/kg doses, there was an increase in absorbance at 541 nm that was >25% above the mean value of the control (Bartnik et al., 1987).

A significant reduction in red blood cell counts was reported for male JCL-ICR mice dosed orally with 500 ($p < 0.05$) or 1,000 ($p < 0.01$) mg/kg Butoxyethanol 5 days/week for 5 weeks. There were no effects on leukocyte counts, mean corpuscular volume, or hemoglobin concentrations (Nagano et al., 1979; NIOSH, 1990).

The results of a fragility test on blood drawn from rabbits 1 h after the injection of 3.0% Butoxyethanol (dose 0.5 g/kg) indicated initial hemolysis in the tube containing 0.60% NaCl and complete hemolysis in the tube containing 0.48% NaCl. At 2 h postinjection, initial hemolysis was observed in the tube containing 0.57% NaCl. The results of a fragility test on blood drawn from rabbits 0.5 h after the injection of 3.0% Butoxyethanol (dose 0.316 g/kg) indicated initial hemolysis in the tube containing 0.52% NaCl and complete hemolysis in the tube containing 0.44% NaCl. At 1 h postinjection, initial hemolysis was observed in the tube containing 0.64% NaCl and complete hemolysis in the tube containing 0.56% NaCl. At 1.5 h postinjection, initial hemolysis was observed in the tube containing 0.72% NaCl and complete hemolysis in the tube containing 0.68% NaCl. The normal values for initial and complete hemolysis of rabbit blood are 0.44 and 0.36% NaCl, respectively (Mellon Institute of Industrial Research, 1952).

The development of tolerance to 2-Butoxyethanol-induced hemolytic anemia was

TABLE 7. *Hematotoxicity of Butoxyethanol*

Animal/human	Dose/concentration	Results	Ref.
6 rats (wts not stated)	Inhalation of 200 ppm daily for 9 days	Increased erythrocyte fragility, followed by recovery	Mellon Institute of Industrial Research (1952)
Rats (no. and wts not stated)	Inhalation of 25 ppm for 7 h	Increased erythrocyte fragility	Mellon Institute of Industrial Research (1952)
4 rats (wts not stated)	Inhalation of 10 ppm for 7 h	Increased erythrocyte fragility in 1 rat	Mellon Institute of Industrial Research (1952)
1 rabbit (wt not stated)	Inhalation of 200 ppm for 10 h	Saline concentrations causing hemolysis of blood samples decreased with time postexposure	Mellon Institute of Industrial Research (1952)
2 dogs (wts not stated)	Inhalation of 665 ppm daily for 3 days	No increase in erythrocyte fragility	Mellon Institute of Industrial Research (1952)
2 dogs (wts not stated)	Single 8-h inhalation exposures to 419 and 624 ppm	No increase in erythrocyte fragility	Mellon Institute of Industrial Research (1952)
4 rats	Single oral dose of 10% aqueous Butoxyethanol (1.48 g/kg)	Increased erythrocyte fragility	Mellon Institute of Industrial Research (1952)
15 Wistar rats (av. wt 16 ± 9 g)	Groups of 3 rats received single dermal doses of 260, 375, and 500 mg/kg; 6 rats dosed with 200 mg/kg	All doses >200 mg/kg caused hemolytic effects	Bartnik et al. (1987)
20 Wistar rats (wts 200 ± 7 g)	Groups of 4 rats received single i.v. doses of 25, 37.5, 50, 62.5, and 75 mg/kg	Hemoglobin detected in sera of rats dosed with 75 mg/kg	Bartnik et al. (1987)
Rabbits (no. and wts not stated)	Injection of 3% Butoxyethanol (dose 0.5 g/kg)	Saline solution concentrations causing initial and complete hemolysis in blood samples were greater than normal	Mellon Institute of Industrial Research (1952)
F344 rats	Blood samples incubated with concentrations of 5, 10, or 20 m*M* Butoxyethanol in whole blood	Only 20 m*M* Butoxyethanol caused significant increase in packed cell volume and hemolysis	Ghanayem (1989)
4 Wistar rats	Blood samples incubated with Butoxyethanol in PBS buffer	175–200 mmol/L Butoxyethanol caused complete lysis of erythrocytes	Bartnik et al. (1987)
Rats, dogs, rabbits, humans	Erythrocyte suspensions added to Butoxyethanol (in saline) concentrations of 0.05–0.5%	Lysis of dog erythrocytes over entire concentration range; erythrocytes from rats, rabbits, and humans lysed at concentrations of ⩾0.25%	Imperial Chemical Industries (1985)
Humans	NB4 human promyelocytic cell line incubated with Butoxyethanol	IC_{50} = 5 m*M* at 6 h and 0.1 m*M* at 96 h; potent hematopoietic toxicity	Boiron (1991)

TABLE 7. *Continued*

Animal/human	Dose/concentration	Results	Ref.
Humans	DA1 human factor-dependent cell line	IC_{50} = 80 μ*M* at 48 h; potent hematopoietic toxicity	Boiron (1991)
Humans	Human myelocytic leukemia NB4 cell line; test concentrations of 0.1 μ*M* to 0.1 *M*	Dose–response curve suggests that cell viability went from normal to nil when concentrations were increased by factors of 4–8	Ruchaud et al. (1992)
Humans	Human umbilical cord cells incubated with Butoxyethanol	Dramatic reduction in growth of hematopoietic colonies; virtually no growth at concentrations >20 m*M*	Ruchaud et al. (1992)
B6/D2 F1 mice	Bone marrow cultures incubated with Butoxyethanol	After incubation with 7.5 m*M*, practically no hematopoietic cells; aplastic cultures with no hematopoiesis obtained at doses between 7 and 15 m*M*; hematopoietic microenvironment destroyed at doses >30 m*M*	Ruchaud et al. (1992)
Fischer 344 rats (10–14 wks old, 6 rats/group)	Doses of 125 mg/kg by gavage; 1 group received single dose and 5 groups repeated doses	Significant hemolysis induced by single dose; with repeated exposures, a time-related increase in erythrocytes and hemoglobin was noted	Ghanayem et al. (1992)
Sprague–Dawley rats (145–350 g)	Doses of 50–500 mg/kg by gavage; physical characteristics of erythrocytes in blood samples determined by scanning electron microscopy	Induced membrane and cellular changes in erythrocytes alter their rheological properties; microvessel transit is compromised, leading to hemolysis	Kurantsin-Mills et al. (1992)

evaluated using five groups of male Fischer 344 rats (10–14 weeks old, 6 rats/group). One group received a single dose (by gavage) of 125 mg Butoxyethanol in 5 ml water/kg body wt, and the remaining five groups received the same dose for 2, 3, 6, and 12 consecutive days, respectively. The control group received 5 ml water/kg body wt daily for 12 consecutive days. Data were presented as the means ± SE of four to five rats. At 24 h postadministration of the last dose, the rats were anesthetized in a CO_2 chamber and blood samples were collected from the retroorbital venous sinus. The administration of 125 mg/kg Butoxyethanol (single dose) resulted in significant hemolysis of red blood cells; decreased numbers of red blood cells and decreased hemoglobin and hematocrit were noted. This effect was more pronounced after the third day of dosing; gradual recovery was observed thereafter. Repeated exposure to Butoxyethanol beyond day 3 caused a gradual increase in red blood cells and hemoglobin; by day 12, these values approached those noted prior to treatment. A similar pattern for changes in the hematocrit was also noted. Adenosine triphosphate concentration, mean corpuscular volume, and the number of reticulocytes increased as dosing continued through day 6. By day 12, these values had declined, but remained greater than those noted in controls. Spleen weight/body weight ratios increased markedly, reaching a maximum after day 6; a decline in this ratio was

noted between days 6 and 12. Liver weight/body weight ratios were minimally affected by repeated doses of Butoxyethanol. A moderate decline in this ratio was noted between days 3 and 6, and an increase over that observed in controls was observed on day 12. The authors concluded that chronic exposure to Butoxyethanol would be expected to result in tolerance to Butoxyethanol-induced hemolytic anemia. Further studies, in vitro and in vivo, involving male Fischer 344 rats (10–14 weeks old) were performed to investigate the underlying mechanism(s) of tolerance to the hematotoxicity of Butoxyethanol. Based on these studies, it was concluded that the mechanisms responsible are likely related to the fact that older cells are more susceptible to the effects of Butoxyethanol and its metabolite, butoxyacetic acid. Furthermore, hemolysis of the older cells during the initial exposure, followed by their replacement with less susceptible, younger cells, may account for the development of tolerance (Ghanayem et al., 1992).

The rheological mechanisms associated with the hematotoxicity of Butoxyethanol were evaluated using Sprague–Dawley rats (weights 145–350 g). Butoxyethanol was administered (by gavage) in doses of 50–500 mg/kg body wt. Blood samples were obtained after 0.5, 2, and 4 h, and the physical characteristics of erythrocytes were determined using scanning electron microscopy. The frequency distribution of erythrocytes was determined by flow cytometry. The results of this study indicate that membrane and cellular changes in erythrocytes that are due to Butoxyethanol or its metabolites alter their rheological properties. Therefore, transit through the microvessels is compromised, resulting in hemolysis (Kurantsin-Mills et al., 1992).

In Vitro Exposure

The hematotoxicity of Butoxyethanol and its metabolites, butoxyacetic acid and butoxyacetaldehyde, was evaluated using F344 male rats (9–13 weeks old). The extent of erythrocyte hemolysis was determined by measuring the concentration of free hemoglobin in the plasma. Blood samples, obtained via cardiac puncture, were pooled and gently mixed. The mixture of blood with solutions of Butoxyethanol (in 0.9% saline) resulted in final concentrations of 5.0, 10.0, or 20.0 m*M* Butoxyethanol in whole blood. The final concentrations of butoxyacetic acid and butoxyacetaldehyde in whole blood were 0.5, 1.0, or 2.0 m*M*, respectively. Mixtures were incubated (37°C) in a shaking water bath for 0.25–4 h. Concentrations of either 5 or 10 m*M* Butoxyethanol caused a slight (statistically insignificant) increase in packed cell volume and no significant hemolysis of erythrocytes. Butoxyethanol (20 m*M*) caused a slight but significant decrease in packed cell volume. This decrease was accompanied by significant erythrocytic hemolysis. Concentrations of butoxyacetaldehyde (0.5, 1.0, or 2.0 m*M*) caused both time- and concentration-dependent swelling of erythrocytes and increased packed cell volume. Statistically significant hemolysis was noted only after 4 h of incubation with 2 m*M* butoxyacetaldehyde. Concentrations of 0.5 or 1.0 m*M* butoxyacetic acid also caused both a time- and concentration-dependent increase in packed cell volume, followed by hemolysis. Butoxyacetic acid (2.0 m*M*) caused a faster time-dependent increase in packed cell volume. The maximum increase was reached at 2 h, and there was nearly complete hemolysis by 4 h (Ghanayem, 1989).

Following the incubation of blood (rats) with [^{14}C]Butoxyethanol according to the

procedure in the preceding paragraph, an HPLC analysis of plasma and blood extracts demonstrated that no Butoxyethanol metabolites had been formed, indicating that rat blood was unable to metabolize Butoxyethanol in vitro. In another experiment, the hemolytic activity of Butoxyethanol in rat blood was not shown in the presence of exogenous alcohol and aldehyde dehydrogenases and their cofactors, NADP and NAD, respectively. Therefore, the addition of exogenous enzymes had no effect on the hemolytic activity of Butoxyethanol in rat blood (Ghanayem, 1989).

In another study, the in vitro hemolytic activities of Butoxyethanol and butoxyacetic acid were evaluated using four male Wistar rats (300–400 g). Blood was collected via heart puncture and erythrocytes were separated via centrifugation. Aliquots of 2% erythrocyte suspensions (12.5 ml) were treated with phosphate-buffered saline (PBS) buffer (12.5 ml) containing Butoxyethanol and then incubated. Erythrocyte suspensions were similarly treated with PBS buffer containing butoxyacetic acid. Hemolytic activity was expressed as the percentage of totally hemolyzed control. After 1 h of incubation, 7.5 m*M* butoxyacetic acid caused complete lysis of rat erythrocytes, whereas 175–200 m*M* of Butoxyethanol was required. The hemolytic activity of Butoxyethanol (200 m*M*) in rat erythrocytes (100%) was much greater than what was observed in human erythrocytes (8.8%) after 1 h of incubation. Also, after 1 h of incubation, butoxyacetic acid (7.5 m*M*) did not cause hemolysis of human erythrocytes (Bartnik et al., 1987).

To assess the susceptibility of human erythrocytes to the hemolytic effects of butoxyacetic acid, a metabolite of Butoxyethanol, blood from healthy male and female volunteers (18–40 years old) was incubated with butoxyacetic acid concentrations of 1.0, 2.0, 4.0, and 8.0 m*M*. There were no significant changes either in packed cell volume or erythrocytic hemolysis at concentrations below 4.0 m*M*. At the maximum concentration tested (8.0 m*M*), there was a significant increase in packed cell volume, followed by significant erythrocytic hemolysis. Erythrocytes obtained from female subjects were slightly more sensitive to the effects of butoxyacetic acid, all concentrations, than were those obtained from male subjects (Ghanayem, 1989).

The effect of Butoxyethanol and its metabolite, butoxyacetic acid, on erythrocyte fragility was evaluated using blood samples from rats, dogs, rabbits, and humans. Blood samples from dogs, rabbits, and humans were obtained via venipuncture; blood samples from rats were obtained via cardiac puncture. In all experiments, 1 ml of erythrocyte suspension was added to 3 ml of test solution. Solutions of both test substances (pH 7.2) were prepared with 0.60% sodium chloride. Erythrocytes from dogs were lysed by Butoxyethanol over the entire range of concentrations tested (0.05–0.5%). Erythrocytes from rats, rabbits, and humans were lysed at Butoxyethanol concentrations of 0.25% and higher. In the presence of butoxyacetic acid, erythrocytes from rats were lysed at concentrations of $\geq$0.05%. However, erythrocytes from dogs, rabbits, and humans were stable at concentrations of butoxyacetic acid up to 2% (Imperial Chemical Industries, 1985).

Butoxyethanol was hematotoxic in the NB4 human promyelocytic cell line. After incubation with Butoxyethanol for 6 h, the IC_{50} (inhibitory concentration, concentration that reduced cell viability in culture by 50.0%) was 5 m*M*; an IC_{50} of 0.1 m*M* was reported at 96 h. In another hemopoietic cell line, the factor-dependent cell line (DA1), the IC_{50} was 80 μM at 48 h. The authors concluded that Butoxyethanol was a potent hematopoietic toxin (Boiron, 1991).

The hematotoxicity of Butoxyethanol was evaluated using the following cell lines: established hematopoietic cell lines (either leukemic or growth-factor-dependent cell lines), murine marrow cells or human cord blood cell suspensions (to evaluate hematopoietic progenitor growth and differentiation), and bone marrow long-term cultures from female B6/D2 F1 mice (to determine the effects on both the hematopoietic and stromal cell tissues). The three leukemic cell lines used were derived from the mouse, rat, and human, respectively: the myelo-monocytic cell line WEHI-3b D^+, the rat promyelocytic cell line IPC-81 derived from the BN rat leukemia, and the human promyelocytic leukemia NB4. The murine factor-dependent cell lines used were NFS-60 and DA1. Butoxyethanol was tested for 48 h at various concentrations between 0.1 μM and 0.1 M, using serial half-dilutions. Cell viability was judged by morphological intactness of cells under phase contrast microscopy, by the ability of cells to exclude 0.2% trypan blue under bright-field microscopy, and by the dimethylthiazoldiphenyl tetrazolium bromide colorimetric assay for mitochondrial dehydrogenase enzymic activity. The dose–response curve for Butoxyethanol in human promyelocytic leukemia NB4 illustrates the common feature of a steep slope on a semilog plot, indicating that cell viability went from normal to nil by increasing concentrations by factors of 4 to 8. This steep slope and sigmoid shape of the curve suggested that Butoxyethanol hindered some cellular mechanisms that are essential for survival. Combined dose–response/time–course analyses were performed using the human promyelocytic leukemia cell line NB4. Butoxyethanol was also tested at concentrations between 0.1 M and 0.1 μM. The results indicated that toxicity increased regularly with time over a 96-h treatment period. The authors stated that, based on these results, there is no indication that very low doses (<100 μM) could be devoid of deleterious effects on growth and survival during long-term (>1 week) exposure. The following IC_{50} values were reported in similar experiments in which cell lines were incubated with Butoxyethanol for 48 h: 0.4 mM (NB4 cells), 2.4 mM (IPC-81 cells), 1.2 mM (WEHI-3b cells), 0.08 mM (DA1 cells), and 0.38 mM (NFS-60 cells). The IC_{50} for 48 h of incubation corresponded to the concentration (mM) for which the cell viability in treated cultures was 50% of the viability in untreated control cultures (Ruchaud et al., 1992).

In another hematotoxicity experiment (Ruchaud et al., 1992), human umbilical cord cells were treated with Butoxyethanol for 15 h prior to assay plating for colony-forming units in culture medium. The cells were then incubated for 14 days with human bladder carcinoma cell line 5637–conditioned medium (source of growth factors) for 14 days. Incubation with Butoxyethanol resulted in a dramatic reduction in the growth of hematopoietic colonies. There was virtually no growth at concentrations of >20 mM.

In a third experiment (Ruchaud et al., 1992), Butoxyethanol was tested on bone marrow long-term cultures from female B6/D2 F1 mice to determine the effects on both the hematopoietic and stromal cell tissues. Two weeks after the cultures were established, hematopoiesis was monitored for 2 months during constant exposure to various concentrations of Butoxyethanol. The acute toxicity of Butoxyethanol was obvious after 4 days of exposure to a concentration of 7.5 mM. Practically no hematopoietic cells were detected in the culture medium, and only a few hematopoietic foci were present within the layer of stromal cells. After 2 weeks of exposure, aplastic cultures were obtained at concentrations between 7 and 15 mM Butoxyethanol. In these cultures, the stromal cell layer was unaffected, but there was no hematopoiesis. The hematopoietic microenvironment was destroyed at doses above 30 mM.

Immunotoxicity

The immunotoxicity of Butoxyethanol was evaluated using four groups of six adult male Fischer 344 rats. The rats were immunized with a single intravenous injection of 0.5 ml of 40 μg/ml trinitrophenyl-lipopolysaccharide (TNP-LPS), in sterile saline, on 2 consecutive days according to the procedure of Smialowicz et al. (1991*a,b*). Two to 4 h later, three groups of rats were dosed (by gavage) with 50, 100, and 200 mg/kg Butoxyethanol, respectively, for 2 consecutive days and the control group was dosed with distilled water. Butoxyethanol was toxic at doses of 200 and 400 mg/kg/day. One of the rats dosed with 200 mg/kg/day died and the other was moribund on the day of the assay. All of the rats that received 400 mg/kg/day died. A significant reduction ($p < 0.05$; four rats) in the serum hemagglutination titer was reported for the 200-mg/kg/day dose group; this was the only alteration in the immune response to TNP-LPS. The authors stated that this alteration was probably due to the overt toxicity of Butoxyethanol at doses of 200 mg/kg/day (Smialowicz et al., 1992).

Four groups (two groups of six males, two groups of six females) of Sprague–Dawley outbred rats received oral doses of Butoxyethanol in deionized drinking water for 21 consecutive days. The concentrations and doses of Butoxyethanol administered were as follows: 1,600 ppm, 204 ± 3 mg/kg/day (six females, weights 225 ± 3 g); 2,000 ppm, 180 ± 8 mg/kg/day (six males, 347 ± 8 g); 4,800 ppm, 444 ± 15 mg/kg/day (six females, 223 ± 4 g); and 6,000 ppm, 506 ± 10 mg/kg/day (six males, 335 ± 8 g). The control group (six males, six females) received deionized water. On days 7 and 13 of Butoxyethanol administration, an aqueous solution of the antigen KLH (keyhole limpet hemocyanin) was subcutaneously injected at the base of the tail of each animal. The purpose of the second injection was initiation of an IgG antibody response. On day 20, heat-aggregated KLH was injected into the right footpad of each animal and saline into the left footpad. The animals were killed via CO_2 asphyxiation on day 21. Serum samples were collected for analysis of antibody levels to KLH, and spleens were used as the cell source for the assessment of natural killer cell cytotoxicity and interleukin-2 and interferon production. There were no microscopic changes in the thymus or testes obtained from experimental or control animals. Liver weights, expressed as a percentage of body weight, were significantly increased ($p \leq 0.05$) in male rats dosed with 2,000 ppm Butoxyethanol. In female rats dosed with 1,600 ppm Butoxyethanol, kidney weight (actual weight) was significantly decreased ($p \leq 0.05$). Microscopic changes were not observed in the liver or kidneys of male and female rats. Natural killer cell cytotoxic responses were enhanced in male rats dosed with 2,000 ppm Butoxyethanol and in female rats dosed with 1,600 ppm Butoxyethanol. No effect on antibody production and delayed-type hypersensitivity reactions were observed in any of the treatment groups. The cellular infiltrate in the KLH-injected area of the footpad consisted chiefly of mononuclear phagocytes; this is typical of a type IV hypersensitivity reaction (Exon et al., 1991).

MUTAGENICITY

Results from several mutagenicity test systems, including Ames tests, sister chromatid exchange (SCE) assays, unscheduled DNA synthesis, chromosome aberrations, point mutations, and forward mutations in CHO cells, are reported. Overall, the results suggest that Butoxyethanol is not mutagenic.

The mutagenicity of Butoxyethanol (99.0% pure) was evaluated using strains TA98, TA100, TA1535, TA1537, and TA97 of *Salmonella typhimurium.* Each strain was tested (with and without metabolic activation) with Butoxyethanol at concentrations ranging from 100 to 10,000 μg/plate. Untreated cultures served as controls. The positive controls in the absence of metabolic activation were sodium azide (strains TA100 and TA1535), 9-aminoacridine (strains TA1537 and TA97), and 4-nitro-*o*-phenylenediamine (strain TA98). The positive control for metabolic activation in all strains was 2-aminoanthracene. Butoxyethanol was not mutagenic at any of the concentrations tested. All of the positive controls were mutagenic (NTP, 1993*a*).

The mutagenicity of a mixture containing Butoxyethanol (18%), isopropyl alcohols (18%), fluorochemical salt (27%), and water (37%) was evaluated (Ames test) using strains TA100, TA98, TA1535, TA1537, and TA1538 of *S. typhimurium* in the presence and absence of metabolic activation. The mixture was tested at concentrations that ranged from 10 to 5,000 μg/plate. Sodium azide, 9-aminoacridine, 2-nitrofluorene, and 2-anthramine served as positive controls, and water served as the negative control. There were no dose-related increases in the number of histidine-independent revertants in the presence or absence of metabolic activation. It was concluded that the mixture was reproducibly nonmutagenic. All positive controls were mutagenic (SRI International, 1985).

In another study, the mutagenicity of a mixture containing 2.0% Butoxyethanol, trichlorobenzene, and anionic emulsifiers was evaluated in *S. typhimurium* and *Escherichia coli* reverse mutation assays. The mixture (concentrations of 312.5–5,000 μg/plate) was tested in strains TA98, TA100, TA1535, and TA1537 of *S. typhimurium* and strain WP2 of *E. coli*, both with and without metabolic activation. The following positive controls were used: sodium azide, 9-aminoacridine, 2-nitrofluorene, *N*-methyl-*N*-nitro-*N*-nitrosoguanidine, and 2-anthramine. Ethanol served as the solvent control. There was no evidence of genetic activity in cultures incubated with the Butoxyethanol mixture. All positive controls were mutagenic (Dow Corning Corp., 1988).

The mutagenic potential of Butoxyethanol was evaluated using the unscheduled DNA synthesis assay. The stimulation of incorporation of tritiated thymidine into both purified hepatocyte nuclei and DNA served as the indicator of chemically induced DNA damage. Butoxyethanol was tested at concentrations ranging from 0.0001 to 0.1% by volume. Dimethyl sulfoxide (DMSO) served as the solvent control and 4-nitroquinoline and dimethylnitrosamine served as positive controls. Statistically significant induction of unscheduled DNA synthesis was observed only at Butoxyethanol concentrations of 0.0001 and 0.001%. In positive control cultures, 4-nitroquinoline caused statistically significant unscheduled DNA synthesis at all concentrations tested. The results were also statistically significant in cultures exposed to concentrations of 100 and 1,000 μg/ml dimethylnitrosamine, but not in those exposed to 300 μg/ml dimethylnitrosamine. Analyses of the data via linear regression indicated that the correlations between the values reported with nuclei and dosage were statistically significant. As a second assessment of unscheduled incorporation of radioactive thymidine, analyses of DNA from aliquots of hepatocyte nuclei isolated from cells in the preceding two experiments were performed. The data resulting from these analyses were essentially identical to the data obtained through analyses of radioactive incorporation into nuclei. However, certain findings made it difficult to determine the biological significance of the results. First of all, linear regression analyses of the dose–response values indicated that the data were statistically significant,

but the correlation coefficient was negative. Next, a maximum effect was observed with 0.0001% Butoxyethanol when DNA was analyzed. However, a maximum effect was observed with 0.001% Butoxyethanol when nuclei were analyzed. In that only the two lowest concentrations tested (0.0001 and 0.001% Butoxyethanol) in the unscheduled DNA synthesis assay resulted in a significant response, it was suggested that the lack of a wider dose–response was due to possible Butoxyethanol-induced inhibition of either uptake or inhibition of incorporation of [^{3}H]thymidine in hepatocytes. The authors concluded that Butoxyethanol was weakly active in the induction of DNA damage in vitro (Bushy Run Research Center, 1980*d*).

The induction of point mutations in vitro by Butoxyethanol was evaluated using CHO cells. Cultures were incubated with Butoxyethanol, concentrations up to 1%, for 5 h in both the presence and the absence of metabolic activation. The incubation period was followed by a 7-day expression period. A single, high mutation frequency was noted in the presence of metabolic activation at the lowest concentration tested. However, the response was not dose-related. The authors concluded that Butoxyethanol was not mutagenic at the HGPRT locus of CHO cells (McGregor, 1984).

The mutagenicity of Butoxyethanol was evaluated in a forward mutations assay using CHO cells. CHO cells were exposed for 5 h to Butoxyethanol concentrations of 0.0625–1.0% vol/vol (without metabolic activation) and 0.03125–0.5% vol/vol (with metabolic activation). Water and DMSO served as solvent and negative controls, respectively, both with and without metabolic activation. Dimethylnitrosamine and ethylmethanesulfonate served as positive controls with and without metabolic activation, respectively. Over the range of concentrations tested, Butoxyethanol did not cause a significant increase in the frequency of mutants per 10^6 viable cells either with or without metabolic activation. The mutation frequencies in both the positive and the solvent control cultures were within the range of historical control values. The statistically significant value for the negative control, DMSO, was within the normal expected variation observed in historical control data and was not considered biologically significant. The authors concluded that a dose–response relationship was not evident in the data and that Butoxyethanol was not mutagenic (Bushy Run Research Center, 1980*d*).

The induction of SCEs in CHO cells exposed to various concentrations of Butoxyethanol was evaluated. In the first experiment (with and without metabolic activation), Butoxyethanol concentrations ranging from 0.0156 to 0.25% were tested. Concentrations ranging from 0.007 to 0.125% were tested in the second experiment (with metabolic activation). Two experiments were performed because there was no statistically significant response to the positive control for metabolically activated cultures in the first experiment. Cultures without metabolic activation were exposed to Butoxyethanol for 5 h, and cultures with metabolic activation were exposed for 2 h. Water and DMSO served as negative and positive controls, respectively, both with and without metabolic activation. Dimethylnitrosamine and ethylmethanesulfonate served as positive controls with and without metabolic activation, respectively. Without metabolic activation, Butoxyethanol did not induce SCEs over the range of concentrations tested (0.0156–0.25%). However, with metabolic activation, statistically significant induction of SCEs was noted only at a concentration of 0.0312% Butoxyethanol. In the second experiment (with metabolic activation), the maximum dose of Butoxyethanol (0.25%) and the positive control dimethylnitrosamine (2,200 μg/ml) was toxic and inhibited the expression of sister chromatid

staining. Within the remaining range of Butoxyethanol concentrations tested (0.007–0.125%), there was no induction of SCEs. Statistically significant induction of SCEs was noted when the dose of the positive control was reduced to 1,800 μg/ml. The authors concluded that Butoxyethanol did not cause reproducible, statistically significant increases in SCEs and thus was not an active mutagenic agent (Bushy Run Research Center, 1980*d*).

Butoxyethanol also did not induce SCEs (in vitro) in CHO cells exposed to concentrations up to 0.25% for 2 h (presence of metabolic activation) and 5 h (absence of metabolic activation). Similar results were reported when experiments were repeated (McGregor, 1984).

The mutagenicity of Butoxyethanol (99.0% pure) was evaluated in another study using CHO cells. Specifically, the potential for induction of SCEs and chromosomal aberrations was evaluated with and without metabolic activation. In the SCE assay (with metabolic activation), Butoxyethanol was tested at concentrations up to 5,000 μg/ml; concentrations up to 3,500 μg/ml were tested without metabolic activation. In the chromosome aberrations assay, Butoxyethanol was tested at concentrations up to 5,000 μg/ml with and without metabolic activation. In both assays, untreated cultures served as negative controls; mitomycin-C and cyclophosphamide served as positive controls with and without metabolic activation, respectively. Butoxyethanol induced a delay in the cell cycle but did not induce SCEs either with or without metabolic activation. In the chromosomal aberrations assay, a weakly positive response was noted at the highest dose tested (5,000 μg/ml) during the second trial. However, this response was not reproduced during a third trial, and it was concluded that Butoxyethanol was not mutagenic in the chromosomal aberrations assay. All positive controls were mutagenic (NTP, 1993*a*).

The results from unpublished NTP mutagenicity studies are summarized as follows. Butoxyethanol was not mutagenic to *S. typhimurium* strains tested with and without metabolic activation. In in vitro cytogenetic assays, Butoxyethanol did not induce chromosome aberrations in CHO cells with or without metabolic activation. Butoxyethanol also did not induce SCEs in CHO cells in the presence of metabolic activation. However, both positive and negative results were noted without metabolic activation (NTP, 1985,1989*a*).

The mutagenicity of Butoxyethanol in bacteriophage T4D was evaluated according to the method of Kvelland (1986). Butoxyethanol was tested at concentrations ranging from 19.6 to 111.1 μl/ml. The number of mutants per 10^4 surviving bacteriophages ranged from 23.95 to 51.39 within the range of concentrations tested. Additionally, a severe toxic effect on bacteriophage yield (range 0.4–0.9%) was observed. The authors concluded that Butoxyethanol was not mutagenic (Kvelland, 1988).

CARCINOGENICITY

The carcinogenicity of a hair dye formulation containing 10% Butoxyethanol was evaluated using random-bred Swiss Webster mice (6 weeks old). The experimental group and the two untreated control groups each contained 60 male and 60 female mice. The dye was applied to each animal (0.05 ml/application) three times weekly for 20 months. Applications were made via a calibrated syringe to an area of skin, clipped free of hair, in the interscapular region. After 9 months of treatment, 10 males and 10 females were randomly selected from each group for clinical tests, hematology, and necropsy. Urine

samples were analyzed for color, pH, occult blood, albumin, and glucose. Blood samples were obtained via cardiac puncture and complete blood counts determined. At 20 months posttreatment, the remaining animals were killed and necropsy performed. Blood samples were obtained from 10 mice (5 males, 5 females) per group and both complete and differential blood counts determined. Results from analyses of the blood and urine indicated no treatment-related effects. Pulmonary adenomas, hepatic hemangiomas, and malignant lymphomas were observed in experimental and control groups. Statistical analyses, χ^2 and Fisher exact tests, of the incidence of hepatic hemangiomas and pulmonary adenomas indicated no significant differences between experimental and control groups. Additionally, no difference in the incidence of malignant lymphomas between experimental and control groups was found (Jacobs et al., 1984).

A chronic (864 days) lifetime skin-painting study of a ''solvent-cutback type rust-preventive'' containing Butoxyethanol was conducted using 50 male C3H/HeJ mice (4–6 weeks old). The composition of the product was as follows: Stoddard solvent (90.9%), Butoxyethanol (2.5%), and a calcium soap of oxidized wax solids blended with a minor amount of calcium petroleum sulfonate (6.6%). The product (25 μl) was applied via a pipette to dorsal skin, clipped free of hair, of each animal three times per week. The positive control group (50 mice) was treated with 10% catalytic-cracked clarified oil in white mineral oil and the negative control group (50 mice) with white mineral oil. At the conclusion of the study, gross and microscopic examinations were performed. Squamous cell carcinomas in or adjacent to the treatment area were diagnosed in 6 (12%) of the 50 mice treated with the product; a papilloma was also observed in 1 of the 6 mice. In the positive control group, neoplasms were observed in 48 mice. Neoplasms were not observed in the negative control group (Exxon Corp., 1984). An Environmental Protection Agency (EPA) status report stated that the data submitted indicated that the product was carcinogenic and added: ''In general it is not necessarily surprising that a product containing approximately 90% Stoddard solvent (a refined petroleum distillate with a typical boiling range of approximately 300–400 °F) is carcinogenic considering the increasing body of evidence which indicates that most (if not all) petroleum derivatives may possess some degree of oncogenic or co-oncogenic potential'' (EPA, 1984).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

The results of 20 different reproductive and developmental toxicity tests, including fertility, reproductive performance, testicular effects, developmental abnormalities, and maternal and fetal toxicity, are reported herein. Oral, inhalation, and dermal exposures are represented. Results from NTP studies are included.

The effect of Butoxyethanol exposure on the testis was evaluated using male Alpk/Ap Wistar-derived rats (weights 250 g). Groups of five animals were exposed (3–4 h) to undiluted Butoxyethanol vapor in glass exposure chambers. A 14-day observation period was initiated at the end of exposure. On day 15, the animals were killed with halothane and subjected to gross examination; testicular weights were determined and compared with controls. Hematuria was observed in exposed animals. However, no evidence of testicular atrophy was found (Doe, 1984).

In another study, the effect of Butoxyethanol (in water) on the testis was evaluated using male JCL-ICR mice (6 weeks old). Groups of five mice were given 500-, 1,000-,

and 2,000-mg/kg doses, respectively, via stomach tube over a period of 5 weeks (5 days/week). Control mice received doses of water according to the same procedure. After administration of the final dose, animals were killed with sodium pentobarbital and subjected to postmortem examination. Testicular weights were determined. For hematological studies, blood was obtained from the posterior vena cava at the time of postmortem examination. No significant difference in testicular weight between experimental and control groups was observed. A decrease in the erythrocyte count was observed in mice of ≥500-mg/kg dose groups (Nagano et al., 1984).

The developmental toxicity of Butoxyethanol was evaluated using female primigravida CD-1 mice (6–8 weeks old). The test substance (in water) was administered via gavage in doses of 1,180 mg/kg/day (10-ml/kg volumes) once daily on gestation days 6–13. Control animals (n = 50) were dosed with water according to the same procedure. In consideration of the expected 10% mortality and potentially low pregnancy rate, based on a preliminary dose range-finding study, initial group size in the reproductive phase was established at 50 time-mated females. This allowed for anticipation of at least 20 surviving pregnant mice at term, a group size considered necessary for adequate statistical analysis. Ten of the 50 dams died. Compared with the control, Butoxyethanol significantly decreased the number of viable litters (i.e., increased the proportion of failed pregnancies). Maternal body weight gain, between gestation day 6 and postnatal day 3, was also significantly less in the experimental group. No significant differences in the number of liveborn per litter, percentage survival, birth weight, and weight gain between experimental and control groups were found (Hardin et al., 1987).

In another study, the reproductive toxicity of Butoxyethanol (≥99% purity) was evaluated using 50 CD-1 mice (6–8 weeks old). During a preliminary dose range-finding study, 10 of 50 dams dosed with 1,180 mg/kg Butoxyethanol in distilled water died. In the reproductive toxicity assay, the test substance (dose 1,180 mg/kg in distilled water) was administered via gavage at a constant dose volume of 10 ml/kg on days 7–14 of gestation. Animals in the control group were dosed with water according to the same procedure. Butoxyethanol administration resulted in a significant reduction (77%) in the number of viable litters produced. No significant differences in the following parameters were observed between experimental and control groups: number of live pups per litter at birth, number of dead pups per litter at birth, pup postnatal survival, pup weight gain over days 1–3 postpartum, and pup birth weight (Schuler et al., 1984).

The toxicity of Butoxyethanol (99.0% pure) was evaluated using F344/N rats (5–7 weeks old, 5 males/dose). The groups received oral doses (in drinking water) of 100, 150, 250, 400, or 650 mg/kg daily for 14 days; the control group received water. None of the rats died or was killed prior to the end of the study. Increases and decreases in absolute and relative testicular weights were noted; however, these changes were not significantly different when compared with the control group. Microscopic examination of the testis and epididymis did not reveal any test substance-induced lesions (NTP, 1993*b*).

The NTP also evaluated the toxicity of Butoxyethanol (99.0% pure) in $B6C3F_1$ mice (5–7 weeks old, 5 males/dose) using the procedure described; the same doses were administered. None of the mice died or was killed prior to the end of the study. Testicular weights in experimental mice were not significantly different from those noted for the control group (NTP, 1993*b*).

In another short-term study, the toxicity of Butoxyethanol (99.0% pure) was evaluated

using F344/N male rats (5–7 weeks old, 30 rats/dose). Doses of 1,500, 3,000, or 6,000 ppm Butoxyethanol were administered in drinking water daily for 60 days (note: during week 6, rats in the 1,500-ppm group mistakenly received 2,500 ppm ethoxyethanol in drinking water). Control rats (n = 30) were dosed with water according to the same procedure. The animals were evaluated at the end of treatment and 30 and 56 days after the termination of treatment. At the end of treatment, 10 rats/dose group were killed. No test-substance-related microscopic lesions were noted in the testis or epididymis (NTP, 1993*b*).

The potential reproductive toxicity of Butoxyethanol (99.0% pure) was also evaluated in 13-week studies involving F344/N rats and B6C3F_1 mice (5–7 weeks old; 10 males, 10 females/dose). The test substance in drinking water was administered daily to groups of rats in doses of 1,500, 3,000, 4,500, or 6,000 ppm; male and female control groups received distilled water. None of the experimental animals died or was killed prior to the end of the study. Compared with the control group, sperm concentration was significantly decreased ($p < 0.01$) in males from all three dose groups; this was the only spermatozoal measurement that was significantly changed. Estrous cycle lengths in experimental groups of females were not significantly different from those noted in the control group. However, compared with the control group, various stages of the estrous cycle were significantly longer in female rats of the 4,500- and 6,000-ppm dose groups. A reduction in size of the uterus in rats from the 4,500- and 6,000-ppm dose groups was the only gross lesion that was considered related to test substance administration (NTP, 1993*b*). None of the mice exposed to the same doses administered to the rats died or was killed prior to the end of the study. The results of sperm morphology and vaginal cytology evaluations indicated no biologically significant changes in any of the reproductive parameters that were evaluated (NTP, 1993*b*).

In a series of three experiments, the reproductive toxicity of Butoxyethanol was evaluated using 11-week-old (COBS) CD-1 (ICR)BR mice. Based upon results from a preliminary dose range-finding study, Butoxyethanol was administered to three groups of 40 mice at concentrations of 0, 0.5, 1.0, and 2.0% in drinking water, respectively. Twenty males and 20 females were tested at each concentration over a period of 18 weeks (1 week prior to cohabitation, 14 weeks of cohabitation, and 3 weeks thereafter). Newborn litters were evaluated and immediately killed. Thirteen of the 20 female mice in the 2.0% dose group died as compared with 1 each in control and 0.5% dose groups and 6 in the 1.0% group. All breeding pairs in the 0.5% dose group were fertile (delivered at least one litter). In the control and 1.0% dose groups, all, except for one pair each, were fertile. In the 2.0% dose group, five of the seven breeding pairs were fertile. Data from pairs in which one or both partners died during the cohabitation phase were excluded from intergroup comparisons and statistical analyses. There was a significant decrease ($p < 0.05$) in the mean number of litters per fertile pair at 1.0 and 2.0% concentrations of Butoxyethanol. Other reproductive parameters significantly affected at these doses were the number of live pups (male, female, as well as combined) per litter ($p < 0.1$), the proportion of pups born alive ($p < 0.01$), and weights of live pups (both absolute and adjusted; $p < 0.05$). The live pup weight values were also significantly affected ($p < 0.01$) in the 0.5% dose group. The preceding results indicate that 1.0 and 2.0% Butoxyethanol (in drinking water) significantly affected fertility (Gulati et al., 1985).

In a second experiment, animals from the 1.0% dose group (same as in preceding study)

were tested in a crossover mating trial to determine whether males, females, or both sexes had compromised reproductive performance when matched with control animals (same as in preceding experiment). The experimental procedure was the same as that stated. Females that were treated with 1.0% Butoxyethanol and cohabited with control males had 46% fertile matings as compared with a control value of 79%. The corresponding value for males (1% dose group) mated with control females was 75%. There was also a decrease with respect to the number of live pups per litter and the proportion of pups born alive, but the difference ($p > 0.05$) was not significant. The average live pup weight (absolute and adjusted) for the offspring of control males mated with females (1% dose group) was significantly affected ($p < 0.05$). These parameters were not affected for pups delivered by control females mated with Butoxyethanol-treated males. The results indicate that the reproductive capacity of female mice is relatively more susceptible to the toxic effects of Butoxyethanol than that of males under the same exposure conditions. At the end of the study, male and female mice were necropsied. Detailed sperm morphology and vaginal cytology evaluations were also performed. The cauda epididymal sperm counts were essentially identical for control and treated male mice. The sperm motility values for the control and treated male mice were 93 and 94%, respectively. The incidence of abnormal sperm was also not affected ($p > 0.05$) by 1.0% Butoxyethanol treatment. Butoxyethanol treatment also did not appear to interfere with the relative frequency of various estrous stages or the average estrous cycle length (Gulati et al., 1985).

A third experiment was designed to evaluate the reproductive performance of the offspring from litters of control and 0.5% dose groups (from the first experiment). The pups initially received Butoxyethanol via lactation until weaning (3 weeks after delivery) and later in drinking water. At 74 ± 10 days of age, 20 second-generation male mice were randomly mated with 20 second-generation female mice in both the control and the 0.5% Butoxyethanol groups. Mating was continued until a copulatory plug was found or for 7 days. The mating index (percent of plug positive/no. cohabited) for both the control and the treated second-generation animals was 85%. The fertility indexes (percent of no. fertile/no. cohabited) for the control and treated pairs were 80 and 75%, respectively. No significant differences ($p > 0.05$) in litter size, proportion of pups born alive, or sex ratio between experimental and control groups were observed. At the end of the experiment, all second-generation animals were necropsied; the reproductive tract and gonadal tissues were weighed. In females, the group mean kidney weight for animals exposed to 0.5% Butoxyethanol was significantly different from that of the control group. Female group mean weights for kidneys and the liver were significantly different from those of controls when organ weight was adjusted for body weight. In males, the group mean organ weights for the liver, right testis, right cauda, right epididymis, prostate, seminal vesicles, and kidneys were not significantly different ($p > 0.05$) from the control group. However, the group mean hepatic weight for males was significantly different from that of the control group when organ weight was adjusted for body weight. Butoxyethanol treatment (0.5% dose) had no significant effect on the incidence of abnormal sperm, average sperm motility, and sperm density. Vaginal smears, from all second-generation female mice, were prepared over a period of 7 days prior to necropsy. The average estrous cycle lengths in control and treated animals were 4.4 and 5.1 days, respectively. No significant differences were noted with respect to the relative frequency of different estrous stages (Gulati et al., 1985).

The embryotoxicity of Butoxyethanol was evaluated using cultures of postimplantation rat embryos. The embryos were cultured in rat serum from day 9.5 to day 11.5 of gestation and then evaluated. Butoxyethanol concentrations that were >12.5 m*M* caused 100% lethality. Concentrations of ethylene glycol ethyl ether and ethylene glycol methyl ether that were higher than 50 and 100 m*M*, respectively, caused 100.0% lethality. The investigators noted that dose-related embryotoxicity and teratogenicity have been observed at lower concentrations; the no-effect levels were as follows: Butoxyethanol (3.12 m*M*), ethylene glycol ethyl ether (6.25 m*M*), and ethylene glycol methyl ether (25 m*M*). The investigators stated that the results of this study indicate that the teratogenic potential of the three solvents is directly related to the alkyl group chain length (Giavini et al., 1992).

The teratogenicity of Butoxyethanol (97% purity) was evaluated using CD-1 mice (8–10 weeks old, weights 17–33 g). The test substance was diluted with distilled water and administered via gavage on days 8–14 of gestation. Five groups of six mice received 350-, 650-, 1,000-, 1,500-, and 2,000-mg/kg doses, respectively; the dose volume was 10 ml/kg/day. The control group was dosed with distilled water. Animals were killed via CO_2 inhalation on day 18 of gestation. Necropsy was performed and uterine contents were examined for numbers of implantation sites, resorptions, and live and dead fetuses. Fetuses were examined for external abnormalities, and fetal weights were measured. Three animals in the 1,500-mg/kg dose group and all animals in the 2,000-mg/kg dose group died. The number of resorptions in 1,500- and 1,000-mg/kg dose groups was significantly greater than that reported for the control group. These were the only statistically significant findings reported. Additionally, cleft palate was observed in one of five litters (4/43 fetuses) of the 1,000-mg/kg dose group and one of three litters (1/25 fetuses) of the 1,500-mg/kg dose group (Wier et al., 1987). In a subsequent postnatal study, two groups of CD-1 mice received 650- and 1,000-mg/kg doses of Butoxyethanol, respectively, according to the procedure previously mentioned. On postnatal day 22, surviving dams and offspring were killed via CO_2 inhalation. Compared with controls, no statistically significant differences in survival or body weight of offspring in either treatment group were found (Wier et al., 1987).

In another study, the teratogenicity of Butoxyethanol was evaluated using 70 female CD-1 mice (weights ~17–32 g). Groups of 19 and 20 mated females (experimental animals) received doses of 650 and 1,000 mg/kg Butoxyethanol, respectively, on days 8 through 14 of gestation. The control group, 30 mated females, received 10,000 mg/kg distilled water. All doses were administered via gavage, and dose volumes were 10 ml/kg/day in all groups. During necropsy of the only animal (1,000-mg/kg dose group) that died, a slight amount of thin red fluid was found in the thoracic cavity and red mottling of the lungs was also observed. The numbers of mated females that gave birth were as follows: 15 of 19 (650-mg/kg group), 19 of 20 (1,000-mg/kg group), and 27 of 30 (control group). In both experimental groups, the number of live pups or the fraction of the litter that remained alive was not significantly different from the control group on days 1, 8, 15, or 22 postpartum. Also, on these days, no significant differences in group mean body weight between experimental and control groups were observed. Cranioschisis in a pup that died (1,000-mg/kg group) and fusion of digits on the right hindpaw (650-mg/kg group) were the only malformations observed in experimental animals. Malformations were not observed in the control group (Exxon Biomedical Sciences, 1986).

The teratogenicity of Butoxyethanol (>99.0% purity) was evaluated using Fisher 344 inbred albino rats. The weights of the dams on gestation day 0 ranged from 150 to 202 g. The purpose of the study was to determine whether the exposure of pregnant rats to Butoxyethanol during critical periods of cardiovascular development adversely affected the structure of the fetal heart and great vessels. Butoxyethanol (in distilled water) was administered via gavage to a total of 298 rats; 104 rats served as controls. The distribution of doses for the 184 animals dosed on days 9 through 11 of gestation was as follows: 46 rats (0 mg/kg/day), 46 rats (30 mg/kg/day), 47 rats (100 mg/kg/day), and 45 rats (200 mg/kg/day). A total of 93 rats (all dose groups included) were killed on day 12 of gestation and the remaining 91 rats on day 20. For the 220 rats dosed on days 11 through 13 of gestation, the distribution of doses was as follows: 58 rats (0 mg/kg/day), 52 rats (30 mg/kg/day), 59 rats (100 mg/kg/day), and 51 rats (300 mg/kg/day). A total of 104 rats (all dose groups included) was killed on day 14 of gestation and the remaining 116 rats on day 20. Also, on gestation day 20, fetuses were killed and examined. Embryo/fetal effects were limited to increased resorptions, nonlive implants, and adversely affected (nonlive plus malformed) implants per litter in the 200-mg/kg/day group dosed on gestation days 9 through 11 and to decreased platelet count in the 300-mg/kg/day group dosed on gestation days 11 through 13. Butoxyethanol exposure during gestation did not increase the incidence of fetal malformations; particularly, no cardiovascular malformations were observed. Maternal body weights, organ weights, and hematology were altered by 200-mg/kg/day doses administered on days 9 through 11 of gestation and 300-mg/kg/day doses administered on days 11 through 13 of gestation (NTP, 1989*b*).

Sleet et al. (1991) also studied the effect of Butoxyethanol on cardiovascular development in the developing fetus from F344 rats. Butoxyethanol, in distilled water, was administered daily (by gavage, gestation days 9–11) in doses of 0, 30, 100, or 200 mg/kg/day and in doses of 0, 30, 100, or 300 mg/kg/day on gestation days 11–13. Doses of $\geqslant$100 mg/kg induced maternal toxicity on gestation days 9–13; reductions in body weight, increased spleen and kidney weight, and hematotoxicity were noted; hematotoxicity was practically reversed by day 20. The viability of embryos was markedly reduced at doses of 200 mg/kg, but not at 300 mg/kg. Developmental toxicity, which included fetal heart and great vessel defects, was not observed on day 20 of gestation following exposures on either gestation days 9–11 or days 11–13.

In an interim report by Eastman Kodak Co. that was submitted to EPA in 1983, the teratogenicity of Butoxyethanol was evaluated in Fischer 344 rats. Three groups of rats inhaled Butoxyethanol vapor concentrations of 100, 200, and 300 ppm, respectively, 6 h/day on days 6 through 15 of gestation. A group of 30 air-exposed rats served as the control. In dams, Butoxyethanol exposure caused a dose-related increase in the frequency of hematuria. In the 300-ppm exposure group, uterine weight was significantly lower than that in the control group and a marked increase in embryo and fetal lethality (manifested by resorptions of concepti) was observed. A more moderate increase in resorption, compared with the control group, was noted in the 200-ppm exposure group. Fetal visceral examinations indicated a dose-related increase in atelectasis. Additionally, fetuses from the various exposure groups had ventricular septal defects, absent innominate arteries, and severely shortened innominate arteries. After analyzing the preceding submission, EPA noted that the data included were incomplete because fetal skeletal examinations and statistical analyses had not been finished (EPA, 1983).

In another study, the teratogenicity of Butoxyethanol (98–99.5%) was evaluated in female Sprague–Dawley rats. The animals were exposed to Butoxyethanol vapor on gestation days 7–15 (7 h/day) in 0.5-m^3 inhalation chambers. Animals were left in the chamber for at least 1 additional h after vapor generation was terminated. Sixteen and 15 rats were exposed to 150 and 200 ppm Butoxyethanol, respectively. The desired concentrations were obtained by mixing the vapor with filtered room air. The untreated control group consisted of 34 dams. On day 20, the animals were killed via chloroform asphyxiation. The entire uterus was removed and numbers of resorption sites (classified as early, middle, or late) and numbers of live fetuses were determined. Fetuses were serially removed, blotted of excess fluids, weighed, and examined for external and visceral malformations. On day 1 of exposure, hematuria was observed in dams in 150- and 200-ppm exposure groups. This was the only adverse effect observed in dams. There were no significant differences in the number of resorptions, fetal weights, and the incidence of malformations between the group exposed to 200 ppm Butoxyethanol and the control group. A statistically significant decrease in fetal weights was observed in the 150-ppm exposure group, but the difference was slight and not observed in the 200-ppm exposure group; therefore, it was not considered to have been of biological significance. One fetus with cephalo-thoracomphalopagus was observed (150-ppm exposure group); the observation was regarded as a chance occurrence (Nelson et al., 1984).

Another rat inhalation teratogenicity study was conducted by Tyl et al. (1984). Four groups of 36 female Fischer 344 rats (100 days old) were exposed to concentrations of 25, 50, 100, and 200 ppm Butoxyethanol (in air), respectively. An untreated group of 36 rats served as the control. For each group, exposures were carried out in a 4,350-L exposure chamber on days 6 through 15 of gestation (6 h/day). On day 21 of gestation, animals were killed via carbon dioxide asphyxiation. Maternal toxicity was observed in 100- and 200-ppm exposure groups. In the 200-ppm exposure group, a significant increase in the number of totally resorbed litters was observed. Additionally, the number of viable implants and the percentage of live fetuses per litter were significantly reduced, and the number of nonviable implants was significantly elevated after exposure to Butoxyethanol (200 ppm). No statistically significant increases in the incidence of external, visceral, skeletal, or total malformations between experimental and control groups were found. However, a significant increase in the number of litters with one or more fetuses with unossified skeletal elements was observed in the 200-ppm group, for example, anterior arch of the atlas and cervical centra 5 and 6. In the 100-ppm group, a significant increase in the incidence of unossified cervical centrum 6 was observed. No evidence of either maternal toxicity, embryotoxicity, or fetotoxicity was found in rats of 25- and 50-ppm exposure groups.

The teratogenicity of Butoxyethanol (99.6%) was also evaluated using 5- to 5.5-month-old New Zealand white rabbits (weights 3–3.5 kg). Four groups of 24 rabbits were exposed to concentrations of 25, 50, 100, and 200 ppm Butoxyethanol (in air), respectively. An untreated group of 24 rabbits served as the control. For each group, exposures were carried out in a 4,350-L exposure chamber on days 6 through 18 of gestation (6 h/day). The animals were killed on day 29 of gestation. Compared with controls, an apparent, but not statistically significant, increase in spontaneous abortions and deaths (dams) was observed in the 200-ppm exposure group. Additionally, a significant reduction in the number of total implants and viable implants per litter was observed in this group.

Treatment-related effects on the number of nonviable implants, preimplantation loss, percent live fetuses, and male and female body weight per litter were not noted. Significant reductions in unossified sternebra 6 and in rudimentary rib (first lumbar vertebra), bilaterally, were also noted in litters of the 200-ppm exposure group. Except for these two findings, no treatment-related changes in the incidence or types of variations identified across treatment groups were found. No statistically significant increase in the number of litters with one or more fetuses with external, visceral, skeletal, or total malformations was observed in any treatment group. In the 100-ppm group, an increase (compared with control) in the number of litters with one or more fetuses having fusion of papillary muscles in the left ventricle was found. This malformation was not observed in other treatment groups and thus was not considered treatment related. Rudimentary rib or extra rib was seen across all groups at a relatively high frequency, with no exposure-related incidence. No evidence of maternal toxicity, embryotoxicity, or fetotoxicity was observed in rabbits of 25- and 50-ppm exposure groups (Tyl et al., 1984).

The developmental toxicity of a test material containing 14.0% nonylphenoxy polyethoxy ethanol (a nonoxynol; no. moles ethylene oxide not stated), 64% tallow fatty acid amine ethoxylate, and 22% Butoxyethanol was evaluated using groups of 25 female Crl:CD BR nulliparous rats (~64 days old). The test substance was administered by gavage in doses of 3, 8, 20, and 50 mg/kg body wt on days 7–16 of gestation; the control group (25 rats) was dosed with deionized water. Maternal toxicity was induced at doses of 20 and 50 mg/kg/day. There were no significant differences in fetal malformations between experimental and control groups at any of the doses tested. The authors concluded that the no-observable-adverse-effect level was 8 mg/kg/day for the dam and >50 mg/kg/day for the conceptus (Anonymous, 1992).

In another study, the teratogenicity of Butoxyethanol was evaluated using primigravida SPF Sprague–Dawley rats. The interscapular region of each animal was shaved on day 5 or 6 of gestation. Undiluted Butoxyethanol (0.35 ml) was then applied four times daily on days 7–16 of gestation. Ethylene glycol monoethyl ether (0.25 ml) and water served as positive and negative controls, respectively. The treatment volume was reduced to 0.12 ml four times daily in the second replicate because marked toxic effects (11 of 12 rats died) were observed when 0.35-ml volumes were administered. On day 21 of gestation, dams were killed via CO_2 asphyxiation and fetuses were weighed and examined for external malformations. No signs of toxicity were observed in the dams when the volume of Butoxyethanol administered was reduced to 0.2 ml four times daily on days 7–16 of gestation. No evidence of embryotoxicity or fetotoxicity was observed in any of the litters. Also, the incidence of visceral and skeletal defects was not significantly different from that of the control (water) group (Hardin et al., 1984).

The mechanism of teratogenicity of Butoxyethanol was evaluated using pregnant Wistar-Porton rats. Embryos (aged 9.5 days) were explanted from the dams and cultured for 48 h with 2 or 5 m*M* butoxyacetic acid. Crown–rump length, head length, and yolk sac diameter were measured at the end of the 48-h culture period, and a morphological scoring system was used to evaluate the degree of differentiation. Butoxyacetic acid (5 m*M*) caused statistically significant reductions in somite number ($p < 0.01$), head length ($p < 0.01$), yolk sac diameter ($p < 0.05$), and protein content of the embryo ($p < 0.05$). Exposure to 2 m*M* butoxyacetic acid did not cause any significant reductions in growth parameters. Irregularity of the neural suture line was another observation in embryos cultured with 5

m*M* butoxyacetic acid; 29% of the embryos were affected. Embryos exposed to butoxyacetic acid also had abnormal otic and somatic development. The authors concluded that none of the preceding observations indicated abnormal development (Rawlings et al., 1985).

CLINICAL ASSESSMENT OF SAFETY

In addition to clinical data on irritation, sensitization, and photosensitization, this section includes human studies of absorption, distribution, and metabolism, with results that parallel those seen in other test systems presented earlier in this report.

Percutaneous Absorption

The percutaneous absorption of Butoxyethanol was evaluated using five healthy male volunteers (weights not stated) who had not been exposed to Butoxyethanol in the workplace. All of the subjects were nonsmokers and indicated either little or no alcohol consumption. One of the subjects was allergic to chlorpromazine and had atopic eczema on the chest, back, face, and cubital fossa. Another subject had neurodermatitis of the left leg. All subjects were instructed not to consume alcoholic beverages 24 h prior to Butoxyethanol exposure or during exposure and also to avoid activities that might cause exposure to organic solvents and damage to the skin. Each subject placed four fingers of the left hand into a polyethylene jar (21°C) filled with undiluted Butoxyethanol. Unexposed fingers served as controls. The fingers were placed through holes in the jar cap. At the conclusion of the 2-h exposure, each subject washed the exposed hand with a mild soap and tap water. There was no evidence of skin irritation; however, exposed fingers appeared wrinkled and somewhat more rigid after exposure. These effects reached a maximum at ~2–4 h postexposure and then gradually disappeared. A dry, reticulate pattern, with small fissures, was also noted within a few hours after exposure. In some instances, slight erythematous reactions were observed in the fissures, disappearing within 1 or 2 days. Additionally, both finger volume and skinfold thickness decreased and then returned to normal. The percutaneous uptake rate of Butoxyethanol into the blood varied from 127 to 1891 μmol. These values corresponded to 7–96 nmol Butoxyethanol/min/cm^2 of exposed area. During the decay phase, the half-time of Butoxyethanol ranged from 0.6 to 4.8 h (geometric mean 1.3 h). A linear regression analysis for all of the experiments suggested that, on the average, 17% of the absorbed dose of Butoxyethanol was excreted in the urine (Johanson et al., 1988).

In another study, the percutaneous absorption of Butoxyethanol vapor was evaluated using four adult men (23–36 years old, weights 75–80 kg). Two men were subjected to two 2-h exposures to 50 ppm Butoxyethanol per experiment. In each experiment, the first 2-h exposure was followed by a 1-h nontreatment period. During the first 2-h exposure, the subjects were seated outside of the exposure chamber and breathed chamber air through a mouth valve. The second 2-h exposure took place inside the exposure chamber. Duplicate capillary blood samples were obtained at regular intervals before, during, and after exposure. In one experiment, the concentration of Butoxyethanol in the blood was lower after dermal exposure. However, in seven of the experiments, the concentration of Butoxyethanol in the blood was 2.4–5.5 times higher after dermal exposure than after oral exposure. Similarly, the high rate of percutaneous absorption (31 μmol/min) was 2.5–5.9

times higher than the respiratory rate. The results of these experiments suggest that the dermal uptake of Butoxyethanol accounts for ~75.0% of the total uptake during whole-body exposure to Butoxyethanol vapor (Johanson and Boman, 1991).

Over a period of between 1 and 30 years, a total of 17 subjects (15 males, 2 females; between 23 and 59 years old) were exposed to Butoxyethanol and other glycol ethers in a varnish production plant. The subjects were involved with the production of varnishes (12 men), worked in the adjacent store (3 men), and worked in the laboratory (2 women) where the quality of the products was tested. To estimate external exposure, air samples were collected during the entire shift via personal air monitors. Measurements of internal exposure were based on concentrations of Butoxyethanol in the blood and butoxyacetic acid in the urine. Based on personal air monitoring, the average concentrations ($n = 17$) of Butoxyethanol in the three areas of the plant were as follows: 1.1 ppm (varnish production), <0.1 ppm (store), and <0.1 ppm (laboratory). Average Butoxyethanol concentrations in the blood at the end of the shift were as follows: 121.3 μg/L (varnish production), 49.4 μg/L (store), and <5.0 μg/L (laboratory). The average concentrations of butoxyacetic acid in the urine before and after the shift, respectively, were as follows: 3.3 and 10.5 μg/L (varnish production), 2.1 and 4.5 μg/L (store), and 0.2 and 4.2 μg/L (laboratory). A plot of Butoxyethanol concentrations in the air versus concentrations of butoxyacetic acid in the urine (17 subjects) indicated that even at small or undetectable concentrations of Butoxyethanol in the air, metabolite (butoxyacetic acid) excretion was high. This urinary metabolite was due to the percutaneous absorption of Butoxyethanol (Angerer et al., 1990).

Metabolism and Excretion

The metabolism and excretion of inhaled Butoxyethanol were evaluated using seven healthy male subjects (21–38 years old, weights 65–85 kg). The subjects had no history of occupational exposure to solvents. Each subject was exposed to Butoxyethanol (20 ppm or 0.85 mmol/m^3 air) during light physical exercise on a bicycle ergometer. Exposures were conducted in an air-conditioned open-system exposure chamber (volume 12 m^3); solvent vapor was dynamically generated at the fresh air side of the ventilation system. Expired air from each subject was collected (4-min period) in polyester-laminated aluminum foil bags at six regular intervals. Solvent uptake during each collection period was defined as the difference between the amount of solvent in inhaled and exhaled air. Total solvent uptake for the entire exposure period was calculated via linear interpolation between the collection periods. Blood samples were taken from one finger during the exposure period and at 3 h postexposure. Urine samples were obtained at 2-h intervals over a period of 6 h. The concentration of Butoxyethanol in the blood increased rapidly and reached a plateau within 1–2 h. The average plateau level of Butoxyethanol in the blood was 7.4 ± 2.0 μmol/L ($n = 21$). This value was significantly greater than the average concentration of Butoxyethanol in the urine, 1.3 ± 0.5 μmol/L ($n = 7$), at the end of exposure. Butoxyethanol was not detected in the blood 2–4 h after the end of exposure. The total amount of Butoxyethanol detected in the urine was <0.03% of the total uptake in all of the experiments. There appeared to have been an increase in the concentration and excretion rate of butoxyacetic acid, metabolite of Butoxyethanol, during the first few hours of exposure. The maximum concentration in the urine and the maximum excretion

rates were noted after 5–12 and 2–10 h of exposure, respectively. Relative to the uptake of Butoxyethanol, the average amount excreted as butoxyacetic acid on an equimolar basis was 41.1% (n = 6). There were no consistent changes in either pulmonary ventilation, respiratory frequency, or heart rate during exposure. Butoxyethanol did not induce toxicity in any of the subjects tested (Johanson et al., 1986*b*).

In another study, butoxyacetic acid was detected (using gas chromatography) in venous blood samples from five male volunteers who were exposed to 20 ppm Butoxyethanol for 2 h during light physical exercise. The protocol for exposure was in accordance with the method of Johanson et al. (1986*a*). Butoxyacetic acid was not detected in blood samples that were obtained prior to exposure. Butoxyacetic acid blood concentrations ranged from 22 to 60 μM (Johanson and Johnsson, 1991).

Three male subjects (ages and weights not stated) were exposed to 60 mg/m^3 (12.6 ppm) and 120 mg/m^3 (25.2 ppm) Butoxyethanol, respectively, at rest for 4 h (50 min/h) using face masks. The three male subjects were also exposed (same procedure) to 60 mg/m^3 Butoxyethanol during 30 W of exercise. The average retention relative to the three exposure conditions was as follows: 68.9% (12.6 ppm exposure), 67.0% (25.2 ppm), and 77.6% (12.6 ppm, during exercise). Respiratory elimination (at rest) of 12.6 and 25.2 ppm Butoxyethanol averaged 0.69 and 0.66%, respectively. During exercise, respiratory elimination of 12.6 ppm Butoxyethanol averaged 0.24%. The average percentages of butoxyacetic acid (expressed as percentage of absorbed Butoxyethanol) recovered were as follows: 27% (12.6 ppm exposure), 27% (25.2 ppm), and 13.6% (12.6 ppm, during exercise) (Van Vlem, 1987).

In another study, the relationship between the excretion of butoxyacetic acid and Butoxyethanol exposure was evaluated using five women who were employees of a silk screening operation. Half-shift personal monitoring was conducted for 5 days, and following a 12-day halt in production, monitoring continued for an additional 7 days. Weekly exposures to Butoxyethanol averaged 0.65 ppm (3.1 mg/m^3). During each of the 5 days, urinary concentrations of butoxyacetic acid were greater at the end of a shift than at the beginning. Following 2 days off from work (third Monday morning of monitoring), no butoxyacetic acid was detected; this indicated that there was complete clearance of butoxyacetic acid during the weekend. A 8.3-h half-life for Butoxyethanol was calculated (Van Vlem, 1987).

Short-Term Inhalation Toxicity

Two adult male subjects (between 30 and 45 years old) and one female subject (24 years old) breathed ~200 ppm Butoxyethanol during two 4-h periods, separated by a 30-min lunch. Blood pressure and pulse rate were determined three times, and erythrocyte fragility tests were conducted twice during the day of exposure. Urinalyses for glucose and albumin were conducted during the morning after exposure, and butoxyacetic acid concentrations were determined in 24-h urine samples that were collected at the end of the day of exposure. One male subject and the female subject excreted considerable amounts of butoxyacetic acid, while the other male subject excreted only trace amounts. All three subjects experienced immediate irritation of the nose and throat, followed by ocular irritation and disturbed taste. The female subject, who excreted the largest amount of butoxyacetic acid, reacted most adversely to the exposure. She acquired a headache that lasted for ~24 h (Mellon Institute of Industrial Research, 1955).

A total of four subjects breathed ~100 ppm Butoxyethanol during two 4-h periods, separated by a 30-min lunch. The ages of three of the subjects (two females and one male) were between 24 and 37 years old. The remaining subject was the male, exposed to 200 ppm Butoxyethanol in the preceding study, who had trace amounts of butoxyacetic acid in the urine. The 100-ppm exposure occurred 20 days after the 200-ppm exposure, and the conditions of exposure were identical. The only significant finding following the 100-ppm exposure was urinary excretion of butoxyacetic acid. The male subject who had been previously exposed to 200 ppm Butoxyethanol excreted significant quantities of butoxyacetic acid in the urine after exposure to 100 ppm; 75.5 mg was eliminated in 24 h. Additionally, the concentration of butoxyacetic acid in the urine of the remaining three subjects was similar to that detected in the subjects exposed to 200 ppm Butoxyethanol. Concerning subjective responses to exposure, the two female subjects experienced greater distress during and after exposure than did the males. Vomiting was induced in one female subject at 7 h postexposure and several times during the following day. The other female subject complained of a headache on the following day (Mellon Institute of Industrial Research, 1955).

Hematotoxicity

Two men were exposed to an average concentration of 113 ppm Butoxyethanol in a 1,250-ft^3 room with a glass front partition. Erythrocytic fragility tests were performed four times on each subject. Two rats served as concurrent positive controls. Signs and symptoms reported during exposure were as follows: nasal and ocular irritation, disagreeable metallic-like taste, slightly increased nasal mucous discharge, and occasional belching. The results of the fragility tests did not deviate from the preexposure normal values of 0.42% NaCl (initial hemolysis) and 0.36% NaCl (complete hemolysis). For the two rats, initial hemolysis values of 0.44 and 0.46% NaCl were reported after 2 h of exposure and values of 0.46 and 0.48% NaCl after 3.5 h of exposure. The sensitivity of humans to Butoxyethanol vapor was said to be less than that of the rat (Mellon Institute of Industrial Research, 1952).

Skin Irritation and Sensitization

The skin sensitization potential of 10.0% (vol/vol) aqueous Butoxyethanol was evaluated using 214 male and female subjects between 18 and 76 years of age. A total of 201 subjects completed the study; withdrawal from the study was not related to administration of the test substance. During induction, nine consecutive patch applications (occlusive patches) of the test substance were made to the back of each subject. The subjects were instructed to remove the patches at ~24 h postapplication and return to the testing facility at 48-h intervals for evaluation of test sites and patch reapplication. Patches that were applied on Friday were removed at 24 h and sites were evaluated on the following Monday. The challenge phase was initiated after a 14-day nontreatment period. Patches were applied to new test sites and removed 24 h later; reactions were scored at 48 and 72 h postapplication. Challenge reactions were observed in 14 subjects. Definite erythema, with no edema, was observed in one subject at 72 h and doubtful (barely perceptible erythema, only slightly different from surrounding skin) responses were observed in 13 subjects: 6 subjects at 48 and 72 h, 6 subjects at 72 h, and 1 subject at 48 h. Eleven of the

14 subjects with challenge reactions also had reactions ranging from doubtful to definite erythema, but with no edema, during the induction phase. Additionally, a total of 52 subjects had reactions only during the induction phase; 35 subjects had doubtful reactions and 17 subjects had reactions ranging from doubtful to definite erythema, but with no edema. The authors concluded that there was no evidence of sensitization to 10.0% aqueous Butoxyethanol (TKL Research, 1992).

Skin Sensitization and Photosensitization

An industrial hygiene survey was conducted in the ''Vac-Pac'' area of a citrus processing plant glass filling department to evaluate personal exposures to organic vapors. Four printer operators and one relief operator who cleaned printing machines with ink wash solvent were evaluated. Printing machines were used for printing the company logo on glass bottles. The ink wash solvent contained 10–50% Butoxyethanol and 50–90% alkyl-substituted benzenes (i.e., methylbenzene). Personal breathing zone samples were collected (1.3- to 2.7-h sampling period) on charcoal tubes and analyzed by gas chromatography to quantify the organic vapors released from the solvents during use. The concentrations of Butoxyethanol detected ranged from 1.73 to 9.75 ppm (five workers). To detect skin sensitization, the workers were patch-tested with the wash solvent (5% in petrolatum). The test substance was applied to the left side of the back of each subject, and the application site was covered with a patch made of nonabsorbent material. Patches were secured with nonirritating tape for 48 h. Sites were scored at 48 and 96 h postapplication according to the following scale: +1 (reddened, raised skin) to +3 (red, raised skin with ulcerations or small, closely set vesicles extending beyond the border of the patch). To detect photosensitization, the test substance was applied to the right side of the back via an occlusive patch according to the same procedure. After 24 h of exposure, patches were removed and sites exposed to sunlight for 30 min to 1 h. Sites on the opposite side of the back were covered with an additional opaque material such that exposure to sunlight was avoided. Sites were scored at 48 and 96 h postapplication according to the same procedure. Four of the workers had +1 reactions at 48 h postapplication. At 96 h postapplication, +1 reactions were observed in three workers. The wash solvent did not induce sensitization or photosensitization reactions in any of the five workers tested (Salisburg and Bennett, 1988).

Occupational Exposure

The 15-min time-weighted average exposure for Butoxyethanol that should not be exceeded at any time during a work day is 360 mg/m^3. The time-weighted average concentration of Butoxyethanol, for a normal 8-h workday and a 40-h workweek, to which nearly all workers may be repeatedly exposed (day after day) without adverse effect is 120 mg/m^3 of air or 25 ppm. Parts per million here means parts of vapor or gas per million parts of contaminated air by volume at 25°C and 760 torr (American Conference of Governmental Industrial Hygienists, 1986).

Three employees at a glass manufacturing facility were evaluated for health effects resulting from inhalation of a solvent vapor mixture containing 3–5 ppm Butoxyethanol. The only employee who was asked to complete a medical questionnaire had the following signs and/or symptoms: occasional headaches, sore throat, and soreness and bleeding of

the nose (Apol, 1987). The following signs and/or symptoms were also observed in one of six employees at a label printing company exposed to a solvent vapor mixture containing 2 ppm Butoxyethanol: headaches, ocular irritation, dry throat, tightness of the chest, and coughing (Daniels, 1982). In another study involving 14 employees of a can manufacturing plant, the following symptoms were observed after exposure to a vapor mixture containing 0.047–0.185 ppm Butoxyethanol: asthma, transient symptoms of respiratory irritation, and abnormal baseline pulmonary functions. The authors stated that the transient symptoms of respiratory irritation were consistent with the effects of an inhaled amine (Gann and Roseman, 1983).

When cellular immune responses were evaluated in nine healthy employees of a parquet manufacturing company who were exposed to an organic solvent mixture containing 24.6 mg/m^3 Butoxyethanol, differences in various lymphocyte subpopulations between experimental and control groups were noted. The authors stated that similar changes in lymphocyte subpopulations are observed in conditions of immunodeficiency and immunogenetic forms of aplastic anemia (Denkhaus et al., 1986).

SUMMARY

Butoxyethanol (CAS no. 111-76-2) is an ether alcohol that is usually produced by the reaction of ethylene oxide with butyl alcohol. The purity of technical-grade Butoxyethanol ranges from 98 to 99.5%. Butoxyethanol has been used as a solvent in cosmetic products at concentrations ranging from ≤0.1 to 10.0%. Frequency of use data submitted to FDA in 1994 indicate that this ingredient is used in 47 hair dyes and colors. Hair dyes and colors containing Butoxyethanol may be marketed with patch test instructions and a caution statement.

The results of animal (rats) and human studies indicated that [^{14}C]Butoxyethanol was percutaneously absorbed and metabolized to butoxyacetic acid (excreted in the urine) after oral, dermal, or inhalation exposure. The in vitro percutaneous absorption of Butoxyethanol was demonstrated using rat, guinea pig, and human skin samples. When [^{14}C]Butoxyethanol was administered orally to rats, the major route of excretion was via the urine, and butoxyacetic acid was the major urinary metabolite. In one study, an analysis of tissues excised subsequent to oral administration indicated that the greatest specific activity was detected in the thymus, followed by the spleen and liver.

In acute inhalation toxicity studies, animal mortality rates were related to both the concentration of Butoxyethanol and the duration of exposure. At a concentration of 800 ppm Butoxyethanol, none of the six rats exposed died at 4 h, but three animals died at 8 h. In mice, 7- and 32-h exposures to 770 ppm Butoxyethanol caused 12.5 and 81.25% mortality, respectively. Seven-hour and 32-h exposures to 1,210 ppm Butoxyethanol caused 68.75 and 100% mortality, respectively.

Observations made in animals subjected to repeated inhalations of Butoxyethanol (concentration range 77–500 ppm) included increased erythrocyte fragility, decreased hemoglobin concentration and erythrocyte count, and pathological changes in the kidneys, liver, and lungs. Increased kidney weight was observed in guinea pigs subjected to repeated inhalation of Butoxyethanol concentrations ranging from 203 to 495 ppm. Effects were not noted in animals exposed to 20 and 62.5 ppm Butoxyethanol.

Butoxyethanol was slightly toxic (mean LD_{50} = 2.8 g/kg) in an acute oral toxicity study involving rats. In oral toxicity studies involving guinea pigs and rabbits, LD_{50} values of 1.20 and 0.35 g/kg, respectively, have been reported. Effects noted in rats that received repeated oral doses of Butoxyethanol (dose range 222–1,000 mg/kg) included the following: decreased erythrocyte count, packed cell volume, and hemoglobin concentration; increased weight of spleen, liver, and kidneys; and decreased weight of the thymus. Hemolytic effects of Butoxyethanol were also observed in a number of in vitro hematotoxicity studies.

Butoxyethanol was slightly toxic (mean LD_{50} = 0.58 g/kg) when administered dermally to rabbits. In dermal toxicity studies involving rats and guinea pigs, mean LD_{50} values of 2.52 and 0.23 ml/kg, respectively, have been reported. Dermal necrosis and hemoglobinuria were observed in rabbits that received repeated applications of Butoxyethanol. Also, in rabbits, Butoxyethanol was an ocular irritant and at most moderately irritating to the skin.

In studies involving rats, Butoxyethanol was nephrotoxic when administered intravenously, but was not nephrotoxic when administered intraperitoneally. Butoxyethanol also did not induce immunotoxicity in rats.

Butoxyethanol was not mutagenic in the Ames test or in point mutation, forward mutation, and chromosomal aberrations assays involving CHO cells. Butoxyethanol also did not induce SCEs in metabolically activated cultures of CHO cells. However, positive and negative results were noted without metabolic activation. Butoxyethanol was weakly mutagenic in an unscheduled DNA synthesis assay.

In dermal carcinogenicity studies involving rats, a hair dye containing Butoxyethanol (10.0%) was not carcinogenic, but a rust-preventive product containing Butoxyethanol (2.5%) was carcinogenic. It must be emphasized that the latter product contained 90.9% Stoddard solvent (a refined petroleum distillate).

In studies investigating reproductive effects, neither oral nor inhaled doses of Butoxyethanol caused testicular atrophy in rats or mice, respectively. However, oral administration of Butoxyethanol to mice resulted in a significant decrease in the number of viable litters. In an in vitro study, 100% embryo lethality was induced at Butoxyethanol concentrations of >12.5 m*M*.

In oral teratogenicity studies, the only significant finding in mice dosed with Butoxyethanol was a significant difference in the number of resorptions between experimental and control groups. In rats, oral administration of Butoxyethanol did not result in any increase in the incidence of fetal malformations over that noted in the control group; embryo/fetal effects included increased resorptions and nonlive implants.

In an inhalation teratogenicity study involving rats, ventricular septal defects and absent or severely shortened innominate arteries were noted in fetuses; results of fetal skeletal examinations and statistical analyses were not available. The results of other inhalation teratogenicity studies involving rats included mixed results regarding significant differences in the number of resorptions. In one of these studies, there were no statistically significant increases in the incidence of external, visceral, skeletal, or total malformations. The inhalation of Butoxyethanol by rabbits caused a significant reduction in unossified sternebra 6, rudimentary rib (first lumbar vertebra), and the number of viable implants per litter. However, there were no statistically significant increases in the number of litters with one or more fetuses with external, visceral, skeletal, or total malformations. Dermal

applications of Butoxyethanol to rats did not cause embryotoxicity or fetotoxicity, and there were no significant differences in visceral or skeletal defects.

The short-term inhalation exposure of Butoxyethanol vapor caused irritation of the nose and throat and ocular irritation in humans. Similar effects were reported in individuals exposed to Butoxyethanol, along with other chemicals, in the workplace. The results of erythrocyte fragility tests indicated that the sensitivity of humans to Butoxyethanol vapor was less than that of the rat.

In a human repeated insult patch test involving 201 healthy subjects, 10.0% Butoxyethanol was not a sensitizer. Definite erythema and barely perceptible erythema were observed in 1 and 13 subjects, respectively, during the challenge phase. Reactions ranging from barely perceptible to definite erythema were observed in 63 subjects during induction. The application of an ink wash solvent, diluted to a concentration of Butoxyethanol in the range of 0.5–2.5%, did not induce sensitization or photosensitization reactions in five individuals who had been previously exposed to Butoxyethanol in the workplace.

DISCUSSION

Current cosmetic product formulation data (1994) submitted to FDA indicate that Butoxyethanol is used in hair and nail products. Data submitted to FDA in 1989 indicate that Butoxyethanol was used in hair colors, bleaches, and shampoos at concentrations up to 10%. At the expected maximum use concentration of 10%, there was no evidence of skin sensitization reactions to aqueous Butoxyethanol in a repeated insult patch test involving 201 subjects. Thus, 10% Butoxyethanol is a safe concentration for product formulations intended for rinse-off or leave-on application to human skin.

The Expert Panel recognizes the severe ocular irritation potential of undiluted Butoxyethanol, but acknowledges that moderate and no corneal injuries were observed at concentrations of 15 and 5% aqueous, respectively. In consideration of these results, the ocular irritation potential of Butoxyethanol is attenuated at concentrations expected in cosmetic product formulations.

CONCLUSION

On the basis of the animal and clinical data included in this report, the Expert Panel concludes that Butoxyethanol is safe in hair and nail products at concentrations up to 10.0%.

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BUTOXYETHANOL

A safety assessment of Butoxyethanol was published in 1996 with the conclusion "safe in hair and nail products at concentrations up to 10.0%" (Andersen 1996). Studies available since that safety assessment was completed, along with the updated information regarding uses and use concentrations, were considered by the CIR Expert Panel. The Panel determined to not reopen this safety assessment.

TABLE 3
Historical and current cosmetic product uses and concentrations for Butoxyethanol

Product category	1984 use (Andersen 1996)	2001 use (FDA 2001)	1984 concentrations (Andersen 1996) (%)	2001 concentrations (CTFA 2001) (%)
Eye shadow	—	—	—	3
Shampoos (noncoloring)	2	—	≤0.1–1	
Hair tonics, dressings, etc.	—	2	—	—
Wave sets	1	—	1–5	
Hair dyes and colors	90	94	1–10	—
Hair tints	—	3	—	—
Shampoos (coloring)	—	8	—	—
Hair bleaches	4	2	1–10	—
Blushers	—	—	—	3
Nail polish and enamel	—	1	—	3
Nail polish and enamel removers	1	—	5–10	50
Total uses/ranges for Butoxyethanol	**121**	**110**	**≤0.1–10**	**3–50**

The Panel noted that the in vitro hematotoxicity study by Ruchaud et al. (1992), cited in the published Final Report, has been retracted and agreed that the deletion of this reference would have no substantive effect on this safety assessment.

The CIR Expert Panel discussion focused on the questionable evidence of carcinogenicity (in rats and mice) in a 2-year inhalation carcinogenicity study on Butoxyethanol that was published by the NTP in 2000. The conclusions for rats and mice were as follows: no evidence of carcinogenic activity in male rats; equivocal evidence of carcinogenic activity in female rats, based on increased combined incidences of benign or malignant pheochromocytoma (mainly benign) of the adrenal medulla; some evidence of carcinogenic activity in male mice, based on increased incidences of hemangiosarcoma of the liver; marginal increase in incidences of forestomach squamous cell papilloma and increase in incidences of hepatocellular carcinoma may have been exposure related; and some evidence of carcinogenic activity in female mice, based on increased incidences of forestomach squamous cell papilloma or carcinoma (mainly papilloma).

After reviewing the NTP study, the Panel noted that the results are not relevant to man for the following reasons: (1) Pheochromocytomas observed in one rodent species were within the historical control range. (2) Liver cancer observed in a male rat was thought to have been due to hemolysis [it has been shown that rodent red blood cells are very sensitive to hemolysis by Butoxyethanol, and that this effect was due to iron overload]. (3) An increased incidence of forestomach cancers was observed in female mice after inhalation exposure. The forestomach is not a relevant organ for evaluation, and this effect was thought to have been due to irritation, but not genotoxicity.

The Panel also considered current data indicating that Butoxyethanol is used at concentrations up to 50% in nail polish and enamel removers, in light of its previous limitation of 10.0% in hair and nail products. However, it was concluded that the increased use concentration was of little concern because the nail plate is made up of dead tissue, and, thus, the amount of absorption through the nail would be negligible.

Butoxyethanol was used in 121 cosmetic products in 1984, and a maximum use concentration of 10% was reported in 1984. Currently, there are 110 reported uses, with nail polish and enamel removers comprising the product category with the highest ingredient use concentration (50% Butoxyethanol). Table 3 presents the available use information.

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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: October 8, 2020

SUBJECT: Concentration of Use by FDA Product Category: Butoxyethanol

Butoxyethanol was included in the July 2020 concentration of use survey. No uses of this ingredient were reported.