Amended Safety Assessment of Alkonium Chlorides and Alkonium Bromides as Used in Cosmetics

Status:	Draft Tentative Amended Report for Panel Review
Release Date:	May 22, 2026
Panel Meeting Date:	June 15-16, 2026

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Bruce A. Brod, M.D., M.H.C.I., F.A.A.D., Donald V. Belsito, M.D.; Samuel M. 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. Previous Panel member involved in this assessment: David E. Cohen, M.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 Priya Ferguson, M.S., Associate Toxicologist/Senior Scientific Analyst/Writer, CIR.

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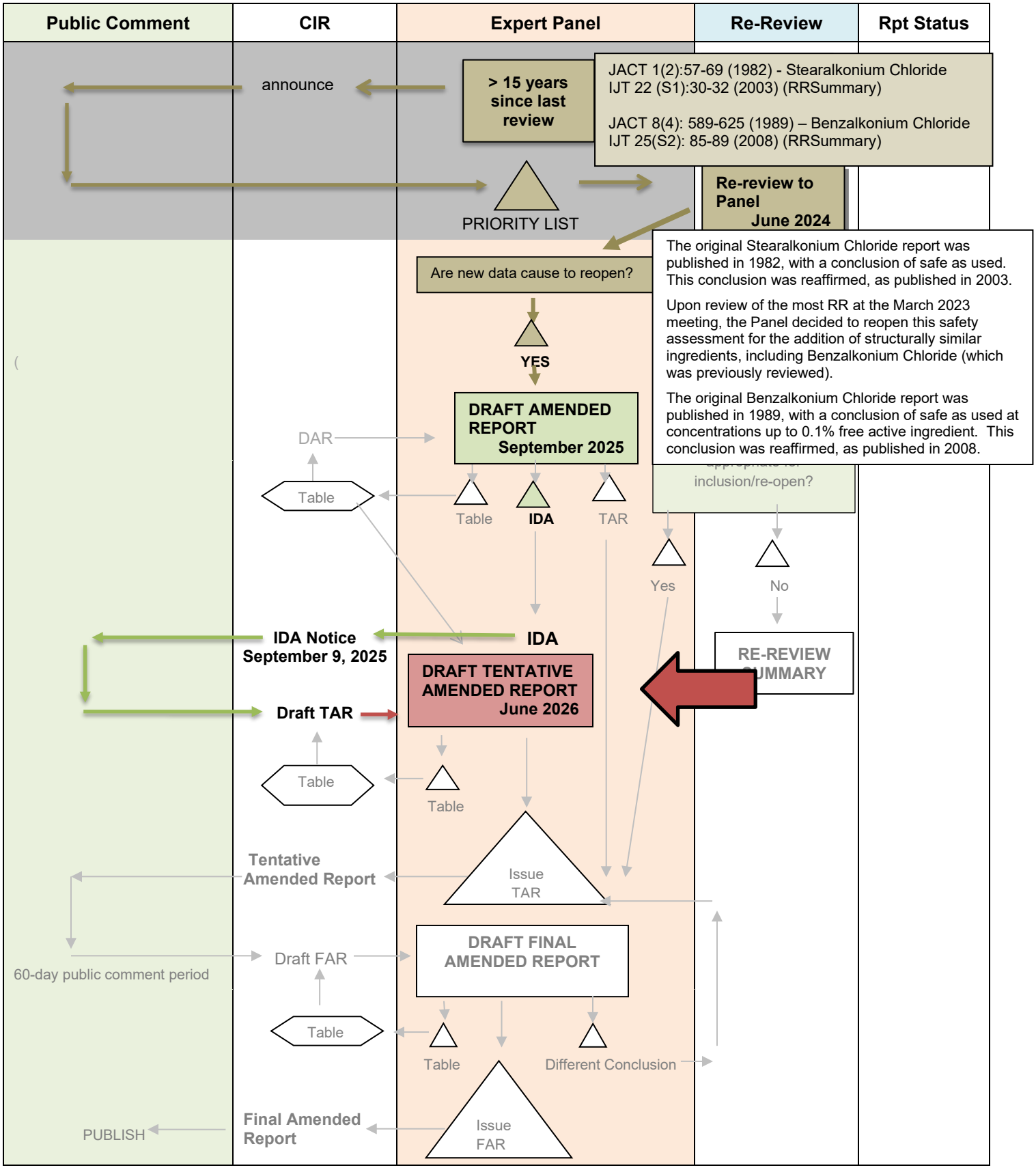
RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Alkonium Chlorides and Bromides

MEETING June 2026



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Priya Ferguson, M.S.
Associate Toxicologist/Senior Scientific Analyst/Writer, CIR
Date: May 22, 2026
Subject: Amended Safety Assessment of Alkonium Chlorides and Alkonium Bromides

Enclosed is the Draft Tentative Amended Report on the Safety Assessment of Alkonium Chlorides and Alkonium Bromides as Used in Cosmetics (it is identified as *report_AlkoniumChlorides_062026* in the report package). At the September 2025 meeting, the Panel determined that the data were insufficient to support the safety of these cosmetic ingredients and issued an Insufficient Data Annoucement (IDA) with the following data needs:

- impurities data on Behenalkonium Chloride, Benzalkonium Bromide, Cetearalkonium Bromide, and Lauralkonium Chloride
- HRIPT on Benzalkonium Chloride at maximum use concentration
- concentration of use of Benzalkonium Chloride and Stearalkonium Chloride in baby products
- concentration of use of Stearalkonium Chloride in products applied near the eye
- ocular irritation data on Stearalkonium Chloride at maximum concentration of use

Since the issuing of the IDA, the following data have been received and incorporated into the report in highlighted text:

- Japanese specifications and use information in medicinal products on Benzalkonium Chloride (*data1_AlkoniumChlorides_062026*)
- impurities data on Stearalkonium Chloride (*data2_AlkoniumChlorides_062026*)
- summary impurities information, in vitro dermal irritation, and in vitro ocular irritation data on Stearalkonium Chloride (*data3_AlkoniumChlorides_062026*)

Also incorporated in highlighted text are changes since the last iteration of this report, along with the addition of new studies found in recent published literature. These studies include toxicokinetics assays on intratracheally administered Benzalkonium Chloride, a study on the effect of Benzalkonium Chloride on gap junction communication, and a study evaluating the inhibitory effects of Benzalkonium Chloride on 11β-HSD2.

Updated 2025 RLD data and 2025 concentration of use (*data4_AlkoniumChlorides_062026*) information have been incorporated into the report. Since 2024, the number of reported uses for this ingredient group has increased; however, reported use concentrations have decreased. Among this ingredient group, the current (2025) maximum reported use concentration is for Stearalkonium Chloride for both rinse-off and leave-on products (2.6% in rinse-off hair conditioners and 2% in leave-on hair preparations). In the previous iteration of this report, the maximum reported concentration was also for Stearalkonium Chloride (it was reported to be used at up to 3.8% in tonics, dressings, and other hair grooming aids).

For comparison purposes, it should be noted that since the re-review of both Benzalkonium Chloride and Stearalkonium Chloride, the number of uses according to 2006/2001 VCRP data and 2023 VCRP data have decreased for both ingredients. The maximum concentrations of use have also decreased since the re-review of these ingredients (from 0.5 to 0.35% for Benzalkonium Chloride and from 7% to 2.6% for Stearalkonium Chloride).

Behenalkonium Chloride, Benzalkonium Chloride, and Stearalkonium Chloride were reported to have uses under category '(17) Other preparations (i.e., those preparations that do not fit another category)' in 2025 RLD. Twenty-six products for Benzalkonium Chloride were co-categorized as either eye makeup preparations (not children's), personal cleanliness products, skin care preparations, or hair preparations (non-coloring). In addition, in several instances, products were only categorized in RLD with a categorization of '(17) Other preparations;' however, the product names were useful in determining product type. For Behenalkonium Chloride, this product type was a hair conditioner. For Benzalkonium Chloride, these product types include makeup removers, moisturizers, toners, disposable wipes, hair masks, shampoos, hair

creams, and deodorant. For one product containing Benzalkonium Chloride, neither the product type nor the area/route of exposure is obvious from the information submitted to the RLD. Lastly, information reported for some of the '(17) Other preparations' for Benzalkonium Chloride and Stearalkonium Chloride suggests that those submitted products might not be considered cosmetic products in the US. We have sent a request to our colleagues in the FDA's OCAC for clarification.

Other documents included in this report package include:

- original 1982 report on Stearalkonium Chloride (*originalreportSAC_AlkoniumChlorides_062026*)
- 2003 re-review of Stearalkonium Chloride (*rereviewSAC_AlkoniumChlorides_062026*)
- original 1989 report on Benzalkonium Chloride (*originalreportBAC_AlkoniumChlorides_062026)*
- 2008 re-review of Benzalkonium Chloride (*rereviewBAC_AlkoniumChlorides_062026*)
- re-review data document on Benzalkonium Chloride (*RRdataBAC_AlkoniumChlorides_062026*)
- flow chart (*flow_AlkoniumChlorides_062026)*
- report history (*history_AlkoniumChlorides_062026*)
- search strategy (*search_AlkoniumChlorides_062026*)
- data profile (*dataprofile_AlkoniumChlorides_062026*)
- minutes from the meetings at which the original reports were discussed (*originalminutes_AlkoniumChlorides_062026*)
- transcripts from recent meetings discussing this amended report (*transcripts_AlkoniumChlorides_062026*)
- comments on the Draft Amended Report from Council that were received prior to the September meeting (*PCPCcomments_AlkoniumChlorides_062026*)
- responses to comments from Council on the Draft Amended Report (*PCPCcomment-reponse_AlkoniumChlorides_062026*)

A draft Abstract and Discussion have been included in this report version. The Panel should carefully consider and discuss the data (or lack thereof) and be prepared to issue a Tentative Amended Report with a safe, safe with qualifications, insufficient data, unsafe, or split conclusion, and identify any additional items for inclusion in the Discussion.

A table is provided to indicate data insufficiencies and availability of the requested data.

Data Insufficiency	Data needs met?	Details
impurities data on Behenalkonium Chloride, Benzalkonium Bromide, Cetearalkonium Bromide, and Lauralkonium Chloride	no	-
HRIPT on Benzalkonium Chloride at maximum use concentration	no	-
maximum concentration of use of Benzalkonium Chloride and Stearalkonium Chloride in baby products	partially	Benzalkonium Chloride is reported to be used at 0.053% in baby lotions, oils, and creams. Stearalkonium Chloride is reported to be used in 1 rinse-off "other baby product"; however, the concentration of use for this preparation is unknown.
maximum concentration of use of Stearalkonium Chloride in products applied near the eye	no*	*Stearalkonium Chloride is not reported to be used in products near the eye
ocular irritation data on Stearalkonium Chloride at maximum concentration of use	yes	in vitro ocular irritation assays provided at final active test concentrations of 3 and 85%

Alkonium Chlorides and Bromides History

1982

Final report on Stearalkonium Chloride published with following conclusion: “Stearalkonium Chloride is safe when incorporated in cosmetic products similar to those presently marketed”

1989

Final report on Benzalkonium Chloride published with following conclusion: Benzalkonium Chloride, at concentrations up to 0.1% free, active ingredient, is safe as a cosmetic ingredient as presently used”

2003

Re-review on Stearalkonium Chloride published – re-affirmed original conclusion

2008

Re-review on Benzalkonium Chloride published – re-affirmed original conclusion

March 2023

Another re-review considered on Stearalkonium Chloride; Panel decided to re-open assessment for addition of structurally similar ingredients (including Benzalkonium Chloride)

September 2025

Comments on Draft Amended Report received from Council

Panel reviews Draft Amended Report on alkonium chlorides and bromides and issues an IDA with the following data needs:

- impurities data on Behenalkonium Chloride, Benzalkonium Bromide, Cetearalkonium Bromide, and Lauralkonium Chloride
- HRIPT on Benzalkonium Chloride at maximum use concentration
- concentration of use of Benzalkonium Chloride and Stearalkonium Chloride in baby products
- concentration of use of Stearalkonium Chloride in products applied near the eye
- ocular irritation data on Stearalkonium Chloride at maximum concentration of use

Unpublished data received: Japanese purity specifications and use data in medicinal products

October 2025

Updated 2025 concentration of use data received

Impurities data received on Stearalkonium Chloride

Summary data received on Benzalkonium Chloride: impurities, in vitro dermal irritation, in vitro ocular irritation

June 2026

Panel reviews Draft Tentative Amended Report

Alkonium Chlorides and Bromides Data Profile* – June 2026 – Writer, Priya Ferguson

				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	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
Behenalkonium Chloride	**X**	**X**		X																									
Benzalkonium Bromide	**X**	**X**																											
Benzalkonium Chloride	**XO**	**XO**	XO		X	XO	X O	X O	X	o	xo	xo		xo	xo	x	x			XO	XO		XO	O		O	XO	X	XO
Cetearalkonium Bromide		**X**																											
Lauralkonium Chloride	**X**	**X**		X		X																							
Stearalkonium Chloride	**XO**	**X**	XO	X				O							x				XO	XO				O		XO	XO		

* "X" indicates that data were available in a category for the ingredient; "O" indicates that data were available in the previous reports on Stearalkonium Chloride or Benzalkonium Chloride

Alkonium Chlorides and Alkonium Bromides

Ingredient	CAS #	PubMed	FDA	CompTox	ChemPort	NIOSH	NTIS	NTP	FEMA	EU	ECHA	SIDS	SCCS	AICIS	FAO	WHO	Web
Behenalkonium Chloride	16841-14-8									X							X
Benzalkonium Bromide	91080-29-4	X															
Benzalkonium Chloride	61789-71-7; 68391-01-5; 68424-85-1; 8001-54-5; 85409-22-9	X	X	X	X					X	X			X			
Cetearalkonium Bromide										X							
Lauralkonium Chloride	139-07-1	X	X	X	X					X	X			X			
Stearalkonium Chloride	122-19-0	X	X							X	X			X			

X = relevant hits found

Search Strategy

PubMed searches done on INCI ingredient names and CAS numbers; INCI ingredient name also searched with qualifiers listed below

Benzalkonium Chloride was searched in PubMed from 2002 onwards and Stearalkonium Chloride was searched from 1998 onwards. All other ingredient searches were not restricted by publishing date.

INCI names and CAS numbers also searched in websites listed below.

Qualifiers

- Toxicity
- Dermal
- Safety
- Skin
- Allergy
- Penetration
- Genotoxicity
- Cancer
- Reproductive
- Developmental
- DART
- Cosmetic

Searched Websites/Search Engines

- Pubmed (- http://www.ncbi.nlm.nih.gov/pubmed)
- wINCI - https://incipedia.personalcarecouncil.org/winci/
- FDA databases http://www.ecfr.gov/cgi-bin/ECFR?page=browse
- FDA search databases: http://www.fda.gov/ForIndustry/FDABasicsforIndustry/ucm234631.htm;,
- EAFUS: http://www.accessdata.fda.gov/scripts/fcn/fcnnavigation.cfm?rpt=eafuslisting&displayall=true
- GRAS listing: http://www.fda.gov/food/ingredientspackaginglabeling/gras/default.htm
- SCOGS database: http://www.fda.gov/food/ingredientspackaginglabeling/gras/scogs/ucm2006852.htm
- Indirect Food Additives: http://www.accessdata.fda.gov/scripts/fdcc/?set=IndirectAdditives
- Drug Approvals and Database: http://www.fda.gov/Drugs/InformationOnDrugs/default.htm

- http://www.fda.gov/downloads/AboutFDA/CentersOffices/CDER/UCM135688.pdf
- FDA Orange Book: https://www.fda.gov/Drugs/InformationOnDrugs/ucm129662.htm
- OTC ingredient list: https://www.fda.gov/downloads/aboutfda/centersoffices/officeofmedicalproductsandtobacco/cder/ucm135688.pdf
- (inactive ingredients approved for drugs: http://www.accessdata.fda.gov/scripts/cder/iig/

- HPVIS (EPA High-Production Volume Info Systems) - https://ofmext.epa.gov/hpvis/HPVISlogon
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FEMA (Flavor & Extract Manufacturers Association) - http://www.femaflavor.org/search/apachesolr_search/

- EU CosIng database: http://ec.europa.eu/growth/tools-databases/cosing/
- ECHA (European Chemicals Agency – REACH dossiers) – http://echa.europa.eu/information-on-chemicals;jsessionid=A978100B4E4CC39C78C93A851EB3E3C7.live1
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- IUCLID (International Uniform Chemical Information Database) - https://iuclid6.echa.europa.eu/search
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- SCCS (Scientific Committee for Consumer Safety) opinions: http://ec.europa.eu/health/scientific_committees/consumer_safety/opinions/index_en.htm
- NICNAS (Australian National Industrial Chemical Notification and Assessment Scheme)- https://www.nicnas.gov.au/

- International Programme on Chemical Safety http://www.inchem.org/
- 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) technical reports - http://www.who.int/biologicals/technical_report_series/en/
- www.google.com - a general Google search should be performed for additional background information, to identify references that are available, and for other general information

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

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

FROM: Kimberly Norman, Ph.D., DABT, ERT
Industry Liaison to the CIR Expert Panel

DATE: September 2, 2025

SUBJECT: Draft Amended Report: Safety Assessment of Alkonium Chlorides and Bromides as Used in Cosmetics (draft prepared for the September 8-9, 2025, meeting)

The Personal Care Products Council respectfully submits the following comments on the draft amended report, Safety Assessment of Alkonium Chlorides and Bromides as Used in Cosmetics.

Key Issues

For the July 2025 concentration of use survey, CIR staff requested that four ingredients not in the current report (Lauralkonium Bromide, Myristalkonium Chloride, Caprylylalkonium Chloride, and Cetalkonium Chloride) be included. Will these ingredients be added to the CIR report? The potential for adding additional ingredients is not mentioned in the report memo.

Non-Cosmetic Use – The permitted use of Benzalkonium Chloride and Behenalkonium Chloride in first aid antiseptic products still needs to be added to this report. See the First Aid Antiseptic Drug Products monograph at Final Administrative Order OTC000030_M003-First Aid Antiseptic products for OTC Human Use_0.pdf
Permitted ingredients include: Benzalkonium chloride 0.1 to 0.13%; Benzethonium chloride 0.1 to 0.2%

Transport and Interaction of Cosmetic Product Material on the Ocular Surface; Summary – Based on the abstract, reference 76 appears to be a hypothesis rather than an experimental study. Please consider removing this study from the CIR report. If it is left in the report, please also add:
Response to: Malik and Claoué - transport and interaction of cosmetic product material within the ocular surface: beauty and the beastly symptoms of toxic tears.

Geis PA, Steinberg D. Cont Lens Anterior Eye. 2013 Jun;36(3):151. doi: 10.1016/j.clae.2013.01.008. Epub 2013 Mar 6.PMID: 23481273 No abstract available.

And Response to Geis et al.

Malik A, Claoué C. Cont Lens Anterior Eye. 2013 Jun;36(3):152. doi: 10.1016/j.clae.2013.03.005. PMID: 23587495 No abstract available.

Risk Assessment – Is the 90-day unpublished dermal rat study (rats treated with 1% Benzalkonium Chloride) used in reference 149 for the point of departure described in the CIR report? If possible, a description of this study should be in the CIR report in the appropriate section, and it should be noted that it has been used in a published risk assessment. If there is not a summary of this study in reference 149 (and if it is not already summarized in the CIR report), the risk assessment section should clearly state that it is not summarized in the CIR report and (if correct) insufficient details were available to summarize the study in the CIR report.

Additional Considerations

Introduction – There was also a re-review of Stearalkonium Chloride. That re-review document is not mentioned in the last paragraph of the Introduction, nor is a reference included in the Reference section.

Cosmetic Use – In addition to describing the use of Benzalkonium and Stearalkonium Chlorides, it would be helpful to describe use (or lack of use) for the other ingredients.

Cosmetic Use; Summary – Whenever a concentration of use is presented, the cosmetic product category for which it was reported should also be stated.

Dermal Penetration – In the text, please provide more details about the dermal penetration study using excised human skin such as the vehicle and the identity of the receptor fluid. It is not clear why the results at 0.03% are presented as the 0.3% concentration seems more relevant to cosmetic use concentrations. Rather than absorbed dose, the delivered dose (amount in skin and receptor fluid) is more relevant. The delivered dose was similar at the concentrations tested (2.22% at 0.03% and 2.16% at 0.3%).

ADME – In reference 34, was 120 µg/g/day a dose (g bw) or a dietary concentration (g diet)?

Acute, old report summary – Were mice treated with both 6.5% and 50% Benzalkonium Chloride? Or should this be "6.5% or 50% Benzalkonium Chloride"?

Acute – The durations of exposure need to be stated for the acute inhalation studies. Stating that the NOEL is <0.049 mg/m^3 is not very useful information. Based on the information in Table 7, it would be helpful to state: "Signs of respiratory irritation (concentration dependent reduction in tidal volume and an increase in respiratory rate) were observed in mice exposed (head-only) for 30 minutes to aerosolized Benzalkonium Chloride at concentrations of 0.049-19 mg/m^3. Inflammatory effects in the lungs were observed at the two highest concentrations (5.3 and 19 mg/m^3)."

Repeated-Dose – What do the 50 and 100 mg/kg values represent? Is this the amount of Benzalkonium Chloride, or the amount of Benzalkonium Chloride solution? Is this a dose (/kg bw) or the amount in the vehicle (/kg water or milk)?

Repeated Dose; Summary; Table 8 – For reference 39 it needs to be made clear that 5000 mg/kg is the dose of the sanitizing agent (containing 0.01% Benzalkonium Chloride) not the dose of Benzalkonium Chloride (0.5 mg/kg/day is the dose of Benzalkonium Chloride).
Please add "hr" after 6 in "6/day"

Development and Reproductive Toxicity, old report summary – On what days of gestation were the female rats treated vaginally with Benzalkonium Chloride?

Effect on Histamine Release, old report summary – Please add the units for the IC_{50} values.

Ocular Toxicity – Is a separate section really needed for these direct eye exposure studies? Perhaps they should be presented in an Ocular Irritation/Toxicity section.

Ocular Toxicity, old report summary – How were the human subjects treated with 0.01% Benzalkonium Chloride? What was the vehicle?

Reduction of Skin Irritation Potential, old report summary – This section should be presented after the skin irritation section. Please correct: "following by" (following" should be "followed")

Ocular Penetration Enhancement and Barrier Disruptions– These sections should be in the ADME section.

Effect of Benzalkonium on Mucociliary Clearance – The section belongs under Clinical Studies.

Dermal Irritation and Sensitization, Table 11 – If available, please provide more details about the results of the LLNA. What were the stimulation indices at each tested concentration? Was an EC_3 value calculated?

Mucous Membrane Irritation, old report study – How many times/day did the subjects use the oxymetazoline hydrochloride spray containing Benzalkonium Chloride?

Mucous Membrane Irritation – What was the vehicle for Benzalkonium Chloride in the sheep study (likely PBS as the controls were treated with PBS) (reference 100)?

Retrospective and Multicenter – What was the route of exposure in the 7 clinical trials?

Case Reports – If available, information on the doses/concentrations of Benzalkonium Chloride should be added to the CIR report.

Summary – Please correct: "more pronounces" to "more pronounced"

Table 6, Reference 30 – The Results column gives results for Benzalkonium Chloride in water, but the vehicle column does not list water as a vehicle (only citric acid, caprylyl glycol, and vitamin E).

Alkonium Chlorides and Bromides – June 2026 – Priya Ferguson	
SUBJECT: Draft Amended Report: Safety Assessment of Alkonium Chlorides and Bromides as Used in Cosmetics (draft prepared for the September 8-9, 2025, meeting) **Comment Submitter**: Kimberly Norman, Ph.D., DABT, ERT, Personal Care Products Council **Date of Submission:** September 2, 2025	
Comment	**Response/Action**
For the July 2025 concentration of use survey, CIR staff requested that four ingredients not in the current report (Lauralkonium Bromide, Myristalkonium Chloride, Caprylylalkonium Chloride, and Cetalkonium Chloride) be included. Will these ingredients be added to the CIR report? The potential for adding additional ingredients is not mentioned in the report memo.	These will not be added to the report. At the time of grouping formation, 2024 RLD was reviewed, and no uses were seen for these ingredients. The concentration of use survey included these ingredients to see if any uses were reported via that survey. Since there are none, these ingredients are not considered to be in use, and therefore are not being reviewed herein.
Non-Cosmetic Use – The permitted use of Benzalkonium Chloride and Behenalkonium Chloride in first aid antiseptic products still needs to be added to this report. See the First Aid Antiseptic Drug Products at Final Administrative Order OTC000030_M003-First Aid Antiseptic products for OTC Human Use_0.pdf Permitted ingredients include: Benzalkonium chloride 0.1 to 0.13%; Benzethonium chloride 0.1 to 0.2%	Addressed – however, it should be noted that Behenalkonium Chloride is not mentioned in the monograph.
Transport and Interaction of Cosmetic Product Material on the Ocular Surface; Summary –Based on the abstract, reference 76 appears to be a hypothesis rather than an experimental study. Please consider removing this study from the CIR report. If it is left in the report, please also add: Response to: Malik and Claoué - transport and interaction of cosmetic product material within the ocular surface: beauty and the beastly symptoms of toxic tears. Geis PA, Steinberg D. Cont Lens Anterior Eye. 2013 Jun;36(3):151. doi: 10.1016/j.clae.2013.01.008. Epub 2013 Mar 6.PMID: 23481273 No abstract available. And Response to Geis et al. Malik A, Claoué C. Cont Lens Anterior Eye. 2013 Jun;36(3):152. doi: 10.1016/j.clae.2013.03.005. PMID: 23587495 No abstract available	Study deleted.
Risk Assessment – Is the 90-day unpublished dermal rat study (rats treated with 1% Benzalkonium Chloride) used in reference 149 for the point of departure described in the CIR report? If possible, a description of this study should be in the CIR report in the appropriate section, and it should be noted that it has been used in a published risk assessment. If there is not a summary of this study in reference 149 (and if it is not already summarized in the CIR report), the risk assessment section should clearly state that it is not summarized in the CIR report and (if correct) insufficient details were available to summarize the study in the CIR report.	Addressed
Introduction – There was also a re-review of Stearalkonium Chloride. That re-review document is not mentioned in the last paragraph of the Introduction, nor is a reference included in the Reference section.	This document could not be referenced as we do not have access to that re-review document, however the RR sum is cited.
Cosmetic Use – In addition to describing the use of Benzalkonium and Stearalkonium Chlorides, it would be helpful to describe use (or lack of use) for the other ingredients.	lack of use reported for ingredient not in use

Alkonium Chlorides and Bromides – June 2026 – Priya Ferguson	
SUBJECT: Draft Amended Report: Safety Assessment of Alkonium Chlorides and Bromides as Used in Cosmetics (draft prepared for the September 8-9, 2025, meeting) **Comment Submitter**: Kimberly Norman, Ph.D., DABT, ERT, Personal Care Products Council **Date of Submission:** September 2, 2025	
Comment	**Response/Action**
Cosmetic Use; Summary – Whenever a concentration of use is presented, the cosmetic product category for which it was reported should also be stated.	Addressed
Dermal Penetration – In the text, please provide more details about the dermal penetration study using excised human skin such as the vehicle and the identity of the receptor fluid. It is not clear why the results at 0.03% are presented as the 0.3% concentration seems more relevant to cosmetic use concentrations. Rather than absorbed dose, the delivered dose (amount in skin and receptor fluid) is more relevant. The delivered dose was similar at the concentrations tested (2.22% at 0.03% and 2.16% at 0.3%).	Addressed; receptor fluid details in table
ADME – In reference 34, was 120 µg/g/day a dose (g bw) or a dietary concentration (g diet)?	Dose; test substance added to the control diet at a dosage of 120 µg/g/d
Acute, old report summary – Were mice treated with both 6.5% and 50% Benzalkonium Chloride? Or should this be "6.5% or 50% Benzalkonium Chloride"?	Addressed
Acute – The durations of exposure need to be stated for the acute inhalation studies. Stating that the NOEL is <0.049 mg/m3 is not very useful information. Based on the information in Table 7, it would be helpful to state: "Signs of respiratory irritation (concentration dependent reduction in tidal volume and an increase in respiratory rate) were observed in mice exposed (head-only) for 30 minutes to aerosolized Benzalkonium Chloride at concentrations of 0.049-19 mg/m^3. Inflammatory effects in the lungs were observed at the two highest concentrations (5.3 and 19 mg/m^3)."	Addressed
Repeated-Dose – What do the 50 and 100 mg/kg values represent? Is this the amount of Benzalkonium Chloride, or the amount of Benzalkonium Chloride solution? Is this a dose (/kg bw) or the amount in the vehicle (/kg water or milk)?	Original text does not make this clear.
Repeated Dose; Summary; Table 8 – For reference 39 it needs to be made clear that 5000 mg/kg is the dose of the sanitizing agent (containing 0.01% Benzalkonium Chloride) not the dose of Benzalkonium Chloride (0.5 mg/kg/day is the dose of Benzalkonium Chloride).Please add "hr" after 6 in "6/day"	Addressed
Development and Reproductive Toxicity, old report summary – On what days of gestation were the female rats treated vaginally with Benzalkonium Chloride?	Information not provided.
Effect on Histamine Release, old report summary – Please add the units for the IC50 values.	Addressed
Ocular Toxicity – Is a separate section really needed for these direct eye exposure studies? Perhaps they should be presented in an Ocular Irritation/Toxicity section.	All "other relevant study" ocular studies have been placed together in the other relevant studies section under relevant headers
Ocular Toxicity, old report summary – How were the human subjects treated with 0.01% Benzalkonium Chloride? What was the vehicle?	Addressed
Reduction of Skin Irritation Potential, old report summary – This section should be presented after the skin irritation	Addressed.

Alkonium Chlorides and Bromides – June 2026 – Priya Ferguson	
SUBJECT: Draft Amended Report: Safety Assessment of Alkonium Chlorides and Bromides as Used in Cosmetics (draft prepared for the September 8-9, 2025, meeting) **Comment Submitter**: Kimberly Norman, Ph.D., DABT, ERT, Personal Care Products Council **Date of Submission:** September 2, 2025	
Comment	**Response/Action**
section. Please correct: "following by" (following" should be "followed")	
Ocular Penetration Enhancement and Barrier Disruptions– These sections should be in the ADME section.	Kept as is as studies do not talk about the ADME of the ingredients, but of the effect this ingredient may have on certain drugs.
Effect of Benzalkonium on Mucociliary Clearance – The section belongs under Clinical Studies	Addressed
Dermal Irritation and Sensitization, Table 11 – If available, please provide more details about the results of the LLNA. What were the stimulation indices at each tested concentration? Was an EC3 value calculated?	Addressed
Mucous Membrane Irritation, old report study – How many times/day did the subjects use the oxymetazoline hydrochloride spray containing Benzalkonium Chloride?	Addressed
Mucous Membrane Irritation – What was the vehicle for Benzalkonium Chloride in the sheep study (likely PBS as the controls were treated with PBS) (reference 100)?	Addressed
Retrospective and Multicenter – What was the route of exposure in the 7 clinical trials?	Addressed
Case Reports – If available, information on the doses/concentrations of Benzalkonium Chloride should be added to the CIR report.	Not done as concentrations are not likely to affect Panel conclusion
Summary – Please correct: "more pronounces" to "more pronounced"	Addressed
Table 6, Reference 30 – The Results column gives results for Benzalkonium Chloride in water, but the vehicle column does not list water as a vehicle (only citric acid, caprylyl glycol, and vitamin E).	Addressed

MARCH 2023 MEETING – FIRST REVIEW/SECOND RE-REVIEW

Belsito Team – March 6, 2023

DR. BELSITO: Okay. So, then, the next one is stearalkonium chloride. This is a rereview. The expert panel first published our safety of stearalkonium chloride in '82, with a conclusion safe when incorporated in cosmetic products in concentrations similar to those presently marketed. We looked at a rereview in 2003, and we didn't reopen. We reaffirm the conclusion. But now it's been another 15 years, so we're back at it again. There were new studies for several tox endpoints. I don't think anything added to what we didn't already know.

So my opinion was, unless there are other stearalkonium chlorides that we can add to this report, like myristalkonium or anything else, and/or we want to further explore EU restrictions, there's no reason to reopen this. Thomas?

DR. GREMILLION: Thank you, Dr. Belsito. I wanted to ask, on Page 9, in the table there, it says in the earlier report, a teratogenicity assay performed using this read-across ingredient did not induce any indication of teratogenicity. My question was whether the more recent study reported in the table did find an indication of teratogenicity?

DR. BELSITO: You're on Page 9?

DR. GREMILLION: Yeah.

DR. BELSITO: That's the old report.

DR. GREMILLION: Wait a sec. Am I -- let's see.

DR. SNYDER: Yeah. The new DART study --

DR. GREMILLION: Oh, no. I'm sorry. Page 6.

DR. SNYDER: The new DART study was negative --

DR. HELDRETH: That's Page 6.

DR. SNYDER: -- 2,000 parts per million, highest dose tested.

DR. GREMILLION: Sorry. It was the first entry in the table on Page 6, under notable new data, the DART study. And there are a few NOAELs stated. So it just seemed like if the existing data didn't find any indication of teratogenicity, and this did, that might be a reason to reopen.

DR. BELSITO: It wasn't teratogenic. The litter size was decreased.

DR. GREMILLION: Okay.

DR. BELSITO: There were NOAEL for reproductive toxicity for F2, 2,000 parts per million due to reduced litter size at the highest concentration.

DR. GREMILLION: I guess, just from a consumer perspective, I read this, and from a consumer advocate perspective, I saw EU is (audio skip), it's been quite a few years, since 1982, when the Panel really looked at it closely. So I was looking for reasons to reopen.

DR. BELSITO: We are looking at it closely, in the sense that we're looking at all the new data plus our old report, right?

DR. GREMILLION: One other thing I noted was the EU reg says that contact with eyes should be avoided. And a lot of uses are like conditioners and shampoos, where it seems like there would be at least kind of incidental contact with the eyes. It shouldn't contain more than 3 percent, according to the EU reg, and here the max concentration of use is 3.8 percent.

DR. BELSITO: Well, it's 3 percent as benzalkonium chloride.

DR. GREMILLION: Okay.

DR. BELSITO: So this is stearalkonium chloride. The way I read it, there must be some residual concentrations of benzalkonium chloride and stearalkonium chloride. Which is why I raised -- one of the issues is we would reopen if there are other alkonium chlorides, like myristalkonium chloride, which is actually what one of the studies was done on, right?

DR. GREMILLION: Yeah, that was the teratogenicity.

DR. BELSITO: Right. Have we reviewed those, Bart?

DR. HELDRETH: We have reviewed some of the alkonium chlorides. I was trying to search right now to bring up which ones we looked at.

DR. BELSITO: Is there a reason to reopen to group with others?

DR. HELDRETH: So the Panel has previously assessed the safety of benzalkonium chloride.

DR. KLAASSEN: That's what I thought.

DR. HELDRETH: Benzalkonium montmorillonite (phonetic), benzalkonium bezipiollite (phonetic).

DR. BELSITO: What about myristalkonium chloride, which is used in cosmetics?

DR. HELDRETH: We have not --

DR. RETTIE: So the exposure of the population, generally, to benzalkonium chloride in hand sanitizers has just exploded, of course, over the last few years because of COVID. And there was some calls, I believe, from regulatory authorities to look more closely at the toxicity of benzalkonium chloride.

DR. BELSITO: Yeah. But that's being used as an OTC there, Allan, not as a cosmetic.

DR. RETTIE: Yeah.

DR. BELSITO: The use in cosmetics has also exploded because of the restrictions on MI, MCI, methyldibromo glutaronitrile and other preservatives. So benzalkonium chloride that you never saw in cosmetic products, now you'll see in the label down low because it's being used as a preservative system in the cosmetic product. But not at the level that it's used in hand sanitizers.

DR. HELDRETH: So, currently, in the VCRP data that we got from FDA this year, myristalkonium chloride only has two reported uses.

DR. BELSITO: I'm surprised. They must be used in -- like, I see them in hair products.

DR. SNYDER: Max concentration use went from 3 to 3.8 percent. Not significant, no new data, do not reopen.

DR. BELSITO: Right. I mean, that's what I said, too, Paul, unless there were other alkonium chlorides and we wanted to further explore the EU restrictions. But there was no reason from the data we saw to do it for EU reasons. And the question is, do we reopen to include other ingredients?

DR. HELDRETH: There's a longer chain one behen (phonetic) alkonium chloride, but it only has ten uses reported this year. And (inaudible) alkonium chloride, which is a shorter one, which only has four uses.

DR. BELSITO: Okay. So, Paul, you're in the not-reopened camp. Allan, Curt?

DR. KLAASSEN: Yeah, the conclusion isn't going to change.

DR. BELSITO: Okay.

DR. KLAASSEN: There is new data, but we don't open just because of new data.

DR. BELSITO: Okay. So we're suggesting not to reopen this, Priya.

DR. GREMILLION: Dr. Belsito, would you mind just saying again what the relationship between stearalkonium chloride and benzalkonium chloride is?

DR. BELSITO: There's no relationship, I mean, other than they're alkonium chlorides. I can only imagine that there may be benzalkonium chloride present in some samples of stearalkonium chloride. I don't know why the SCCS is pointing out benzalkonium chloride levels, and why they're equating stearalkonium chloride to benzalkonium chloride. I'm not a chemist in that regard.

DR. GREMILLION: I'm just reading the line on the first page of the memorandum. It says it should be noted that, according to the EU, stearalkonium chloride is used in rinsed-off hair products, and ready-to-use preparations should not contain more than 0.3 percent, parentheses as benzalkonium chloride. I don't understand.

DR. BELSITO: I don't either, quite honestly. That's what I was saying to Bart. I see it in a lot of haircare products. I think it's used as an antistatic. It's used, particularly, in hair conditioners is where I see these stearalkonium chloride, and myristalkonium chloride.

DR. GREMILLION: I mean, it's an order of magnitude under the concentration of use for stearalkonium chloride. And then it says stearalkonium chloride is safe for use as a food additive. It seems like --

DR. ANSELL: Well, that's an incorrect statement. It's actually 3 percent.

DR. BELSITO: I'm sorry. What's 40 percent, Jay?

DR. ANSELL: No, the CosIng at 0.3 percent is incorrect. The actual regulation allows it up to 3.0 percent.

MS. CHERIAN: The number in the table is correct. The number in the memo is 0.3. It should be 3.0.

DR. BELSITO: What pages are we on here?

MS. CHERIAN: PDF Page 2, in the memo, it says 0.3 for ready-to-use preparations and rinse-off hair products. And, in the table and PDF Page 3, it says maximum concentration in ready-to-use preparations should not contain more than three percent.

DR. ANSELL: That goes back to --

DR. GREMILLION: It says that according to European Union, ready-to-use preparation should not contain more than 0.3 percent. From Dr. Ansell, that should read 3 percent? But, then, I also don't understand what it means, it says stearalkonium chloride should not contain more than -- or hair products should not contain more than 0.3 percent, and then, parentheses, as benzalkonium chloride.

DR. BELSITO: No, 3.0 percent is the correct number. Right, Priya?

MS. CHERIAN: Yes.

DR. GREMILLION: Then what does it mean to have in parentheses as benzalkonium chloride?

DR. ANSELL: No guidance on that, I was just pointing out the correct number.

DR. BELSITO: I don't know.

DR. KLAASSEN: The only thing I could come up with, is maybe they are quantitating this by looking at how much alkonium chloride. It's a relatively nonspecific analytical method. That is, quantitating the alkyl chloride rather than the other compound or the other part of the molecule. Do you think that could be possible? I have no experience.

DR. BELSITO: If you look at the impurities data that we have from the old table, there's no mention of benzalkonium chloride. Three to 6 percent sterile alcohol, 1.5 percent to 4 percent sterile dimethylamine hydrochloride and sterile dimethylamine.

So I don't know why they're going to -- I mean, with formaldehyde it made sense because formalin is 37.5 percent formaldehyde in water. And we wanted to get away from the confusion between what formalin and formaldehyde was, and when added, it had to be formaldehyde equivalents.

That makes sense to me. Benzalkonium chloride in here makes zero sense to me. It's almost like they're using it as a surrogate, like Curt said. I don't know, Thomas. I can't answer your question.

DR. HELDRETH: I don't know if this informs the situation.

DR. GREMILLION: It could be a reason to reopen.

DR. HELDRETH: The Panel safety assessment of benzalkonium chloride, in 1989, states that the ingredient is used in cosmetic products as a foaming, cleansing bacterial side agent at concentrations up to 5 percent. The compound was non-mutagenic in several different cell assays. It is a skin and ocular irritant at concentrations greater than 0.1 percent. The cosmetic ingredient is not a sensitizer to normal humans at concentrations of 0.1 percent, but may be to individuals with diseased skin.

It is concluded that benzalkonium chloride can be safely used as an antimicrobial agent at concentrations up to 0.1 percent. Maybe the concern has to do with the irritancy of benzalkonium chloride. I'm not sure.

DR. BELSITO: So sort of a class effect like the FDA does where, if a (audio skip) inhibitor given systemically causes an effect when put topically, it has to be labeled that same way, despite the fact that there's zero absorption and any concern for any of the systemic tox. I don't know. I personally am not worried about it and think we shouldn't reopen.

DR. ANSELL: Yeah. We would agree. The fact that we can't explain the analysis doesn't suggest there's data that would affect us requiring a reopening.

DR. BELSITO: Plus, we've already restricted benzalkonium chloride to 0.1 percent, which is essentially what the EU did.

DR. GREMILLION: It's not clear what the EU did.

DR. BELSITO: Yeah, I know.

DR. GREMILLION: Yeah.

DR. BELSITO: Except that they said there shouldn't be more than 0.1 percent benzalkonium chloride. Which, if you go to our benzalkonium chloride report, we've already said that.

While Thomas is thinking about that, Priya, just a question. Here on PDF Page 4, under the ADME dermal, you say that the maximum systemic absorption, feces, urine, carcass, and skin site, was 50 and 51.1 percent for males and females. Test substance uniformly distributed plasma. So I'm having a hard time to really get to 50 percent.

I think most of it was stuck in the skin, right, given all the other data we have on the poor absorption, unless the animals were really licking this stuff off. So was the predominant amount found in the skin and the carcass, which I presume would have contained skin?

MS. CHERIAN: I can look back at the study and see if it differentiated the amount between carcass and skin type.

DR. BELSITO: Yeah. I think the amount that would be present in plasma and blood would be extraordinarily low. And here it gives the impression that it could be 50 to 50.1 percent.

So it'd be nice to get some definition of where they really found it in the results overview there. Because all the other data would suggest that they found it in the stratum corneum, the skin, the carcass, but not the blood. That was it. But I didn't think we needed to reopen. Thomas?

DR. GREMILLION: Yeah. I don't know. I was hoping I could find the EU report or language to understand that better, but maybe it'll come out tomorrow.

Cohen Team – March 6, 2023

DR. COHEN: So stearalkonium chloride was first reviewed in 1982, with a conclusion of safe. And was re-reviewed and reaffirmed in 1982 -- they reaffirmed the 1982 conclusion in 2003, so it's been 15 years. A couple of notes that I highlighted; the EU has stearalkonium chloride as used in rinse off hair products, and ready to use preparations, not containing more than 0.3 percent as benzalkonium chloride. And FDA has stearalkonium chloride as safe as a food additive, antimicrobial agent, adhesive, and slimicide.

We have updated use data with stearalkonium chloride in 88 formulations up to 3.8 percent in 2001. And it's reported use in 151 formulations up to 7 percent now and 3 percent in leave-on. Looks like the skin irritation/sensitization studies from the original report were at 1 percent. There's also some more recent literature on an increase in benzalkonium chloride reactivity in contact dermatitis.

So, before I discuss what I thought we should with this, I just wanted to open it up for the rest of you. Is this a reopen or not?

DR. ROSS: I thought it was a reopen.

DR. BERGFELD: I did, too.

DR. ROSS: Based on the EU conclusion. I mean, I think we're on record as -- I think previously, at least the way I read it, 2001, it was at 7 percent and according to --

DR. COHEN: Oh, did I reverse it?

DR. ROSS: Yeah.

DR. TILTON: Yeah.

DR. ROSS: 2022 was 3.8 percent. And so, I think it's on record as safe as used at 7 percent and just based on that I thought we should reopen.

MS. CHERIAN: I just wanted to clarify that the 0.3 in the memo should be 3 percent. In the table it says 3 percent. So, max concentration in ready to use preparations should not contain more than 3 percent.

DR. COHEN: Oh. Was that in the verbiage as 0.3 percent?

MS. CHERIAN: In the memo.

DR. COHEN: Okay.

MS. CHERIAN: It should've been 3.

DR. COHEN: Okay.

DR. ROSS: Alex pointed it out I think in the Wave 3. Yeah. In the text it was 0.3, in the table it was 3 and so it's 3.

DR. SLAGA: So, it's 0.3, right?

MS. CHERIAN: Three.

DR. ROSS: No, it's three.

DR. COHEN: It's three.

DR. SLAGA: Three.

DR. ROSS: And so, I think it's still on record as safe as used at 7 percent from the previous conclusion of this committee which is why I felt that we should reopen it, based on that a new conclusion if we're still up at 7 percent and the EU has got a finding at 3 percent.

DR. SLAGA: Yeah.

DR. BERGFELD: We also have a lot of new tox data in this one, I think.

DR. COHEN: I came to the same conclusion that we should reopen it.

DR. SLAGA: Yeah.

DR. BERGFELD: Me too.

DR. COHEN: It's a pretty commonly used product and I think as we heard from the discussion this morning, that commercial uses the ingredients are going to be disclosed. Benzalkonium chloride gets -- these quaternary ammoniums get used a lot so it's time for a refresh on this. And just, I guess, one question. Monice, is a hand protectant used in industry a cosmetic agent?

MS. FIUME: So, I'm not sure, David, what you're asking. Are you asking if it's something that is required for use as they're using it or as a moisturizer type protector?

DR. COHEN: It's probably being used as a moisturizer or hand sanitizer or something like that, but within industry, not for retail use, but like as a GOJO or something like that. Does that fall under this purview?

MS. FIUME: I am not sure. Carol, do you have an answer to that question because I don't want to answer it incorrectly? Or does FDA have an answer to that question?

MS. EISENMANN: I mean, it depends on partly how the product is labeled. But I'm not aware that it's an official OTC material for that use.

DR. COHEN: Yeah, okay. Okay. So, we'll reopen this and have further discussion tomorrow.

MS. FIUME: And then, I think I have one further clarification just for the purposes of this discussion tomorrow. Am I reading the limitation, the limitation is 3 percent for --

DR. COHEN: EU.

MS. FIUME: But for the rinse off hair products, what is the limit, Priya?

MS. CHERIAN: Three percent.

MS. EISENMANN: No, it has a limit of 0.1 for products other than rinse off hair products. So other products is 0.1 percent.

MS. FIUME: And then rinse off hair products is 3 percent?

MS. EISENMANN: Correct.

MS. FIUME: Okay, thank you.

DR. COHEN: Yeah. I guess, I was a little confused with that too because "ready to use preparations" kind of threw me off. So final product concentrations of benzalkonium chloride, bromide, and saccharinate with an alkyl chain of C_{14} or less must not exceed 0.1 percent as benzalkonium chloride. And then David's point is the old reports way higher than that. So, we really need to relook at this. Okay. Any further comments?

DR. BERGFELD: Well, just to mention that in the summary in the old report, that they had rabbit studies at 25 percent and showed some irritations. I mean, they had some animal studies, not just human.

DR. COHEN: Yeah. Well, I think that would come into a new report anyway, right? So, we pull that up.

DR. BERGFELD: Well, that's the old report. Yeah.

DR. COHEN: Yeah. Okay. I mean, we don't often see benzalkonium chloride still in the North American standard series anymore, but I test to it routinely. Okay.

DR. COHEN: Okay. Any other comments on propylene carbonate? It's used as an excipient in tacrolimus ointment.

Full Panel – March 7, 2023

DR. BERGFELD: Okay. Next item is Dr. Cohen again. Stearalkonium chloride.

DR. COHEN: Okay. So stearalkonium chloride was reviewed by the panel in 1982, with a conclusion of safe. It was re-reveiwed again in 2003, reaffirming that. It's listed in Annex 3 with some concentrations in rinse off and ready to use products of 3 percent as benzalkonium chloride and in other final products as 0.1. It's additionally cleared by FDA as a food additive anti-microbial agent, adhesive, and slimicide.

We had some updated data, including concentration of use of 3.8 percent. There's been numerous publications about contact dermatitis to BAK. There's a lot of data out there discussing contact dermatitis to this -- to quaternary ammoniums and this report is 40 years old. And we discussed reopening this to update this report.

DR. BERGFELD: Is that a motion?

DR. COHEN: That's a motion.

DR. BERGFELD: Don, any support of that motion or discussion?

DR. BELSITO: We didn't feel that it needed to be reopened, unless we were going to add other alkonium chlorides like myristalkonium, behenyl alkonium chloride (phonetic) to the report. That was our team's conclusion.

DR. COHEN: Don, can you give us -- because I don't know -- a little history? Were those previously reviewed?

DR. BELSITO: Bart would have to give that history. We asked about myristalkonium, it was reviewed. Is that correct, Bart?

DR. HELDRETH: Myristalkonium chloride, behenyl alkonium chloride have not been reviewed. They both have VCRP entries of ten or fewer frequency of use. The only alkonium chloride that the panel has performed a safety assessment on so far is the benzalkonium chloride.

DR. BELSITO: I mean, if we can add in those other alkonium chlorides, and you want to reopen it to look at sensitization data, I don't have a problem with that. I also don't understand the EU sort of making the data as benzalkonium equivalents. I don't understand at all where that language comes from. I understand with like formaldehyde because formalin is 37.5 percent formaldehyde. I understand that clarification that we made in our report. I don't understand the EU.

But if we can add in all the other alkonium chlorides in the dictionary, even if they have low use and you want to look at the sensitization data, I'm happy with that.

DR. COHEN: I think that's a great idea. I hadn't even thought of that, but that would make this more robust.

DR. RETTIE: Can I ask for some clarification on nomenclature here? My internet dropped out and I missed this one yesterday, so I would've asked this then. My understanding is we were talking about benzalkonium chloride as if it's one thing.

DR. COHEN: Right, right.

DR. RETTIE: My understanding is that the BAKs are a family that includes the C_{18} chainline, which is -- I can't say this word -- stearalkonium chloride, which is the single thing we're talking about here.

So, I'm just confused because I don't understand if benzalkonium chlorides that we've looked at in the past include this or not. Because it seems to me that they should.

DR. COHEN: Allan, I was very confused by it as well, because some of the limits are as benzalkonium chloride. And when you look up stearalkonium chloride, it says it is a benzalkonium chloride. So, this report is 40 years old. It might be nice to update the chemistry, the nomenclature, for people to use. And I thought Don's idea was great.

DR. BERGFELD: So are you adding that to your motion, by the way?

DR. COHEN: Yes.

DR. BERGFELD: Okay. Bart, do you want to respond in any way?

DR. HELDRETH: Yeah. No, I completely agree with Allan, it is very confusing. We have these, essentially, tertiary ammonium chloride salts, which is supposed to only vary by the quote/unquote alkonium piece. The -- how man -- you know, what the alkyl groups are that come off of this. And then the other -- the fourth group on that tertiary ammonium is either stearal -- in the case of stearalkonium chloride, or benzal in the case of benzal and so forth.

So I agree, we will -- when we create this draft amended report, we will try to flesh out those details to make it a little bit clearer.

DR. BERGFELD: Would you be also expanding the chemical groups, you think?

DR. HELDRETH: Yeah. That's what I'm hearing that the motion will now include adding the alkyl alkonium chlorides. Not benz, but things like myristal and behenyl that are just different chain links.

DR. BERGFELD: Okay. Okay. Allan, did you have something to say? I'm sorry.

DR. RETTIE: No, it was off-topic. Forget it.

DR. BERGFELD: Okay. Okay. It looks like we have a second, is that true, Don, to reopen this ingredient and expand it slightly?

DR. BELSITO: Yes.

DR. BERGFELD: Okay. And we have an approval by Bart to do so. So we have a second. I'm going to call the question. All those opposing reopening? Abstaining? So, this ingredient is reopened.

SEPTEMBER 2025 MEETING – SECOND REVIEW/DRAFT REPORT REVIEW

Belsito Team – September 8, 2025

DR. BELSITO: Moving on to Alkonium Chlorides. Okay. So, we reviewed Stearalkonium Chloride, concluded that it was safe when incorporated in cosmetics similar to those presently marketed. That was in 1982. And, in 2003, we reaffirmed the conclusion. It was 15 years since that re-review, so we took a look at it again in March of 2023. We reopened it for the addition of structurally similar ingredients.

Benzalkonium Chloride was also previously reviewed by the Panel on a safety assessment in '89, with the conclusion that at concentrations up to 0.1 percent free active ingredient, it was safe in cosmetic products. We reaffirmed that in '89. It was published in 2008. So, now we're looking at a Draft Amended Report on these ingredients and four previously unreviewed ingredients, including Behenalkonium Chloride, Benzalkonium Bromide, Cetearalkonium Bromide and Lauralkonium Bromide.

So, Benzalkonium and Stearalkonium were previously reported to be used in 79 as of 2006, and 151 as of 2001, so they were going down. According to 2023 VCRP, they're even further down, 69 and 88. Maximum reported concentration of Benzalkonium has remained consistent with up to 0.5 percent reported. Maximum concentration of use of Stearalkonium has decreased. And Stearalkonium is currently used in 885 formulations, Benzalkonium in 565. Lots of studies.

So, in terms of those studies, we now have evidence of penetration enhancement, so that would need to be in our Discussion. We have a repro NOAEL. Repro NOAEL was 2,000 parts per million or 30 milligrams per kilogram body weight per day, which we could use for marginal exposure if we needed. We have eye makeup preps at 0.1 percent.

And just a discussion for the team, while we're in ocular, the transport and interaction of cosmetic products materials on the ocular surface, which was a separate heading under miscellaneous, would that be better under ocular toxicity as a discussion or maintained separate?

DR. RETTIE: Sounds like it's under ocular.

DR. SNYDER: I agree. I think it should be under ocular.

DR. BELSITO: And then, the same as accumulation following ocular administration to accumulate, that's PDF Page 25. In ocular penetration enhancement, I just thought it might all be better under ocular.

DR. RETTIE: Yep.

DR. SNYDER: I think it makes more sense to keep it all together.

DR. BELSITO: Yeah. Okay. So, just a little rearrangement there. And, in terms of the penetration enhancement, I just made a note that the Benzalkonium Chloride and Prostaglandin eye drops can increase absorption. So, we need to bring that study over to the Prostaglandin study, Prostaglandin Report, whomever going to be doing that.

Endocrine activity, this is PDF Page 26. I thought we should put it -- we need to discuss it. But I thought, given the dose, they're really irrelevant to the use of these alkonium chlorides in cosmetic products.

DR. SNYDER: I agree with that.

DR. BELSITO: Curt, Allan?

DR. KLAASSEN: Yes. Fine.

DR. RETTIE: Sure.

DR. BELSITO: Okay.

DR. RETTIE: I'm looking at it.

DR. BELSITO: Sensitization, I think, is okay. They can be irritating. So, final conclusion would be to formulate to be non-irritating. This phototoxicity I found curious given the result, the lack of absorption. I wasn't overwhelmed with the phototox in Table 11. I don't know if anyone else was. Synergistic effects were observed when UV was administered with Benzalkonium Chloride. Anyone else have concerns about that?

DR. SNYDER: I didn't pick up on that, so I don't.

DR. RETTIE: Yeah. I'm with Paul, I didn't either.

DR. BELSITO: It's PDF Page 28. You see where I am under phototoxicity?

DR. SNYDER: Is that for in vitro, Don? It's kind of confusing there because it says that it's in vitro in cultures. But then it says, after administration. So, do they mean after?

DR. BELSITO: Yeah. I took it that way. It's in vitro.

DR. SNYDER: Yeah.

DR. BELSITO: I don't make anything of it in terms of cosmetic use. We have absorption showing that there's really a lack of absorption. And I don't think phototoxicity is an issue with cosmetic use.

DR. SNYDER: We just need to put that in the Discussion.

DR. BELSITO: Right. Then, on PDF Page 30, it says, the bottom of the page, the NOAEL chosen for risk characteristics was 20 milligrams per kilogram per day, which was derived from a 90-day unpublished dermal rat study treated with topical 1 percent. I couldn't find that study in our report.

MS. FERGUSON: It was an unpublished study that I couldn't find either. This MOS calculation was performed by someone else, and they used an unpublished study to create it.

DR. BELSITO: Who was it performed by, 149, Choi, Roh, Lim, Kacew, Kim? And, in the Journal of Toxicology Environmental Health Critical Reviews, it doesn't say how they got that value?

MS. FERGUSON: I don't believe so, but I can go back and double check.

DR. BELSITO: Yeah. If we could, check the primary reference. So, having said all that, penetration enhancement in our Discussion, discuss endocrine disruption studies, doses not relevant to humans, formulate to be non-irritating. Do our own margin of exposure calculations or not?

Use the whole Discussion regarding Benzalkonium Chloride binders and effective free concentration. That's all I had. And, basically, safe as used when formulated to be non-irritating.

DR. SNYDER: I agree with the discussion points and the conclusion of safe when used, formulated be non-irritating.

DR. RETTIE: Agreed.

DR. KLAASSEN: Agreed.

DR. BELSITO: Okay. Brown algae.

MS. FIUME: Don, I'm sorry. I know it's late, but before we move on.

DR. BELSITO: No. That's fine.

MS. FIUME: Just as we go forward with the report, did I understand that you want the ocular irritation, the irritation studies that were under clinical assessment that were done in patients and the -- where is the other one -- accumulation following ocular administration all together?

DR. BELSITO: Under ocular.

MS. FIUME: So, where do you want that in the report? Do you want an under other relevant studies? Because we've sort of gone by the premise that the things under, like, the irritation, either the dermal irritation and sensitization or ocular, are true studies not on patients. And we've always broke them out between clinical and those irritation sections.

And things that really weren't irritation, we had put under the other relevant studies. So, just for our use, as we go forward, where would you like to see all of that combined in the report?

DR. BELSITO: I know that we have a set format, and I'm not sure what usually comes after ocular. I guess, in this case, mucous membrane irritation. But maybe in this case, other relevant ocular studies right after the ocular section, so it's not that far removed.

MS. FIUME: Okay. So, keep ocular irritation as its own subsection, and then the other ocular studies as a second subsection?

DR. BELSITO: Yeah. So that they're essentially together. Although, if you want to bracket ocular irritation, as we always do, do that, and then make a next subheading of other relevant ocular studies. We'll discuss with the whole Panel tomorrow anyway.

MS. FIUME: Yeah. That would be appreciated because I know sometimes we move things, and then someone else says I can't find it. If we could discuss that tomorrow, that'd be great.

DR. BELSITO: The other team gets upset. Yeah.

MS. FIUME: Yeah.

DR. BELSITO: Okay.

MS. FIUME: Thank you.

DR. RETTIE: I just mentioned we explicitly call out a mixture for one of these, but they're all mixtures. And I wondered if we could have a clarification in table -- let me find it. Or I assume they're all mixtures. Certainly Benzalkonium is a mixture. And we say that the cetyl and stearyl are a mixture, the R16 and the R18 chain links, but nothing about any of the others.

So, I was just curious if we knew what the ratios were because we have some information about ratios for Benzalkonium Chloride. So, it's just really a clarification that all of these are actually mixtures, and any percentage data that might exist, particularly for the combined cetyl, stearyl one. Probably getting a bit down in the weeds there, but that was my only other comment.

DR. BELSITO: Okay. And then, in the tables, Priya, Table 10 Genotoxicity, this is PDF Page 54. The second study on Benzalkonium Chloride, you have mean olive tail movement. It should be moment. You see where I'm at?

MS. FERGUSON: Yep, I see what you're saying. Thank you.

DR. BELSITO: And, in the Table 13, where you give the patch test concentrations, if it's available, I think it's important to add the vehicle as some centers use Benzalkonium Chloride in water, others use it in PET. And Benzalkonium Chloride in water is more irritating, will lead to more false positives on patch testing. So, it would be important to see if there are differences, like 0.5 versus 4.3, whether the 4.3 was water and the 0.5 PET.

Because it's tricky to patch test these alkonium chlorides because you get a lot of weak positive patch tests that are really more irritant than sensitization. And those were the only other comments that I had on these.

MS. FERGUSON: For the margin of exposure calculation, which endpoint would you like that one?

DR. BELSITO: I had that here someplace.

MS. FERGUSON: Did you originally say repro? I think I heard that in the very beginning.

DR. BELSITO: Yeah, except that I couldn't find the 20 milligram per kilogram body weight per day for the DART endpoint. Let me see. But it's that 90-day unpublished dermal study that we can't find.

So, it'd be nice if we can just see if they say how they got that endpoint. But I think that would be the one to use if we can find out how it was derived. Any other questions, Monice, Priya, team?

MS. FIUME: No, thank you.

DR. BELSITO: This is going to be a first. It's now 5:06. We've made it past 5:00.

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DR. DAVID COHEN: Okay. So, we'll go to Alkonium Chlorides. We first published a review of the safety of Stearalkonium chloride in 1982, and the Panel concluded that it is safe when incorporated into cosmetic products similar to those presently marketed. That's a very old Conclusion. The Panel considered a rereview of this report and reaffirmed the '82 Conclusion in 2003.

It's been 15 years and in accordance with the CIR procedure, we again reviewed this agreement in March 2023. We decided to reopen it for the addition of structurally similar ingredients. One of the additions, Benzalkonium Chloride, was also previously reviewed by the Panel in the safety assessment with the Conclusion that Benzalkonium Chloride at concentrations up to 0.1 percent free active ingredient is safe in cosmetic ingredients as presently used.

The Panel considered rereview of the ingredient and reaffirmed the 1989 conclusion in 2008. We have a Draft Amended Report on these ingredients as well as four unreviewed ingredients, which is Behenalkonium Chloride, Benzalkonium Bromide, Cetearalkonium Bromide, and Lauralkonium Bromide.

Benzalkonium Chloride and Stearalkonium Chloride were previously reported to be used in 79 products as of 2006, and 151 formulations in 2001. The VCRP, in 2023, have them in 69 and 88 respectively. So, the Benzal is 69, the Stearal is 88.

Maximum use concentration of Benzalkonium Chloride has remained at 0.5 percent reported in both 2006 and 2023, and Stearalkonium Chloride has decreased from the reported 7 percent. They have, in 2024, lots of uses. And numerous studies on Benzalkonium Chloride were identified in the literature.

So that's just the opening recitation; there's a lot to discuss here. Any comments? Any nitrosamine issues with these that we have to talk about?

DR. ROSS: Yeah. Go ahead.

DR. DAVID COHEN: David, you can start. It's okay.

DR. ROSS: I was pulling up the dossier here. Yeah. There are, I think. Let's see.

DR. BERGFELD: Can we read across with these extra ingredients?

DR. ROSS: Oh, I knew that question was going to come up. I predicted it would be the first question, and it was pretty close after nitrosamines. So, I'll leave the nitrosamines for a second. Susan, maybe you can get to that. But the read-across, I mean I thought when I first looked at this that there was no reason not to read across. But then at second take, as the great Dr. Cohen just said there, there were some real issues that cropped up here.

So, if you look at them, the Benzalkonium Chloride, for example, is a mixture of variable length alkyl chain, C8-C18. It's optimally designed to disrupt membranes, essentially, so it's a great biocide, and that's one of the reasons you use it. If you take Stearalkonium chloride, it's a single long C18 alkyl chain. I mean, it will disrupt membranes, but not particularly well relative to Benzalkonium Chloride, and it's actually a better conditioner. So, if you actually compare the data of Benzalkonium and Stearalkonium across this dossier, you'll see that there's more toxicity and irritancy with Benzalkonium Chloride than there is with Stearalkonium and that's the reason.

I had an initial conversation with Dr. Rettie about this and, initially, I felt that we could read across, but then when I dug into it, I wasn't sure. However, I think if we can clear both Stearalkonium and Benzalkonium chlorides for ocular, dermal, and mucous membrane effects, if we can clear them both then I think you can read across to the others because you've got representatives of both classes of compound basically. And so, I think that would be the way I would go with this on the read-across.

Susan, do you want to do a nitrosamines while I find it?

DR. TILTON: I am looking, too. I just wanted to comment on the read-across.

DR. ROSS: Okay, go ahead.

DR. TILTON: I was actually going to bring this up in our group meeting last week. So, I think that's a good summary. I mean, I do notice that we don't have things like impurities for the new compounds. I was questioning because of the concerns about dermal and ocular irritation, whether or not that was needed. But thinking of it from this perspective, as we kind of have this -- I don't know if you would consider it a range of response since we have two ingredients, but because we have data from these groups, and if we can go through and clear based on that, I agree that it could clear the other compounds.

I mean those are some of the primary concerns due to how we know these to work. The fact that we know they're going to be cytotoxic.

DR. ROSS: Yeah. I mean, bottom line is on the nitrosamines, yeah, you want some comment about nitrosamine levels in here, David. And I think we've got standard text on those nitrosamines, but that would have to be in there.

DR. TILTON: Is that in here?

DR. ROSS: Yeah, it's not in there right now but -- at least I didn't see it.

DR. TILTON: I guess it shows up in reactivity. Just the description. And that's derived from the original report.

DR. ROSS: Yeah. So, we want some indication of the levels of nitrosamines. And I think you'll get the same thing from Allan from this report.

DR. DAVID COHEN: Are we having an IDA on this?

DR. ROSS: No, I think it would be an insufficiency. I've got a long list of things here.

DR. DAVID COHEN: So, we need impurities on the new compounds. Can you just call them out so I can have in my --

DR. ROSS: Yeah.

DR. DAVID COHEN: Since we're presenting this.

DR. ROSS: You could just say the new compounds, but there's Behenalkonium Chloride, there's --

DR. DAVID COHEN: I guess I could go to the four that we've just added.

DR. TILTON: Yeah, it's for those.

DR. DAVID COHEN: I got that. Okay. What else?

DR. ROSS: I thought you needed dermal irritation and sensitization for Benzalkonium chloride at the max use of 0.25 percent.

DR. DAVID COHEN: Maybe this is the time to discuss this.

DR. ROSS: What do you think?

DR. DAVID COHEN: I really, really spent a lot of time on this report trying to come to terms with it. So, we have a Conclusion. Benzalkonium Chloride at concentrations up to 0.1 percent free active ingredient is safe as a cosmetic when presently used. And then we have the maximum reported concentration has remained consistent at 0.5 percent. I couldn't wrap my head around that. Now I don't know how to predict free versus total. Does anybody? Do you chemists know how to do that?

DR. ROSS: No. Well, Susan might but I don't. But with respect to the --

DR. BERGFELD: I think that referred to the free was active.

DR. ROSS: Yeah. The 0.5 percent was in a wipe, and I think the previous discussion, the previous approval, said that a lot of it would be bound in the wipe and there wouldn't be as much free, so they were okay with that. So, I don't think you can go with that 0.1 percent anyway.

I mean, I think for other reasons, that I'll get to in a minute, you might have to go non-irritating with this one. But anyway, I thought you needed the dermal irritation and sensitization at max for Benzalkonium Chloride. But I was going to defer to you, Dr. Cohen, of whether you thought we may need them.

DR. DAVID COHEN: I knew you would. Look, we have in irritation, right, 7 of 30 subjects at 0.1 percent had positive patch tests. So, it's curious to me how that wasn't cleared to be non-irritating. Number two, there's no human sensitization data there, but there is a remarkably large body of data demonstrating that Benzalkonium Chloride tested at 0.1 percent on patch tests is a top 10 to 20 allergen in the United States with Don Belsito as an author.

So, the question is, what is the proper max concentration of this product to avoid sensitization? I really don't know the answer to that.

DR. BERGFELD: To what the threshold is? Looking for threshold of sensitization?

DR. DAVID COHEN: Listen, it comes up all the time. It's reported constantly. There's no question that the North American Group, these folks on there that believe that a lot of those patch test reactions are irritant reactions. But in one of the papers that Don is on, it says 13 percent of stasis dermatitis patients -- that's a dermatitis on the legs -- reacted to 0.1 percent Benzalkonium Chloride. Among patients with stasis dermatitis diagnosis, the top ten allergens with highest proportions of a positive allergic patch test were fragrance mix and bacitracin and then Benzalkonium Chloride like came pretty close in these top numbers.

So, I was a little surprised when I read the report the first time that we didn't have human data on this. We only had human irritation data, and we didn't have sensitization data on it, yet the world literature on allergic reactions is very, very large.

DR. ROSS: Oh, good. I'm pleased you thought we needed that also. I mean, I think the general previous conclusion of up to 0.1 percent, I couldn't agree with that right now because it would be obviously problems with the ocular and there are potential problems in the mucous membrane of those concentrations. So, we're going to have to amend that conclusion anyway. I mean, that was the first one I needed dermal irritation and sensitization for Benzalkonium Chloride at max.

DR. DAVID COHEN: We have irritation on it.

DR. ROSS: Okay.

DR. DAVID COHEN: We have human irritation. We don't have it for Stearalkonium Chloride, and the question is, can we read across on that or do we ask for it?

MS. FERGUSON: There's some Benzalkonium Chloride HRIPT data in the old data.

DR. ROSS: Yeah.

DR. DAVID COHEN: In the old data, right. It's in the old data, it's not in the tables though, right?

MS. FERGUSON: Yeah. Right.

DR. DAVID COHEN: It's in the old data and it shows no sensitization from my recollection, right?

MS. FERGUSON: Right, right.

DR. DAVID COHEN: Which is a problem. And that was at 0.1, right?

MS. FERGUSON: 0.1 to 0.13 percent, HRIPTs.

DR. DAVID COHEN: Right. So, the question is this 3.1 versus the 0.5, I think is an issue.

DR. ROSS: But hang on a second. Are we talking Benzalkonium or Stearalkonium? I'm a bit confused.

DR. DAVID COHEN: Benzalkonium. I think Priya is talking to us about Benzalkonium. It's in the (inaudible) stuff.

DR. TILTON: And there is HRIPT for both. That concentration range was specifically for Benzalkonium.

Yes, Stearalkonium is much higher. It's 1 to 20 percent and mild erythema in some cases, but no sensitization. And HRIPTs had Stearalkonium. But you're right, Benzalkonium is currently okay but only up to 0.13 percent.

DR. DAVID COHEN: If that were the case, though -- see this is contradictory to what we're seeing in real life, right? That this HRIPT data is not predicting what we're seeing.

DR. TILTON: David, is that sensitization from current case reports, is that specifically in patients with dermatitis?

DR. DAVID COHEN: Yes.

DR. TILTON: So that wouldn't be covered here.

DR. DAVID COHEN: Well, the issue is the dermatitis may be caused by this contact allergen. So, I think when you have no activity on the HRIPT, you might not expect that chemical to come up as a top allergen in a North American study. You'd expect a little bit more protection there.

DR. SAM COHEN: David, can you explain that? I'm not sure I follow.

DR. DAVID COHEN: I think if you take a group of people and you patch test them, and you're saying there's no positives in, you know, a hundred people. I'm trying to remember what this one was. Dermal irritation and sensitization -- at 50. There's an assumption that people who use it regularly wouldn't get allergic contact dermatitis from it. But in survey patch testing, and people who have dermatitis, or have some other problem, would you expect that product to come in as a top 10 or 15 allergen in the patch testing?

Don may disagree with that, but I think we see a lot of reactions to it. There's definitely going to be disagreement within the North American Group, I would expect about what those really mean. Carol, I know you had your hand up.

DR. EISENMANN: Listening to this conversation, I'm a little concerned about using read-across for the sensitization endpoint from Benzalkonium and Stearalkonium, and they're also used very differently. So, if you set a limit for Benzalkonium, I don't think this should be applied to the larger -- and especially even the larger Behenalkonium Chloride, which is 22, I think.

DR. ROSS: Maximum 23. Yeah. I can't remember. Yeah. Yeah. No, I agree with that, Carol. Yeah, I mean, that's why I said, we approach them sort of separately and it's sort of like two groups. You've got the Benzalkonium Chloride, which is a mix of compounds; then you've got the longer chain, Stearalkonium, which is better as a --

DR. EISENMANN: Hair conditioning. Right.

DR. ROSS: Yeah. I know it's a biocide. Yeah. So, I think you're looking at that, read-across on irritation would be difficult.

DR. EISENMANN: And sensitization too, I think.

DR. ROSS: Yeah. Yeah. Irritation. Yeah, definitely. So, you would need that data separately for both Benzalkonium and Stearalkonium.

DR. EISENMANN: Right. So, if you set a limit for Benzalkonium, it might not apply to the larger ones. Okay, just wanted to confirm that. Thanks.

DR. DAVID COHEN: So, if that's the case, I guess our Conclusion could still be okay, because the concentrations of the individual chemicals will be in the report. And we can put that in the Discussion. It doesn't preclude us from having them all in the same report, I suppose.

DR. ROSS: Correct. That's what I interpreted.

DR. SAM COHEN: But do we ask for data?

DR. ROSS: Yeah, we need data. I mean, that's --

DR. SAM COHEN: On sensitivity and sensitization? I mean, sensitization and irritation?

DR. ROSS: We had the dermal sensitization for Benzalkonium Chloride. I think we also need --

DR. SAM COHEN: No, but for the other ones? For these other ones.

DR. ROSS: Yeah?

DR. SAM COHEN: The four new ones.

DR. ROSS: No, I don't think you need those. I think if you can clear them on Stearalkonium, and you clear them on Benzalkonium, you can read across from one of those to all of the other compounds. That would be my take on it. Others may disagree, but I think that's probably the way to go. But you know my insufficiencies, David, dermal sensitization for Benzalkonium. I felt we needed maximum concentration of ocular use of Stearalkonium and some data.

DR. DAVID COHEN: Max ocular irritation.

DR. ROSS: Of Stearalkonium Chloride. And some data demonstrating lack of ocular effect in max. Right now, I don't think we have that.

DR. DAVID COHEN: So, isn't that ocular irritation?

DR. ROSS: Yeah. I mean, we have two sections in here, ocular toxicity and ocular irritation. And I understand why that was done because the ocular toxicity is looking at effects on the back of the eye and also nerve effects, and I think it's clear in the sections, but it would be nice to have them together. I don't know if that's possible, Priya.

DR. DAVID COHEN: Do we need ocular toxicity and irritation of Stearalkonium?

DR. ROSS: Well, I think it'd be hard to integrate the sections. I thought initially we could, but then looking at it, I'm not sure that's the best approach. Yeah. So anyway.

DR. DAVID COHEN: The insufficiency is for both?

DR. ROSS: No, I think -- my notes -- my summary was just for Stearalkonium we needed it.

DR. DAVID COHEN: No, no, but I mean tox and irritation or -- for Stearalkonium?

DR. ROSS: Just irritation. Just irritation, I think. Yeah, I mean Benzalkonium in the ocular studies is generally at 0.01 percent. And that looked okay at about 0.01 percent in animals. In humans, there was slight conjunctival hyperemia at 0.02. Ocular irritation at greater than 0.01 in rabbits. So, we're probably okay with Benzalkonium Chloride and its ocular data in there.

DR. DAVID COHEN: Yeah, yeah.

DR. ROSS: This is another reason why we can't stay with that 0.1 percent overall conclusion, because we've got ocular irritation with Benzalkonium greater than 0.01.

DR. DAVID COHEN: Well, if you pulled out the 0.1 percent, it would be as described in this report and then the max concentration in the eye is --

DR. ROSS: 0.01 for Benzalkonium Chloride.

DR. DAVID COHEN: Right. So that would take care of that, right?

DR. ROSS: It would. Stearalkonium is not the case. You need a maximum ocular use concentration.

DR. DAVID COHEN: And how much of it used in eye?

DR. ROSS: We don't have a maximum use.

DR. SAM COHEN: It says that it's used in ophthalmic solutions a lot, but do we know what concentrations it's in?

DR. ROSS: I didn't see it in the table, Sam.

DR. DAVID COHEN: So, we want concentration of use in ocular products?

DR. ROSS: Yeah, and some data demonstrating lack of ocular effect at max -- lack of ocular irritation at max. And I had some concerns on -- I don't have anybody else did -- on the mucous membrane effects, continuing our discussion from previous dossiers. And effects were clearly demonstrated in this dossier in nasal and vaginal studies at doses lower than the currently stated max of 0.33 percent for mucous membrane exposure with Benzalkonium Chloride.

Our dossier quotes 0.14 percent, by the way, for Benzalkonium Chloride in the introduction for exposure to mucous membranes at the 0.3 percent of the tables. I don't know, it's probably from mouthwashes I expect. But anyway.

DR. DAVID COHEN: Mucus membranes at 0.5, right?

DR. ROSS: I thought it was at 0.3, but 0.5 is even worse.

DR. DAVID COHEN: No, it says mucous membrane -- oh, that's an old one. It's 0.3 in the 2023 survey.

DR. ROSS: Oh, vindicated. Yeah. Okay, 0.3. Yeah.

DR. DAVID COHEN: You are.

DR. ROSS: I always like vindication.

DR. SAM COHEN: But it's also listed for baby products.

DR. ROSS: Yeah, yeah.

DR. DAVID COHEN: But without a concentration of use, right?

DR. SAM COHEN: Right. Right.

DR. ROSS: Yeah.

DR. SAM COHEN: So, we need the information on concentration of use there.

DR. ROSS: And this is another reason why we have to go with non-irritating when we eventually get to a conclusion with this, because not only are we extending the data across to these other materials that we don't have a lot of data -- we're using read across -- but you look at some of these mucous membrane effects.

All right and I need some help with this one from Bart and Priya. There's a section on something we don't often see in these reports, which is ototoxicity. And we have use of Benzalkonium Chloride in ear drops and I don't -- do we have that? I guess that's my question. Do we have that, and where would I find it in the Use tables? Because ototoxicity is quite important. You know, you think of the gentamycin analogues and you think of other things, but I haven't seen it our reports very much and so I wondered where that was covered. Priya?

MS. FERGUSON: The eardrops would be in the non-cosmetic Use section. It wouldn't be in the table because it's not cosmetic.

DR. ROSS: Okay. So, we wouldn't be covering it in here, then, at all? Is that right? I mean, eardrops even if you're using them to get rid of, I don't know, wax or whatever, that's not in our --

DR. SAM COHEN: It's not cosmetic.

DR. ROSS: It's not cosmetic, no.

DR. HELDRETH: Right. For both the ear and the eye, we're just looking at incidental exposures because cosmetics aren't intended to be put in either.

DR. ROSS: No. So basically, I don't have to worry, then, about the ototoxicity concentrations of use and potential effects?

DR. DAVID COHEN: But the ocular irritation and products used around the eyes is appropriate?

DR. ROSS: Yeah, no question.

DR. DAVID COHEN: Did you finish your list, David?

DR. ROSS: Yeah, I think so. I mean, we've talked about the two ocular sections. I can comment on the MOEs if needed, but I'll leave it there.

DR. SAM COHEN: I have one very minor point, and that is listed for the past reports and things is March 2025, Tom Slaga is still listed as a participant, so that can't be right.

DR. ROSS: It's not, yeah. I got that as well, yeah.

DR. SAM COHEN: It's a different date. I don't know what date it was, but it certainly wasn't in March of 2025.

DR. ROSS: I put the date in. I dug it out; it's in there somewhere -- 2023.

DR. DAVID COHEN: Susan, any --

DR. TILTON: No, I think we've covered everything.

DR. DAVID COHEN: Now, before Wilma asks me, I'm going to read out our insufficiencies. Did I catch that right, Wilma? See, I'm learning.

Our Insufficient Data Announcement is impurities on the new compounds, the four new ones, human sensitization data on Benzalkonium Chloride. I have to figure out how I'm going to deal with that because as Priya appropriately pointed out, we have it. The question is, what do we do with it? We have the data, we have it at 0.1 percent, we have max use at 0.5 percent, but the 0.1 percent is showing no sensitization.

DR. ROSS: Dermal use max is 0.25 I think, right?

DR. DAVID COHEN: For Benzalkonium?

DR. ROSS: Yeah, let's have a look.

DR. SAM COHEN: No, I thought it was 0.1.

DR. ROSS: I'm all over the place.

DR. DAVID COHEN: Wait, max use is 0.5.

DR. ROSS: Yeah. Dermal.

DR. BERGFELD: I have it at 0.5.

DR. DAVID COHEN: Leave on is 0.47.

DR. ROSS: Where am I getting 0.25 from then on that dermal?

DR. TILTON: As you go further down, there's a dermal contact.

DR. ROSS: Dermal contact, 0.25, 2023, VRCP.

DR. DAVID COHEN: I'm not sure we're vindicating you for that one, David, because a leave on product is dermal contact.

DR. ROSS: But why do those numbers -- why don't they agree with -- yeah?

DR. EISENMANN: It could be a leave on hair product or a nail product. I don't know, I haven't looked. I do have a new survey underway now -- concentration of use survey. So, you'll be getting new information, hopefully, by the next time you see it.

DR. DAVID COHEN: Okay, that'll help.

DR. ROSS: Yeah, it will. You could just say at maximum concentration of use, David, tomorrow.

DR. DAVID COHEN: Yeah, for sensitization, right?

DR. ROSS: Yeah.

DR. DAVID COHEN: At max to use. Irritation and sensitization of Stearalkonium Chloride. Max use ocular irritation on Stearalkonium Chloride, concentration of use in ophthalmic products for Stearalkonium Chloride. Concentration of use in baby products for both.

DR. SAM COHEN: And then, David, can you explain where states that Benzalkonium is readily penetrant in the dermal studies, but there's no systemic exposure? It seems a little contradictory. And elsewhere it says it's not absorbed from oral intramuscular/intrarectal administration. But there was a study there that said that it was dermally penetrant. How can it be penetrant and have no systemic exposure?

DR. ROSS: My notes, Sam, say variable data on absorption, but it's low in the study in humans with -- I guess it was a soap -- at 0.13 percent Benzalkonium Chloride.

DR. EISENMANN: Instead of saying ophthalmic products, could you say eye area?

DR. DAVID COHEN: Yes, yes. that's better. Thank you. Because it really isn't ophthalmic products.

DR. ROSS: No, it's not. Yeah.

DR. SAM COHEN: But it's used in ophthalmic products, and it would be useful to know what concentrations they allow in ophthalmic medications/drops for comparison with exposure to around the eye.

DR. DAVID COHEN: Right, but that may not be the group that Carol's representing, right? Those are drug uses.

DR. SAM COHEN: Yeah, I understand that, but it would give us information that we could use for assessing any risk that would be around the eye. I mean, if they're using at a very high concentrations in the drops, we don't have to worry about it around eye use.

DR. DAVID COHEN: I guess the question is, who are we asking?

MS. FERGUSON: I found a literature source that says it's used up to 0.02 percent. I'm not sure how all-encompassing that is, with all the products out there, but 0.02 percent. And I can put that in the non-cosmetic use section.

DR. DAVID COHEN: Okay, that would be good. Acts as a preservative system, I assume.

DR. SAM COHEN: Yeah.

MS. FERGUSON: Yes.

DR. DAVID COHEN: I saw a lot of this come into ophthalmic products when Thimerosal was coming out of mercury thiosalicylate that's getting a lot of attention. Okay. Any other further comments?

DR. ROSS: So, could you just reiterate your insufficiencies there again, David, so we've got them?

DR. DAVID COHEN: Sure. Impurities on the new compounds, sensitization data on Benzalkonium Chloride at max use. Irritation and sensitization of Stearalkonium Chloride. Ocular irritation at max use for Stearalkonium Chloride. Concentration of use in eye area products for Stearalkonium Chloride and concentration in baby products for both.

DR. ROSS: So, we don't have -- you're talking about -- you want dermal for Stearalkonium Chloride? Is that the third item in there?

DR. TILTON: Yeah, I was going to clarify too because I think we were talking ocular.

DR. ROSS: Yeah.

DR. DAVID COHEN: Oh, I thought that's what you asked for, David. I must have misinterpreted.

DR. ROSS: No, I think Stearalkonium Chloride dermal looks okay. Both irritation and sensitization. Stearalkonium Chloride's non-irritant when tested neat and reconstructed human epidermis even at 40 percent. Only moderate irritation in rabbits with respect to sensitization. HRIPT is 1 to 20 percent, only mild erythema in some cases but no sensitization. So, I came down okay with that.

DR. DAVID COHEN: Okay, that's not in the table, but hold on.

DR. ROSS: Yeah. It's a little old data, right, probably. It's just distinction between the old and the new again. Priya, this was a massive document. It must have been a terrible thing to put together.

MS. FERGUSON: It was something.

DR. ROSS: Yeah. It went on forever this thing.

DR. DAVID COHEN: I just want to find the Stearalkonium Chloride. Stearalkonium undiluted was turning non-irritating…..okay.

DR. ROSS: Yeah. So, it's a PDF -- have you got it?

DR. DAVID COHEN: I'm not on a PDF, but I can go into a PDF. Just what section is it under and where?

DR. ROSS: Dermal irritation and sensitization.

DR. DAVID COHEN: Very good. That's where I am. First paragraph or second paragraph?

DR. ROSS: Second paragraph. Got it?

DR. DAVID COHEN: Even non- -- the second line is non-irritant and reconstructed human epidermis.

DR. ROSS: Yeah. One, two, three, four -- line five. Yeah, line one, two, three, four.

DR. DAVID COHEN: Yeah. We had read it before, it said 1 to 20, but I can't find it.

DR. ROSS: Moderate irritation was observed, blah, blah, blah, using a mixture containing 40 percent Stearlkonium Chloride tested neat.

DR. DAVID COHEN: That was an irritation study.

DR. ROSS: Yeah.

DR. DAVID COHEN: You see sensitization data? That's what I couldn't find. Priya, do you remember sensitization data for Stearalkonium Chloride?

MS. FERGUSON: In the old data up to 20 percent.

DR. DAVID COHEN: That's what --

DR. TILTON: That's in the first paragraph.

DR. ROSS: Last line in the first paragraph, David. HRIPTs --

DR. DAVID COHEN: One to 20 percent produce mild erythema in some cases.

DR. ROSS: Yeah. That's what I got. Yeah.

DR. DAVID COHEN: It's one line. It's not referenced. And it's not in the table.

DR. ROSS: Usually, that's how the old data is. You go back to the previous studies and you're just abstracting the Conclusion from the old reports.

DR. DAVID COHEN: But this is a brand-new report amalgamating a lot of things, right?

DR. ROSS: All I'm saying is I think that's usually where we get this stuff. It's abstracted from the old reports and it's in a summary form. If you want to dig down into the data, you can go back into the old report.

DR. DAVID COHEN: I'll cross it out. Max use ocular irritation on Stearalkonium Chloride, concentration of use in eye area products for Stearalkonium, and the concentration of use in baby products for both.

DR. ROSS: Okay.

DR. TILTON: I guess even if the data isn't described in the new report, is it still possible to include the references?

DR. HELDRETH: We absolutely can. I mean, everything is essentially referenced back to the original report. You know that whole italics. However, even if there's a study in there that the Panel is going to be basing their Discussion or the Conclusion on, we can bring that study forward and we can find the original reference and put it in the report, you know, if it's going to help the Panel.

Typically, or historically, I should say, you know we've kept it this way where we're referring to the old report and just kind of not putting it in our tables, and not doing the detail on it, because it prevents issues with getting it into the publication and the Journal not saying well, hey, you're republishing something that published all these years ago. But when there's something that's of importance to the Panel onto their Discussion, there's no problem with us bringing that study forward and giving the original citation if you find it helpful.

DR. DAVID COHEN: But I think in light of Carol's comment about not reading across sensitization, it is going to be part of our new Conclusion. I would just bring that one forward.

DR. HELDRETH: We can do that.

DR. DAVID COHEN: All right. So, should we call it right now for lunch, but should we get back at one? Does anyone need a little more time?

DR. BERGFELD: How about 1:15.

DR. DAVID COHEN: 1:15 it is.

DR. SRINIVASAN: David, I had a quick question. Jannavi here. I was hearing in the discussions about the impurities that you're missing for all four, but I did not hear anything about the bromides for the other studies that you were asking in your IDA. I just wanted to ask if that was something missed or that the bromides are okay and you're only focusing on the chlorides? Thank you.

DR. ROSS: I think we're assuming that you could read across from, for example, the Benzalkonium Chloride to the Benzalkonium Bromide. And that wouldn't be an issue.

DR. BERGFELD: No, I think that would be a discussion item for our Discussion in our manuscript, the read across.

DR. DAVID COHEN: Is that what you were asking?

DR. SRINIVASAN: Yep.

DR. DAVID COHEN: Okay.

DR. SRINIVASAN: Thank you.

DR. DAVID COHEN: All right. Why don't we break? I'll see everyone at 1:15.

Full Panel – September 9, 2025

DR. DAVID COHEN: Okay. We need your help here too, Don. We first reviewed the safety of Stearalkonium Chloride in 1982, with a conclusion of safe when incorporated in cosmetic products similar to those presently marketed. The Panel considered a re-review of this report and reaffirmed the 1982 conclusion as published in 2003.

Because it had been 15 years since the re-review was published, the Panel again re-reviewed this ingredient in March 2023. We agreed to reopen with the addition of structural similar ingredients.

One of the additions, Benzalkonium Chloride was also previously reviewed by the Panel, and a safety assessment published with the conclusion that Benzalkonium Chloride at concentrations up to 0.1 percent free active ingredient is safe as a cosmetic ingredient as presently used. The Panel reconsidered re-review and reaffirmed that conclusion again in 2008. A Draft Amended Report of these as well as four previously un-reviewed ingredients, Behenalkonium Chloride, Benzalkonium Bromide, Cetearalkonium Bromide, and Lauralkonium Bromide are here as well.

Benzalkonium and Stearalkonium Chloride were previously reported to be used in 79 and 151 total formulations many years ago. According to the 2023 VCRP, there are 69 and 88 formulations, respectively. Benzalkonium Chloride remains consistent with the use of 0.5 percent in both 2006 and 2023. And Stearalkonium Chloride decreased from 7 percent in 2001, to 3.8 percent in 2022. The RLD for Stearalkonium Chloride has 885 formulations, while Benzalkonium Chloride has 565.

We had some issues with the read-across, but I'll go right to our motion. Our motion is an Insufficient Data Announcement with the following needs: impurities on the new compounds Behenalkonium Chloride, Benzalkonium Bromide, Cetearalkonium Bromide, and Lauralkonium Bromide. We need human sensitization data on Benzalkonium Chloride at max use. The current concentration, while the prior ones were 0.1, the use table shows 0.5. Max use ocular irritation of Stearalkonium Chloride, as well as the concentration of use in eye area products for Stearalkonium Chloride, and concentration of use in baby products for both. That's my motion and I certainly expect some discussion.

DR. BERGFELD: Dr. Belsito.

DR. BELSITO: Well, we thought we could go safe as used when formulated to be nonirritating.

DR. DAVID COHEN: I wasn't surprised by that, Don. Don, a couple of things, we have in the Use tables, 0.5 percent as max use all over the place. And, we do have this older type of conclusion of 0.1 percent free active ingredient, which is a bit of an older type of conclusion.

And, Don, I know you know all too well, and I'm sure the North American group, you argue about it all the time, but it's so many article relating to vary frequent reactivity on patch testing. You got a 2022 article with 13 percent of stasis dermatitis patients reacting to 0.1 percent Benzalkonium Chloride. And in there it says positive allergic patch tests, not irritants. They all has it as a top 15 allergen as of 2023.

And so, if we have max use at 0.5, and we have sensitization data at 0.1, showing no sensitization, and we have all of this data that you have published on it being a frequent allergen, do we need sensitization data at 0.5 percent, or can you -- I'm really asking this sincerely. Is there another way for us to address this data with a conclusion other than nonirritating?

DR. BELSITO: Yeah, like one of the things I asked Priya to do in the chart, where she was going through all of the data, was to also put in the vehicle whether it was being tested in water or petrolatum. Because we know that it's much more likely to be irritant in water than it is in petrolatum. And positive allergic patch tests are in the eye of the beholder, as you know.

DR. DAVID COHEN: I know, I know.

DR. BELSITO: And I would like to know how many of those positive allergic patch tests were questionable, meaning erythema without any edema, or were one plus. Benzalkonium Chloride, Stearalkonium Chloride, they're very dermal to patch tests. They cause a lot of irritation. It's under occlusion, which is not how these products are being used in cosmetics. So, I have my concerns about that data.

DR. DAVID COHEN: I knew you would. Don, I knew you would. And the only thing that complicated it was that you were an author on some of these, right. But, there are many leave-on products at very high concentration, 0.47, that will have water.

DR. BELSITO: Yeah.

DR. DAVID COHEN: Right? So, I'm not trying to frame this as a gotcha on this. And I wasn't surprised by your team's conclusion, but we are faced with this contradiction, right. We're seeing a lot of reports with Benzalkonium Chloride coming out as a top 10 or top 20 allergen. We have 0.1 percent sensitization data in the report that shows no sensitization. And there's so much data showing that there's some relevance to these reactions. And it's popping up in lots of places.

So, how do we address that? Right? It's not immutable, my opinion. But what about the IDA issues, the impurities for the new ones, ocular irritation for Stearalkonium Chloride, would like concentration of use in eye areas, concentration of use in baby products? And the question is, do you have any issues with those IDAs? And then, could we think a little bit more about the sensitization data that's floating out there.

DR. BELSITO: My team obviously felt we didn't need all of those. This is the first time we're looking at this, really, right?

DR. DAVID COHEN: It is.

DR. BELSITO: That is these ingredients. So, I'm not necessarily averse to putting out the IDA. But, I mean, this is reporting on what my initial impression was and that of my team's. So I'll ask Curt and Paul and Allan to comment.

DR. SNYDER: Well, as you stated, Don, this is the first look. And so, if we go another round and ask for this data, I'm fine with that to give some comfort level to the other team.

DR. RETTIE: Yeah, I agree with that. Benzalkonium Chloride is front and center for a lot of reasons these days, so I think it deserves another look.

DR. KLAASSEN: Yes, I would support that. I found it interesting that, you know, the local lymph node assay came out as being a sensitizer, while the humans was not. Of what relevance that is, I don't know, but there was something there.

DR. DAVID COHEN: That didn't fall short on me that you had nonhuman data showing as sensitizer. The HRIPT looked really clean. And then, you have dozens of reports of positive patch tests at 0.1 percent. Absolutely what Don says, I agree with. It's under occlusion. It's 48 hours of occlusion.

It's not simulating that, but at the same time we have real use leave-on data at 5x the patch tests concentration. And I suspect many with water in them. And I suspect on the face and near the eyes.

DR. ROSS: We also -- sorry, go ahead. I'll just --

DR. BERGFELD: No, continue. I was just going to restate the motion and get everybody's agreement.

DR. ROSS: No, I think that's fine. Go ahead, Wilma, let's get this show on the road.

DR. BERGFELD: All right, so, we have -- this is your item Dr. Cohen, so could you restate your motion and see if we can get agreement on what you've requested as IDA?

DR. DAVID COHEN: The IDA is impurities on the newer compounds, before new ones, the Behenalkonium Chloride, Benzalkonium Bromide, Cetearalkonium Chloride, and Lauralkonium Chloride, human sensitization data for Benzalkonium Chloride at max use, ocular irritation at max use for Stearalkonium Chloride, concentration of use in the eye area for Stearalkonium Chloride, and concentration of use in baby products for Benzalkonium and Stearalkonium Chloride.

DR. BERGFELD: Is there a second to this IDA?

DR. BELSITO: Second.

DR. BERGFELD: Any further discussion?

DR. BELSITO: Yeah, just in formulating our Discussion, it is irritating so that will have to be in our final conclusion. It's a penetration enhancer. We need to discuss the endocrine disruption data, which is really done at doses not relevant to human exposure in cosmetics.

If needed, we need to do our own margin of exposure. We didn't really feel that would be needed, but there was an endpoint that was reported that we couldn't find in our study. I asked Priya to look at the original study (audio skip), PDF Page 30.

DR. DAVID COHEN: Which study was that Don? You were breaking up a little.

DR. BELSITO: On PDF Page 30, derived from a 90-day unpublished dermal rat study with topical 1 percent Benzalkonium Chloride. And they used a NOAEL of 20 mg/kg/d. And it's not clear how they got that.

And, Priya, maybe you can comment, I don't remember exactly. So this is just the report you saw but you didn't see the original, is that correct?

MS. FERGUSON: Right. It was a report that did their own MOE calculation, and they used that NOAEL based off of an unpublished study that I don't have access to.

DR. BELSITO: So the study was not published?

MS. FERGUSON: No, not the study that they used for the NOAEL.

DR. BELSITO: And did they at least give any detailed information on what that NOAEL was based off of?

MS. FERGUSON: No, other than it saying it was a dermal rat study.

DR. ROSS: This was for the Benzalkonium Chloride, Priya, that you're talking about?

DR. BELSITO: Yes. It's at the bottom of PDF-30. It was for DART endpoint, but.

DR. ROSS: Um-hm. And this came from that ECS -- SCCS European Cosmetology community, or whatever it was, from 1980?

MS. FERGUSON: No, I think it was a 2017 study performed by Choi et al.

DR. ROSS: Okay, that's a different one. I was looking --

DR. BELSITO: It's Reference 146.

DR. ROSS: Okay.

DR. KLAASSEN: What was the NOAEL? Was it 20?

DR. BELSITO: 20 mg/kg/d, yes.

DR. KLAASSEN: In our material that was sent there is a NOAEL of 30.

DR. BELSITO: Right.

DR. KLAASSEN: Which we could use to calculate.

DR. ROSS: Yes.

DR. KLAASSEN: So as far as some solid data we have in our report.

DR. BELSITO: Right.

DR. ROSS: Yeah, I'm fine with that.

DR. BELSITO: The 20 is lower than 30.

DR. KLAASSEN: Exactly.

DR. ROSS: It sounds like, Allan, you were reading across. I just have some quick questions. Our opinion was that there were two very different kinds of groups of compounds here, the stearalkoniums and the benzalkoniums. Benzalkoniums has been optimize for biocidal; Stearalkoniums is more of a conditioning type of molecule.

DR. RETTIE: Yeah, I would agree there are two subgroups in there. They are distinct, or are somewhat distinct, not just based on the uses or the (audio skip). The Panel was the one that was, of course, so far out for me, and we had so little data on that.

DR. BELSITO: So, if you think there are two groups of molecules, shouldn't they be reviewed in the same report? Can we read across? Is this a no-brainer?

DR. ROSS: I think that -- sorry, do you want to take it, Allan, or?

DR. RETTIE: Go ahead, Dave, you were ahead of me.

DR. ROSS: I thought that we were pretty close to clearing the Stearalkonium and pretty close to clearing the Benzalkonium. And I thought if we cleared both of those, they encompass both types of molecules and you can then read across to the other. You know, you could then.

DR. RETTIE: Yeah, kind of a bookend.

DR. ROSS: Yeah.

DR. RETTIE: I agree with that.

DR. BELSITO: So we're keeping them in the same report, at least that's the impression I'm getting.

DR. BERGFELD: Yes.

DR. BELSITO: Okay.

DR. BERGFELD: And there's a proposal to go out as an IDA?

DR. BELSITO: Yeah, I think we heard that the rest of my team pretty much agrees with that, so I'll second that.

DR. BERGFELD: Okay.

MS. FERGUSON: I had a quick question.

DR. BERGFELD: Okay.

MS. FERGUSON: I had a quick question about the IDA. So, one of the request was max use ocular irritation data on Stearalkonium Chloride. In Table 12, we have some animal data at 40 percent Stearalkonium Chloride. It's a mixture containing 40 percent.

DR. DAVID COHEN: Which table?

MS. FERGUSON: Table 12.

DR. BELSITO: What PDF, Priya?

MS. FERGUSON: 57.

DR. ROSS: 57, yeah.

DR. DAVID COHEN: 57, corrosive to eyes, irreversible ocular damage. That sounds not so good.

DR. KLAASSEN: Isn't that at 100 percent?

DR. BELSITO: Yeah, 100 percent.

MS. FERGUSON: Yes.

DR. RETTIE: Yeah, neat.

DR. ROSS: Yeah.

DR. DAVID COHEN: Well, it's not neat; it's 40 percent Stearalkonium Chloride.

DR. ROSS: Yeah, 40 percent neat.

DR. DAVID COHEN: With 40 percent water. Right, so it's neat mixture, but it's 40 percent Stearalkonium Chloride with pretty bad outcome.

DR. ROSS: Yeah.

DR. DAVID COHEN: So, maybe the IDA should stay the way it is.

DR. BELSITO: Yeah, I'm fine with that.

DR. BERGFELD: Okay.

DR. BELSITO: I mean we didn't use to ask for ocular because it involved animal testing. Now there are so many OECD protocols that are in vitro, I think it's fine asking for it.

DR. DAVID COHEN: Yeah, Don, that's our impression too.

DR. BERGFELD: I think the discussion has ended. Can we call the question? All those against an IDA please indicate by stating your name. Opposed? This is approved and going out as an IDA. Moving on to the next ingredient the Sodium Borate and Boric Acid, Dr. Belsito.

STEARALKONIUM CHLORIDE

Draft Report Review (November 1979)

The Report contained the following Conclusion: It is the opinion of the Expert Panel based on the evidence at hand, which it believes to be relevant and accumulated in a reasonable manner, that the cosmetic ingredient, Stearalkonium Chloride, is safe when incorporated in concentrations similar to those presently marketed.
Dr. Beyer instructed that, in view of the suggested revisions, the document be revised and mailed to the panel for further review. Upon adoption, the document will be issued as a Tentative Report for a 90-day comment period.

Draft Tentative Report Review (March 1980)

Stearalkonium Chloride. Subject to minor revision, the Draft Tentative Report setting out the safety of this ingredient was adopted by the Expert Panel. Dr. Fine voted against the adoption because, is his opinion, there is a lack of human safety data available.

Draft Final Report Review

Not available

First Re-Review (November 2001)

Dr. Belsito stated that a CIR Final Report with the following conclusion on this ingredient was published in 1982: On the basis of the evidence at hand, the Expert Panel concludes that the cosmetic ingredient, Stearalkonium Chloride, is safe when incorporated in cosmetic products in concentrations similar to those presently marketed.
Dr. Belsito noted that very little new information on Stearalkonium Chloride has entered the published literature since the Final Report was published, and that his Team concluded that the Final Safety Assessment on this ingredient does not need to be reopened.
The Panel unanimously concluded that the CIR Final Safety Assessment on Stearalkonium Chloride does not need to be reopened.

BENZALKONIUM CHLORIDE

Draft Report Review

Not available

Draft Tentative Report Review (April 1988)

Dr. Bergfeld reported that Benzalkonium Chloride is an antibacterial agent and has produced ocular and dermal irritation as well as sensitization at concentrations greater than 0.1%. She noted that this had been addressed in the discussion of the report and was the basis for a recommendation of a concentration limit of 0.1% for use in cosmetics. A UV spectrum also had been requested and received; Benzalkonium Chloride had a peak absorption maximum of 262 nm and did not absorb UV light at wavelengths of 300 nm and above.

Dr. Schroeter noted his team's concern with the fact that, in solution, Benzalkonium Chloride bears a net charge and therefore might be bound by proteins or other agents, possibly leaving no free Benzalkonium Chloride to act as a preservative. His team concurred with the 0.1% concentration limit but wanted the limit to apply to the free active ingredient.

After noting that the discussion of the report should reflect the Panel's concerns as to Benzalkonium Chloride's irritation and sensitization potential and its behavior in solution, the Panel unanimously approved Benzalkonium Chloride as safe as a cosmetic ingredient at concentrations up to 0.1% of the free, active ingredient. The revised and corrected report will be mailed to the Panel for a two-week review, after which the tentative final report will be announced for a 90-day comment period.

Draft Final Report Review

Not available

First Re-Review (June 2006)

Dr. Marks stated that a CIR Final Report with the following conclusion was published in 1989: On the basis of the data presented in this report, the CIR Expert Panel concludes that Benzalkonium Chloride, at concentrations up to 0.1% free, active ingredient, is safe as a cosmetic ingredient as presently used. He then noted that his Team determined that the Final Report should not be reopened.

Amended Safety Assessment of Alkonium Chlorides and Alkonium Bromides as Used in Cosmetics

Status:	Draft Tentative Amended Report for Panel Review
Release Date:	May 22, 2026
Panel Meeting Date:	June 15-16, 2026

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Bruce A. Brod, M.D., M.H.C.I., F.A.A.D., Donald V. Belsito, M.D.; Samuel M. 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. Previous Panel member involved in this assessment: David E. Cohen, M.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 Priya Ferguson, M.S., Associate Toxicologist/Senior Scientific Analyst/Writer, CIR.

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ABBREVIATIONS

AICIS	Australian Industrial Chemicals Introduction Scheme
ATP	adenosine triphosphate
BALF	bronchoalveolar lavage fluid
CIR	Cosmetic Ingredient Review
CFR	Code of Federal Regulations
Cx43	connexin 43
Council	Personal Care Products Council
DEREK	Deductive Estimation of Risk from Existing Knowledge
Dictionary	*International Cosmetic Ingredient Dictionary*
DNA	deoxyribonucleic acid
DMSO	dimethyl sulfoxide
EC	European Commission
EC1.8	concentration inducing a stimulation index of 1.8
EC3	effective concentration causing a 3-fold increase in lymph node cell proliferation
ECHA	European Chemicals Agency
EPA	Environmental Protection Agency
ER	estrogen receptor
ET_{50}	exposure time required to reduce tissue viability to 50%
EU	European Union
FDA	Food and Drug Administration
FEV_1	forced expiratory volume in 1 sec
GD	gestation days
GPER1	G-protein coupled estrogen receptor 1
HRIPT	human repeated-insult patch test
IC_{50}	half-maximal inhibitory concentration
ID/g	injected dose per gram of tissue
IgE	immunoglobulin E
IL	interleukin
IL-6	interleukin-6
IL-1α	interleukin-1alpha
IFNα	interferon alpha-2b
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LLNA	local lymph node assay
l.o.	leave-on
MALDI-TOF	matrix assisted laser desorption ionization time-of-flight
MCF-7	Michigan Cancer Foundation
MMAD	mean mass aerodynamic diameter
MOE	margin of exposure
MOS	margin of safety
MoCRA	Modernization of Cosmetics Regulation Act of 2022
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NA	not applicable
NACDG	North American Contact Dermatitis Group
NOAEL	no-observed-adverse-effect-level
NOEL	no-observed-effect-level
NR	not reported
OECD	Organisation for Economic Co-operation and Development
OPP	Office of Pesticide Programs
OPPTS	Office of Prevention, Pesticides, and Toxic Substances
OTC	over-the-counter
Panel	Expert Panel for Cosmetic Ingredient Safety
PBS	phosphate-buffered saline
PoD	point of departure
QSAR	quantitative structure-activity relationship
RIVM	National Institute for Public Health and the Environment
RLD	Registration and Listing Data
RNA	ribonucleic acid
r.o.	rinse-off
ROS	reactive oxygen species

SCC	Scientific Committee on Cosmetology
SCCS	Scientific Committee on Consumer Safety
SED	systemic exposure dose
SDS	sodium dodecyl sulfate
SLS	sodium lauryl sulfate
TG	test guidelines
US	United States
UV	ultraviolet
ZO-1	tight junction zonula occludens-1

DRAFT ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) reassessed the safety of Benzalkonium Chloride and Stearalkonium Chloride, along with 4 additional structurally-related ingredients that had not been reviewed. All of the alkonium chlorides and bromides reviewed in this report are reported to function as antistatic agents in cosmetics. The majority of these ingredients are also reported to function as cosmetic biocides. The Panel reviewed the relevant data to determine the safety of these ingredients. Accordingly, the Panel issued an amended report…[to be determined].

INTRODUCTION

This assessment reviews the safety of the following 6 alkonium chlorides and bromides as used in cosmetic formulations:

Behenalkonium Chloride	Cetearalkonium Bromide
Benzalkonium Bromide	Lauralkonium Chloride
Benzalkonium Chloride*	Stearalkonium Chloride*

*previously reviewed

According to the web-based *International Cosmetic Ingredient Dictionary* (*Dictionary*), all of these ingredients are reported to function as antistatic agents (Table 1).[1] The majority of these ingredients (4 of the 6) are also reported to function as cosmetic biocides.

Stearalkonium Chloride was previously reviewed by the Panel in a safety assessment published in 1982, with the conclusion that Stearalkonium Chloride is safe when incorporated in cosmetic products at concentrations similar to those presently marketed (as indicated in that report).[2] The Panel also considered a re-review of this report and re-affirmed the 1982 conclusion, as published in 2003.[3] In March 2023, this ingredient was again considered for re-review, and the report was re-opened for the addition of structurally similar ingredients.

It should be noted that Benzalkonium Chloride was also previously reviewed by the Panel in a safety assessment published in 1989 with the conclusion that Benzalkonium Chloride, at concentrations up to 0.1% free, active ingredient, is safe as a cosmetic ingredient as presently used (as indicated in that report).[4] The Panel considered a re-review of this ingredient and re-affirmed the 1989 conclusion, as published in 2008.[5]

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; a search was last conducted April 2026. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Some of the data included in this safety assessment was found on the European Chemicals Agency (ECHA)[6,7] and Australian Industrial Chemicals Introduction Scheme (AICIS) websites.[8] Please note that these websites provide summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when ECHA or AICIS is cited.

It should be noted that Benzalkonium Chloride consists of mixtures of alkylbenzyldimethylammonium chlorides, and may therefore have varying chain lengths.[9] They are typically commercialized as compounds ranging from C_8 – C_{18}, with higher biocide activity for C_{12} and C_{14} derivatives.[10] Some review sources (e.g., ECHA and AICIS) list test substances in studies by chain length (e.g., C_{12} - C_{16} alkylbenzyldimethylammonium chloride).[8,11,12] As these mixtures are equivalent to Benzalkonium Chloride, the test substance for these studies will be listed as Benzalkonium Chloride, with the addition of a notation stating the chain length (e.g., Benzalkonium Chloride (C_{12} – C_{16})). This distinction is not made in text summarizing studies in tables; but have been indicated in the "Test Article" column of tables, as appropriate.

Excerpts from the 1982 report on Stearalkonium Chloride, the 1989 report on Benzalkonium Chloride, and the re-review document[13] presented to the Panel in June 2006 are disseminated throughout the report, as appropriate, and are *identified by italicized text*. (This information is not included in the tables or the Summary section.) Accordingly, for Stearalkonium Chloride and Benzalkonium Chloride, an exhaustive search of the world's literature was performed for studies dated 1999 and 2003 forward, respectively, and relevant new studies were included.

CHEMISTRY

Definition and Structure

The definitions and structures of the ingredients included in this review are provided in Table 1.[1] These ingredients are quaternary ammonium halides, specifically, alkylbenzyldimethylammonium salts with chloride or bromide counterions, as shown in Figure 1.[8]

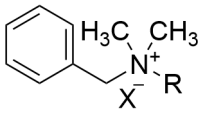


Figure 1. Alkonium halides, wherein R is an even numbered alkyl chain (C_{10} – C_{22}) and X is bromide or chloride.

These cationic surfactants have varying carbon chain lengths of the hydrophobic alkyl moieties (C_{10} – C_{22}; e.g., Stearalkonium Chloride (CAS No. 122-19-0) has a chain length of C_{18}). Benzalkonium Bromide (CAS No. 91080-29-4) and Benzalkonium Chloride (CAS No. 61789-71-7; other CAS numbers for this ingredient may be found in Table 1) are mixtures of alkylbenzyldimethylammonium chlorides.[9] Benzalkonium Chloride preparations specifically comprise homologous compounds with alkyl chain lengths typically ranging from C8 – C18.[10]

Chemical Properties

Benzalkonium Chloride exists as a white or yellowish-white amorphous powder or gel that is soluble in water, acetone, and alcohol, and has an average formula weight of 360 g/mol.[4] Chemical properties of Benzalkonium Chloride and the other ingredients reviewed in this report may be found in Table 2.

Method of Manufacture

The quaternary ammonium compounds evaluated in this report are synthesized through a quaternization reaction involving *N*,*N*-dimethylbenzylamine and the respective 1-bromoalkanes or 1-chloroalkanes.[14] In a typical process, *N*,*N*-dimethylbenzylamine is combined with the selected 1-bromoalkane or 1-chloroalkane (each differing by alkyl chain length (behenyl C_{22}, cetearyl C_{16} - C_{18}, lauryl C_{12}, stearyl C_{18}, or mixed chains)) in dry ethanol. The reaction mixture is heated to facilitate quaternization, where the tertiary amine is converted into a quaternary ammonium salt. After reaction completion, solvent removal is followed by purification steps including recrystallization from acetone, washing with ether, and drying at room temperature.

Composition and Impurities

Benzalkonium Chloride is sold commercially as 50 or 80% solutions in water or alcohol.[4] *It may also be sold in a mixture of water, ethyl alcohol, and isopropyl alcohol. The total alkylbenzyldimethylammonium chloride content of Benzalkonium Chloride is not less than 97%.*

There are several known impurities of Stearalkonium Chloride.[2] *These include stearyl alcohol (3 – 6%) and stearyl dimethylamine hydrochloride (1.4 – 4%).*

Benzalkonium Chloride

According to a supplier, Benzalkonium Chloride (50% aqueous) may contain impurities including benzyl chloride (< 200 ppm), benzyl alcohol (< 2000 ppm), and benzal chloride (< 100 ppm), with heavy metals limited to < 2 ppm mercury.[15] According to a specification for manufacturing in Japan, Benzalkonium Chloride should not evolve ammonia upon alkalization and heating and is limited to ≤ 20 ppm heavy metals and ≤ 2 ppm arsenic.[16]

Stearalkonium Chloride

Stearalkonium Chloride may contain a mixture of free amine and free amine hydrochloride at levels below 4.25%.[17] It may may also contain toluene at levels below 0.025%.

Reactivity

These cationic compounds can be used to precipitate sulfated hydrocolloids at critical temperature and pH values.[2] *These substances may lower surface tension, making possible many chemical reactions (e.g., hydrolysis of carboxylic acid esters of polyvinyl alcohol). In the presence of nitrites, nitrogen oxides, or other nitrosating agents, alkylbenzyldimethylammonium chlorides will give rise to traces of N-nitrosamines. The significant impurities, alkyldimethylamines, are easily nitrosated to N-nitrosamines.*

Ultraviolet (UV) Absorption

In a UV spectral analysis of Benzalkonium Chloride, the absorption peak was well below 300 nm.[13] *No measurable absorption was observed above 310 nm, with a maximum UV absorption occurring at 262 nm.* [4]

USE

Cosmetic

The safety of the cosmetic ingredients 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 alkonium chlorides and bromides in cosmetics. Registration and Listing Data (RLD) obtained from the FDA report frequency of use, and responses to a survey conducted by the Personal Care Products Council (Council) indicate maximum reported concentrations of use; it is these values that define the present practices of use and concentration that are assessed by the Panel. Since 2024, as a result of the Modernization of Cosmetics Regulation Act of 2022 (MoCRA), manufacturers and processors are required to register facilities and list their products (and ingredients therein) with the FDA (i.e., RLD). An exception is made for small

businesses (average gross annual sales in the US of cosmetic products for the previous 3-yr period is less than $1,000,000, adjusted for inflation), which are exempt from MoCRA reporting for most cosmetic product categories. Eye area products, injected products, internal use products, or products that alter appearance for more than 24 h, and the facilities that manufacture these products, are not included in this exemption.[18] Another change resulting from MoCRA is the addition of tattoo preparations (permanent tattoo inks, temporary tattoo inks, and other tattoo products) to the product categories for which companies need to list their products with FDA. However, evaluating the safety of ingredients as used in tattoo preparations is not within the purview of the Panel; accordingly, such use is not included as part of the present practices of use that are assessed by the Panel.

According to RLD obtained from the FDA in 2025, Stearalkonium Chloride has the highest number of uses; it is used in 1136 total formulations (Table 3).[19,20] This ingredient is also reported to have the highest reported maximum concentration of use in rinse-off and leave-on products. It is used at up to 2.6% in rinse-off hair conditioners and 2% in leave-on, non-coloring "other hair preparations".[21] One ingredient, Cetearalkonium Bromide, is not reported to be in use in the RLD, and concentration of use data were not submitted.

These ingredients may result in incidental ingestion, incidental ocular exposure, and mucous membrane exposure as they are reported to be used in formulations used in the mouth (e.g., Benzalkonium Chloride is used in dentifrices at 0.1%), near the eye (e.g., Benzalkonium Chloride is used in eye makeup removers 0.015%), and other mucous membranes (Benzalkonium Chloride is used in disposable wipes at up to 0.35%). In addition, these ingredients are reported to be used in baby products (e.g., Benzalkonium Chloride is used in baby lotions/powders/creams at 0.053%).

These ingredients are used in products that may be incidentally inhaled (e.g., Benzalkonium Chloride is used in body and hand spray at up to 0.1% and in face powders and foot powders and sprays (concentrations not reported)). In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

It is possible that some products containing alkonium chlorides and bromides may be marketed for use with airbrush delivery systems. With the advent of MoCRA and the current product categories outlined therein, 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 these ingredients as listed in the RLD include a designation using airbrush application, so it is possible that these ingredients are used with airbrush delivery systems, but not reported as such. Additionally, the concentration of use surveys are conducted based on product categories as stated in the RLD, but airbrush use was not reported in response to the survey. No consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with airbrush technology, 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. If these ingredients were to be used in airbrush formulations, the data are insufficient to evaluate the exposure resulting from cosmetics applied in such a manner.

Benzalkonium Bromide and Benzalkonium Chloride are listed in the European Union (EU) Cosmetic Regulation 1223/2009 Annex V (list of preservatives allowed in cometic products) with a maximum concentration of 0.1% in cosmetic formulations.[22] Additionally, these substance are also referenced in Annex III, Entry 65, which permits certain quaternary ammonium compounds in rinse-off hair products at concentrations up to 3%, provided the total content of benzalkonium compounds with alkyl chains of C_{14} or less do not exceed 0.1% (as Benzalkonium Chloride). Although Behenalkonium Chloride, Cetearalkonium Bromide, Lauralkonium Chloride, and Stearalkonium Chloride are not individually listed in Annex III, they fall under the scope of Entry 65 due to their chemical similarity to listed quaternary ammonium compounds, and are subject to the same restrictions.

Non-Cosmetic

Benzalkonium Chloride is commercially available in aqueous solutions up to 5%, and is used at concentrations of up to 0.1% as a germicide and sanitizer.[4] *Benzalkonium Chloride is also used as a preservative in ophthalmic solutions, medications, and contact lenses, and as a spermicide. It is used in both prescription and non-prescription drug products. Some over-the-counter (OTC) products that Benzalkonium Chloride may be used in include antimicrobial soaps, handwashes, preoperative skin preparations, skin antiseptics, wound protectant, wound cleansers, surgical hand scrub, dandruff treatment, astringents, and insect bite/sting treatment.*

Benzalkonium Chloride and Stearalkonium Chloride have reported non-industrial uses.[8] These uses include excipients in pharmaceutical formulations and in hospital or household/commercial grade disinfectants. Benzalkonium Chloride is permitted by the FDA for use in OTC first aid antiseptic products at concentrations of 0.1 to 0.13%.[23] Stearalkonium Chloride is reported to be used as an inactive ingredient in an FDA-approved topical drug at 3.15%.[24] Benzalkonium

Chloride is reported to be used in both OTC and prescription formulations (e.g., glaucoma medications (up to 0.02%), eye/ear drops, nasal sprays).[8,25] These chemicals may be used in cleaning products at up to 20% as well as in the manufacture of building materials, paper products, and textiles, and in insect spray, pool care products, pet care products, paints, lacquers, varnishes, adhesives, fillers, and lubricant. In scientific assays, Benzalkonium Chloride may be used to induce cytotoxicity, dry eye disease, or ocular irritation/inflammation.[26-30] Several Code of Federal Regulation (CFR) citations permitting the use of some of these ingredients for certain uses (e.g., food additive for sugarcane juice, use in food packaging) have been established. A listing of these citations may be found in Table 4.

According to unpublished Japanese data, Benzalkonium Chloride is used at concentrations up to 3% in medicated soaps, shampoos, rinses, and depilatories.[16] Also according to these data, Benzalkonium Chloride is used at up to 0.05% in hair growth agents, other medicated cosmetics (including deodorants), medicated lip products, and bath agents and up to 0.01% in medicated toothpastes.

TOXICOKINETIC STUDIES

The average value of the membrane cell integral diffusion coefficient for Benzalkonium Chloride was 0.000008 cm^2/s at 25° C, indicating low diffusivity across lipid membranes.[13] *There was no significant effect of alkyl chain length on the measured value of the membrane cell integral diffusion coefficient.*

According to an assay evaluating the metabolism of Benzalkonium Chloride homologues (C_{10}–C_{16}) by human hepatic cytochrome P450 enzymes, metabolic stability in human liver microsomes increased with alkyl chain length.[31] The study demonstrated that Benzalkonium Chloride undergo nicotinamide adenine dinucleotide phosphate-dependent ω- and (ω-1)-hydroxylation, producing a range of oxidized alkyl chain metabolites, including ω-hydroxy, diol, ketone, and ω-carboxylic acids, with no *N*-dealkylation observed. Shorter-chain benzalkonium chlorides were metabolized more rapidly than their longer-chain counterparts, as indicated by increasing half-lives in the order of $C_{10} < C_{12} < C_{14} < C_{16}$.

Dermal Penetration

The dermal absorption and penetration studies summarized herein can be found in Table 6. Benzalkonium Chloride (0.123%) was observed to readily penetrate through the epidermis and into the dermis in an assay performed using excised human skin.[32] In this assay, penetration was affected by vehicle, with the highest penetration occurring when citric acid was used as the vehicle. An average of 15% of the applied dose was absorbed in an assay in which rats were dermally treated with radiolabeled Benzalkonium Chloride for 72 h under occlusive conditions.[33] Dermal delivery was determined to be 2.16% in an assay in which Benzalkonium Chloride (80.5% active ingredient; in water; radiolabeled and non-radiolabeled) was applied to human skin samples at 0.3%.[11] Systemic exposure to Benzalkonium Chloride (0.13%) via hand soap was determined to be very low in a study performed in 32 subjects.[34]

Penetration Enhancement

Permeation of cyclosporin A through intact and de-epithelialized human vaginal mucosa was enhanced by 0.01% Benzalkonium Chloride.[13] *Specimens were obtained from excess tissue removed from 8 post-menopausal patients.*

Details regarding the penetration enhancement study summarized below may be found in Table 6. The effect of Benzalkonium Chloride (0.5 – 5%) on lorazepam permeation was evaluated in rat skin.[35] An increased steady-state flux of lorazepam was observed when applied to the skin with Benzalkonium Chloride, compared to controls; however, this effect was not observed in a dose-dependent manner.

Absorption, Distribution, Metabolism, and Excretion (ADME)

The lung and kidneys were target organs of Benzalkonium Chloride in an assay in which 12 - 18 rats (sex not stated) were administered Benzalkonium Chloride via catheter (either jugular vein or femoral artery administration; dose of 15 mg/kg 1% Benzalkonium Chloride over 1 min).[13] *Blood Benzalkonium Chloride levels and kinetics were similar among the different routes of administration; however, the lung and kidney levels were higher in jugular vein-administered rats.*

A commercial mixture of alkylbenzyldimethylammonium chlorides (predominantly C_{12}, C_{14}, and C_{16}) was administered orally, rectally, or intramuscularly to rabbits, dogs, and cats, at 10 times the lethal dose.[2] *After oral administration, most of the compound remained in the upper gastrointestinal tract, with small concentrations found in the liver and blood. After rectal administration, nearly all of the compound was recovered from the lower bowel, with small amounts in the blood, liver, and kidney tissue. Following intramuscular administration, nearly all of the mixture remained at the injection site.*

No Benzalkonium Chloride was detected in blood or breast milk samples at any time point (15 min – 24 h after application) following the use of tampons containing Benzalkonium Chloride (60 mg; n = 3 – 4 women).[4]

Benzalkonium Chloride was found in the corneal epithelium, endothelium, stroma, and in the bulbar and palpebral conjunctivae of rabbits (number and sex not stated) following administration of a 50 µl eyedrop containing [^{14}C]-Benzalkonium Chloride.[4] *Measurable levels of Benzalkonium Chloride were present in ocular tissues for up to 120 h. No radioactive material was found in the aqueous humor or any other tissues.*

The ADME studies summarized herein can also be found in Table 5. After a single dermal application of radiolabeled 0.1% Benzalkonium Chloride (49.9% active ingredient) to rats, mean radioactivity levels were below quantifiable limits in all

tissues and organs except for intestines and stripped skin.[12] Rapid absorption of the test substance (in limited amounts) was observed in a 3-part experiment in which rats were orally administered (via diet (at 100 ppm) or gavage (at 1 or 5 mg/ml)) Benzalkonium Chloride (30% active ingredient; radiolabeled and non-radiolabeled).[11] Following a single oral dose of 200 mg/kg bw radiolabeled Benzalkonium Chloride (49.9% active ingredient), radioactivity levels were highest in the intestines of rats while trace levels were found in other tissues and organs (e.g., abdominal fat, heart, lungs).[12] The maximum amount of Lauralkonium Chloride observed in the blood and liver were determined to be 1000 and 550 nM, respectively, following administration of 120 µg/g/d via diet to mice.[36] In an assay in which rats were intratracheally administered ^{14}C-labeled Benzalkonium Chloride, the lungs were the primary site of deposition, showing the highest retention at 3 h and still containing measurable material at 168 h post-dosing.[37] In a similar study, prolonged retention of radioactivity was observed in the pancreas and heart of rats following ^{14}C-labeled Benzalkonium Chloride administration.[38]

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Of 96 mice receiving dermal applications of 6.5 or 50% Benzalkonium Chloride, 29 died within 72 h after application.[4] *Other effects at these concentrations included permanent bald spots, ear necrosis, and weight loss, while lower concentrations (0.1 – 1.5%) produced mild, temporary effects. A dermal median lethal dose (LD_{50}) of 525 mg/kg was established in an acute oral toxicity assay in which Benzalkonium Chloride was given to white rats. No toxic effects were observed in an assay in which a cream containing 0.13% Benzalkonium Chloride (5 ml/kg) was administered to rats via gavage. No deaths were observed in an assay in which a cream containing 0.1% Benzalkonium Chloride (7 ml/kg) was given to rats via gavage. Adverse effects such as difficulty breathing, diarrhea, and death were observed in rats (number and sex not stated) treated with 250 mg/kg Benzalkonium Chloride via gavage.*[13]

LD_{50}s of greater than 0.5 g/kg but less than 1.25 g/kg were determined in experiments in which 25% aqueous solutions of Stearalkonium Chloride was administered to rats via gavage.[2] *An aqueous solution containing 20% Stearalkonium Chloride yielded an LD_{50} of 4 ml/kg in rats (method of oral administration not stated). In a different study, pure Stearalkonium Chloride administered via gavage (type of animals used not stated) yielded an LD_{50} greater than 0.0625 g/kg but less than 1.25 g/kg. An oral LD_{50} of 0.76 g/kg was established in an assay performed using pure Stearalkonium Chloride in mice (method of oral administration not stated).*

Details regarding the acute toxicity studies summarized below may be found in Table 6. LD_{50}s were determined to be 3.56 ml/kg bw (assay performed in rabbits) and 930 mg/kg bw (species not stated) Benzalkonium Chloride in acute dermal irritation assays.[8,11] The lowest oral LD_{50} for Benzalkonium Chloride was reported to be 234 mg/kg following administration of 10% Benzalkonium Chloride to rats.[11,12,39,40] A concentration-dependent reduction in tidal volume and an increase in respiratory rate was observed in mice exposed (head-only) for 30 min to Benzalkonium Chloride at concentrations of 0.049 - 19 mg/m^3.[41] Inflammatory effects were observed at the two highest concentrations (5.3 and 19 mg/m^3). In a different acute inhalation toxicity assay performed in rats, a median LC_{50} of 52.84 mg/m^3 Benzalkonium Chloride was determined.[8,42]

Repeated-Dose Toxicity Studies

The short-term toxicity of 1.5% Benzalkonium Chloride was evaluated in female mice (3/group) dermally treated daily, 5d/wk, for 8 wk.[13] *An intense inflammatory infiltrate in the dermis resulted in a migration of neutrophils through the epidermis to form a huge exudate within a parakeratotic horny layer (keratinocytes showed marked cytotoxic changes). Benzalkonium Chloride solutions (50 and 100 mg/kg (doses were 1:20 and 1:10 dilutions of 10% Benzalkonium Chloride, respectively)) diluted in water or milk were administered to rats (10 males/group), via gavage, once daily, for 12 wk.*[4] *Two rats receiving 100 mg/kg died (vehicle not specified). Growth suppression was observed in rats treated with the aqueous solution at 100 mg/kg. No signs of toxicity were observed at the lower dose, regardless of the vehicle. No adverse effects were observed in a 14-wk inhalation toxicity assay in which rats (n = 12 females) and hamsters (n = 12 (sex not stated)) were exposed to an aerosolized hair conditioner containing 0.1% Benzalkonium Chloride (9.9 mg/m^3 of air) for 4 h/d, 5 d/wk. Beagle dogs (6/group; sex not stated) were given 10% Benzalkonium Chloride (12.5 – 50 mg/kg) diluted in either milk or water, daily, for 52 wk, via gavage.*[4] *Deaths were observed in groups given the water dilutions at 25 and 50 mg/kg. Slight to moderate hyperemia of the small intestine and pyloric stomach was observed in animals given milk dilutions at 50 mg/kg. Moderate to severe gastrointestinal irritation was observed at all water-diluted doses.*

Details regarding the repeated-dose toxicity studies summarized below may be found in Table 7. A dermal no-observed-adverse-effect-level (NOAEL) of 20 mg/kg/d was determined in a 90-d assay in which Sprague-Dawley rats (number and sex not stated) were topically treated with up to 20 mg/kg/d Benzalkonium Chloride (81% active ingredient).[43] An oral NOAEL of 5000 mg/kg/day of a sanitizing agent was determined in a 91-d study in Sprague-Dawley rats (10/sex/group) administered via gavage.[44] The sanitizing agent contained 0.01% Benzalkonium Chloride, corresponding to an active ingredient dose of 0.5 mg/kg/day Benzalkonium Chloride. A no-observed-effect-level (NOEL) of 500 ppm was determined in an assay in which Sprague-Dawley rats (15/sex/dose; 95 – 96 d) were treated with up to 4000 ppm Benzalkonium Chloride (79.7 – 80.5% active ingredient) via diet due to adverse effects observed at higher concentrations (e.g., gross lesions, hepatocellular atrophy).[11] No treatment-related adverse toxicological effects were observed in a 13-wk assay in which Beagle dogs (4/sex/group) were treated with up to 3000 ppm Benzalkonium Chloride (50% active ingredient).[12] Statistically significant increased lung weight and increased immunoglobulin E (IgE) and interleukin-6 (IL-6)

values were observed in female Wistar rats (5/sex/group) exposed to aerosolized Benzalkonium Chloride at 30 mg/m^3 (6 h/d; 3 d).[42] Similar results, along with statistically increased absolute liver mass and histopathological adverse effects of the lungs were observed in female Wistar rats (7 – 10/group) given aerosolized Benzalkonium Chloride (35 mg/m^3; 6 h/d exposure; 5 d).[45] A no-observed-adverse-effect concentration (NOAEC) of < 0.08 mg/m^3 was determined in a 14-d assay in which Fischer 344 rats (5/sex/dose) were treated with aerosolized Benzalkonium Chloride at concentrations up to 20 mg/m^3 (6 h/d exposure).[46]

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY (DART) STUDIES

Female mice (number not stated) were given Benzalkonium Chloride (3, 10, and 30 mg/kg), daily, via gavage, on days 0 – 6 of gestation.[13] *No significant adverse effects were observed in dams. The number of implantations, live fetuses, sex ratio, and body weights in fetuses were not significantly affected; however, a tendency toward a slight decrease in the pregnancy rate of dams in the 10 and 30 mg/kg treated group, and a slight increase in the number of dead or resorbed fetuses in the 30 mg/kg treated group, was observed. No significant differences were noted in treated and control dams and fetuses in an assay in which female rats (number not stated) were treated with up to 100 µg/kg on days 0 – 6 of gestation or up to 50 µg/kg on days 0 – 18 of gestation (method of administration not stated). A teratogenicity assay was performed in female rats (6 – 8/group) administered aqueous Benzalkonium Chloride (25 – 200 mg/kg) into the vagina.*[4] *Dams were killed on day 21 of gestation and fetuses were removed for examination. Reductions in maternal body weight and inflammation in the vagina were observed at doses of 100 mg/kg and higher. Statistically significant, dose-dependent reductions in the mean number of live fetuses and litter weights were noted in rats dosed with 50 mg/kg and higher, compared to controls. Sternal defects were observed in fetuses of mothers treated with 100 mg/kg and higher. The mean number of implantations in dams treated with 200 mg/kg was significantly reduced compared to controls ($p < 0.05$).*

Details regarding the DART studies summarized below can be found in Table 8. Statistically significant decreases in sperm viability and motility were observed in an assay in which human sperm were treated with up to 0.02 mmol/l Benzalkonium Bromide.[47] Similar effects were observed when human sperm was treated with 6.81 mM Benzalkonium Chloride.[48] In a prenatal developmental toxicity study using female CD rats (25/group) administered Benzalkonium Chloride (81.09% active ingredient; up to 100 mg/kg/d; gavage treatment on gestation day (GD) 6 - 15), no significant effects were observed regarding maternal weight, food survival, or skeletal development.[49] However, isolated fetal abnormalities were observed across dosed groups. A reproductive NOAEL of 2000 ppm for F_0, F_1, and F_2 rats was determined in a 2-generation reproductive toxicity assay in which Sprague-Dawley rats (28/sex/dose) were treated with Benzalkonium Chloride (81.09% active ingredient; via diet; treatments before and during mating, and throughout gestation, parturition, and lactation).[11] Similarly, a reproductive NOAEL of 2000 ppm was established in a 2-generation assay in which Sprague-Dawley rats (25/sex/group) were given Benzalkonium Chloride (49.9% active ingredient) in the diet at up to 4000 ppm, before, during, and after mating, until pups were weaned.[12] In a prenatal developmental toxicity study performed in female New Zealand White rabbits (16/group) at doses of up to 9 mg/kg/d (treatment during GD 6 – 18) of Benzalkonium Chloride (81.09% active ingredient), no treatment-related effects were observed relating to maternal weight, fetal survival, or malformations; however, isolated fetal variations occurred across all groups, including controls.[49] A developmental NOAEL of 30 mg/kg bw/d was established in a prenatal developmental toxicity assay in which female New Zealand White rabbits (22/dose) were administered up to 30 mg/kg bw/d Benzalkonium Chloride (49.9% active ingredient) on GD 6 – 28.[8,50]

GENOTOXICITY STUDIES

Benzalkonium Chloride (concentration not stated) was not mutagenic to Salmonella typhimurium strains TA1535, TA1536, TA1537, and TA1538 and Escherichia coli strains B/r WP2 hcr$^+$ and WP2 hcr in rec-assays in combination with reverse mutation systems.[4] *The mutagenic activity of Benzalkonium Chloride (concentration not stated) was not demonstrated in reversion assays involving strains S. typhimurium TA1535, TA1536, TA1537, and TA1538. Similarly, the deoxyribonucleic acid (DNA)-damaging capacity of Benzalkonium Chloride (10%) was not positively detected when evaluated in a S. typhimurium TA98 and TA100.*[13] *In a plate incorporation assay, Benzalkonium Chloride (concentration not stated) was not mutagenic to S. typhimurium strains TA98, TA1538, TA1537, and TA100. In an E. coli DNA polymerase assay, Benzalkonium Chloride induced repairable DNA damage in strains W3110 (polA$^+$) and p3478 (pol A$^-$). No genotoxicity was observed (with and without metabolic activation) when the genotoxicity potential of Benzalkonium Chloride (up to 5 µg/ml) was evaluated in an umu assay using S. typhimurium TA1535/pSK. Benzalkonium Chloride (up to 30 µm) produced negative results in a chromosomal aberration assay using Syrian hamster embryo cells (with and without metabolic activation). Treatment of L5178 with Benzalkonium Chloride (concentration not stated) alone or in combination with UVA irradiation did not induce any mutations.*

Details regarding the genotoxicity studies summarized below may be found in Table 9.

In studies evaluating gene mutations, Benzalkonium Chloride (0.01% in a sanitizing solution or 50 – 80% active ingredient) yielded negative results in multiple Ames assays (tested at up to 110 µg/plate; assays performed in *E. coli* and/or *S. typhimurium*; performed with and without metabolic activation).[11,44,51] No mutagenicity was observed in mammalian cell gene mutation assays performed on Benzalkonium Chloride (81.09% active ingredient; up to 100 µg/ml) using Chinese hamster cells.[11,12] Stearalkonium Chloride (up to 5000 µg/plate) also yielded negative results when evaluated in Ames assays

performed with and without metabolic activation in *E. coli* and/or *S. typhimurium*.[12,52] When evaluating the ability to induce chromosomal damage, Benzalkonium Chloride (50 – 99.24% active ingredient; using Chinese hamster ovary cells (at up to 98 µg/ml) and human lymphocyte (at up to 24 µg/ml)) and 10% Benzalkonium Chloride (up to 0.0003% in human dental pulp cells) yielded negative results in chromosomal aberration assay performed with and without metabolic activation.[11,12,53] Statistically significant increases in micronuclei formation (compared to controls) were observed at 1 mg/l in a micronucleus assay (performed without metabolic activation; peripheral human lymphocytes) using Benzalkonium Chloride.[51] When examining effects on other endpoints, positive results were observed in alkaline comet assays performed in human corneal epithelial cells and human Chang conjunctival cells using Benzalkonium Chloride (0.00001 – 0.001%).[54,55] Similarly, Benzalkonium Chloride resulted in positive results in single cell gel electrophoresis assays (in human bronchial cells at 0.002 – 0.02%; in primary rat hepatocytes at 1 mg/ml).[51,56] Conversely, Benzalkonium Chloride (up to 2 mM) was non-mutagenic in a single-cell gel electrophoresis assay performed in human hepatocellular carcinoma and liver cancer cells.[57] Negative results were obtained in in vivo micronucleus assays performed using rats given either 0.01% Benzalkonium Chloride (up to 20 ml/kg) or 400 mg/kg Benzalkonium Chloride.[8,44]

CARCINOGENICITY STUDIES

The tumorigenicity of Benzalkonium Chloride was evaluated in a dermal assay using 100 female mice and 10 rabbits (male and female).[4] *Animals were treated with either 8.5% or 17% Benzalkonium Chloride (solvent either methanol or acetone), dermally, 2x/wk, for 80 – 90 wk. No animals survived the full treatment period. The test substance induced ulceration and inflammation in both species but did not cause tumors.*

OTHER RELEVANT STUDIES

Effect on Histamine Release

The effect of Benzalkonium Chloride on histamine release was evaluated using mixed cellular suspensions (11% mast cells) from peritoneal cavities of rats.[4] *Low concentrations of Benzalkonium Chloride (0.3 and 3 µg/ml) inhibited histamine release by 70 and 14%, respectively. Higher concentrations of Benzalkonium Chloride (10 and 30 µg/ml) stimulated histamine release by 70 and 90%, respectively. In a similar study, Benzalkonium Chloride (1 and 5 µg/ml) produced a concentration-dependent inhibition of histamine secretion in rat mast cells (2 -10% purity).*

Benzalkonium Chloride inhibited histamine release from rat peritoneal macrophages induced plasma bradykinin, oligomer-specific Datura stramonium agglutin, synthetic compound 48/80, and polyethyleneimine, resulting in half-maximal inhibitory concentrations (IC_{50}s) of 0.63, 3.04, 1.56, and 0.48 µg/ml, respectively.[13]

Ototoxicity

Benzalkonium Chloride (0.026 and 0.05%) resulted in statistically significant mild to moderate mucosal thickening of the tympanic membrane when instilled into the bulla of juvenile male guinea pigs (number not stated) for 7 d (compared to saline control).[13] *Applications of 0.1% Benzalkonium Chloride in water or 70% alcohol to the round window membrane of the middle ear of 13 guinea pigs (5 – 8/group; sex not stated) for 10 - 60 min resulted in severe, time-dependent ototoxicity.*[4] *These effects include fibrosis, inner hair cell loss, and vestibular damage.*

Ocular Toxicity

In vitro and in vivo studies demonstrate concentration- and exposure-dependent corneal and retinal toxicity.[4] *No morphological changes were observed in excised rabbit corneas exposed to 0.0001% Benzalkonium Chloride for 2 h. However, 0.01 – 0.02% Benzalkonium Chloride caused endothelial cell damage, mitochondrial disruption, and persistent edema in rabbit corneas. Dose-dependent endothelial swelling occurred at ≥ 0.000065% Benzalkonium Chloride. Subconjunctival injections of 0.007 – 0.01% Benzalkonium Chloride for 2 wk caused retinal elevation and detachment. Corneal epithelial necrosis and leukocytic infiltration were observed at ≥ 0.5% Benzalkonium Chloride in rabbits and guinea pigs; at 0.1%, only rabbits were affected. Progressive damage was noted within 6 h after a single 0.01% Benzalkonium Chloride instillation. In contrast, no corneal endothelial abnormalities were observed in human subjects topically treated with one drop of 0.01% Benzalkonium Chloride (0.1 mg/ml) 2x/d for 2 wk (vehicle not stated).*

Significantly reduced stromal nerve fiber density and aqueous tear production and increased inflammatory cell infiltration was observed in an assay in which *Thy1*-YFP+ mice (5/group; sex not stated) were topically treated with Benzalkonium Chloride (0.01 or 0.1%; ocular treatment 1x/d for 7 d).[58] Severe corneal epithelial defects were observed in an assay performed in C57BL/6J mice (n = 72) treated with topical Benzalkonium Chloride (0.2%) for 7 d. In the same study, 0.1% Benzalkonium Chloride induced punctate fluorescent staining without detriment to corneal smoothness, disorganized basal corneal epithelial cells with enlarged cytoplasmic halos, and decreased goblet cells.[59] Topical exposure to 0.01% Benzalkonium Chloride in male Wistar rats (n = 6) caused permanent functional impairment of corneal cold-sensitive trigeminal neurons and structural degeneration of intraepithelial nerve fibers.[60]

Benzalkonium Chloride (0.1%) applied to the eyes of male New Zealand albino rabbits (8/group) resulted in significant increases in central corneal thickness, endothelial carboxy fluorescein permeability, endothelial cell damage, and disruption of the perijunctional actomyosin ring (application 2x/d for 4 d).[61] Dose-dependent corneal toxicity with significant epithelial disruption was observed at concentrations ≥ 0.01% in an assay performed in male New Zealand White rabbits (3/group) using

commercially available Benzalkonium Chloride (C_{12} – C_{16}).[62] Rabbits received 10 instillations (every 30 min) at concentrations ranging from 0.001 – 0.03% over 10 d. Topical administration of eye drops containing 0.01 and 0.1% Benzalkonium Chloride (4x/d for 14 d) to male New Zealand White rabbits (6/group) resulted in decreased goblet cell density and histopathological changes.[63] Conversely, no local ocular toxicity was observed in assay in which 0.005 and 0.01% Benzalkonium Chloride was applied to the eyes of *Macaca fascicularis* monkeys (n = 6 – 10) and Dutch rabbits (n = 6 – 18).[64] Applications occurred multiple times per day (6 – 8 x/d) for several weeks (3 – 52 wk).

Accumulation Following Ocular Administration

Ocularly applied Benzalkonium Chloride is reported to accumulate in the cornea, lens, conjunctiva, iris, ciliary muscle epithelium, and trabecular meshwork.[65] Because of this, exacerbated ocular toxicity may be apparent in patients using eyedrops containing Benzalkonium Chloride repetitively.

Ocular Penetration Enhancement

An in vitro assay was performed in rabbit eyes (n = 25) to evaluate the penetration enhancement of Benzalkonium Chloride (0.01 or 0.05%) on betamethasone 21-phosphate solution (solutions of betamethasone 21-phosphate with and without Benzalkonium Chloride; continuously administered to sclera via osmotic pump for 1 wk).[13] *Benzalkonium Chloride increased concentrations of betamethasone 21-phosphate in the vitreous and retina-choroid compared with the control.*

The permeability enhancement ability of Benzalkonium Chloride in ocular pharmaceutical formulations may enhance toxicity. For example, in a rabbit model, topical latanoprost preserved with 0.02% Benzalkonium Chloride significantly increased corneal epithelial permeability (measured by carboxyfluorescein uptake) and caused loss of tight-junction integrity, compared to both untreated controls and travoprost preserved without Benzalkonium Chloride.[66,67]

Barrier Disruption

The effect of Benzalkonium Chloride (0.05% in water) on stratum corneum organization and permeability of the protein drug interferon alpha-2b (IFNα) was evaluated in human skin samples (full-thickness breast skin) in vitro.[68] The test substance decreased both short and long lamellar periodicity distances. In addition, a high degree of lateral packing disruption was observed. Although significant lipid disordering was observed, IFNα permeation remained low (total IFNα permeation approximately 1 ng/cm^2 of skin; PBS resulted in permeation of approximately 1.5 ng/cm^2 skin). In addition to skin barrier disruption, Benzalkonium Chloride has been observed to result in corneal epithelial disruption (effects observed at 0.005 – 0.4% Benzalkonium Chloride).[61,69-71]

Effect on Gap Junction Communication

Topical administration of Benzalkonium Chloride (0.05% and 0.1%) to the eyes of male New Zealand albino rabbits (n = 12/group; application 2x/d for 7 d) resulted in altered connexin43 (Cx43) and tight junction zonula occludens-1 (ZO-1) distribution and reduced Cx43 expression.[73] The test substance also induced significant increases in Cx43 phosphorylation status concomitant with decreases in the Cx43-ZO-1 protein-protein interaction. These effects are associated with a decline in gap junction intercellular communication activity. In a similar study in which 0.02% Benzalkonium Chloride in saline was applied to the eyes of male Japanese white rabbits (n = 3/group) every 5 min for 20 min, ZO-1 expression was discontinuous and fragmented in exposed corneas.[74] Evaluation of tissue sections indicated that exposure to the test substance also altered ZO-1 localization.

Cytotoxicity

Cytotoxicity was observed in multiple cell types (rat prostatic epithelial cells, rat cardiac fibroblasts, human conjunctival cells, corneal epithelial cells, human neutrophils) in in vitro assays performed using Benzalkonium Chloride at varying concentrations (0.00001% - 0.15%).[4,13] *Cytotoxicity from Benzalkonium Chloride was also observed in murine tumor cell lines (at concentrations of 0.6 – 7.2 ppm), red blood cells of rabbits (at 0.042 mM), Chinese hamster cells (at concentrations as low as 10 µg/ml), human oral and skin keratinocytes (at concentrations as low as 0.001 mM), and 3T6 fibroblasts (at concentrations as low as 1 µg/ml).*

Many cytotoxicity assays using Benzalkonium Chloride were found in the literature. Cell types evaluated, as well as concentrations at which Benzalkonium Chloride induced cytotoxicity are as follows: human alveolar epithelial A549 cells (at ≥ 1 µg/ml), human and rabbit corneal epithelial cells (at ≥ 0.001%), human trabecular meshwork cells (at ≥ 0.001%), human respiratory epithelial cells (at ≥ 0.005%), reconstructed human epidermis (at ≥ 0.5 mg/ml), human corneal stromal fibroblasts (at ≥ 0.00000001%), human meibomian gland epithelial cells (at ≥ 50 µg/ml in one assay and at ≥ 0.005 µg/ml in a different assay), human conjunctival cells (at ≥ 0.005 µg/ml), human vaginal cells (at ≥ 0.05 mM), porcine corneal explants (at 0.01%), in human nasal epithelial cells (at ≥ 0.01%), T-lymphocytic Jurkat cells (at ≥ 0.0001%), human limbal epithelial stem cells (at ≥ 0.00002%), rat neural progenitor cells (at 1 µM), human primary subcutaneous pre-adipocytes (at ≥ 0.005%), human gingival fibroblasts (at ≥ 0.05 mM), and Sertoli cells (at 0.02%).[54,56,71,75-88]

Reactive Oxygen Species (ROS) Generation

A stimulation of ROS (dioxygen and hydrogen peroxide) production was observed in an in vitro assay at concentrations of 0.00001% and higher following cell (human conjunctival cell line) human treatment with Benzalkonium Chloride.[13]

Arachidonic acid release was evaluated in murine Swiss albino 3T6 fibroblasts treated with Benzalkonium Chloride. Benzalkonium Chloride induced significant [^{3}H]arachidonic acid release at a concentration of 1µg/ml.

The effect of Benzalkonium Chloride (0, 2, 5, 10, 20 and 40 µg/ml on ROS formation was evaluated using human type II alveolar epithelial (A549) cells.[89] Treatment with the test substance did not induce production of extracellular ROS as low concentrations (≤ 5 µg/ml); however, a slight increase was observed at the 10 µg/ml concentration (not statistically significant). Statistically significant increases in extracellular ROS levels were observed following treatment with ≥ 20 µg/ml. Similarly, Benzalkonium Chloride (1 µM) resulted in a 9-fold increase in ROS generation when evaluated in rat neural progenitor cells compared to the vehicle-control group (0.1% dimethyl sulfoxide (DMSO)).[84]

Effect on Cytokines

In an assay performed in human keratinocytes, Benzalkonium Chloride (0.1 µg/ml) stimulated the production and intracellular accumulation of interleukin-1α (IL-1α).[13] *The release of IL-1α was also observed when evaluated in mouse keratinocytes were exposed to Benzalkonium Chloride. Benzalkonium Chloride (concentrations up to 30% in distilled water) elicited neutrophils to the site of chemical application in mouse skin (number and sex not stated) in an in vivo assay; however, time-dependent and chemical-specific patterns of inflammation were not detected.*

The effect of Benzalkonium Chloride (0.25 – 2 mg/ml) on the differential release of IL-1α and interleukin (IL)-8 was evaluated in reconstructed human epidermis (20 h incubation).[76] Reconstructed human epidermis treated with the test substance released large amounts of IL-1α and IL-8 (increases were observed in a dose-dependent manner). Measurable increases of IL-6 and IL-8 were observed in organ culture fluids in a similar assay in which tissue (hip skin via human punch biopsies) was exposed to 5 and 10 µM Benzalkonium Chloride.[90] In a different assay, immortalized human conjunctival and corneal epithelial cells exposed to 0.1% Benzalkonium Chloride for 1 h resulted in induction of significant amounts of IL-1, tumor necrosis factor, and moderate amounts of C-reactive protein, IL-10, and IL-12 in both cell types.[91]

Intranasal Products

A published literature review evaluated conflicting reports of damage to human nasal epithelia and/or rhinitis medicamentosa exacerbation associated with intranasal products containing Benzalkonium Chloride.[92] This review considered 18 studies (11 in humans, 3 in animals, and 4 in vitro) with concentrations of Benzalkonium Chloride ranging from 0.00045 to 0.1%. Ten studies concluded that Benzalkonium Chloride resulted in degenerative changes in human nasal epithelia or exacerbation of rhinitis medicamentosa; however, only 2 of these studies were supported by statistically significant differences between Benzalkonium Chloride and the control group. In these 2 studies, oxymetazoline was used in some or all subjects (oxymetazoline is associated with rhinitis medicamentosa). No toxic effects were observed in 8/18 of the reviewed studies, including a 6-mo and 1-yr study.

Effect on DNA, RNA, and Protein Synthesis

Inhibition of DNA, RNA, and protein synthesis was observed in a dose-dependent matter in Chinese hamster lung fibroblast cells treated with Benzalkonium Chloride (3 – 30 µg/ml).[13] *Cells were exposed to the test substance for 2 h.*

Neurotoxicity

Markedly reduced, damaged, or degenerating nerves, loss of mitochondrial cristae, damaged organelles, and cell membrane lysis were observed in an assay in which the serosal surface of small intestines of male rats (number not stated) were exposed to 0.062% Benzalkonium Chloride.[13] *A significant reduction in the neuron number of the myenteric plexus, and thickening of smooth muscle was observed following serosal application of a 0.2% solution of Benzalkonium Chloride to the jejunum of male rats (n = 87). Denervation was apparent following local treatment to the bladders of female rats (n = 15) with 0.3% Benzalkonium Chloride. Topical Benzalkonium Chloride (0.5%) application to the muscularis of the esophagus of 38 rats (sex not stated) resulted in distal esophageal aganglionosis, characterized by distal narrowing, proximal dilation, decreased food intake, and limited weight gain (compared to controls).*

The potential neurotoxicity of Benzalkonium Chloride (0 – 10 µg/ml) on human-derived SH-SY5Y astrocyte cultures was evaluated via a neurite outgrowth assay.[93] The neurite length and cell number were evaluated following a 24-h exposure to the test substance. Complete inhibition of neurite outgrowth and cell number occurred at 2.5, 5, and 10 µg/ml.

Endocrine Activity

A H295R steroidogenesis assay was performed to evaluate the effect of Benzalkonium Chloride (0, 0.5, 1, and 1.5 mg/l; 48-h incubation) on estrogen synthesis.[94] Exposure to the test substance at concentrations of 1 and 1.5 mg/l significantly increased estradiol production of H295R cells in a concentration-dependent manner. In addition, transcription of 4 steroidogenic genes, 3β-HSD2, 17β-HSD-1, 17β-HSD4, and CYP19A, was statistically significantly enhanced by Benzalkonium Chloride (at all concentrations tested; compared to controls). A Michigan Cancer Foundation-7 (MCF-7) proliferation assay was performed using estrogen-responsive breast cancer MCF-7 cells incubated with Benzalkonium Chloride (0, 0.5, 1, and 1.5 mg/l; 48-h incubation). In ER-positive MCF-7 cells, exposure to the test substance at all concentrations significantly increased the cell proliferation rate in a dose-dependent manner ($p < 0.01$; compared to controls). The protein expression levels of ERα and G-protein coupled estrogen receptor 1 (GPER1) were statistically significantly increased by all concentrations of Benzalkonium Chloride. In the MCF-7 cells exposed to the highest concentration, the expressions of ERα and GPER1 were 2.1-fold and 2.3-fold of the controls, respectively. Conversely, no estrogen-stimulating

effects were observed in an assay in which recombinant human breast cancer cells (cells containing stably integrated ER-responsive firefly luciferase reporter plasmid) were incubated with Benzalkonium Chloride at concentrations up to 10 µM for 24 h.[95]

The inhibitory effects of Benzalkonium Chloride homologs with varying alkyl chain lengths (C1 – C18) on human and rat 11β-HSD2 were evalauted.[96] Inhibition was dependent on alkyl chain length, with C10 and longer homologs exhibiting activity against the human enzyme and C12 and longer homologs against the rat enzyme. C16 Benzalkonium Chloride showed the greatest potency, with IC_{50} values of 4.22 µM for the human enzyme and 24.74 µM for the rat enzyme.

Effect of Lauralkonium Chloride on Gut Microbiome and Bile Acid Profile

The effect of orally-ingested Lauralkonium Chloride (120 µg/g/d; 1-wk treatment; via diet; controls fed untreated diet) on the gastrointestinal microbiota and bile acid profiles of C57BL/6J mice (4 - 6/sex/group) was studied.[36] Effects on the microbiome were evaluated using 16S ribosomal RNA gene sequencing using cecum intestinal content and bile acids were evaluated via feces and liver extraction. Treatment with the test substance resulted in statistically significant decreased alpha diversity and differential composition of gut bacteria, with notability decreased actinobacteria phylum (compared to controls). In addition, statistically significant decreases in secondary bile acids were observed in treated mice. All effects were more prominent in female versus male mice.

Effect of Benzalkonium Chloride on Atopic Dermatitis

The effect of Benzalkonium Chloride (0.2%) on atopic dermatitis was evaluated in male NC/Nga mice (72 total mice; number/group not stated) subcutaneously injected with mite allergen to induce atopic dermatitis-like skin lesions.[97] Benzalkonium Chloride-exposed mice exhibited markedly elevated clinical dermatitis scores, significant increases in eosinophil infiltration and mast cell accumulation, and degranulation in the subcutaneous tissue. Test substance exposure also resulted in upregulated expression of inflammatory cytokines and elevation of serum immunoglobulin E (IgE). These effects were statistically significant compared to controls (control group 1 was treated with saline (in place of mite allergen) + vehicle (vehicle not stated); control group 2 treated with mite allergen + vehicle).

DERMAL IRRITATION AND SENSITIZATION STUDIES

Multiple studies report concentration-dependent dermal irritation associated with Benzalkonium Chloride.[4,13] *In rats and rabbits, Benzalkonium Chloride concentrations ≥ 0.1% caused erythema, necrosis, edema, or eschar formation, particularly under occlusive conditions. Severe skin damage was observed in rabbits exposed to 2% Benzalkonium Chloride on intact and abraded skin. Human patch testing with 0.1 – 5% Benzalkonium Chloride demonstrated dose-dependent irritation, with erythema and edema increasing at higher concentrations. However, repetitive daily application of 0.1% Benzalkonium Chloride showed no cumulative irritation in rabbits or humans. Sensitization was observed in 2/10 guinea pigs in a modified Buehler's assay (induction: 10%; challenge: 0.5%), and significant ear swelling was noted in a murine local lymph node assay (LLNA)/irritancy assay at concentrations up to 5%. In contrast, human repeated-insult patch testing (HRIPT) (n = 101 – 155) with creams containing 0.1 – 0.13% Benzalkonium Chloride showed no sensitization. Stearalkonium Chloride was mildly irritating at concentrations ≥ 1.25% in rabbits, with primary irritation indices up to 6.0 at 25%.*[2] *A 20% solution of Stearalkonium Chloride induced minimal swelling in guinea pig stratum corneum. In HRIPTs (n = 50), 1 – 20% solutions produced mild erythema in some cases but did not result in sensitization.*

The irritation and sensitization studies summarized below can be found in Table 10. Dermal irritation was evaluated using reconstructed human epidermis models, with Stearalkonium Chloride tested at final concentrations of 0.49, 3, and 100%; all concentrations were found to be non-irritating.[12,15] Benzalkonium Chloride (50 – 80% active ingredient) was irritating/corrosive in a dermal irritation assay performed in rabbits (n = 6; occlusive conditions; tested neat).[11] Moderate irritation was observed in a 24-h dermal irritation assay performed in rabbits (n = 2; occlusive conditions) using a mixture containing 40% Stearalkonium Chloride (tested neat).[99] Seven out of 30 subjects had a positive patch test reaction to 0.1% Benzalkonium Chloride in an irritancy patch test assay (no positive reactions at lower concentrations).[100] Benzalkonium Chloride was determined to be sensitizing in a 2-stage modified LLNA performed in female mice (4/group) at concentrations of 0.5 and 1%.[101] Conversely, Benzalkonium Chloride (50%; tested at 0.1% for induction and challenge) active ingredient was non-sensitizing in a modified Draize assay performed in guinea pigs (6/group).[12] In a sensitization assay performed in 50 subjects, the test substance (20% Stearalkonium Chloride in water and stearyl alcohol; occlusive conditions) was considered to be non-sensitizing.[2] Erythema was observed in some subjects following the initial patch application, which the study authors suggested may have been due to impurities in the test material or irritation from the stearyl alcohol vehicle.

Computational Predictions

The Deductive Estimation of Risk from Existing Knowledge (DEREK) Nexus system was used to predict the skin sensitization of ingredients in this group.[8] Alerts for skin sensitization by quaternary ammonium cations (hapten acting though ion pair formation) were reported; however, insufficient data were available to make an effective concentration causing a 3-fold increase in lymph node cell proliferation (EC3) prediction, and the confidence level of the prediction was equivocal. The molecular initiating event leading to dermal sensitization in the OECD adverse outcome pathway for dermal sensitization is covalent binding of a relatively low molecular weight electrophilic chemical with key nucleophilic sites of

skin proteins.[8,102] Due to their chemical structures, it is unlikely that the chemicals in this group would bind to proteins in this manner.

Reduction of Skin Irritation Potential

The potential for Benzalkonium Chloride (1%) to reduce the skin irritation of 20% sodium dodecyl sulfate was evaluated in 54 subjects.[13] *Sodium dodecyl sulfate was applied for 2 h under occlusion, then Benzalkonium Chloride was applied to the same site (water used instead of Benzalkonium Chloride as control). Forty subjects had some reaction to sodium dodecyl sulfate following by either Benzalkonium Chloride or water. In comparison to the water control, 20/40 subjects had a weaker irritation reaction, and 4 had a stronger reaction.*

Phototoxicity

Details regarding the following phototoxicity assay may be found in Table 11. The combined toxic effect of UV radiation (0.17 J/cm^2) and Benzalkonium Chloride (0.001 - 0.004%) was evaluated in cultured human corneal epithelial cells.[103] UV administrations occurred at different times (before, at the same time, or after Benzalkonium Chloride administration). In all 3 exposure orders, synergistic effects were observed when UV was applied with Benzalkonium Chloride at all concentrations.

Ocular Irritation Studies

Benzalkonium Chloride exhibited concentration-dependent ocular irritation in animal studies.[4,13] *No irritation was observed in rabbits following a single instillation of 0.1% Benzalkonium Chloride. Minimal-to-weak and reversible irritation was noted at concentration of 0.01 – 0.3%. Severe ocular effects, including conjunctival necrosis and iritis, were reported in rabbits administered 2% Benzalkonium Chloride twice daily for 7 d. While 0.01 – 0.1% Benzalkonium Chloride did not cause ocular damage at low frequency, repeated instillation (e.g., 0.1% Benzalkonium Chloride 5x/d), resulted in corneal endothelial damage. Corneal damage percentages were 50.55 and 57% in mice and rabbits, respectively, following a single administration of 0.5% aqueous Benzalkonium Chloride. Draize scores ranged from 6 (50% Benzalkonium Chloride at 24 h; scale not provided) to 40/110 (concentration unspecified; 3 h post-application). In humans, 0.02% Benzalkonium Chloride produced slight conjunctival hyperemia in 1/51 subjects.*

In rabbits, Stearalkonium Chloride caused transient irritation at concentrations ≤ 1.25%.[2] *A 25% solution was classified as a severe ocular irritant. A 4:1 mixture of Stearalkonium Chloride to stearyl alcohol was non-irritating at a threshold of 0.04% Stearalkonium Chloride and 0.01% stearyl alcohol in 2/5 animals.*

Details regarding the ocular irritation assays summarized below can be found in Table 11. No prediction of irritation could be made in an EpiOcular™ assay performed using undiluted Benzalkonium Chloride; however, cell viability was significantly reduced compared to controls. Stearalkonium Chloride (final test concentration of 2%) was predicted to be moderately irritating in an EpiOcular™ assay and irritating when tested at a final test concentration of 3% in a SkinEthic™ human corneal epithelial model.[15,62] An undiluted Stearalkonium Chloride mixture (85% in glycerin) tested in a reconstructed human cornea-like epithelium assay was also predicted to be irritating. Ocular irritation was observed at concentrations ≥ 0.01% Benzalkonium Chloride when evaluated in male rabbits (3/group).[62] A mixture containing 40% Stearalkonium Chloride was observed to be corrosive to the eyes in an assay performed in rabbits (n = 2).[99] Mild conjunctival hyperemia was observed in a Draize assay performed in rabbits (n = 7) treated with 0.002% Stearalkonium Chloride.[8]

MUCOUS MEMBRANE IRRITATION STUDIES

Cervical erythema, vaginal erythema, and vaginal epithelial disruption was noted in female monkeys (n = 50) treated intravaginally with a spermicide containing 1.2% Benzalkonium Chloride (treatment 1x/d for 4 d).[13] *Cervical biopsy specimens revealed acute inflammatory infiltrates with occasional plasma cells and lymphoid follicles.*

Histological changes and nasal lesions were observed in the male rats (5/group) treated intranasally with 0.01% Benzalkonium Chloride for 1 or 2 wk (effects worsened in a time-dependent manner).[13] *The long-term use of Benzalkonium Chloride (0.01 and 0.1%) solutions on rat nasal respiratory mucosa was evaluated in rats (9/group; sex not stated). Solutions were administered to the nasal cavities through both nostrils for 2 – 4 wk (duration of each administration not stated). Nazal wheezing was observed at the higher concentration. Histological changes (e.g., severe degrees of proliferation of intraepithelial glands, vascular hyperplasia) were observed in treated groups. In a study performed in humans, the use of an oxymetazoline nasal spray containing Benzalkonium Chloride (0.1 mg/ml) resulted in nasal swelling (n = 10; subjects treated 3x/d for 10 d). In a different assay, no rebound swelling was observed in subjects (n = 18) that administered oxymetazoline hydrochloride nasal spray containing Benzalkonium Chloride (0.5 mg/ml) for 10 d (number of uses per day not stated).*

In a nasal irritation assay using male Sprague-Dawley rats (number of animals not stated), animals were intranasally administered 0.2% Benzalkonium Chloride in saline (25 µl).[104] Approximately 20 min post-dose, the cavity was lavaged with a bolus of saline, and fluid was evaluated for total protein, lactate dehydrogenase, and IL-1α (20 min lavage collection time). Test substance administration resulted in strong mucosal irritation and significant increases in all nasal lavage biomarkers compared to controls treated with a saline solution ($p < 0.05$). In an assay performed in rat, cat, and pig vaginal

models, 2% Benzalkonium Chloride was used as a positive control to stimulate known mucosal irritant effects (vaginal inflammation, epithelial damage, and cytokine elevation were observed following vaginal administration).[105]

The effect of Benzalkonium Chloride (0.02, 0.2, and 2%) on the vaginal surface of female Swiss Webster mice (n = 9 - 14/group) was evaluated by endoscopic colposcopy and histology imaging following vaginal instillation (controlled treated with PBS only).[106] Images from 0.02% Benzalkonium Chloride-treated animals were similar to controls; however, vascular changes (petechiae and blood vessel size and number increase) were observed in animals treated with 0.2% Benzalkonium Chloride. Vaginal surfaces appeared blanched and epithelial disruption was apparent in animals treated with the highest concentration. Treatment with 0.2% Benzalkonium Chloride significantly increased vascular injury scores ($p < 0.01$; compared to controls) and treatment with 2% significantly increased epithelial disruption ($p < 0.01$; compared to controls). Erythema scores were comparable across all treatment groups. Epithelial denuding was statistically significantly increased in the mid- and high-dose treatment groups, compared to controls. Similarly, in a different assay, vaginal treatment with 2% Benzalkonium Chloride in female New Zealand White rabbits (3/group) resulted in multifocal damage of the vaginal mucosa with congestion, edema, inflammatory infiltrate, and epithelial disruption.[88] The total rabbit vaginal irritancy score was statistically significantly higher in Benzalkonium Chloride-treated animals compared to controls treated with saline.

The potential for 0.2% Benzalkonium Chloride (single 8 ml administration; controls treated with PBS; vehicle not stated) to cause mucosal disruption was evaluated in an assay in which yearling female sheep (4/group) were treated rectally.[107] Colonoscopy and imaging were performed at baseline and after treatment. At baseline, erythema or petechiae were seen in 3/8 sheep. After treatment, erythema was seen in both control and treated groups, and Benzalkonium Chloride-treated animals had superficial mucosal disruption, primarily noted in the distal colon. Treatment with Benzalkonium Chloride also resulted in epithelial disruption and a hyporeflective granular appearance of the mucosa and submucosa. Histological investigations revealed that Benzalkonium Chloride treatment caused crypt disruption and inflammatory changes. Inflammation and necrosis were statistically-significantly more prominent in the Benzalkonium Chloride-treated group compared to controls.

CLINICAL STUDIES

Retrospective and Multicenter Studies

A meta-analysis was performed evaluating 7 controlled clinical trials to compare the incidence of punctate keratitis among patients assigned to ophthalmic treatment of latanoprost or timolol.[108] In all studies, treatment solutions contained Benzalkonium Chloride; however, the amount of Benzalkonium Chloride in the latanoprost solution was twice that of the timolol solution. A total daily dose of up to 14.2 µg of Benzalkonium Chloride in latanoprost-treated patients was not associated with an increased incidence of punctate keratitis compared with the daily dose of 6.2 µg of Benzalkonium Chloride in timolol-treated patients.

Effect of Benzalkonium Chloride on Mucociliary Clearance

A double-blind, placebo-controlled, randomized, single-center trial with a 3-wk washout period was performed in order to determine the effect of a saline nasal spray containing 0.01% Benzalkonium Chloride on nasal mucociliary clearance rate.[98] Healthy subjects (n = 43) were instructed to use a nasal spray for 2 separate periods of 3x/d for 3 wk, with a 3-wk washout period in between periods 1 and 2. Subjects were randomized and given either saline or the nasal spray containing Benzalkonium Chloride during period 1. After the 3-wk washout period, subjects were given the spray that they did not use during period 1. Evaluations were performed at baseline and the end of each period by γ-scintigraphy with technetium^{99m}-labeled strontium. No differences were observed between baseline and post-treatment nasal mucociliary rate values after treatment with saline; however, the nasal mucociliary rate was determined to be significantly impaired following treatment with spray containing Benzalkonium Chloride ($p < 0.01$ compared to baseline and after saline period).

Ocular Irritation/Toxicity in Patients

A retrospective study was performed using glaucoma patients treated with 0.5% timolol preserved with or without 0.01% Benzalkonium Chloride (n = 15 - 17).[13] *Analyses revealed a significant increase in inflammatory markers (human leukocyte antigen and intercellular adhesion molecule-1) and decrease in goblet cell density in the groups treated with timolol and Benzalkonium Chloride versus timolol alone. Twelve out of 19 eyes that underwent routine cataract surgery developed permanent corneal decompensation post-operatively. Investigation revealed that the cause was inadvertent intracameral use of a balanced salt solution preserved with 0.013% Benzalkonium Chloride.*

Benzalkonium Chloride used as a preservative in topical ophthalmic medications has been reported to result in a higher rate of ocular surface disease.[109,110] Increased dosing of treatments containing Benzalkonium Chloride correlates with increased ocular surface disease prevalence and severity. Symptoms of Benzalkonium Chloride-induced ocular toxicity in these patients include pain, discomfort, dryness, inflammation, tearing, increased staining of conjunctival and corneal epithelial surfaces, increased tear break-up time, and lower Schirmer scores.

The effect on Benzalkonium Chloride-preserved timolol versus preservative-free timolol on inflammation in the anterior chamber of the eye was evaluated in a randomized clinical trial (measured by flare values).[111] Patients with ocular hypertension (n = 26) received Benzalkonium Chloride-preserved timolol in one eye and unpreserved timolol in the other eye (applications 2x/d for 1 mo). Flare values were obtained at baseline and following treatment. Treatment with eyedrops

containing Benzalkonium Chloride as a preservative resulted in statistically significantly higher flare values than eyes treated with unpreserved drops ($p = 0.0013$).

Case Reports

Many case reports were found in the literature reporting adverse dermal effects, bronchoconstriction, and allergic disease.[13] *These effects were observed in patients following use of products (disinfectants, detergents, nebulized solutions, eyedrops, plaster casts, intraarticular medications, perfume, antifungal solutions, creams, and shampoos) containing Benzalkonium Chloride. Patch testing with Benzalkonium Chloride in many of these cases, but not all, yielded positive results.*

Case reports on the ingestion of Benzalkonium Chloride in humans is reported to result in caustic burns in the digestive tract, hypersalivation, vomiting, hematemesis, diarrhea, confusion, hypotension, shock, respiratory paralysis/failure, cough, irritability, fever, dehydration, convulsions, coma, and cardiorespiratory arrest, and death.[8,112] In addition, topical exposure in humans has been reported to cause severe skin and eye irritation and corrosion. Many case reports have been found in the literature regarding adverse effects following the use of products containing Benzalkonium Chloride. These adverse effects include contact dermatitis, eczema, corrosive balanoposthitis, bronchospasms, granular parakeratosis, anaphylaxis, rhinorrhea, nasal obstruction.[113-130]

Occupational Exposure

Adverse effects such as dermatitis or asthma were observed in nurses, a cleaner, a varnish maker, and an ophthalmology department worker, all of whom were exposed to substances containing Benzalkonium Chloride in the workplace.[13] *Patch testing (using Benzalkonium Chloride at 0.05 – 1%)/inhalation sensitization assays (using disinfectants containing 10 or 40% Benzalkonium Chloride) resulted in positive results in the majority, but not all of these individuals. In a study evaluating occupational irritant and allergic contact dermatitis in healthcare workers, 7/360 patients showed positive patch test results to Benzalkonium Chloride.*

Occupational exposure to Benzalkonium Chloride (typically in the healthcare, cleaning, and manufacturing industries) has been associated with irritant effects, predominantly dermatitis.[131,132] Other effects such as asthma, as well as stomach, respiratory, and ocular irritation have also been reported in the occupational setting.[133,134]

Patch Testing in Patients

Multiple patch testing studies performed in patients with various diseases (conjunctivitis, otitis, allergic disorders, contact dermatitis, leprosy, leg ulcers) demonstrate that Benzalkonium Chloride is a contact sensitizer with concentration-dependent allergic potential.[4,13] *In studies using 0.1% Benzalkonium Chloride, positive reaction rates ranged from 0.9% (1/110 patients w) to 5.5% (126/2295 patients with allergic contact dermatitis). A North American Contact Dermatitis Group (NACDG) assay reported 4.3% positive reactions in 4892 patients. Lower concentrations also showed sensitization potential (e.g., 6% of conjunctivitis patients reacted to 0.07% and some individuals reacted to concentrations as low as 0.005% Benzalkonium Chloride). Higher concentrations of Benzalkonium Chloride (0.5 – 10%) resulted in reactions such as primary irritant dermatitis (observed in 12 – 13 patients (total number of patients not stated), erythema (in 47% of leprosy patients at 2.5%), and pustular/bullous reactions (in 47% of patients at 0.5 – 2%). Patients with pre-existing allergic conditions demonstrated higher reaction rates (27 – 40%) when tested with 1% aqueous Benzalkonium Chloride.*

Many clinical assays (n = 10 – 42,898 subjects) were found in the literature regarding patch tests performed using 0.1% Benzalkonium Chloride (patch tests predominantly performed in patients with confirmed or suspected dermatitis; Table 12).[8,100,132,135-150] Positive patch test results were seen in 0.5 – 32.2% of these populations. It should be noted that according to some sources, although positive reactions are noted in many studies, these supposed allergic reactions are likely misinterpreted irritant responses.[151,152]

Benzalkonium Chloride Cross-Reaction in Patients

In one study, 6 out of 8 patients with allergy to Benzalkonium Chloride (positive patch test to 0.1 and/or 0.15% Benzalkonium Chloride) had a cross reaction with benzethonium chloride (positive patch test reaction to 0.15 and/or 0.5% benzethonium chloride).[153] Data from a patch testing center indicate that of 17 Benzalkonium Chloride patch test-reactive patients, 11.8% were positive to quaternium-15 (concentrations of test substances not stated).[154]

Benzalkonium Chloride Administration to Asthma Patients

Airway response to Benzalkonium Chloride and histamine was evaluated in 12 asthmatic patients.[13] *Airway caliber was measured before and during the inhalation challenge as the forced expiratory volume in 1 second (FEV_1). Benzalkonium Chloride (dissolved in 0.9% sodium chloride to produce a range of doubling concentrations of 0.4 to 50 mg/ml) and histamine caused concentration-related decreases in FEV_1 in all subjects, with Benzalkonium Chloride being 7.4 times less potent as a bronchoconstrictor agonist than histamine.*

The effects of inhaled aqueous solutions containing Benzalkonium Chloride (600 µg nebulized Benzalkonium Chloride; inhalations repeated every 20 min until FEV_1 decreased by 15% or more, or 3 doses were administered) were evaluated in 30 subjects with bronchial asthma (10 healthy patients used as control).[155] FEV_1 was measured at baseline and 15 min after each dose. FEV_1 reductions following inhalation of the test substance was significantly higher in asthmatics than normal subjects

($p < 0.05$; mean percent fall in FEV_1 in asthmatics after the first, second, and third inhalation was 2.69, 5.36, and 5.30%, respectively). Benzalkonium Chloride induced bronchoconstriction in 6 asthmatics. No significant changes were observed in FEV_1 values in control groups at baseline and after treatment. Conversely, no evidence of bronchoconstriction was observed in a meta-analysis in which 631 asthma and 1538 chronic obstructive pulmonary disease patients using a soft-mist inhaler containing approximately 0.44 µg Benzalkonium Chloride per actuation.[156]

RISK ASSESSMENT

Margin of exposure (MOE) is a quantitative ratio calculated for cosmetic ingredients by dividing the point of departure (PoD) for an ingredient in an animal experiment by the estimated systemic exposure dose (SED) for the ingredient in humans, generally according to US Environmental Protection Agency (EPA) and European Commission (EC) Scientific Committee on Consumer Safety (SCCS) guidelines. An MOE value greater than 100 has traditionally been considered an indication of safety. The basis for this MOE value of 100 comes from two multiplication factors: a 10-fold factor for extrapolating data from test animals to human beings (interspecies extrapolation), and an additional 10-fold for differences among the human population (intraspecies extrapolation). Notably, the MOE value is sometimes referred to as the margin of safety (MOS) despite the parameters being definitionally different.

A risk assessment was found in the literature evaluating Benzalkonium Chloride in certain cosmetic products (hair conditioners, shampoos, and lotion (on various body sites)).[43] Systemic exposure doses (SEDs) were estimated to be approximately 0.0081 – 0.032 mg/kg bw/d (for hair conditioners used at up to 2%) and 0.044 – 0.176 (for shampoos used at up to 2%) based on the following parameters: a maximum daily usage amount of up to 528.4 g/d, an average use frequency of approximately 1.1 applications/d, 10% dermal absorption, 60 kg average adult body weight, and a retention factor of 0.01 (to account for wash-off/dilution effects on wet skin or hair). The NOAEL chosen for risk characterization was 20 mg/kg/d (derived from a 90-d unpublished dermal rat study summarized in the Repeated Dose Toxicity Studies section of this report). MOE values were between 621 – 2483 for hair conditioners and 114 – 454 for shampoos. The MOE calculation performed for body lotion and face cream were calculated by using a maximum concentration of 0.1%, a NOAEL of 20 mg/kg/d (based on the same study as above), a 60 kg average adult body weight, dermal absorption of 10%, and SED values ranging from 0.016 – 0.128 (dependent on location of application). The MOE remained above 100 across all body sites (MOE values reported for hands, arms, feet, legs, neck/throat, back, and other parts were determined to be 156, 217, 348, 298, 769, 1271, and 826, respectively. The MOS for face cream was determined to be 323.

An MOE of 372 was calculated by the EC Scientific Committee on Cosmetology (SCC) based on daily bioavailability when used in preservatives, intimate hygiene, skin, and hair products.[33] This calculation was based on a daily bioavailability of 0.08, maximum concentrations of 0.1 - 3% (maximum concentrations vary by product), average adult body weight of 60 kg, cutaneous penetration of 15% (derived from a rat study using radiolabeled Benzalkonium Chloride) and an NOAEL of 30 mg/kg/d (based on absence of systemic toxicity in several oral toxicity assays performed using Benzalkonium Chloride).

In a different risk assessment, an MOE calculation for Benzalkonium Chloride as a cosmetic ingredient was calculated to be 260.2.[157] This calculation was based on 100% dermal absorption, a maximum concentration of 0.1%, an estimated daily exposure of 269 mg/kg bw/d, an SED of 0.269, and an NOAEL of 70 mg/kg bw/d (based on a subchronic oral toxicity assay; no other details provided).

CIR staff performed MOE calculations for Benzalkonium Chloride based on the maximum concentration of use reported for disposable wipes (leave-on) at 0.35%, according to the 2025 concentration of use survey conducted by the Council (Table 3). Although disposable wipes may be used for a variety of purposes and do not necessarily contact mucous membranes, the National Institute for Public Health and the Environment (RIVM) intimate hygiene wipe exposure scenario was selected as a conservative estimate for wipe use that may involve the genital area and potential mucous membrane contact.[158] In this scenario, the default use frequency is 463 uses/yr, and the amount of product available for exposure is 0.75 g/use, resulting in an estimated daily product amount of 0.95 g/d.

Using this estimated daily product amount, a maximum concentration of use of 0.35%, and a default adult body weight of 60 kg, the SED was calculated to be 0.028 mg/kg bw/d, assuming 50% absorption in the absence of absorption data relevant to mucous membrane contact. When a dermal NOAEL of 20 mg/kg bw/d, derived from a 90-d topical study in rats treated with Benzalkonium Chloride (81% active ingredient; C12 - C16), was applied,[43] the resulting MOE was 714. For a more conservative estimate, an additional calculation assuming 100% absorption was performed. Under this assumption, the estimated SED was 0.056 mg/kg/day, resulting in an MOE of 357. Both MOE values were greater than 100 and were considered protective.

SUMMARY

The quaternary ammonium compounds evaluated in this report are reported to function in cosmetics as antistatic agents and cosmetic biocides. Stearalkonium Chloride was first reviewed by the Panel in a safety assessment published in 2003, with the conclusion that Stearalkonium Chloride is safe when incorporated in cosmetic products similar to those presently marketed. The Panel re-affirmed this conclusion in a re-review as published in 2003. In March 2023, this ingredient was again re-reviewed, and the report was re-opened for the addition of structurally similar ingredients. One of these ingredients,

Benzalkonium Chloride, was also previously reviewed by the Panel in a safety assessment that was published in 1989 with the conclusion that Benzalkonium Chloride, at concentrations up to 0.1% free, active ingredient, is safe as a cosmetic ingredient. This conclusion was re-affirmed in re-review as published in 2008.

According to RLD obtained from the FDA in 2025, Stearalkonium Chloride has the highest number of uses (it is used in 1136 total formulations). This ingredient is also reported to have the highest concentration of use in rinse-off and leave-on products. It is used at up to 2.6% in rinse-off hair conditioners and up to 2% in leave-on, non-coloring "other hair preparations".

Benzalkonium Chloride homologues are metabolized by microsomal enzymes via NADPH-dependent ω- and (ω–1) hydroxylation, producing various oxidized metabolites. Metabolic stability increases with alkyl chain length, with shorter chains (e.g., C10) metabolizing more rapidly than longer ones (e.g., C16). Benzalkonium Chloride (0.123%) extensively penetrated the epidermis and dermis when citric acid was used as the vehicle. An average of 15% of the applied dose was absorbed in an assay in which rats were dermally treated with radiolabeled Benzalkonium Chloride for 72 h under occlusive conditions. Dermal delivery was determined to be 2.16% in an assay in which Benzalkonium Chloride (80.5% active ingredient) was applied to human skin samples at 0.3%. A study performed in 32 subjects found systemic exposure from hand soap to be very low. Benzalkonium Chloride (0.5 – 5%) enhanced transdermal flux of lorazepam, though not in a dose-dependent manner.

After a single dermal application of radiolabeled 0.1% Benzalkonium Chloride (49.9% active ingredient) in rats, detectable radioactivity was limited to the intestines and stripped skin. Oral administration in rats (via diet or gavage) with Benzalkonium Chloride (30% active ingredient) showed limited but rapid absorption. Following a single oral dose of 200 mg/kg bw (Benzalkonium Chloride (49.9% active ingredient) in rats, the highest radioactivity was found in the intestines, with trace levels in other tissues. In mice, dietary exposure to Lauralkonium Chloride (120 µg/g/d) resulted in maximal blood and liver levels of 1000 and 550 nM, respectively. Following intratracheal administration of ^{14}C-Benzalkonium Chloride to rats, the lungs were the primary site of deposition, with measurable retention through 168 h post-dosing. In a similar study, prolonged retention of radioactivity was observed in the pancreas and heart of rats following ^{14}C-labeled Benzalkonium Chloride administration.

Acute dermal LD_{50} values for Benzalkonium Chloride were reported as 3.56 ml/kg bw in rabbits and 930 mg/kg bw (species not specified). The lowest oral LD_{50} was 234 mg/kg in rats given 10% Benzalkonium Chloride. A concentration-dependent reduction in tidal volume was observed in mice exposed (head-only) for 30 min to Benzalkonium Chloride at concentrations of 0.049 - 19 mg/m^3. An LC_{50} of 52.84 mg/m^3 was determined in rats exposed to aerosolized Benzalkonium Chloride for 4 h.

In repeated-dose toxicity studies, a dermal NOAEL of 20 mg/kg/bw was determined in a 90-d topical study using rats and an oral NOAEL of 5000 mg/kg (sanitizing agent) was determined in a 91-d gavage study in rats, corresponding to 0.5 mg/kg/d Benzalkonium Chloride. In a 95 – 96-d dietary study in rats, a NOEL of 500 ppm was established due to adverse effects at higher doses. No adverse toxicological effects were observed in Beagle dogs treated with up to 3000 ppm Benzalkonium Chloride for 13 wk. Inhalation studies showed increased lung weight, IgE, IL-6, liver mass, and lung histopathology at 30 – 35 mg/m^3 Benzalkonium Chloride. A NOAEC of < 0.08 mg/m^3 was identified in a 14-d inhalation study in rats exposed to up to 20 mg/m^3 Benzalkonium Chloride.

Benzalkonium Bromide (0.02 mmol/l) and Benzalkonium Chloride (6.81 mM) reduced human sperm viability and motility in vitro. In rats, prenatal exposure (up to 100 mg/kg/d) showed no significant maternal or developmental effects; however, isolated fetal abnormalities were observed. Two 2-generation studies in rats established reproductive NOAELs of 2000 ppm. In rabbits, no treatment-related effects were seen at doses up to 9 mg/kg/d, with isolated fetal variations across all groups. In a prenatal developmental toxicity study, a developmental NOAEL of 30 mg/kg/d was identified in rabbits.

In genotoxicity assays, Benzalkonium Chloride and Stearalkonium Chloride were negative in multiple Ames assays, with Benzalkonium Chloride tested at up to 110 µg/plate and Stearalkonium Chloride at up to 5000 µg/plate. Benzalkonium Chloride was also negative in mammalian cell gene mutation assays up to 100 µg/ml. For chromosomal damage, Benzalkonium Chloride was generally negative in chromosomal aberration assays (up to 98 µg/mL in CHO cells, 24 µg/mL in human lymphocytes, and 0.0003% in dental pulp cells), but a statistically significant increase in micronucleus formation was observed in human lymphocytes at 1 mg/L Benzalkonium Chloride. Benzalkonium Chloride showed positive results in comet assays at 0.0001 – 0.001% and in single-cell gel electrophoresis assays at 0.002 – 0.02% and 1 mg/ml, but was non-mutagenic up to 2 mM in another assay. In vivo rat studies with doses up to 20 ml/kg (using Benzalkonium Chloride at 0.01%) and 400 m/kg. Negative results were obtained in in vivo assays performed using rats given either 0.01% Benzalkonium Chloride (up to 20 ml/kg) or 400 mg/kg Benzalkonium Chloride.

Topical application of Benzalkonium Chloride caused dose-dependent ocular toxicity in animals. In mice, 0.01 – 0.2% applied for 7 d led to nerve damage, inflammation, epithelial defects, and goblet cell loss. In rats, 0.001% Benzalkonium Chloride caused permanent nerve impairment. In rabbits, ≥ 0.01% (up to 0.03%) Benzalkonium Chloride caused corneal and epithelial damage, with effects worsening at higher doses and frequency. However, no ocular toxicity was observed in monkeys or rabbits treated with 0.005 – 0.01% up to 8 x/d for 3 – 52 wk.

Ocularly-applied Benzalkonium Chloride is reported to accumulate in the cornea, lens, conjunctiva, iris, ciliary muscle epithelium, and trabecular meshwork. Because of this, exacerbated ocular toxicity may be apparent in patients using eyedrops containing Benzalkonium Chloride repetitively.

The permeability enhancement ability of Benzalkonium Chloride in ocular pharmaceutical formulations may increase toxicity. For example, in a rabbit model, topical latanoprost preserved with 0.02% benzalkonium chloride significantly increased corneal epithelial permeability and caused loss of tight-junction integrity.

Benzalkonium Chloride (0.05%) disrupted stratum corneum organization and lipid packing in human skin but did not significantly increase IFNα permeation. Additionally, Benzalkonium Chloride has been shown to cause corneal epithelial disruption at concentrations as low as 0.005%.

Topical Benzalkonium Chloride (0.05% and 0.1%, 2x/d for 7 d) in rabbits disrupted Cx43 and ZO-1 distribution, reduced Cx43 expression, increased its phosphorylation, and weakened Cx43–ZO-1 interaction. These changes were linked to reduced gap junction intercellular communication. In a similar study, discontinuous and fragmented ZO-1 expression was observed in the corneas of rabbits treated with 0.02% Benzalkonium Chloride (administration every 5 min for 20 min).

Numerous cytotoxicity assays report that Benzalkonium Chloride induces toxicity across a wide range of human and animal cell types (e.g., human corneal stromal fibroblasts, human respiratory epithelial cells). Cytotoxic effects were observed at concentrations from as low as 0.00000001%.

Benzalkonium Chloride induced significant extracellular ROS formation in human alveolar epithelial cells at concentrations ≥ 20 µg/ml, with only slight, non-significant increases at lower doses. Similarly, a 1 µM concentration of Benzalkonium Chloride caused a 9-fold increase in ROS generation in rat neural progenitor cells compared to controls.

Benzalkonium Chloride (0.25 – 2 mg/ml) induced dose-dependent increases in IL-1α and IL-8 release in reconstructed human epidermis after 20 h. Similarly, exposure to Benzalkonium Chloride (5 – 10 µM) in human skin biopsies and 0.1% in conjunctival and corneal epithelial cells triggered elevated levels of various inflammatory cytokines, including Il-1, tumor necrosis factor, and IL-8.

A published literature review of 18 studies on intranasal products with Benzalkonium Chloride (0 – 0.1%) found mixed results regarding nasal epithelial damage and rhinitis medicamentosa exacerbation. While 10 studies reported adverse effects, only 2 showed statistically significant differences, both involving oxymetazoline use. Eight studies, including long-term ones, found no toxic effects.

Benzalkonium Chloride (2.5 – 10 µg/ml) completely inhibited neurite outgrowth and reduced cell number in human derived astrocyte cultures. Cultures were exposed for 24 h.

Benzalkonium Chloride (0.5 – 1.5 mg/ml) increased estradiol production and upregulated steroidogenic gene transcription in human adrenal cells, and significantly enhanced proliferation and estrogen receptor protein expression in ER-positive MCF-7 breast cancer cells. No estrogenic effects were observed in a luciferase reporter assay using recombinant breast cancer cells at concentrations up to 10 µM. Benzalkonium Chloride homologs inhibited human and rat 11β-HSD2 in an alkyl chain length–dependent manner, with C16 showing the greatest potency (IC_{50} = 4.22 µM in human and 24.74 µM in rat enzyme assays).

Oral exposure to Lauralkonium Chloride (120 µg/g/d for 1 wk) in mice caused significant reductions in gut microbiota diversity and lowered secondary bile acid levels. These effects were more pronounced in males compared to females.

Benzalkonium Chloride (0.2%) worsened atopic dermatitis in mice. Significant increases in clinical dermatitis scores, eosinophil infiltration, mast cell activation, inflammatory cytokines, and serum IgE levels were observed compared to controls.

Stearalkonium Chloride was non-irritating in reconstructed human epidermis assays when tested at final concentrations of 0.49, 3, and 100%, though moderate irritation was observed in rabbits with a 40% mixture. Benzalkonium Chloride (0.1 – 1%) caused irritation and sensitization in some animal and human assays, but was non-sensitizing in a modified Draize assay using guinea pigs. In a sensitization assay performed in 50 subjects, the test substance (20% Stearalkonium Chloride in water and stearyl alcohol) was considered to be non-sensitizing. The combined effects of UV radiation and Benzalkonium Chloride (0.001 – 0.004%) were tested in human corneal epithelial cells. Benzalkonium Chloride and UV treatment combined, regardless of exposure order, produced synergistic cytotoxic effects.

Undiluted Benzalkonium Chloride significantly reduced cell viability in an EpiOcular™ assay, while concentrations ≥ 0.01% caused ocular irritation in rabbits. In in vitro assays, Stearalkonium Chloride was moderately irritating at a final test concentration of 2% and irritating at final active concentrations of 3 and 85%. In animals, Stearalkonium Chloride was corrosive at 40%, and caused mild conjunctival hyperemia at 0.002%.

In nasal and mucosal irritation assays, Benzalkonium Chloride caused dose-dependent irritation and tissue damage across multiple species and sites. Specifically, 0.2% intranasal dosing in rats induced strong mucosal irritation and increased biomarkers, while vaginal application of 0.2 – 2% in mice and rabbits led to vascular injury, epithelial disruption, and inflammation. Additionally, 0.2% rectal treatment in sheep caused mucosal disruption, crypt damage, and inflammation.

A meta-analysis of 7 controlled clinical trials found no increased incidence of punctate keratitis in patients ocularly treated with latanoprost, which contains twice the amount of Benzalkonium Chloride compared to the timolol solution. Daily Benzalkonium Chloride doses of up to 14.2 µg in the latanoprost solution did not raise keratitis risk compared to the 6.2 µg in timolol solution-treated patients.

A placebo-controlled trial was performed in 43 healthy subjects testing a nasal spray containing 0.01% Benzalkonium Chloride over 2 3-wk periods separated by a washout period (saline spray used as control). Results showed that while saline had no effects, Benzalkonium Chloride spray significantly impaired nasal mucociliary clearance ($p < 0.01$).

Benzalkonium Chloride used as a preservative in topical eye medications is linked to a higher incidence and severity of ocular surface disease, causing symptoms like pain, dryness, inflammation, and tear film disruption. In a clinical trial with ocular hypertension patients, eyes treated with Benzalkonium Chloride-preserved timolol showed statistically significantly increased anterior chamber inflammation compared to those treated with preservative-free timolol.

Ingestion of Benzalkonium Chloride in humans can cause severe digestive burns, respiratory failure, shock, and even death. Topical exposure has been linked to severe skin and eye irritation, as well as various allergic and inflammatory reactions.

Occupational exposure to Benzalkonium Chloride (typically in the healthcare, cleaning, and manufacturing industries) has been associated with irritant effects, predominantly dermatitis. Other effects such as asthma, as well as stomach, respiratory, and ocular irritation have also been reported in the occupational setting.

Clinical assays using 0.1% Benzalkonium Chloride showed positive reactions in 0.5 – 32.2% of patients with suspected dermatitis. However, some sources suggest many of these positive results may actually be irritant responses rather than true allergy.

In one study, 6/8 allergic to Benzalkonium Chloride also showed cross-reactivity with benzethonium chloride. Additionally, data from a patch testing center found that 11.8% of 17 Benzalkonium Chloride-reactive patients tested positive for quaternium 15.

In a study of 30 asthmatic subjects, inhalation of nebulized Benzalkonium Chloride (600 µg doses) caused significant reductions in FEV_1 and bronchoconstriction in 6 patients, while no changes were seen in healthy controls. However, a meta-analysis of over 2000 patients using a soft-mist inhaler containing Benzalkonium Chloride (approximately 0.44 µg per actuation) found no bronchoconstriction.

A risk assessment of Benzalkonium Chloride in cosmetics estimated systemic exposure doses (SEDs) ranging from 0.0081 to 0.176 mg/kg bw/day for products like hair conditioners (up to 2%) and shampoos (up to 2%), with MOE values well above 100. Additional assessments using different exposure and NOAEL assumptions yielded MOE values between 260 and 372, supporting the safety of Benzalkonium Chloride at typical cosmetic use concentrations (up to 0.1 – 3%). CIR staff performed an MOE calculation for Benzalkonium Chloride (as used in disposable wipes at 0.35%). When assuming 100% absorption, the resulting MOE was 357.

DRAFT DISCUSSION

[Note: This Discussion is in the draft form, and changes will be made following the Panel meeting.]

In accordance with its Procedures, the Panel re-evaluates the conclusions of previously issued reports approximately every 15 years. In 1982, the Panel published a final report on Stearalkonium Chloride, concluding that this ingredient is safe when incorporated in cosmetic products at concentrations similar to those presently marketed (as indicated in that report). A re-review on this ingredient was published in 2003 re-affirming the conclusion from the original report. In 2023, another re-review was considered, and this report was reopened for the addition of structurally similar ingredients (i.e., Behenalkonium Chloride, Benzalkonium Bromide, Benzalkonium Chloride, Cetearalkonium Bromide, and Lauralkonium Chloride). It should be noted that Benzalkonium Chloride was also previously reviewed by the Panel in a safety assessment published in 1989 with the conclusion that Benzalkonium Chloride, at concentrations up to 0.1% free, active ingredient, is safe as a cosmetic ingredient as presently used (as indicated in that report). This conclusion was re-affirmed in a re-review published in 2008). The Panel reviewed the data on these alkonium chlorides and bromides and concluded [to be determined].

The Panel noted that these ingredients can enhance the penetration of other ingredients through the skin, eyes, and mucous membranes. The Panel cautioned that care should be taken in formulating cosmetic products that may contain these ingredients in combination with any ingredients whose safety was based on their lack of absorption data, or when absorption was a concern.

The Panel was concerned that the potential exists for dermal irritation with the use of products formulated using alkonium chlorides and bromides. The Panel specified that products containing these ingredients must be formulated to be non-irritating. The Panel further noted that the irritation potential of these ingredients may be influenced by the formulation vehicle. Accordingly, the Panel emphasized that formulators should exercise caution when developing products containing these ingredients, as irritation potential may vary depending on the composition and characteristics of the vehicle.

Data suggesting potential endocrine activity of Benzalkonium Chloride in in vitro studies were also reviewed; however, the levels required or reported for such activity are not relevant to cosmetic use. For further explanation of what qualifies as endocrine activity or disruption, please refer to the CIR resource document: https://www.cir-safety.org/supplementaldoc/cir-precedents-endocrine-activity.

The Panel expressed concern regarding heavy metals that may be present in these ingredients. They stressed that the cosmetics industry should continue to use the necessary procedures to minimize impurities in cosmetic formulations according to limits set by the US FDA and EPA.

The Panel noted that some tested formulations contained Benzalkonium Chloride at concentrations exceeding 0.1%. However, in systems containing proteins or other binding components, the effective concentration of free Benzalkonium Chloride may be lower due to binding interactions, necessitating higher total concentrations to achieve 0.1% free active substance.

The Panel discussed the issue of incidental inhalation exposure resulting from these ingredients (e.g., Benzalkonium Chloride is used in body and hand spray at up to 0.1% and in face powders and foot powders and sprays (concentration not reported)). Inhalation toxicity was observed in available data. However, the Panel noted that the majority of droplets/ particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the low concentrations at which these ingredients are used (or expected to be used) in potentially inhaled products, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

The Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern. Although it is known that these ingredients are used in products applied via airbrush technology, no data are available for consumer habits and practices thereof, product particle size, or other relevant particle data (e.g., diameter). As a result of deficiencies in these critical data needs, the data profile is incomplete, and the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Accordingly, the Panel has concluded the if these ingredients are used in airbrush formulations, the data are insufficient to support the safe use when applied with such delivery system.

CONCLUSION

To be determined.

TABLES

Table 1. Definitions, idealized structures, and reported functions[1,CIR Staff]

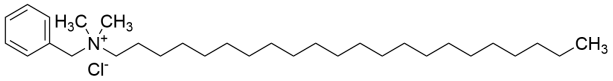
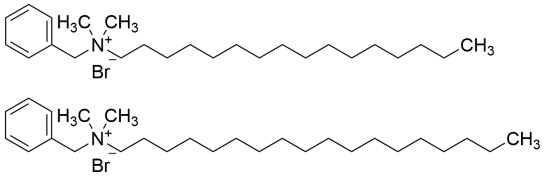
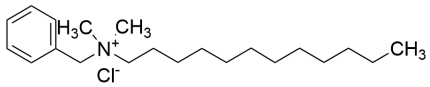
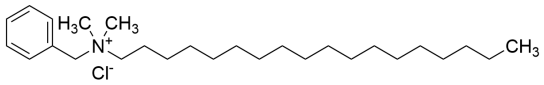
Ingredient/CAS No.	Definition	Function(s)
Behenalkonium Chloride 16841-14-8	Behenalkonium Chloride is the quaternary ammonium salt that conforms to the structure: 	Antistatic Agents
Benzalkonium Bromide 91080-29-4	Benzalkonium Bromide is the mixture of alkylbenzyldimethylammonium bromides that conforms generally to the structure in Figure 1, wherein R represents a mixture of even numbered alkyl chains, including all or some of the group beginning with capryl and extending through higher homologs, with lauryl, myristyl, and cetyl predominating; and X is bromide.	Antistatic Agents Cosmetic Biocides Deodorant Agents
Benzalkonium Chloride 61789-71-7 68391-01-5 68424-85-1 8001-54-5 85409-22-9	Benzalkonium Chloride is a mixture of alkylbenzyldimethylammonium chlorides that conforms generally to the structure in Figure 1, wherein R represents a mixture of even numbered alkyl chains, including all or some of the group beginning with capryl and extending through higher homologs, with lauryl, myristyl, and cetyl predominating; and X is chloride.	Antimicrobial Agents Antistatic Agents Cosmetic Biocides Deodorant Agents Pesticides
Cetearalkonium Bromide	Cetearalkonium Bromide is the quaternary ammonium salt that conforms generally to the mixture of structures derived from cetyl and stearyl alcohol: 	Antistatic Agents Cosmetic Biocides
Lauralkonium Chloride 139-07-1	Lauralkonium Chloride is a quaternary ammonium salt that conforms to the structure: 	Antistatic Agents Cosmetic Biocides
Stearalkonium Chloride 122-19-0	Stearalkonium Chloride is the quaternary ammonium salt that conforms generally to the structure: 	Antistatic Agents

Table 2. Chemical properties

Property	Value	Reference
Behenalkonium Chloride		
Formula Weight (g/mol)	480.2	159
Vapor pressure (mmHg @ 25ºC)	0 (estimated; modified grain method)	160
Melting Point (ºC)	284 (estimated)	160
Boiling Point (ºC)	654 (estimated)	160
Water Solubility (mg/l @ 25ºC)	0.0002 (estimated; WSKOW v1.42)	160
log K_{ow} (@ 25ºC)	7.74 (estimated; WSKOW v1.42)	160
Benzalkonium Bromide		
Formula Weight (g/mol; median (range))	440.0 (355.4 – 524.7)	161
Benzalkonium Chloride		
Physical Form	amorphous powder or gelatinous pieces	4
Color	white or yellowish-white	4
Odor	aromatic	4
Formula Weight (g/mol; average (range))	360 (310.9 – 480.2)	4
Water Solubility	very soluble in water	4
Other Solubility (g/l)	very soluble in acetone and alcohol; slightly soluble in benzene; almost insoluble in ether	4
UV Absorption (λ) (nm)	262	4
Cetearalkonium Bromide		
Formula Weight (g/mol; range)	440.6 – 468.6	161
Lauralkonium Chloride		
Physical Form	solid	11
Color	white	11
Formula Weight (g/mol)	340	162
Density (g/ml @ 20ºC)	1.03	11
Vapor pressure (mmHg @ 20ºC)	0	11
Melting Point (ºC)	45.2	11
Boiling Point (ºC)	162.7	11
Water Solubility (g/l)	65 - 175	11
log K_{ow} (@ 24ºC)	1.1	11
Stearalkonium Chloride		
Physical Form	solid	12
Color	white	12
Formula Weight (g/mol)	424.2	161
Odor	sweet	12
Specific Gravity (@ 20ºC)	1.02	12
Melting Point (ºC)	63.4	12
Water Solubility (mg/l @ 20ºC)	20.8	12
log K_{ow} (@ 20ºC)	3.89 (estimated)	12

Table 3. Frequency and concentration of use according to likely duration and exposure and by product category[19-21]

	# of Uses	Max Conc of Use	# of Uses	Max Conc of Use	# of Uses	Max Conc of Use
	RLD (2025)	% (2025)	RLD (2025)	% (2025)	RLD (2025)	% (2025)
	Behenalkonium Chloride		Benzalkonium Bromide		Benzalkonium Chloride	
Totals*	**59**	**0.011 – 1.9**	**6**	**NR**	**923**	**0.005 – 0.35**
summarized by likely duration and exposure**						
Duration of Use						
Leave-On	*6*	*0.48*	*3*	*NR*	*717*	*0.005 – 0.35*
Rinse-Off	*54*	*0.011 – 1.9*	*2*	*NR*	*302*	*0.015 – 0.13*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*3*	*NR*
Unknown	*1*	*NR*	*NR*	*NR*	*109*	*NR*
Exposure Type						
Baby Products	NR	NR	NR	NR	42	0.053
Children's Makeup	NR	NR	NR	NR	NR	NR
Eye Area	NR	0.011	NR	NR	92	0.015
Incidental Ingestion	NR	NR	NR	NR	NR	0.1
Mucous Membrane	NR	NR	NR	NR	394	0.1 – 0.35
Incidental Inhalation-Spray	43[a]; 3[b]	0.48	4[a]	NR	23; 91[a]; 191[b]	0.009 – 0.1; 0.11[a]; 0.005 – 0.13[b]
Incidental Inhalation-Airbrush	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Powder	3[b]	NR	NR	NR	1; 191[b]; 2[c]	0.005 – 0.13[b]; 0.53[c]
Dermal Contact	1	0.011	7	NR	750	0.005 – 0.35
Deodorant (underarm)	NR	NR	1	NR	4	NR
Hair - Non-Coloring	44	0.48 – 1.9	NR	NR	252	0.009
Hair-Coloring	15	NR	NR	NR	5	NR
Nail	NR	NR	4	NR	8	NR
Other Preparations (Unknown Exposure Type)	1	NR	NR	NR	109	NR
as reported by product category						
Baby Products						
Baby Lotions/Oils/Powders/Creams					2	0.053
Baby Wipes					39	NR
Other Baby Products					1 (r.o.)	NR
Bath Preparations (diluted for use)						
Bubble Baths					2	NR
Other Bath Preparations					1	NR
Eye Makeup Preparations (not children's)						
Eyebrow Pencil					4	NR
Eye Lotion					1	NR
Eye Makeup Remover	NR	0.011			38	0.015
Mascara					2	NR
Eyelash and Eyebrow Adhesives/Glues/Sealants					18	NR
Eyelash and Eyebrow Preparations (primers, conditioners, serums, fortifiers)					17	NR
Other Eye Makeup Preparations					12	NR
Fragrance Preparations						
Perfumes					10	NR
Other Fragrance Preparation					2	NR
Hair Preparations (non-coloring)						
Hair Conditioners	2 (l.o.); 9 (r.o.)	0.48 (l.o.); 1.9 (r.o.)			28 (l.o.); 42 (r.o.)	NR
Hair Sprays (aerosol fixatives)	NR	0.48 (pump spray)			11	0.009 (pump spray)

Table 3. Frequency and concentration of use according to likely duration and exposure and by product category[19-21]

	# of Uses RLD (2025)	Max Conc of Use % (2025)	# of Uses RLD (2025)	Max Conc of Use % (2025)	# of Uses RLD (2025)	Max Conc of Use % (2025)
Hair Straighteners					8	NR
Permanent Waves					4	NR
Rinses (non-coloring)	1	NR			4	NR
Shampoos (non-coloring)					4 (l.o.); 39 (r.o.)	NR
Tonics, Dressings, Other Hair Grooming Aids	2	NR			46	NR
Wave Sets						
Other Hair Preparations	1 (l.o.); 29 (r.o.)	NR			56 (l.o.); 10 (r.o.)	NR
Hair Coloring Preparations						
Hair Dyes and Colors (all types requiring caution statements and patch tests)	1	NR			1	NR
Hair Tints						
Hair Rinses (coloring)					1 (r.o.)	NR
Hair Shampoos (coloring)						
Hair Lighteners with Color						
Other Hair Coloring Preparation	14 (r.o.)	NR			1 (r.o.)	NR
Makeup Preparations (not eye or children's)						
Face Powders					1	NR
Foundations					13 (traditional application)	NR
Makeup Bases					NR	0.11 (traditional applications)
Other Makeup Preparations					8 (traditional applications)	NR
Manicuring Preparations						
Cuticle Softeners					1	NR
Nail Polish and Enamel			2	NR		
Other Manicuring Preparations			2	NR	7	NR
Oral Hygiene Products						
Dentifrices					NR	0.1
Personal Cleanliness						
Bath Soaps and Body Washes					46	0.13
Deodorants (underarm)			1	NR	4	0.025 – 0.11 (not spray)
Douches					1	NR
Feminine Deodorants					1 (l.o.); 4 (r.o.)	NR
Disposable Wipes					216	0.35
Other Personal Cleanliness Products					60 (l.o.); 24 (r.o.)	0.13 (r.o. hand wash)
Shaving Preparations						
Beard Softeners	1	NR				
Pre-shave Lotions (all types)					1	NR
Shaving Cream (aerosol, brushless, lather)					1	NR
Skin Care Preparations						
Cleansing			2	NR	60	0.015 – 0.11
Depilatories						
Face and Neck (excluding shaving preps)					19 (l.o.) 6 (r.o.)	0.005 – 0.054 (l.o.)
Body and Hand (excluding shaving preps)					23 (l.o.); 2 (r.o.)	0.04 (l.o.); 0.13 (r.o.); 0.1 (body and hand spray)
Foot Powders and Sprays					4	NR

Table 3. Frequency and concentration of use according to likely duration and exposure and by product category[19-21]

	# of Uses RLD (2025)	Max Conc of Use % (2025)	# of Uses RLD (2025)	Max Conc of Use % (2025)	# of Uses RLD (2025)	Max Conc of Use % (2025)
Moisturizing			2	NR	41	0.11 (not spray)
Night			1	NR		
Paste Masks (mud packs)					NR	0.097
Skin Fresheners			1	NR	21	0.11
Other Skin Care Preparations					42 (l.o.); 5 (r.o.)	NR
Suntan Preparations						
Indoor Tanning Preparations					5	NR
Other Preparations (i.e., those that do not fit another category)	1				109	
	Lauralkonium Chloride		**Stearalkonium Chloride**			
Totals*	**3**	**NR**	**1136**	**0.1 – 2.6**		
summarized by likely duration and exposure**						
Duration of Use						
Leave-On	*3*	*NR*	*236*	*0.1 – 2*		
Rinse-Off	*NR*	*NR*	*1003*	*1.3 – 2.6*		
Diluted for (Bath) Use	*NR*	*NR*	*3*	*NR*		
Unknown	*NR*	*NR*	*4*	*NR*		
Exposure Type						
Baby Products	2	NR	1	NR		
Children's Makeup	NR	NR	NR	NR		
Eye Area	NR	NR	NR	NR		
Incidental Ingestion	NR	NR	NR	NR		
Mucous Membrane	2	NR	8	NR		
Incidental Inhalation-Spray	1[s]	NR	4; 117[a]; 143[b]	0.1 – 2[b]		
Incidental Inhalation-Airbrush	NR	NR	NR	NR		
Incidental Inhalation-Powder	NR	NR	143[b]	0.1 – 2[b]		
Dermal Contact	3	NR	60	NR		
Deodorant (underarm)	NR	NR	NR	NR		
Hair - Non-Coloring	NR	NR	831	0.1 – 2.6		
Hair-Coloring	NR	NR	351	1.5 – 2.5		
Nail	NR	NR	NR	NR		
Other Preparations (Unknown Exposure Type)	NR	NR	4	NR		
as reported by product category						
Baby Products						
Baby Lotions/Oils/Powders/Creams						
Baby Wipes	2	NR				
Other Baby Products			1 (r.o.)	NR		
Bath Preparations (diluted for use)						
Bubble Baths			1	NR		
Other Bath Preparations			2	NR		
Eye Makeup Preparations (not children's)						
Eyebrow Pencil						
Eye Lotion						
Eye Makeup Remover						
Mascara						
Eyelash and Eyebrow Adhesives/Glues/Sealants						
Eyelash and Eyebrow Preparations (primers, conditioners, serums, fortifiers)						

Table 3. Frequency and concentration of use according to likely duration and exposure and by product category[19-21]

	# of Uses RLD (2025)	Max Conc of Use % (2025)	# of Uses RLD (2025)	Max Conc of Use % (2025)	# of Uses RLD (2025)	Max Conc of Use % (2025)
Other Eye Makeup Preparations						
Fragrance Preparations						
Perfumes						
Other Fragrance Preparation						
Hair Preparations (non-coloring)						
Hair Conditioners			66 (l.o.); 464 (r.o.)	1.1 – 1.8 (l.o.); 1.3 – 2.6 (r.o.)		
Hair Sprays (aerosol fixatives)			4	NR		
Hair Straighteners			8	NR		
Permanent Waves						
Rinses (non-coloring)			48	NR		
Shampoos (non-coloring)			1 (l.o.); 26 (r.o.)	NR		
Tonics, Dressings, Other Hair Grooming Aids			57	0.1 – 0.14		
Wave Sets			3	NR		
Other Hair Preparations			73 (l.o.); 81 (r.o.)	2 (l.o.)		
Hair Coloring Preparations						
Hair Dyes and Colors (all types requiring caution statements and patch tests)			187	1.5		
Hair Tints			93	1.5		
Hair Rinses (coloring)			2 (r.o.); 5 (r.o.)	2.5 (r.o.)		
Hair Shampoos (coloring)			3 (r.o.)	NR		
Hair Lighteners with Color			3	NR		
Other Hair Coloring Preparation			2 (l..o.); 6 (r.o.)	NR		
Makeup Preparations (not eye or children's)						
Face Powders						
Foundations						
Makeup Bases						
Other Makeup Preparations						
Manicuring Preparations						
Cuticle Softeners						
Nail Polish and Enamel						
Other Manicuring Preparations						
Oral Hygiene Products						
Dentifrices						
Personal Cleanliness						
Bath Soaps and Body Washes			4	NR		
Deodorants (underarm)						
Douches						
Feminine Deodorants						
Disposable Wipes						
Other Personal Cleanliness Products			1	NR		
Shaving Preparations						
Beard Softeners			1	NR		
Pre-shave Lotions (all types)						
Shaving Cream (aerosol, brushless, lather)			8	NR		
Skin Care Preparations						
Cleansing			9	NR		
Depilatories						

Table 3. Frequency and concentration of use according to likely duration and exposure and by product category[19-21]

	# of Uses RLD (2025)	Max Conc of Use % (2025)	# of Uses RLD (2025)	Max Conc of Use % (2025)	# of Uses RLD (2025)	Max Conc of Use % (2025)
Face and Neck (excluding shaving preps)			2 (l.o.)	NR		
Body and Hand (excluding shaving preps)			8 (l.o.); 2 (r.o.)	NR		
Foot Powders and Sprays						
Moisturizing	1	NR	19	NR		
Night						
Paste Masks (mud packs)						
Skin Fresheners						
Other Skin Care Preparations			1 (l.o.); 1 (r.o.)	NR		
Suntan Preparations						
Indoor Tanning Preparations						
Other Preparations (i.e., those that do not fit another category)			4			

NR – not reported
l.o. – leave-on; r.o. – rinse-off
*The sum of the counts given for duration of use and by exposure type, and the sum of the frequency reported by product category, may not equal the sum of total uses because each ingredient may be used in cosmetic formulations that are reported under more than one product category.
**Likely duration and exposure are derived from survey data based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)
[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.
[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
[c] It is possible these products are powders, but it is not specified whether the reported uses are powders.

Table 4. CFR citations

CFR Citation	Regulation Information	Ingredient(s)
21CFR172.165	These ingredients may be used safely as a food additive under the following conditions: 1) pH (5% active solution) 7.0 – 8.0; total amines, maximum 1% as combined free amines and amine hydrochlorides 2) used as an antimicrobial agent in raw sugar cane juice; added before clarification when further processing of the sugar cane juice must be delayed 3) additive is applied to sugarcane juice in the following quantities: 1.6 – 6.0 ppm Stearalkonium Chloride 0.25 – 1.0 ppm Lauralkonium Chloride	Lauralkonium Chloride Stearalkonium Chloride
21CFR173.320	May be used as an agent controlling microorganisms cane sugar and beet sugar mills according to the following conditions: 1) they are applied to the sugar mill grinding, crusher, and/or diffuser systems 2) used at 0.05 ± 0.005 ppm for Lauralkonium Chloride and 0.30 ± 0.030 for Stearalkonium Chloride 3) may adhere to proper labeling	Lauralkonium Chloride Stearalkonium Chloride
21CFR175.105	May be used in food-contact adhesives under strict use conditions.	Benzalkonium Chloride Lauralkonium Chloride Stearalkonium Chloride
21CFR176.300	May be used as a slimicide in processing paper of paperboard used for food-contact materials, controlling microbial slime.	Benzalkonium Chloride Lauralkonium Chloride Stearalkonium Chloride
21CFR178.1010	May be used in sanitizing solutions for food-contact surfaces, provided residuals are drained and concentrations meet prescribed limits.	Benzalkonium Chloride
21CFR310.201	May be used at 0.02% in specific OTC ophthalmic solutions containing 0.25% tyloxapol, under defined formulation and labeling conditions.	Benzalkonium Chloride
21CFR310.545	Recognizes Benzalkonium Chloride as an ingredient in certain OTC topical drug products (e.g., insect bite, dandruff products); inadequate data to establish the general recognition of the safety and effectiveness of Benzalkonium Chloride for the specified uses	Benzalkonium Chloride
40CFR180.940	Exempts Benzalkonium Chloride (C12 – C18) from tolerance requirements when used in food contact sanitizing solutions, up to 400 ppm	Benzalkonium Chloride

CFR = Code of Federal Regulations; OTC = over-the-counter

Table 5. Dermal absorption/penetration, penetration enhancement, and ADME studies

Test Article	Vehicle	Test Population	Concentration/Dose	Protocol	Results	Reference
DERMAL ABSORPTION/PENETRATION						
Benzalkonium Chloride (C_{12} – C_{14})	water, citric acid, caprylyl glycol, and vitamin E (evaluated separately)	excised human skin (n = 3 – 6/substance evaluated)	0.123%	MALDI-TOF mass spectrometry imaging; ex vivo application using Franz diffusion cells; test substance evaluated in different vehicles; 30 sec single application; penetration of Benzalkonium Chloride evaluated by using MALDI imaging heat maps of ions m/z 304 and m/z 332 (penetration depths determined for both ions); Benzalkonium Chloride in water was also evaluated as the positive control	Average ion m/z 304 and m/z 332 penetration into the dermis based on vehicle: Benzalkonium Chloride in water: 760 and 730 µm Benzalkonium Chloride in citric acid: 1450 and 1280 µm Benzalkonium Chloride in caprylyl glycol: 360 and 300 µm Benzalkonium Chloride in vitamin E: 450 and 400 µm Overall, the study showed that Benzalkonium Chloride readily penetrates through the epidermis and into the dermis, and penetration is affected by the vehicle.	32
radiolabeled and non-radiolabeled Benzalkonium Chloride (80.5% active ingredient; C_{12} – C_{16})	water	full-thickness human skin samples	0.03 and 0.3%	OECD TG 428; automated flow-through diffusion cell system; 24 h diffusion; receptor fluid: tissue culture medium containing bovine serum albumin, streptomycin, and penicillin G	At the low concentration level, the mean total unabsorbed dose was 96.8% of the applied dose. The bulk of the radioactivity (30.26%) was recovered in the outermost tape strips (strips 1 – 5). The absorbed dose was approximately 0.05%. Dermal delivery was determined to be 2.22% (sum of the absorbed dose and exposed skin). At the high concentration level, the mean total unabsorbed dose was 94.7% of the applied dose. The bulk of the radioactivity (10.86%) was recovered in the outermost tape strips (strips 1 – 5). The absorbed dose was approximately 0.03%. Dermal delivery was determined to be 2.16%.	11
radiolabeled Benzalkonium Chloride	NR	rats (6/sex; strain not stated)	0.4 ml; concentration not reported	test substance applied to shaved skin of rats under occlusive conditions for 72 h; amount of material excreted in feces and urine measured; amount remaining in carcass measured	In female animals, approximately 0.7, 6.1, and 7% were obtained for urine elimination, fecal elimination, and amount in carcass, respectively. In male animals, the corresponding values were 0.8, 9.9, and 5.2%, respectively. The bulk of the applied dose remained on the treated skin/ Approximately 14% of the applied dose was absorbed in females and approximately 16% was absorbed in males.	33
hand soap containing 0.13% Benzalkonium Chloride	none	32 subjects	100%	blood levels of Benzalkonium Chloride evaluated following use of soap 30x/d for 5 d (60 sec hand wash); blood plasma collected on 32 occasions over the study period; plasma samples valuated for the C_{12} and C_{14} homologues of Benzalkonium Chloride	Among the 32 subjects, the C_{12} homolog was detected above the lower limit of quantification in only 4/1024 plasma samples (at 117.8 – 191.7 ng/l) and the C_{14} homolog was detected in only 1/1024 samples (at 59.5 mg/l). The systemic exposure to Benzalkonium Chloride via hand soap was determined to be very low.	34
PENETRATION ENHANCEMENT						
Benzalkonium Chloride	NR	rat abdominal skin	0.5 – 5%	effect of Benzalkonium Chloride on the permeation of lorazepam (in water and propylene glycol) evaluated in rat skin; Franz diffusion cells used with diffusional area of 5.3 cm^2; receptor fluid collected at time intervals ranging from 0.25 to 24 h after application	The steady-state flux of lorazepam in the presence of Benzalkonium Chloride at concentrations of 0.5, 1, 2.5, and 5% was 0.49, 0.95, 0.62, and 0.89 µg/cm^2/h, respectively. Control samples were not treated with Benzalkonium Chloride and yielded a steady-state flux of 0.12 µg/cm^2/h. The highest enhancement ratio for Benzalkonium Chloride was determined to be 7.66 (observed at 1% Benzalkonium Chloride). The enhancement ratio for the negative control was 1.	35

Table 5. Dermal absorption/penetration, penetration enhancement, and ADME studies

Test Article	Vehicle	Test Population	Concentration/Dose	Protocol	Results	Reference
ADME						
Dermal						
radiolabeled and non-radiolabeled Benzalkonium Chloride (49.9% active ingredient; $C_{12} – C_{16}$)	water	Sprague-Dawley rats (64/group/sex)	0.1 and 1%; 1.5 ml/kg	OECD TG 417; test substance applied rat skin for 6 h followed by washing; samples of blood, urine, feces, bile, expired air, organs, carcass, and skin at intervals from 0 – 168 h after administration	Total recovery was approximately 87.2% in males and 91% in females. The minimum percutaneous absorption (in feces, urine, and intestines) was approximately 46.4% in males and 47.4% in females. The maximum systemic absorption (in feces, urine, carcass, and skin site) was approximately 50 and 50.1% in male and females, respectively. The test substance was uniformly distributed in the stratum corneum. The mean plasma and blood levels for males and females at the 0.1% concentration remained below quantifiable limits at all time points, except from the 7 and 8 time points for blood (average levels in males and females were 3.96 and 2.97 ng-eq./g at the 7 and 8 h time points, respectively). For the 1% concentration, values above quantified limits in the blood were observed at 8 h and 24 h (average levels in males and females were 69.4 and 58.65 ng-eq./g at the 8 and 24 h time points, respectively). Following single dermal application of 0.1%, mean radioactivity levels were below quantifiable limits in all tissues and organs except for the intestines and stripped skin.	12
Oral						
radiolabeled and non-radiolabeled Benzalkonium Chloride (30% active ingredient; $C_{12} – C_{16}$)	water	Sprague-Dawley rats (5/sex/group)	experiment 1: 1 mg/ml experiment 2: 100 ppm experiment 3: 5 mg/ml	EPA OPP 85-1; 3 different experiments; experiment 1: oral gavage with single low dose; experiment 2: dietary with repeated low dose (14 d); experiment 3: gavage with single high dose tissues and body fluids sampled: urine, feces, blood, plasma, cage washes; samples collected at intervals ranging from 0 – 168 h post administration	Following oral administration, the test substance was rapidly absorbed, although in very limited amounts as indicated by low blood levels. Blood concentrations declined to approximately 25% of the peak value within 24 h. Residual radioactivity in tissues was negligible after administration of test substance in all experiments. total recovery in experiment 1 (averaged amounts in males and females): urine: 6.33% feces: 94.9% total: 101% total recovery in experiment 2 (averaged amounts in males and females): urine: 5.25 % feces: 96.2% total: 102% total recovery in experiment 3 (averaged amounts in males and females): urine: 7.35% feces: 88.8% total: 96.5%	11

Table 5. Dermal absorption/penetration, penetration enhancement, and ADME studies

Test Article	Vehicle	Test Population	Concentration/Dose	Protocol	Results	Reference
radiolabeled and non-radiolabeled Benzalkonium Chloride (49.9% active ingredient; C_{12} – C_{16})	water	Sprague-Dawley rats (3/sex/time/group)	50 and 200 mg/kg bw; 10 ml/kg	OCED TG 417; gavage administration; single and repeated doses performed using 50 mg/kg bw/d; single dose performed using 200 mg/kg bw/d; samples of blood, urine, feces, bile, expired air, organs, carcass, and skin at intervals from 0 – 168 h after administration	Approximately 3 – 4% of the orally administered substance was excreted in the urine, and 3.75 – 4.58% was excreted in the bile. Approximately 70-80% of the test compound was excreted, primarily in the feces, 24 h after administration. After a single dose of 50 mg/kg bw, radioactivity levels were below the limit of quantification at all timepoints, and in all organs except for the liver and intestines. Following a single oral dose of 200 mg/kg bw, radioactivity levels were highest in the intestines. Trace levels were found in abdominal fat, heart, kidney, liver, lungs, and pancreas.	12
deuterated Lauralkonium Chloride	diet	C57BL/6J mice (4 - 6/sex/group)	120 µg/g/d	animals given test substance in diet for 1 wk; following 1-wk administration, liver, feces, and blood analyzed for metabolites; analysis via ultra-high performance liquid chromatography-tandem mass spectrometry	The highest levels of the test substance were observed in fecal samples (maximum amounts of approximately 1800 µM in female feces). The maximum amount of the test substance observed in the blood and liver was approximately 1000 and 550 nM, respectively (in females). Quantifiable levels of ω- and (ω-1)- hydroxy metabolites were observed (predominantly in the liver). Even numbered, chain-shortened carboxylic acids (C_6, C_{18}, C_{10}, and C_{12}) were quantified in the blood, liver, and feces (C_{10} observed in highest amounts; maximum amount reported in male and female feces; approximately 475 µM).	36
Inhalation						
^{14}C-labeled Benzalkonium Chloride	saline	male Sprague-Dawley rats (4/group)	293 µg/kg/bw	rats anesthetized using an inhalation system and administered test substance via catheter (intratracheal administration); rats euthanized at predetermined time intervals (5 min and 3, 24, 48, and 168 h post-administration); blood samples obtained and organs evaluated	The lungs showed the highest initial %ID/g (approximately 30%) values, peaking at 3 h post-dosing. A considerable fraction remained even at 168 h post-dosing (approximately 3.6% of the peak value). Rapid clearance was observed in the liver, kidney, spleen, gastrointestinal tract, with peak levels observed within the first 3 h. The adrenal gland showed a pronounced and sustained increase in retained fraction, peaking at 168 h. The pancreas and heart also exhibited substantial accumulation.	37
^{14}C-labeled Benzalkonium Chloride	NR	male Sprague-Dawley rats (4/group)	50 µl; concentration not stated	rats anesthetized using an inhalation system and administered test substance via catheter (intratracheal administration); rats euthanized at predetermined time intervals (5 min and 24, 48, and 168 h post-administration); blood samples obtained and organs (heart, kidney, large intestine, liver, lung, pancreas, small intestine, spleen, stomach, trachea) evaluated	Over the 7-d evaluation period, notable accumulation and prolonged retention of [^{14}C] Benzalkonium Chloride was observed in the pancreas and heart. Among the observed organs, the heart and pancreas showed the highest levels of radioactive concentrations 168 h post-exposure, with values of 198 and 186 ng equivalents of Benzalkonium Chloride/g tissue, respectively. At 48-h post-exposure the tissue-to-blood concentration ratios for the pancreas and heart reached their highest levels (62 and 104, respectively). These values remained notably high at 168 h (41 and 43, respectively).	38

EPA = Environmental Protection Agency; ID/g = injected dose per gram of tissue; MALDI-TOF = matrix assisted laser desorption ionization time-of-flight; NR = not reported; OECD = Organisation for Economic Cooperation and Development; OPP = Office of Pesticide Programs; TG = test guidelines

Table 6. Acute toxicity studies

Test Article	Vehicle	Animals/Group	Concentration/Dose	Protocol	LD_{50}/LC_{50}/Results	Reference
DERMAL						
Benzalkonium Chloride (50 – 80% active ingredient; $C_{12} – C_{16}$)	none	rabbit (4/sex/group; strain not specified)	3, 4, and 5 ml/kg bw	EPA OPPTS 870.1200; test substance applied to abraded and intact skin, under occlusive conditions, for 24 h	LD_{50} = 3.56 ml/kg bw (after correcting for 100% active test substance, the LD_{50} was calculated to be 2730 mg/kg bw)	11
Benzalkonium Chloride	NR	NR	82.26%	NR	LD_{50} = 930 mg/kg bw	8
ORAL						
Benzalkonium Chloride (50 – 80% active ingredient; $C_{12} – C_{16}$)	propylene glycol (for low doses); high doses tested undiluted	Albino rats (5/sex/group)	0.25, 0.32, 0.40, 0.50, 1, 2, 4, 8, and 16 ml/kg bw	single gavage administrations; 14-d observations	LD_{50} = 0.43 ml/kg bw (after correcting for 100% active test substance, the LD_{50} was determined to be 344 mg active ingredient/kg bw)	11
10% Benzalkonium Chloride	NR	male Sprague-Dawley rats (n = 3/group)	250 and 1250 mg/kg	single gavage administration; animals observed for 24 h after administration	LD_{50} = 234 - 525 mg/kg	39
Benzalkonium Chloride	saline	male Balb/c mice (6/group)	100, 150, 200, 300, and 400 mg/kg	single oral administration; method of oral administration not stated	LD_{50} = 241.7 mg/kg	40
Benzalkonium Chloride (50% active ingredient; $C_{12} – C_{16}$)	water	Sprague-Dawley rats (5/sex/group)	500, 794, 1260, and 2000 mg/kg bw	OECD TG 401; gavage administration; 14-d observations	LD_{50} = 795 mg/kg bw	12
INHALATION						
aerosolized Benzalkonium Chloride	air	female BALB/cJ mice (7-8/group)	0, 0.049, 0.19, 0.57, 1.8, 5.3, and 19 mg/m^3	head only exposure for 30 min; respiratory parameters (respiratory rate, inspiration/expiration times, tidal volume, mid-expiratory flow rates) evaluated; following a 16-h recovery period, BALF was collected	NOEL < 0.049 mg/m^3 A concentration-dependent reduction in tidal volume and an increase in respiratory rate was observed (compared to baseline). Statistically significantly increased numbers of inflammatory cells in BALF were seen at the highest exposure level (compared to controls). No inflammation was seen in fluid from mice exposed to concentrations of 1.8 mg/m^3 and below.	41
aerosolized Benzalkonium Chloride (95% purity)	air	female Wistar rats (5/group)	37.64 and 52.84 mg/m^3	head/nose inhalation; 4 h single exposure; 14-d observation	LC_{50} = 52.84 mg/m^3; out of the 5 rats, 2 died within the first 24 h after exposure to 52.84 mg/m^3	8,42

EPA = Environmental Protection Agency; LC_{50} = medial lethal concentration; LD_{50} = medial lethal dose; NOEL = no-observed-effect-level; NR = not reported; OECD = Organisation for Economic Cooperation and Development; OPPTS = Office of Prevention, Pesticides, and Toxic Substances

Table 7. Repeated dose toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
DERMAL							
Benzalkonium Chloride (81% active ingredient; $C_{12} – C_{16}$)	NR	Sprague-Dawley rats (number of animals and sex not stated)	90 d	0, 2, 6, or 20 mg/kg/d	topical application to the back for 6 – 8 h/d; no other details provided	A dose-dependent decrease in reticulocyte numbers were observed in 6- and 20-mg/kg/d-treated female rats; however, this was not concomitant with other hematological parameters, and similar effects were seen in male controls. A significant rise in hyperkeratosis was observed in females of the highest-dosed group; however, it was also noted in all treated, and vehicle-treated males. The NOAEL waws determined to be 20 mg/kg/d.	43
ORAL							

Table 7. Repeated dose toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
2% dilution of a sanitizing agent containing Benzalkonium Chloride (diluted test substance contained 0.01% Benzalkonium Chloride)	none	Sprague-Dawley rats (10/sex/group)	91 d	0, 500, 1000, or 5000 mg/kg bw/d (corresponding to 0, 0.05, 0.1, and 0.5 mg/kg bw/d Benzalkonium Chloride)	OECD TG 408; once daily gavage administration; controls given water	NOAEL = 5000 mg/kg (corresponding to 0.5 mg/kg bw/d Benzalkonium Chloride) All animals survived until study termination. No clinical or ophthalmological toxic effects observed. Food consumption was similar among treated and control groups. Minor body weight differences between control and treated groups were observed but were not considered to be related to the test substance. An increase in the number of nitrite-positive urine was observed in high-dose animals; however corresponding increases in bacteria or white blood cells in urine were not observed. A statistically significant decrease in serum aspartate aminotransferase was observed in mid-dose females compared to controls. The only statistically significant difference in organ weight between treated and control groups were decreased heart/body weight and lung/body weight ratios in low-dose males, and increased epididymides/brain weight ratio in high dose males. All gross and microscopic findings were considered to be of no toxicological relevance.	44
Benzalkonium Chloride (79.7 - 80.5% active ingredient; C_{12} – C_{16})	diet	Sprague-Dawley rats (15/sex/dose)	95 - 96 d	0, 100, 500, 1000, 4000, or 8000 ppm (equivalent to 0, 6, 31, and 62 mg/kg bw/d in males and 0, 8, 38, and 77 mg/kg bw/d in females); due to mortality in the 4000 and 800 ppm groups, the corresponding daily intakes could not be calculated	OECD TG 408; animals administered test substance via diet; controls given untreated diet	male and female NOEL: 500 ppm (equivalent to 25 mg active ingredient/kg bw/d for males and 30 mg active ingredient/kg bw/d for females) All animals in the 8000 ppm group died. In the 4000 ppm group, 12/15 males and 11/15 females died. Treatment-related clinical findings were observed in the 4000 and 8000 ppm groups (general cachexia, loose feces, decrease in body, weight, gross lesions, organ/tissue congestion, mucosal cell degeneration in the gastrointestinal tract, splenic contraction, hepatocellular atrophy)	11
Benzalkonium Chloride (50% active ingredient; C_{12} – C_{16})	diet	Beagle dogs (4/sex/group)	13 wk	0, 500, 1500, 3000 ppm (corresponding to approximately 0, 8, 25, 50 mg active ingredient/kg bw/d in males and 0, 9, 26, and 45 mg active ingredient/kg bw/d in females); from week 8 the test substance concentration was reduced to 2500 ppm in the high dose female group due to low food intake	OECD TG 409; animals administered test substance via diet; controls given untreated diet	NOAEL in males: 50 mg active ingredient/kg bw/d (1500 ppm); NOAEL in females: 45 mg active ingredient/kg bw/d (1250 ppm) No treatment-related toxicologically significant effects were observed throughout the study. A mean body weight loss was observed in females in the high dose group (not dose-related and correlated to the decrease of food consumption). Mean body weight gain was similar to controls once the dosing was reduced to 2500 ppm.	12
INHALATION							
aerosolized Benzalkonium Chloride (95% purity)	air	female Wistar rats (5/group)	3 d	30 mg/m^3	6 h/d exposure; exposure via exposure chamber; BALF collected from exposed and control (unexposed) animals immediately after termination of exposure and 18 h after exposure	Lung weight, total protein, lactate dehydrogenase activity in BALF, and BALF IL-6 and IgE concentrations were statistically significantly increased (compared to controls) immediately after exposure at both test concentrations.	42

Table 7. Repeated dose toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
aerosolized Benzalkonium Chloride (95% purity)	air	female Wistar rats (7 – 10/group)	5 d	0 and 35 mg/m^3	6 h/d exposure; exposure via exposure chamber; food intake and body weight evaluated on day 7, 14, and 21; animals were challenged after a 2-wk no-treatment period, with the same test substance; biological material (including BALF) collected from animals immediately after termination of exposure (day 21) and 18 h after challenge inhalation	All animals survived treatment. At all measurement time points, food intake and body weight of animals exposed to the test substance were statistically significantly lower compared to controls. Macroscopic examinations did not reveal any significant changes between exposed and control animals. A statistically significant reduction in absolute liver mass and a statistically significant increase of the absolute and relative mass of the lungs was noted in treated animals compared to controls. A statistically significant increase in total protein concentration, leukocyte count, and macrophage inflammatory protein-2 in BALF was observed (compared to controls). Statistically significantly higher IL-6 and IgE concentrations and a statistically significant decrease in CC16 concentrations in BALF were observed immediately after treatment (compared to controls). Minimal perivascular, interstitial edema, focal aggregates of alveolar macrophages, interstitial mononuclear cell infiltrations, thickened alveolar septa, and marginal lipoproteinosis were observed in the lungs of treated rats.	45
aerosolized Benzalkonium Chloride	air	Fischer 344 rats (5/sex/dose)	14 d	0.8, 4, 20 mg/m^3	6 h/d exposure; MMAD of 1.1 – 1.61 µm; no other details regarding treatment provided	NOAEC = < 0.8 mg/m^3 Nasal discharge was observed in all treated groups. Deep breathing and rales were reported in male rats in the high dose group. Significant reductions in body weight were observed in mid and high dose animals. Significant differences in hematology and organ weights (lung and spleen) were observed in mid and high dose groups. Adverse effects in the respiratory tract were observed in mid and high dose groups (degeneration and regeneration of terminal bronchiolar epithelium, smooth muscle hypertrophy of the bronchoalveolar junction, hypertrophy and hyperplasia of mucous cells in the bronchi and cell debris in the alveolar lumens. Ulceration with suppurative inflammation, squamous metaplasia and erosion with necrosis were observed in the respiratory tract and transitional epithelium in all treated groups. Atrophy of olfactory epithelium was observed in the high dose group only.	46

BALF = bronchoalveolar lavage fluid; IgE = immunoglobulin E; IL-6 = interleukin-6; MMAD = mean mass aerodynamic diameter; NOAEC = no-observed-adverse-effect-concentration; NOAEL = no-observed-adverse-effect-level; NOEL = no-observed-effect-level; OECD = Organisation for Economic Cooperation and Development; SDS = sodium dodecyl sulfate; TG = test guidelines

Table 8. Developmental and reproductive toxicity studies

Test Article	Vehicle	Test Population	Dose/Concentration	Procedure	Results	Reference
				IN VITRO		
Benzalkonium Bromide	NR	sperm from normozoospermic men	up to 0.02 mmol/l	in vitro assay evaluating human sperm viability, motility, mitochondrial status, capacitation, acrosomal status, and calcium movements; 60 or 180 min exposure; untreated negative control	Statistically significant decreases in sperm viability were observed at concentrations of 0.01 mmol/l (for 60 min incubation) and 0.025 (for 180 min incubation) respectively, compared to untreated control. Statistically significant decreases in sperm motility were observed at 0.0085 mmol/l (for 60 min incubation) and 0.015 mmol/l (for 180 min incubation) compared to untreated control. A significant loss of mitochondrial membrane potential was observed at all test concentrations. A strong inhibition of capacitation was observed following exposure to the test substance at a concentration of 0.02 mmol/l for 180 min ($p < 0.05$). No effects on sperm acrosomal status were observed. Significantly increased sperm intracellular calcium concentration in 20.2% of cells ($p < 0.05$).	47
Benzalkonium Chloride	none	sperm from normozoospermic men (n = 15)	6.81 mM	in vitro assay evaluating human sperm motility, viability, acrosomal status, and ability to penetrate cervical mucus; 10 min exposure; PBS used as control	Exposure to the test substance resulted in reduced acrosome integrity, total immobilization, and complete loss of sperm viability ($p < 0.001$; compared to controls). The test substance also significantly decreased sperm penetration ability ($p < 0.0110$; compared to controls).	48
				ANIMAL		
Benzalkonium Chloride (81.09% active ingredient; $C_{12} – C_{16}$)	water	female CD rats (25/group)	0, 10, 30, or 100 mg/kg/d	prenatal developmental toxicity assay; animals administered test substance via gavage to pregnant animals on GD 6-15; animals necropsied on GD 21; control group treated with water	All animals survived until scheduled necropsy. Treatment-related effects were observed at the highest dose level (peroral wetness, audible respiration, unkempt appearance, loose feces, urine stains). Audible respiration was observed in 2 animals at the 30 mg/kg/d dose level. Mean maternal body weights and body weight gains were unaffected by the test substance. Mean maternal food consumption in rats at 100 mg/kg/d was significantly ($p < 0.05$) lower compared to control during the first 3 d of treatment. A single rat dosed at 100 mg/kg/d had the following macroscopic findings: gas -filled gastrointestinal tract, ulceration of the stomach, color changes in the liver and lymph nodes, and a small spleen. No treatment-related effects were observed regarding terminal body weight, gravid uterine weight, net body weight, net body weight change, or liver weight. Intrauterine growth and survival (as determined by preimplantation loss, total number implants, viable implants, nonviable implants, percent live fetuses, mean fetal body weights, and fetal sex ratios) were similar in treated and control groups. One rat fetus in the high dose group had gastroschisis, and another fetus in this group had a thread-like tail and imperforate anus. An absent innominate artery was observed in one rat fetus at 10 mg/kg/d. Hydronephrosis and/or hydroureter observed in all groups (including 1 animal in the control group). No skeletal malformations were observed in fetuses.	49
Benzalkonium Chloride (81.09% active ingredient; $C_{12} – C_{16}$)	diet	Sprague-Dawley rats (28/sex/dose)	0, 300, 1000, or 2000 ppm (equivalent to 0, 16 – 31, 51 – 102, and 100 – 188 mg/kg in males and 0, 21 – 32, 67 – 106, and 139 – 198 in females)	EPA OPP 83-4; 2-generation reproductive toxicity assay; P0 animals underwent a 10-wk pre-breed exposure period, also exposed during 3-wk mating period and continued through gestation, parturition, and lactation; F1 generation treated for 10 wk pre-breed period and paired with P1 to produce F2, animals continued treatment during mating, gestation, parturition, and lactation; F2 generation treated until weaning; control animals given untreated diet	reproductive NOAEL for F_0, F_1, and F_2 rats: 2000 ppm The rat NOAEL for systemic toxicity for both parental and offspring generation was considered to be 1000 ppm. Reproductive parameters were not affected in either of the two breeds (F1 or F2). However reduced body weight gains were noted in F1 and F2 pups treated with 2000 ppm. No treatment-related observations or histopathological findings were observed in adult animals or pups at any dose.	11

Table 8. Developmental and reproductive toxicity studies

Test Article	Vehicle	Test Population	Dose/Concentration	Procedure	Results	Reference
Benzalkonium Chloride (49.9% active ingredient; $C_{12} – C_{16}$)	diet	Sprague-Dawley rats (25/sex/group)	0, 500, 2000, or 4000 ppm	OECD TG 416; 2-generation reproductive toxicity assay; animals exposed for 10 wk before mating, 2 wk during mating, and until after weaning of the pups; controls given untreated diet	reproductive NOAEL: 2000 ppm At the 2000 ppm level, P0 males and P1 males and females showed marginally to slightly lower body weight gains and reduced food consumption. Necropsy of parents of both generations revealed dilatation of the caecum in some animals (associated with lower liver weights in parental animals of both generations). At the highest concentration, in P0 and P1 generations, the number of implantation sites and litter size at birth were reduced, and the progenies showed lower pup weights. Upon necropsy, dilatation of the caecum with feces was observed in 4/25 males and 2/25 females in the F2 generation. Test substance treatment had no effect on mating, fertility, and behavioral parameters in P0 and P1 rats at levels up to 2000 ppm. No effect on litter parameters and pre- and post-natal development was observed for either generation at 2000 ppm. The systemic toxicity NOAEL for P1 was determined to be 500 ppm due to adverse effects relating to body weight, food consumption, organ weight, and gross pathology.	12
Benzalkonium Chloride (81.09% active ingredient; $C_{12} – C_{16}$)	water	female New Zealand White rabbits (16/group)	0, 1, 3, or 9 mg/kg/d	pregnant animals treated via gavage on GD 6 – 18; animals necropsied GD 29; control group treated with water	All animals survived treatment. Treatment related effects were observed in 2 animals at the highest dose (hypoactivity and labored respiration). No effects were observed regarding mean maternal body weight or body weight gain. No treatment-related effects gross macroscopic findings were observed. The terminal body weight, gravid uterine weight, net body weight, net body weight change, and liver weight were unaffected by treatment. Intrauterine growth and survival (as determined by preimplantation loss, total number implants, viable implants, nonviable implants, percent live fetuses, mean fetal body weights, and fetal sex ratios) were similar in control and treated groups. No treatment-related fetal malformations were observed. No external malformations observed in fetuses. A herniated diaphragm was observed in one fetus at 1 mg/kg/d. Dilated lateral ventricle of the brain with tissue depression were observed in 2 rabbits of the 3 and 9 mg/kg/d groups. Skeletal malformations were observed in one control rabbit fetus with absent lumbar centrum and arches and one rabbit litter in the 9 mg/kg/day group with one fetus exhibiting fused thoracic centra and ribs and another fetus presenting an extra rib between ribs 10 and 11.	49
Benzalkonium Chloride (49.9% active ingredient; $C_{12} – C_{16}$)	NR	female New Zealand White rabbits (22/dose)	0, 3, 10, or 30 mg/kg bw/d	prenatal developmental toxicity assay; treatment on GD 6 – 28; animals necropsied GD 29; gavage administration	maternal NOAEL: 3 mg/kg bw/d developmental NOAEL: 30 mg/kg bw/d Three animals in the high dose group did not survive treatment. Gross pathological findings included edema in stomach mucosa, dilated intestines, and gall bladder, in some mid and high dose animals. No treatment related effects on any litter parameters, fetal development, or fetal malformations, were reported.	8,50

EPA = Environmental Protection Agency; GD = gestation days; LD_{50} = median lethal dose; NOAEL = no-observed-adverse-effect-level; NR = not reported: OECD = Organisation for Economic Cooperation and Development; OPP = Office of Pesticide Programs; PBS = phosphate-buffered saline; TG = test guidelines

Table 9. Genotoxicity studies

Test Article	Vehicle	Concentration/Dose	Test System	Protocol	Results	Reference
IN VITRO						
Gene Mutation						
2% dilution of a sanitizing agent containing Benzalkonium Chloride (diluted test substance contained 0.01% Benzalkonium Chloride)	none	0, 1.56, 3.13, 6.25, 25, 50, 100 µg/plate	*S. typhimurium* TA1535, TA 1537, TA98, TA100 and *E. coli* WP2uvrA	OECD TG 471; Ames assay performed with and without metabolic activation; appropriate positive and negative controls used	non-mutagenic; controls gave expected	44
Benzalkonium Chloride (50 – 80% active ingredient; C_{12} – C_{16})	water	0, 0.15, 0.5, 1.5, 5, 15, and 50 µg/plate	*S. typhimurium* TA1535, TA 1537, TA98, TA100, and TA102	OECD TG 471; Ames assay performed with and without metabolic activation; appropriate positive and negative controls used	non-mutagenic; controls gave expected results	11
Benzalkonium Chloride	NR	0, 0.011, 0.110. 1.10, 11, 110 µg/plate	*S. typhimurium* TA98, TA100, and TA102	*Salmonella*/microsome assay; 48 h incubation; number of his^+ colonies manually counted; performed with and without metabolic activation; appropriate positive and negative controls used	non-mutagenic; controls gave expected results	51
Benzalkonium Chloride (C_{12} – C_{16})	NR	without metabolic activation: 0, 5, 10, 12.5, 15, 17.5, and 20 nl/ml with metabolic activation: 0, 15, 30, 45, 60, 75, and 90 nl/ml	Chinese hamster ovary cells	OECD TG 476; in vitro mammalian cell gene mutation assay performed with and without metabolic activation; target gene: HGPRT locus; appropriate positive and negative controls used	non-mutagenic; controls gave expected results	12
Benzalkonium Chloride (81.09% active ingredient; C_{12} – C_{16})		experiment 1 without metabolic activation: 0, 1, 5, 10, 13, 16, 20, 25, 35, 50, and 65 µg/ml experiment 1 with metabolic activation: 0, 1, 5, 10, 20, 30, 40, 50, 65, 85, and 100 µg/ml experiment 2 without metabolic activation: 0, 1, 5, 10, 12, 14, 16, 18, 20, and 24 µg/ml experiment 2 with metabolic activation: 0, 10, 20, 22, 24, 26, 28, 30, 40, and 50 µg/ml	Chinese hamster ovary cells	EPA OPPTS 870.5300; mammalian cell gene mutation test performed with and without metabolic activation; target gene: HGPRT locus; appropriate positive and negative controls used; 2-part experiment	non-mutagenic; controls gave expected results	11
Stearalkonium Chloride	water	0, 0.02, 0.05, 0.16, 0.50, 1.6, 5.0, 15.8, 50, 158.1, 500, 1581, and 5000 µg/plate	*S. typhimurium* TA98, TA100, TA1535, and TA1537 and E. coli WP2 uvrA	OECD TG 471; Ames assay performed with and without metabolic activation; appropriate positive and negative controls used	non-mutagenic; controls gave expected results	12
Stearalkonium Chloride (93.9% purity)	NR	0, 1, 3, 10, 33, 100, 166, 333, 666, 1000, 1666, 3333 µg/plate	*S. typhimurium* TA97, TA98, TA100, TA1535, and TA1537	Ames assay performed with and without metabolic activation; appropriate positive and negative controls used	non-mutagenic; controls gave expected results	52

Table 9. Genotoxicity studies

Test Article	Vehicle	Concentration/Dose	Test System	Protocol	Results	Reference
			Chromosomal Damage			
Benzalkonium Chloride (50 – 80% active ingredient; C_{12} – C_{16})	cell culture medium	experiment 1: 0, 4, 8, 16, 20 µg/ml experiment 2: 0, 4, 8, 12, 16, 24 µg/ml	human lymphocytes	OECD TG 473; chromosomal aberration assay performed with and without metabolic activation; appropriate positive and negative controls used; 2-part experiment	non-clastogenic; controls gave expected results	11
Benzalkonium Chloride (99.24% active ingredient; C_{12} – C_{16})	NR	without metabolic activation: 0, 0.36, 1.09, 3.27, and 9.8 µg/ml with metabolic activation; 0, 6.1, 3, 12.25, 24.5, 49, and 98 µg/ml	Chinese hamster ovary cells	OECD TG 473; chromosomal aberration assay performed with and without metabolic activation; appropriate positive and negative controls used	non-clastogenic; controls gave expected results	12
10% Benzalkonium Chloride in PBS	NR	0, 0.0001, 0.0001, and 0.0003%	human dental pulp cells	chromosomal aberration assay performed with and without metabolic activation; up to 30 h incubation; appropriate positive and negative controls used	non-clastogenic; controls gave expected results	53
Benzalkonium Chloride	NR	0, 0.11, 0.33, and 1 mg/l	peripheral human lymphocytes	micronucleus assay; 60 min incubation; micronucleus formation and nuclear division indices evaluated; appropriate positive and negative controls used; performed without metabolic activation	Significant increase in micronucleus formation observed only at 1 mg/l ($p \leq 0.05$); nuclear division indices were similar to control at all test concentrations; controls gave expected results	51
			Other			
Benzalkonium Chloride	culture medium	0.00005, 0.0001, 0.0005, and 0.001%	human corneal epithelial cells	alkaline comet assay with immunofluorescence microscope detection of the phosphorylated form of histone variant H2AX foci; use of metabolic activation not stated; 30 min incubation; control treated with culture medium only	Benzalkonium Chloride at 0.00005, 0.0001, 0.0005, and 001% resulted in 2.56-, 2.74-, 2.95-. and 3.24-fold increases in single-strand breaks compared to the control ($p < 0.001$).	54
Benzalkonium Chloride	culture medium	0.00001, 0.00005, 0.0001, 0.0005, and 0.001%	human Chang conjunctival cells	alkaline comet assay; mean olive tail movement evaluated as marker for DNA damage; use of metabolic activation not stated; control treated with culture medium only	All concentrations of Benzalkonium Chloride induced a significant increase in olive tail movement compared to the negative control ($p < 0.01$).	55
Benzalkonium Chloride (C_{12} – C_{16})	NR	0, 0.002, 0.005. 0.007, 0.01, 0.02, and 0.05%	human bronchial epithelial cells	single-cell gel electrophoresis assay; 2 h incubation; tail movement used as marker for DNA damage; use of metabolic activation not stated; appropriate positive and negative controls used	Concentrations of 0.002 – 0.02% resulted in a stepwise increase of tail movements compared to negative controls ($p < 0.001$). This effect was not observed at 0.05%.	56
Benzalkonium Chloride	NR	0, 0.11, 0.33, and 1 mg/l	primary rat hepatocytes	single-cell gel electrophoresis assay; 60 min incubation; DNA migration evaluated via computer-aided system; use of metabolic activation not stated; appropriate positive and negative controls used	DNA migration significantly affected only at 1 mg/l ($p \leq 0.05$); controls gave expected results	51
Benzalkonium Chloride	PBS	0, 0.5, 1, 2 mM	human hepatocellular carcinoma cells and liver cancer cells	single-cell gel electrophoresis assay; DNA tail intensity evaluated as marker for DNA damage; performed with and without metabolic activation: appropriate positive and negative controls used;	non-mutagenic; controls gave expected results	57

Table 9. Genotoxicity studies

Test Article	Vehicle	Concentration/Dose	Test System	Protocol	Results	Reference
IN VIVO						
Chromosomal Damage						
2% dilution of a sanitizing agent containing Benzalkonium Chloride (diluted test substance contained 0.01% Benzalkonium Chloride	none	0, 5, 10, and 20 ml/kg	Sprague-Dawley rats (5-7/sex/group)	OECD TG 474; mammalian erythrocyte micronucleus test; appropriate positive and negative controls used	non-genotoxic	44
Benzalkonium Chloride (C_{12} – C_{16})	NR	400 mg/kg	rat bone marrow	in vivo micronucleus assay; no other details provided	non-genotoxic	8

EPA = Environmental Protection Agency; NR = not reported; OECD = Organisation for Economic Co-operation and Development; OPPTS = Office of Prevention, Pesticides, and Toxic Substances; PBS = phosphate-buffered saline; SDS = sodium dodecyl sulfate; TG = test guidelines

Table 10. Dermal irritation, sensitization, and phototoxicity studies

Test Article	Vehicle	Concentration/Dose	Test Population/System	Protocol	Results	Reference
IRRITATION						
IN VITRO						
cosmetic product containing 0.58% of a Stearalkonium Chloride mixture (85% active in glycerin)	NR	100%; final concentration of Stearalkonium Chloride tested: 0.49%	reconstructed human epidermis (number of samples not stated)	in vitro dermal irritation assay; 20 h exposure; no other details provided	non-irritant	15
85% Stearalkonium Chloride in glycerin	water	3.5% (final concentration of Stearalkonium Chloride tested: 3%)	reconstructed human epidermis (number of samples not stated)	in vitro dermal irritation assay; 20 h exposure; no other details provided	non-irritant	15
Stearalkonium Chloride	none	100%; 16 ± 2 mg	reconstructed human epidermis (n = 3)	OECD TG 439; reconstructed human epidermis assay; PBS used as negative control; SDS used as positive control; 42 min exposure	non-irritant; 96.1% tissue viability; controls gave expected results	12
ANIMAL						
Benzalkonium Chloride (50 – 80% active ingredient; C_{12} – C_{16})	none	100%; 0.5 ml	rabbit (n = 6; sex and strain not stated)	EPA OPPTS 870.2500; dermal irritation assay in abraded and intact skin; 24-h exposure period under occlusive conditions	irritating/corrosive; severe erythema and edema observed in all test animals in abraded and intact sites; mean primary irritation index calculated to be 6.29/8	11
mixture consisting of 40% Stearalkonium Chloride, 39% water, 10% 1-octadecanol, and 11% 2-propanol	none	100%; 0.5 ml	rabbits (n = 2; sex and species not stated)	24-h dermal irritation assay in intact and abraded skin; occlusive conditions; sites evaluated 24 and 72 h and 7 d after exposure	moderately irritating; primary irritation score of 4.3 (irritation scores between 2 and 5 considered moderately irritation)	99
HUMAN						
Benzalkonium Chloride	water	0.01, 0.025, 0.05, and 0.1%	30 subjects	irritancy patch test; Finn chamber taped to upper arm; removed after 4 d; readings 3 and 4 d after removal; no other details provided	7/30 subjects had positive patch test reading to 0.1% Benzalkonium Chloride on day 4; no positive reaction were observed at other test concentrations	**100**

Table 10. Dermal irritation, sensitization, and phototoxicity studies

Test Article	Vehicle	Concentration/Dose	Test Population/System	Protocol	Results	Reference
SENSITIZATION						
ANIMAL						
Benzalkonium Chloride	acetone and olive oil	0, 0.25, 0.5, and 1%	female BALB/c mice (2 – 4/group)	2-stage LLNA: Daicel alternative method; ears first treated with 1% SLS followed by application of test substance (at highest concentration) or vehicle (treatment performed on days 1, 2, 3 and 7); on day 8, mice killed and auricular lymph nodes removed and evaluated; in stage 2, test substance was evaluated according to OECD TG 442	sensitizing; statistically significant increased ATP content at 0.5 and 1%; stimulation indices at 0, 0.25, 0.5, and 1% were 1, 1.43, 2.17, and 6.19, respectively; EC1.8 = 0.28	101
Benzalkonium Chloride (50% active ingredient; C_{12} – C_{16})	water	0.1% (for induction and challenge)	guinea pigs (6/group; sex and species not stated)	modified Draize assay; induction phase: test substance injected intradermally; procedure repeated every other day for 9 total injections; 2-wk no treatment period followed by challenge injection; no positive or negative controls used; ATP content used to assess lymphocyte proliferation	non-sensitizing	12
HUMAN						
Stearalkonium Chloride	water and stearyl alcohol	20%	50 subjects	cotton patch saturated with test substance applied to inner surface of forearm and covered with aluminum foil and tape; patch removed after 48 h and sites evaluated; 2 wk after first patch was applied, procedure was repeated on other arm to evaluate for sensitization	erythema observed in some subjects (number not reported) after first patch; non-sensitizing according to study authors, the Stearalkonium Chloride used in this study was not a highly purified material, and the primary irritation may have been due to impurities in the material or to the stearyl alcohol vehicle	2

ATP = adenosine triphosphate; EC1.8 = concentration inducing a stimulation index of 1.8; EPA = Environmental Protection Agency; LLNA = local lymph node assay; OECD = Organisation for Economic Co-operation and Development; OPPTS = Office of Prevention, Pesticides, and Toxic Substances; PBS = phosphate-buffered saline; SDS = sodium dodecyl sulfate; SLS = sodium lauryl sulfate; TG = test guidelines

Table 11. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Protocol	Results	Reference
IN VITRO						
Benzalkonium Chloride	none	100%; 50 mg	EpiOcular™ tissues (n = 2)	OECD TG 492; reconstructed human cornea-like epithelium assay; appropriate positive and negative controls used; 6 h exposure	potentially irritating (viability was determined to be 5.7%, well below the threshold (≤60%), indicating no prediction could be made for classification under the globally harmonized system; percent viability negative control:100.3%; percent viability positive control: 10.5%	12
25% Stearalkonium Chloride	NR	8% (final test concentration of Stearalkonium Chloride: 2%)	EpiOcular™ tissues (n = 2)	EpiOcular™ assay and MTT assay; up to 30 min incubation	moderately irritating (ET_{50} = 28.3%)	163
85% Stearalkonium Chloride in glycerin	water	3.52% (final test concentration of Stearalkonium Chloride: 3%)	SkinEthic™ reconstructed human corneal epithelial model (number of tissues not stated)	SkinEthic™ human corneal epithelium eye irritation assay; exposure for 1, 3 and 24 h	irritating	15
85% Stearalkonium Chloride in glycerin	none	100%	reconstructed human cornea-like epithelium (number of tissues not stated)	OECD TG 492; reconstructed human cornea-like epithelium assay	irritating	15
ANIMAL						
Benzalkonium Chloride (C_{12} – C_{16})	saline	0.001, 0.003, 0.005, 0.01, and 0.03; 50 µl	male New Zealand White rabbits (3/group)	test substance administered to eyes 10x (every 30 min); eyes observed 30 min after last application	irritation not observed at concentrations ≤ 0.005%; irritation observed at concentrations ≥ 0.01%, with severity increasing in a dose-dependent manner.	62
mixture consisting of 40% Stearalkonium Chloride, 39% water, 10% 1-octadecanol, and 11% 2-propanol	none	100%; 0.1 ml	rabbits (n = 2; sex and strain not stated)	animals administered test substance into conjunctival sac; evaluations occurred 4, 24, 48, 72, 96 h, and 7, 14, and 21 d post-dosing	corrosive to eyes; irreversible ocular damage	99
Stearalkonium Chloride	NR	0.002%	New Zealand White rabbits (n = 7; sex not stated)	Draize assay; test substance applied to eyes; no other details provided	mild conjunctival hyperemia reported 4 h after treatment; effects were reversible within 24 h	8
PHOTOTOXICITY						
Benzalkonium Chloride	NR	0.001, 0.002, 0.003, and 0.004%	culture human corneal epithelial cells	combined toxic effect of UV radiation (0.17 J/cm^2) and Benzalkonium Chloride evaluated; UV administrations occurred at different times (before, at the same time, or after Benzalkonium Chloride administration; cells also exposed to Benzalkonium Chloride (at all concentrations), alone, or UV administration, alone (phosphate-buffered saline (PBS) also used as control)	Benzalkonium Chloride alone reduced the metabolic activity and cell viability of the corneal epithelial cells in a dose- and time-dependent manner. UV alone had little toxicity on cells and was not significantly different than the PBS control. In all 3 exposure orders, synergistic effects were observed when UV was administered with Benzalkonium Chloride at all concentrations.	**103**

ET_{50} = exposure time required to reduce tissue viability to 50%; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NR = not reported; OECD = Organisation for Economic Co-operation and Development; SDS = sodium dodecyl sulfate; TG = test guideline

Table 12. Patch testing in patients using Benzalkonium Chloride

Patch Test Concentration	Vehicle	Number of Patients/Patient Type	Number of Positive Reactions (% of total)	Reference
0.15%	water	10 patients (8 patients with history of rash or positive patch test to Benzalkonium Chloride; 2 patients receiving routine patch testing)	2 patients (20%)	135
0.15%	petroleum	10 patients (8 patients with history of rash or positive patch test to Benzalkonium Chloride; 2 patients receiving routine patch testing)	0 patients (0%)	135
0.1%	water	30 patients using inhaled or intranasal corticoid steroids for allergic conditions	2 patients (6.7%)	136
0.1%	water	91 dermatitis patients	5 patients (5.5%)	137
0.1%	water	131 cheilitis patients	17 patients (13%)	138
0.1%	water	149 dermatitis patients	5 patients (3.4%)	139
0.1%	water	215 periorbital dermatitis patients	10 patients (4.7%)	140
0.1%	water	429 dermatitis patients (evaluating for late delayed positive reactions)	2 patients (0.5%)	141
0.1%	water	584 dermatitis patients	71 patients (12.2%)	100
0.1%	water	615 dermatitis patients	198 patients (32.2%)	132
0.1%	petroleum	1389 dermatitis patients	48 patients (3.5%)	142
0.1%	NR	2295 dermatitis patients	126 patients (5.5%)	8
0.1%	water	2546 dermatitis patients	176 patients (6.9%)	143
NR	NR	2611 dermatitis patients	47 patients (1.8%)	144
0.1%	water	2694 dermatitis patients	164 patients (6.1%)	145
0.1%	water	3043 dermatitis patients	37 patients (1.2%)	146
0.1%	petroleum	4116 dermatitis patients	21 patients (0.5%)	147
0.1%	water	4892 dermatitis patients	210 people (4.3%)	148
0.1%	water	7390 dermatitis patients	108 patients (1.5%)	149
0.1%	petroleum	42,898 dermatitis patients	361 patients (0.8%)	150

NR = not reported

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4

Final Report on the Safety Assessment of Stearalkonium Chloride

Stearalkonium Chloride is a cationic quaternary ammonium salt used in cosmetic products at concentrations of ≤0.1 to 5%. It is used in cosmetics predominantly for its surfactant and antimicrobial properties.

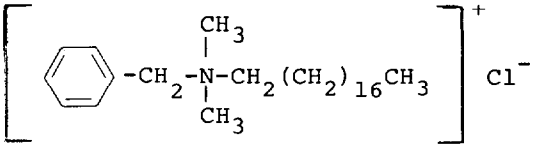
Studies have failed to establish with certainty the oral LD50 in rats of Stearalkonium Chloride, the value falling between 0.5 and 1.25 g/kg. In mice, an LD50 value of 0.760–0.113 g/kg was reported in a seven-day oral study. Single application dermal studies with concentrations of up to 25% have shown Stearalkonium Chloride to produce minor irritation in rabbits. Acute eye studies in rabbits have shown a 25% solution of the material to be a severe irritant. Concentrations of 1.25% and less are slightly and transiently irritating to the rabbit eye.

A repeated insult patch test with a 1% aqueous solution of Stearalkonium Chloride on 50 subjects showed the material to be neither a primary irritant nor a sensitizer. A single 48-hour patch test with challenge two weeks later indicated that 20% Stearalkonium Chloride is not a sensitizer.

On the basis of the evidence at hand, it is concluded that Stearalkonium Chloride is safe when incorporated in cosmetic products in concentrations similar to those presently marketed.

CHEMICAL PROPERTIES

Stearalkonium Chloride is a quaternary ammonium salt. The compound consists of an aliphatic hydrophobic portion and a nitrogenous hydrophilic portion. Because of this amphoteric property and also because of the fact the compound carries a positive charge upon ionization, Stearalkonium Chloride is classified as a cationic surfactant. It has the following structural formula:[1,2]



The respective structures of Cetalkonium and Myristalkonium Chlorides, two compounds closely related to Stearalkonium Chloride, are the same as above, except that the stearyl ($-CH_2(CH_2)_{16}CH_3$) moiety is replaced by cetyl ($-CH_2(CH_2)_{14}CH_3$) or myristyl ($-CH_2(CH_2)_{12}CH_3$) moieties. The safety of Cetalkonium and Myristalkonium Chlorides is not under review in this report. Information and data pertaining to these two compounds have been included to permit a more complete appraisal of the safety of Stearalkonium Chloride. The three alkonium compounds are prepared by the quaternization of the appropriate alkyldimethylamine (stearyl, cetyl, or myristyl) with benzyl chloride. Each is a free flowing powder normally sold as a dispersion in isopropyl alcohol and/or water.[3]

Physical Properties

The melting points of a homologous series of this class of compounds decrease sharply from chain lengths of C_8 to C_{11} and gradually increase with longer alkyl groups. Those with chain lengths of C_8 to C_{13} are soluble in water. The odd-numbered compounds are more soluble in 95% ethanol than even-numbered ones.[3] The pH ranges for 1% and 10% aqueous solutions of Stearalkonium Chloride are 3.5–6.5 and 3–4, respectively.[4,5] The ability to lower the surface tension of water increases with increasing chain length (C_8 to C_{19}) until a minimum of 42–43 dynes/cm is approached.[3]

Aqueous solutions of Cetalkonium Chloride and other quaternary ammonium compounds at concentrations above their critical micelle concentration (CMC) were studied as a function of monovalent electyrolyte concentration and temperature. At a given temperature, there is a critical electrolyte concentration above which the material separates into two phases; the top layer is virtually free of the quaternary ammonium salt, and the bottom layer shows the characteristics of an oil. The volume of the bottom layer decreases with increasing electrolyte concentration. Before separation, turbidity and dissymmetry of light scattering rise sharply with increasing eletrolyte concentration. The phenomenon of two-phase formation in Cetalkonium Chloride and other cationic soap systems shows a pronounced specificity to the anions of the added electrolyte. Small temperature changes produced marked changes in the volume of each layer in the two-phase systems.[6]

The electrical conductance of long-chain alkyldimethylbenzylammonium chlorides (C_{10} to C_{16}) has been studied through the use of a Wheatstone Bridge with an oscilloscope detector. The resulting conductance curves were used to determine C values (Table 1). Calculated values indicate that an increase in chain length by one methylene group changes the free enthalpy of micellization by a constant value.[7]

In a series of studies in which viscosity and conductance measurements were

TABLE 1. Critical Micelle Concentration Values.[a]

Ingredient	*CMC mole/dm³*
Stearalkonium Chloride	Not available
Cetalkonium Chloride	2.9×10^{-4}
Myristalkonium Chloride	1.9×10^{-3}

[a]Data from Ref. 8.

made in molten pyridinium chloride, Bloom and Peinsborough[8,9] determined the CMC of Cetalkonium Chloride to be 0.06–0.07 *M* at 155 °C. Another method has been described for determining micellar charge using the osmotic response of permeable, charged membranes.[10] With increased alkyl chain-length, alkylbenzylammonium chlorides exhibit increasing ability to lower surface tension in the presence of excess electrolyte; this increase adheres closely to Traube's rule. (The surface tension of dilute solutions of certain organic compounds decreases with the increase of the carbon chain length within homologous series.) There is significant deviation in these materials' ability to lower surface tension in the absence of electrolyte.[11]

Reactivity

The cationic charge possessed by these materials enables them to react with the anionic charge of other substances. This permits these compounds to precipitate carrageenan and other sulfated hydro-colloids at critical temperatures and pH values. These materials form water insoluble precipitates when combined with tannic, gallic, and salicylic acids. Their property of lowering surface tension makes possible many chemical reactions, including the basic hydrolysis of carboxylic acid esters of polyvinyl alcohol.[12-14]

It can be expected that in the presence of nitrites, nitrogen oxides, or other nitrosating agents, alkylbenzyldimethylammonium chlorides will give rise to traces of N-nitrosamines. Furthermore, the significant impurities, alkyldimethylamines (Table 2), are easily nitrosated to N-nitrosamines.

Analytical Methods

Quantitative determinations of all cationic surfactants can be accomplished by a two-phase titration with thymolphthalein, eosin, or methylene blue, as indicated. They can also be identified by paper chromatography.[15,16]

Cationic surfactants react with thymolsulfonaphthalein dyes to form large cation–anion complexes. Following a series of extractions, photometric determination of the cationic surfactant complex with thymolsulfonaphthalein is made by the colorimetry at 555 nm.[17]

Spectrophotometry, employing a sulfuric acid blank, for both anionic and cationic compounds of this type has been described by Spada et al.[18] A gravimetric method employs conversion of the quaternary compounds to insoluble reineckates.[19,20] An alkali-metric method in which salts of organic bases are precipitated as tetraphenylboron compounds and then titrated with acid has been described as accurate between +1.6% and −3%, but most errors were much smaller.[21]

A gas–liquid chromatography method utilizing lithium aluminum hydride (LAH) has been described. The long-chain quaternary ammonium salts are reduced to tertiary amines with LAH. Subsequently, the amines are analyzed by temperature-programmed gas chromatography.[22] A method has also been developed for the rapid identification of quaternary ammonium derivatives; this involves (1) the use of a silver nitrate-nitric acid solution to detect the halide; (2) the determination of halide type; and (3) the determination of the halide's melting point to make the final differentiation.[23] It is possible to detect five cationic quaternary ammonium compounds by nuclear magnetic resonance. However, this methodology is more adapted to anionic compounds.[24] In order to identify quaternary compounds in the presence of many others, a semi-microtitration

technique has been developed using sodium lauryl sulfate in a chloroform/water two-phase system. The compounds are first separated on an ion-exchange column.[25]

Impurities

Table 2 lists the reported known impurities contained in Stearalkonium Chloride.

TABLE 2. Impurities.[a]

Chemical names of impurities	*Percent present in material*
Stearyl Alcohol	3–6
Stearyl Dimethylamine Hydrochloride	1.5–4 (combined)
Stearyl Dimethylamine	

[a]Data from Ref. 3.

PURPOSE AND FREQUENCY OF USE IN COSMETICS

In cosmetic products, Stearalkonium Chloride is primarily used as surface-active and antimicrobial agents. Because it has a high affinity for proteins, this material is quite serviceable in cosmetic products intended for use on the hair. Properties relevant to such use are presented in Table 3.

The categories of cosmetic products and the concentrations in which Stearalkonium Chloride is used appear in Table 4. The cosmetic product formulation computer printout which is made available by the Food and Drug Administration (FDA) is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations (1979). Ingredients are listed in prescribed concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the true, effective concentration found in the finished product; the effective concentration in such a case would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework

TABLE 3. Cosmetic Properties of Stearalkonium Chloride.[a]

Property	*Product type(s)*
Improvement of wet combing	Rinses, conditioners
Increased luster	Rinses, conditioners
Improvement of feel	Setting lotions, bleaches
Improvement of dry combing	Setting lotions, rinses, conditioners
Wetting power (leveling action)	Bleaches, dyes, setting lotions
Antistatic effect	All hair products
Foaming power	Special purpose shampoos
Hydrophobizing effect	All hair products

[a]Data from Ref. 1.

TABLE 4. Product Formulation Data on Stearalkonium Chloride.[a]

Cosmetic Product Type	*Concentration (%)*	*No. of product formulations*
Hair conditioners	>1–5	52
	>0.1–1	18
	≤0.1	8
Hair sprays (aerosol fixatives)	>0.1–1	4
	≤0.1	5
Hair straighteners	>0.1–1	1
Permanent waves	>1–5	1
	>0.1–1	3
	≤0.1	2
Rinses (noncoloring)	>1–5	55
	>0.1–1	5
Tonics, dressings, and other hair grooming aids	>1–5	1
	>0.1–1	2
	≤0.1	1
Wave sets	≤0.1	8
Other hair preparations	>0.1–1	2
	≤0.1	3
Hair dyes and colors (all types requiring caution statement and patch test)	>1–5	6
	>0.1–1	4
	≤0.1	11
Hair rinses (coloring)	>1–5	3
	>0.1–1	38
	≤0.1	6
Nail creams and lotions	>0.1–1	1
Aftershave lotions	≤0.1	1
Cleansing (cold creams, cleansing lotions, liquids and pads)	>1–5	1
	>0.1–1	1
Moisturizing	>1–5	4
	>0.1–1	1
Other skin care preparations	>1–5	1

[a]Data from Refs. 26, 27.

of preset concentration ranges also provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to ten-fold error in the assumed ingredient concentration. The compounds are found in a variety of formulations, but are particularly prevalent in hair care products. Concentrations of use for Stearalkonium Chloride range from ≤0.1 to 5%.[26,27]

Stearalkonium Chloride is used in formulations that are applied to all areas of the skin, nails, and hair, and around the body orifices. Formulations containing this ingredient may be applied to the body as infrequently as once each month (hair dyes and colors) or as frequently as once or twice a day (tonics, dressings, and hair grooming aids). They may be in contact with various areas of the body for as little as a few minutes or as much as a few days. Occasional or daily use may extend over a period of years.

Potential Interactions with other Ingredients

The quaternary ammonium salt, Stearalkonium Chloride, is insoluble in water; it can be solubilized by adding an excess of anionics or cationics.[13] However, these solubilized materials cease to have the characteristic properties

of Stearalkonium Chloride. The cationic portion of the quaternary ammonium complex loses its microbial activity, while the anionic portion loses its foaming characteristics.[28] Stearalkonium Chloride is compatible with nonionic ingredients or compounds, is stable in hard water, and is a good emulsifying agent.

BIOLOGICAL PROPERTIES

General Effects

The antibacterial activity of cationic quaternary ammonium compounds varies wih the length of the alkyl chain, the greatest activity being associated with the C_{16} or C_{18} chain length (depending on the organism tested). This activity may increase with increased charge on the nitrogen atom, but may decrease if excessive atoms are clustered around it. Bactericidal activity tends to increase with critical micelle concentration,[29] although no direct correlation has been reported between the surfactant activity and bactericidal action.[29,30]

Standard antibacterial and antifungal tests were performed on a series of alkyldimethylammonium chlorides of C_{8-19} chain length. The most consistent amount of bactericidal and fungistatic activity was seen in compounds of C_{12-16} chain-length. The bactericidal action of a series of these compounds on a myxobacterium, pathogenic to fish, was greatest for the Hexadecyl compound.[31,32]

A 1% solution of Stearalkonium Chloride inhibited bacterial growth in a study of this material's germicidal activity. When tested for bacteriostatic efficiency against *Salmonella typhosa, Staphylococcus aureus,* and *Bacillus anthracis,* Stearalkonium Chloride was found to be an effective bacteriostat, particularly against *S. aureus.*[33,34]

Secondary Effects

The adjuvant activity of 203 aliphatic nitrogenous bases was evaluated through the use of diphtheria toxoid in guinea pigs. The toxoid was administered subcutaneously in the abdominal wall twice, at 28-day intervals. Dilutions were made to achieve a dose of 1 LF in 0.2 ml per injection. (LF = limit flocculation: that amount of dyphtheria toxoid which gives the most rapid flocculation when incubated with one standard unit of dyphtheria antitoxin.) A single 0.1 ml dose of each adjuvant was administered at the time of the first toxoid dose. Adjuvant activity required a combination of basicity and a long aliphatic chain length (C_{12}). Active compounds were hemolytic and produced damage to monkey kidney or human epitheloid (HEp^2) tissue culture mono-layers. Stearalkonium Chloride was highly active by virtue of its long alkyl chain.[35]

Concentrations of Stearalkonium Chloride producing 100% and 50% hemolysis of isolated erythrocytes from rabbits and sheep, respectively, have been determed to be 2.4×10^{-5} *M* and 3.0×15^{u} *M*.[36,37]

Absorption, Metabolism, Storage, and Excretion

A commercial mixture of alkylbenzyldimethylammonium (ABMA) chlorides (predominantly C_{12}, C_{14}, C_{16}) was administered orally, rectally, or intramuscularly to rabbits, dogs, and cats at 10 times the lethal dose. The concentrations in blood and various tissues were determined. After oral administration, most of the compound remained in the upper gastrointestinal tract, with small concentrations be-

ing found in the liver and blood. After rectal administration, nearly all the ABMA chloride was recovered from the lower bowel with small amounts from blood, liver, and kidney tissue. Following intramuscular administration, nearly all the mixture remained at the injection site. These results indicate that the ABMA chlorides are poorly absorbed and poorly distributed in the tissues.[38]

Animal Toxicology

General Studies

Oral toxicity: acute

Studies have failed to establish with certainty the LD50 of Stearalkonium Chloride in rats. Two separate experiments have been reported (Table 5). A 25% aqueous solution of pure Stearalkonium Chloride introduced by stomach tube into rats produced an LD50 value of greater than 0.5 g/kg but less than 1.25 g/kg. A second study reported Stearalkonium Chloride administered by gavage to have an LD50 value greater than 0.0625 g/kg but less than 1.25 g/kg for the pure ingredient.[39,40]

A seven-day oral LD50 in mice has been reported to be 0.76 ± 0.11 g/kg, according to the method of Hoppe and Lands, for pure Stearalkonium Chloride.[30] An aqueous solution containing 20% Stearalkonium Chloride and 5% stearyl alcohol was determined to have an LD50 of 4.0 ± 0.1 ml/kg in rats.[41]

Eye irritation: acute

The Draize procedure was used to determine the eye irritation index in rabbits of Stearalkonium Chloride at various concentrations. Table 6 presents a summary of the data from these experiments. The 25% solution is a severe irritant to the eye, while solutions of 1.25% or less are slightly and transiently irritating, with the effects being limited to the conjunctivae; these effects disappear after 3–4 days.[33,42-45]

A study was undertaken to determine the highest concentration of an aqueous solution containing a 4:1 ratio of Stearalkonium Chloride to stearyl alcohol that did not produce irritancy to rabbit eye mucosa in three or more of five test animals used. This threshold concentration was determined to be 0.04% Stearalkonium Chloride and 0.01% stearyl alcohol.[41]

Dermal irritation: acute

Adult rabbits were used in determining Stearalkonium Chloride's potential for skin irritation. Primary dermal irritation indices were calculated according to the Draize procedure for 25%, 2.5%, and 1.25% concentrations of the material.

TABLE 5. Oral LD50 in Rats.

Dose (g/kg pure Stearalkonium Chloride)	*Animals Dead/Total*	*LD50 (g/kg)*	*Ref.*
0.5	0/6	>0.5	39
1.25	4/6	<1.25	39
2.5	5/6	<2.5	39
0.0625	3/10	>0.0625	40
1.25	9/10	<1.25	40

TABLE 6. Primary Eye Irritation Scores in Rabbits.[a]

Concentration (%)	No. of Animals	Days 1	2	3	4	7	Ref.
Unwashed							
25.	6	33.5	37.8	35.5	36.8	73.8	41
1.25	6	14.7	10.0	3.2	1.0	0.0	41
2.5	6	10.7	6.7	3.0	–	0.0	42
2	3	7.3	4.0	0.67	0.0	0.0	43
4	3	28.0	24.0	24.0	–	–	44
2.5	6	max score of 16.7–blindness after 7th day					32
0.5	6	max score of 2.0–cleared after 3 days					32
Washed							
2.5	6	max score of 5.3–cleared after 4 days					32
0.5	6	0.0	0.0	0.0	0.0	0.0	32

[a]Total score possible/animal/observation interval = 110.

Applications of 0.5 ml of the test solutions were made to clipped areas of intact and abraded skin. The treated areas were covered with gauze and wrapped to keep the test material in contact with the skin and to decrease the rate of vaporization. The wrapping and test material were removed 24 hours following application and the sites examined and scored separately for erythema and edema at 24 and 72 hours. The mean scores for 24- and 72-hour readings were averaged to determine the irritation index. Primary irritation indices were calculated to be 6.0, 2.4, and 1.0 for the 25%, 1.25%, and 2.5% solutions, respectively.[46,47]

The effect of Stearalkonium Chloride on skin swelling was studied using guinea pigs. After being soaked in water for one hour, squares of stratum corneum were lifted out of the water and their dimensions were determined. The squares were then immersed in a 20% solution of Stearalkonium Chloride for 16 hours, after which their dimensions were again measured. Swelling was expressed as the percentage increase in area after exposure to the second solution. Twenty percent Stearalkonium Chloride produced swelling of 1.6%, while sodium lauryl sulfate at a concentration of 13.5% produced swelling of 13.1%.[48]

Fish toxicity

Blueback salmon fingerlings (2 inch) were exposed to solutions of Stearalkonium Chloride at 19 °C for one hour and then placed in fresh water for observation. The concentration at which all fish survived exposure for two days was 1:800,000.[32]

Subchronic studies: dermal irritation

Hair was clipped from the backs and sides of six albino rabbits. Two ml of an aqueous solution of a trade product containing 0.2% Stearalkonium Chloride and 0.05% stearyl alcohol was applied to the clipped area of the skin once daily, five days a week for four weeks. The condition of the skin was monitored carefully, as were signs of toxicity and weight loss in the animals. At the conclusion of the experiment, the animals were sacrificed and representative tissues were examined histopathologically. The product caused a mild and transient erythema of the skin, but in no case were systemic effects apparent.[41]

Myristalkonium Chloride was applied to rabbits in a 20-day subchronic der-

mal test. Two rabbits each were used at the dose levels of 4 and 1 ml/kg. Two control animals each received a dose of 400 ml/kg of water. The hair was clipped from the backs and flanks of the rabbits, and one-half of each test area was abraded while the remainder was left intact. An aqueous solution containing 800 ppm (0.08%) of pure Myristalkonium Chloride was applied daily for 20 consecutive days to 10% of the total body surface. After each application of the test material, the torsos of the rabbits were wrapped with a rubberized fabric to prevent possible ingestion and/or inhalation of the material. The animals were observed for 14 days after the last application. Minimal erythema appeared in the 4 ml/kg group on Day five, with minimal edema being evident on Day nine. On Day 11, the erythema became more pronounced, and it persisted, along with minimal edema, through the rest of the treatment. At the 1 ml/kg dose level, there was minimal hyperemia with no edema. Though the hyperemia increased slightly in intensity on Day 15, it became minimal again on Day 19 and remained so through the rest of the treatment. Minimal edema was observed in this group on Days 17–21. Minimal hyperemia was observed in the controls from Days 11–21. All rabbits showed complete recovery within four days after treatment was stopped.[49]

Chronic studies

An unidentified alkyldimethylbenzyl ammonium chloride surfactant compound at concentrations of 0.063, 0.125, 0.25, or 0.5% was fed to four groups of 12 male rats in their diet for two years. An equal number of rats were used as controls. The animals that received 0.5% died early in the study. As Table 7 shows, weight gains for the first year were reduced among those surviving animals that received the lower doses. The only gross or microscopic pathologic changes were ". . . produced by irritation of the gastrointestinal tract. To an extent which depended on the concentration of the surfactant agents in the diet, this irritation prevented proper nutrition. In severe cases of irritation, death resulted."[50]

Special Studies

Teratology

Albino rats were used to evaluate the teratogenic potential of a 50% solution of Myristalkonium Chloride. Virgin, adult female rats were mated with young adult males, and the detection of vaginal sperm plug was considered to be Day 0 of gestation. Beginning on the sixth day and continuing through Day 15 of gestation, each rat received an appropriate quantity of test material to achieve a dose

TABLE 7. Chronic Feeding of an Undiluted Alkyldimethylbenzylammonium Chloride.[a]

Dietary Dose (%)	*No. of animals*	*Mean wt gain (g)*	*Standard error of mean*	*Significance probability*
0	11	471.9	±13.2	–
0.063	10	455.5	±21.6	–
0.125	10	417.4	±16.4	<0.05
0.25	7	297.8	±31.2	<0.001

[a]Data from Ref. 49.

of 0, 10, 25, or 50 mg/kg/day. The gavage vehicle was water. Water and aspirin were used for the negative and positive controls, respectively. On Day 20 of gestation, each dam was sacrificed and the fetuses removed. Among the treated groups, neither reproduction performance of the dam nor fetus weights differed from those of the control animals. The incidences of any skeletal abnormality and soft tissue abnormalities were no greater in the Myristalkonium Chloride groups than in the control groups. The incidence of both types of abnormalities was significantly greater in the aspirin-treated group. On the basis of this study, investigators concluded that daily oral doses of 10, 25, or 50 mg/kg of Myristalkonium Choloride during days six through 15 of pregnancy did not produce any indication of teratogenicity.[51]

Clinical Assessment of Safety

Skin Irritation and Sensitization: The Shelanski repeated insult patch test was used to determine the irritation/sensitization potential of Stearalkonium Chloride in humans. Fifty volunteers were treated with a 1% solution in water for 15 applications and then given a challenge application. Zero readings were obtained for all subjects, for all induction applications, and for the challenge dose. At this concentration, the material was shown to be neither a primary irritant nor a sensitizer. In a 50-subject test, it is possible to achieve 95% certainty that the test material will only sensitize 0–6% of the population if none of the 50 subjects show any indication of sensitization. Since all readings were zero, it was concluded that this material at the specified concentration was safe for use in contact with the human skin.[52]

In a second study, a cotton patch saturated with an aqueous solution of 20% Stearalkonium Chloride and 5% stearyl alcohol was placed on the inner surface of the forearm of 50 human subjects. The patch was covered with aluminum foil which was held in place with adhesive tape. Forty-eight hours following application, the patch was removed and the area inspected for signs of primary irritation. The solution produced a definite erythema in some subjects (number not reported). Two weeks after the first patch had been applied, the procedure was repeated on the other arm to test for sensitization; none resulted. The Stearalkonium Chloride used in this study was not a highly purified material. The primary irritation may have been due to impurities in the material or to the stearyl alcohol vehicle;[41] however, the latest (1979) diagnostic patch-test data from the North American Contact Dermatitis Group indicate that 30 percent stearyl alcohol is at most a minimal sensitizer.[53] A 0.8% Stearalkonium Chloride solution did not provoke irritation or sensitization.[41]

SUMMARY

Stearalkonium Chloride is a cationic quaternary ammonium salt used in cosmetic products at concentrations of ≤ 0.1–5%. It appears in cosmetics primarily for its surfactant and anti-microbial properties.

Studies have failed to establish with certainty the oral LD50 of Stearalkonium Chloride in rats, the value falling between 0.5 and 1.25 g/kg. In mice, an LD50 value of 0.760–0.113 g/kg was reported in a seven-day oral study. In single application dermal studies with concentrations of up to 25%, Stearalkonium

Chloride produced minor irritation in rabbits. According to acute eye studies in rabbits, a 25% solution of the material was a severe irritant. Concentrations of 1.25% and less were slightly and transiently irritating to the rabbit eye.

A repeated insult patch test with a 1% aqueous solution of Stearalkonium Chloride on 50 human subjects showed the material to be neither a primary irritant nor a sensitizer. A single 48-hour patch test with challenge two weeks later indicated that 20% Stearalkonium Chloride was not a sensitizer.

No subchronic, chronic, carcinogenicity, mutagenicity, or teratogenicity animal testing data were available to the Panel, nor was there substantial information on the absorption, metabolism, storage, and excretion of Stearalkonium Chloride.

Human safety data, namely irritation and sensitization studies are limited, and there is an absence of photosensitization studies.

CONCLUSION

On the basis of the evidence at hand, the Expert Panel concludes that the cosmetic ingredient, Stearalkonium Chloride, is safe when incorporated in cosmetic products in concentrations similar to those presently marketed.

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TABLE 29
Stearalkonium Chloride use

Product category	1976 use (Elder 1982)	2001 use (FDA 2001)	1976 concentrations (Elder 1982)	2001 concentrations (CTFA 2001)
Bubble baths	—	5	—	—
Hair conditioners	78	107	≤0.1%–5%	0.7%–7%
Hair sprays (aerosol fixatives)	9	3	≤0.1%–1%	—
Hair Straighteners	1	—	>0.1%–1%	—
Permanent waves	6	2	≤0.1%–5%	—
Rinses (noncoloring)	60	5	>0.1%–5%	3%
Shampoos (noncoloring)	—	4	—	2%
Hair tonics, dressings, etc.	4	14	≤0.1%–5%	2%–3%
Wave sets	8	2	≤0.1%	—
Other hair preparations	5	3	≤0.1%–1%	2%
Hair dyes and colors	21	—	≤0.1%–5%	0.5%–2%
Hair rinses (coloring)	47	—	>0.1%–5%	—
Hair bleaches	—	—	—	0.4%
Nail creams and lotions	1	—	>0.1%–1%	—
Nail polish and enamel	—	1	—	—
Other personal cleanliness products	—	1	—	—
Aftershave lotions	1	—	≤0.1%	—
Skin cleansing preparations	2	—	>0.1%–5%	—
Body and hand skin care preparations	—	2	—	—
Moisturizing skin care preparations	5	1	>0.1%–5%	0.3%
Other skin care preparations	1	1	>1%–5%	—
Totals/ranges	249	151	≤0.1%–5%	0.3%–7%

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TABLE 30
Wheat Germ Glycerides use

Product category	1976 use (Elder 1980a)	2001 use (FDA 2001)	1976 concentrations (Elder 1980a)	2001 concentrations (CTFA 2001)
Eyeliner	—	—	—	0.05%–2%
Eye shadow	3	—	>0.1%–1%	2%
Other eye makeup preparations	4	—	≤0.1%–1%	—
Hair conditioners	—	—	—	0.001%
Hair tonics, dressings, etc.	—	—	—	0.1%
Face powders	2	—	>0.1%–1%	—
Foundations	9	—	≤0.1%–1%	2%
Lipstick	114	126	≤0.1%–5%	0.3%–25%
Makeup bases	6	—	≤0.1%–1%	—
Other makeup preparations	—	1	—	0.3%
Cuticle softeners	—	1	—	2%
Deodorants (underarm)	1	—	>0.1%–1%	—
Aftershave lotions	—	—	—	0.4%
Cleansing preparations (cold creams, cleansing lotions, liquids, and pads)	8	—	≤0.1%–1%	—
Face and neck skin care preparations[a]	12	—	>0.1%–5%	—
Body and hand skin care preparations[a]		—		—
Hormone (creams, lotions)[b]	1	—	>0.1%–1%	—
Moisturizing preparations[c]	24	—	≤0.1%–1%	—
Wrinkle smoothing (removers)[c]	1	—	≤0.1%	—
Night (creams, lotions)	11	—	≤0.1%–5%	—
Skin fresheners	1	—	≤0.1%	—
Other skin care preparations	15	—	>0.1%–1%	—
Totals/ranges	212	128	≤0.1%–5%	0.001%–25%

[a]Originally, Face and Neck and Body and Hand were combined as one category, but now they are separated.
[b]No longer a product category.
[c]Wrinkle smoothing (removers) are now part of the Moisturizing category.

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STEARALKONIUM CHLORIDE

A safety assessment of Stearalkonium Chloride was published in 1982 with the conclusion that this ingredient is “safe when incorporated in cosmetic products in concentrations similar to those presently marketed” (Elder 1982). New studies, 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.

In 1976, Stearalkonium Chloride was used in 249 cosmetic products, with the largest single use in rinses (noncoloring) in the concentration range of >0.1% to 5%. In 2001, Stearalkonium Chloride was used in 151 products (FDA 2001), with the largest single use reported for hair conditioners. The highest concentration of use was also in hair conditioners (0.7% to 7%) in 2001 (CTFA 2001). Table 29 presents the available use information.

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TABLE 31
Triticum Vulgare (Wheat) Gluten use

Product category	1976 use (Elder 1980a)	2001 use (FDA 2001)	1976 concentrations (Elder 1980a)	2001 concentrations (CTFA 2001)
Mascara	1	2	≤0.1%	—
Other shaving preparations	—	1	—	—
Other skin care preparations	—	2	—	—
Totals/ranges	1	5	≤0.1%	—

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WHEAT GERM GLYCERIDES AND WHEAT GLUTEN, WHEAT FLOUR AND WHEAT STARCH, AND WHEAT GERM OIL

Safety assessments of Wheat Germ Glycerides and Wheat Gluten were published in 1980 with the conclusion that these two ingredients were "safe when incorporated in cosmetic products and constitute no risk to the public in its present cosmetic use of these products" (Elder 1980a). Wheat Flour and Wheat Starch were found to be "safe as cosmetic ingredients in the present practices of use and concentration" (Elder 1980b). Wheat Germ Oil was also found "safe as a cosmetic ingredient in the present practices of use and concentration" (Elder 1980c). New studies, along with the updated information below regarding uses and use concentrations, were considered by the CIR Expert Panel. The Panel determined to not reopen these safety assessments.

TABLE 32
Triticum Vulgare (Wheat) Starch use

Product category	1976 use (Elder 1980b)	2001 use (FDA 2001)	1976 concentrations (Elder 1980b)	2001 concentrations (CTFA 2001)
Hair conditioners	—	1	—	0.01%–0.6%
Hair sprays (aerosol fixatives)	—	1	—	0.001%
Permanent waves	—	—	—	0.001%–0.2%
Shampoos (noncoloring)	—	—	—	0.001%–0.2%
Hair tonics, dressings, etc.	—	5	—	0.1%
Hair dyes and colors	—	19	—	—
Face powders	4	2	>5%–25%	0.1%
Foundations	—	—	—	3%
Bath soaps and detergents	—	—	—	25%
Skin cleansing preparations	—	1	—	0.03%
Face and neck skin care preparations	—	1	—	—
Body and hand skin care preparations	—	3	—	0.1%
Night skin preparations	—	1	—	—
Paste masks (mud packs)	—	4	—	—
Other skin care preparations	—	1	—	—
Totals/ranges	4	39	>5%–25%	0.001%–25%

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2

Final Report on the Safety Assessment of Benzalkonium Chloride

Benzalkonium Chloride is a mixture of alkylbenzyldimethylammonium chlorides. The ingredient is used in cosmetic products as a foaming cleansing and bactericidal agent at concentrations up to 5.0%. The compound was nonmutagenic in several different cell assays. It is a skin and ocular irritant at concentrations greater than 0.1%. This cosmetic ingredient is not a sensitizer to normal humans at concentrations of 0.1%, but may be to individuals with diseased skin. It is concluded that Benzalkonium Chloride can be safely used as an antimicrobial agent at concentrations up to 0.1%.

CHEMISTRY

Benzalkonium Chloride (CAS No. 8001-54-5), is the U.S. Pharmacopeia (USP) name for alkyldimethylbenzylammonium chloride.[1] It is a mixture of alkylbenzyldimethylammonium chlorides that conforms generally to the formula[2] in Figure 1.

The R represents a mixture of alkyls, including all or some of the group beginning with n-C_8H_{17} and extending through higher homologs, with n-$C_{12}H_{25}$, n-$C_{14}H_{29}$, and n-$C_{16}H_{33}$ making up the major portion.[3] On the anhydrous basis, the content of n-$C_{12}H_{25}$ is not less than 40.0% and the content of n-$C_{14}H_{29}$ is not less than 20.0% of the total alkylbenzyldimethylammonium chloride content. The amounts of n-$C_{12}H_{25}$ and n-$C_{14}H_{29}$ together make up not less than 70.0% of the total. The total alkylbenzyldimethylammonium chloride content of Benzalkonium Chloride is not less than 97%.[3] Properties of Benzalkonium Chloride are listed in Table 1.

Benzalkonium Chloride is sold commercially as 50.0% or 80.0% solutions in water or alcohol, or in mixtures of water, ethyl alcohol, and isopropyl alcohol.[4] Solutions of Benzalkonium Chloride are alkaline and foam strongly when shaken.[5] Benzalkonium Chloride, in water or in methanol, has an ultraviolet (UV) absorption maximum at 262 nm; it does not absorb UV light at wave-

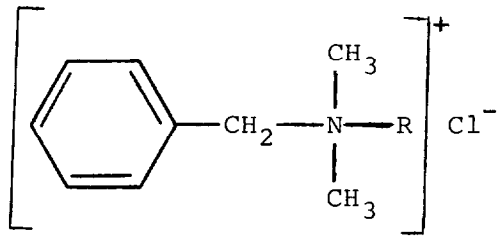

FIG. 1. Benzalkonium Chloride.

TABLE 1. Properties of Benzalkonium Chloride

Variables		*Reference*
Avg. molecular weight	360	3
Form	White or yellowish-white amorphous powder or gelatinous pieces	8
Odor	Aromatic	8
Taste	Very bitter	8
Solubility	Very soluble in water, alcohol, and acetone; slightly soluble in benzene. Almost insoluble in ether	8
Optimum pH	4.0–10.0; 1.0% solution pH 6.0–8.0	9
Stability	Stable at 121°C for 30 min	9
Flash point	482°F	10
Residue on ignition	Not more than 2.0%	3

lengths of 300 nm and above at concentrations up to 1.527 g/L.[6] Properties of 50.0% and 80.0% Benzalkonium Chloride solutions are listed in Table 2.

METHODS OF PRODUCTION

Benzalkonium Chloride is prepared by treating a solution of *N*-alkyl-*N*-methylbenzylamine in a suitable organic solvent with methyl chloride, the solvent being so chosen that the quaternary compound precipitates as it is formed.[5] It can also be made by preparing a primary amine from a fatty acid or a blend of fatty acids, then methylating the primary amine to form dimethyl alkylamine. This tertiary amine is then quaternized with benzyl chloride.[7]

TABLE 2. Properties of 50% and 80% Benzalkonium Chloride[4]

	50% Benzalkonium Chloride	*80% Benzalkonium Chloride*
Assay (%)	49.0–52.0	78.0–82.0
Free amines (% max)	1.0	1.6
pH		
5% aqueous	7.0–9.5	6.0–9.0
10% aqueous	6.5–8.5	7.2–8.0
Residue on ignition (% max)	2.0	2.0
Specific gravity		
at 20/20°C	0.97	0.92–0.94
at 25/25°C	0.93–0.96	—

REACTIVITY

The interaction of label adhesives with Benzalkonium Chloride was demonstrated in ophthalmic solutions packaged in plastic bottles. A component of each adhesive (monomeric plasticizer) migrated through the plastic and reacted with Benzalkonium Chloride in the ophthalmic solution. This reaction involved the loss of Benzalkonium Chloride, the formation of a turbid solution, and the deposition of blue-colored residues on the interior wall of the container. Because of the proprietary nature of commercial adhesives, neither the qualitative nor quantitative composition of adhesives tested was provided by the suppliers. Some of the plasticizers commonly used in adhesives are polypropylene (isotactic form), dibutyl phthalate, and polyisobutylene.[11]

A white precipitate is formed in a 1:3000 aqueous solution of Benzalkonium Chloride when nitrates are present at concentrations greater than the equivalent of 0.5% ammonium nitrate.[8]

Benzalkonium Chloride is incompatible with anionic detergents and some inorganic salts. It is unstable in the presence of strong oxidizing or reducing agents. When Benzalkonium Chloride is stored in closed containers, its stability is indefinite.[4]

ANALYTICAL METHODS

Benzalkonium Chloride has been identified by the following methods: gas chromatography,[12–14] high performance liquid chromatography (HPLC),[15–17] thin layer chromatography,[18] chemical ionization mass spectroscopy,[19] and a direct spectrophotometric assay using bromthymol blue.[20]

IMPURITIES

Information concerning impurities in Benzalkonium Chloride is not available.

USE

Purpose in Cosmetics

Benzalkonium Chloride has the following cosmetic uses: foaming and cleansing agent, conditioner, and bactericide.[7]

Scope and Extent of Use in Cosmetics

The cosmetic formulation listing made available by the Food and Drug Administration (FDA) is compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations, 1979. Ingredients are listed in prescribed concentration ranges under specific product type categories. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the value reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration in such a case would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a 2- to 10-fold error in the assumed ingredient concentration. Data submitted to the FDA in 1986 by cosmetic firms participating in the voluntary cosmetic registration program indicated that Benzalkonium Chloride was an ingredient in 83 cosmetic formulations at the following concentrations of use: ≤ 0.1% (21 products), > 0.1–1% (60 products), and > 1.0–5.0% (2 products) (Table 3).

TABLE 3. Product Formulation Data[21]

Product category	*Total no. of formulations in category*	*Total no. containing ingredient*	*No. of product formulations within each concentration range (percentage)*		
			> 1–5	*> 0.1–1*	*≤ 0.1*
Baby products	33	4	—	1	3
Eye make-up preparations	414	6	—	1	5
Hair shampoos, rinses, tonics, conditioners, and related preparations	2212	45	2	40	3
Personal cleanliness products	506	11	—	10	1
Skin cleansing preparations (cold creams, lotions, liquids, and pads)	729	6	—	5	1
Moisturizing skin care preparations	802	4	—	1	3
Skin care preparations (nonspecified)	1901	7	—	2	5
1986 Totals		83	2	60	21

Surfaces to which Applied

Cosmetic products containing Benzalkonium Chloride are applied to the skin, hair, and vaginal mucosa and may come in contact with the nasal mucosa and eyes.

Frequency and Duration of Application

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

Noncosmetic Use

Benzalkonium Chloride is commercially available as 2.0–5.0% aqueous solutions; it is employed at use concentrations of 0.1% (and less) as a germicide and sanitizer for chemically clean surfaces.[1] In sanitizing aqueous solutions, Benzalkonium Chloride consists principally of 12–16 carbon alkyl groups and contains not more than 1.0% of groups with 8 and 10 carbon

TABLE 4. OTC Panel Recommendations for Benzalkonium Chloride

Pharmaceutical use	*Advisory review panel*	*Category*[a]
Antimicrobial soap	Antimicrobial	PR, II SE
Health care personnel handwash	Antimicrobial	PR, III SE
Preoperative skin preparation	Antimicrobial	PR, III SE
Skin antiseptic	Antimicrobial	PR, III SE
Skin wound protectant	Antimicrobial	PR, III SE
Skin wound cleanser	Antimicrobial	PR, I
Surgical hand scrub	Antimicrobial	PR, III SE
Dandruff	Miscellaneous external drug products	ANPR, III E
Astringent	Miscellaneous external drug products	ANPR, II SE
Insect bite and sting treatment	Miscellaneous external drug products	ANPR, II SE
Antimicrobial	Oral cavity	ANPR, III SE
Minor irritations	Contraceptives	ANPR, III SE

[a]Category I: conditions under which OTC drug products are generally recognized as safe and effective and are not misbranded; category II: conditions under which OTC drug products are not generally recognized as safe and effective or are misbranded; category III: conditions for which the available data are insufficient to permit final classification at this time as category I or II.

ANPR, Advanced Notice of Proposed Rulemaking; PR, Proposed Rule; FR, Final Rule; S, Safety; E, Effectiveness.

atoms; the aqueous solutions may contain either ethyl or isopropyl alcohol as an optional ingredient.[22] Benzalkonium Chloride is commonly used as a preservative in ophthalmic medications and solutions for contact lenses, and is also a potent spermicide.[23,24] It appears in the list of inactive ingredients for approved prescription drug products prepared by the FDA.[25] Some over-the-counter drug uses of Benzalkonium Chloride and their respective safety evaluations are listed in Table 4.[26]

BIOLOGICAL PROPERTIES

Absorption and Distribution

The absorption of Benzalkonium Chloride by the vaginal mucosa was evaluated in three women (ages 23, 27, and 36 years old) using tampons containing Benzalkonium Chloride (60 mg). Venous blood samples (10 ml) were drawn 15 min prior to tampon application and during the following intervals after application: 15 min, 1 h, 3 h, and 24 h. The content of Benzalkonium Chloride in each blood sample was determined via HPLC (detection sensitivity < 50 ng/ml of blood). Benzalkonium Chloride was not detected in blood samples at any time during the study.[27]

In another study, the recovery of Benzalkonium Chloride from the blood and breast milk of four women using tampons containing Benzalkonium Chloride (60 mg) was evaluated. Venous blood and breast milk samples were taken 15 min prior to tampon application and 3 and 24 h after application. The HPLC method mentioned above was used for determining the content of Benzalkonium Chloride in each sample. Benzalkonium Chloride was not detected in any of the four subjects.[28]

A 50 μl drop of [^{14}C]-Benzalkonium Chloride solution was placed on the corneal surface (lids held open) of young and adult New Zealand albino or Dutch belted rabbits. Normal blinking resumed after the lower lid was elevated to prevent fluid runoff. At various intervals after administration, the eye was washed with at least 1 ml saline and the following tissues and fluids were removed: bulbar and palpebral conjunctiva, aqueous humor, corneal epithelium, endothelium and stroma, iris–ciliary body, lens, vitreous, retina, and choroid. A plasma sample was obtained by direct cardiac puncture. Multiple drops of the radioactive solution were also applied. After administration of one drop, Benzalkonium Chloride was found in the corneal epithelium, endothelium, and stroma, and in the bulbar and palpebral conjunctivae. At no time was radioactive material found in the aqueous humor or any other tissues. Benzalkonium Chloride loss from ocular tissues was such that about one-third to two-thirds of its concentration (depending on the tissue) at 30 min remained after 24 h; measurable values existed for as long as 120 h. The administration of multiple drops led to continued accumulation of Benzalkonium Chloride.[29]

Effect on Histamine Release

The effect of Benzalkonium Chloride on histamine release was evaluated using mixed cellular suspensions (11.0% mast cells) from peritoneal cavities of

Wistar rats (200–400 g). Benzalkonium Chloride concentrations of 0.3 and 3.0 μg/ml caused approximately 70.0 and 14.0% inhibition of histamine release from mast cells, respectively, in the presence of a potent histamine releaser. Concentrations of 10.0 and 30.0 μg/ml caused approximately 70.0% and 90.0% increases in histamine release, respectively. Benzalkonium Chloride also antagonized histamine release induced by ATP, bradykinin, polylysine, and curare, but not that induced by antigens, enzymes, monoamines, or detergents.[30] In another study, the challenge of rat mast cells (2.0–10.0% purity) with Benzalkonium Chloride concentrations of 1.0 and 5.0 μg/ml produced a concentration-dependent inhibition of histamine secretion.[31]

Effect on Enzymatic Activity

The effect of Benzalkonium Chloride on the hydrolysis of *p*-toluenesulfonyl-L-arginine methyl ester hydrochloride (substrate for trypsin and chymotrypsin) was evaluated. In separate experiments, trypsin and chymotrypsin (0.275 mg/ml) were each incubated with various concentrations of Benzalkonium Chloride (0.005–0.040 M) at 30°C and pH 3.0. Aliquots (10 μl) of the mixtures were removed over the next 48 h to assay for remaining activity. The substrate concentration in each assay was 0.0015 M. A sigmoid curve was obtained when the percentage inhibition of trypsin was plotted against Benzalkonium Chloride concentration and showed 50.0% inhibition with 0.015 M Benzalkonium Chloride. A similar curve was obtained for chymotrypsin, with 50.0% inhibition occurring at a 10-fold lower Benzalkonium Chloride concentration.[32]

ANIMAL TOXICOLOGY

Subchronic Inhalation Toxicity

An inhalation toxicity study of an aerosolized hair conditioner containing 0.2% of a 50.0% Benzalkonium Chloride solution (effective Benzalkonium Chloride concentration = 0.1%) was conducted with 12 female albino rats of the CD strain (mean weight 216 g) and 12 Syrian Golden hamsters (mean weight 88 g). Exposures were carried out in 500 L dynamic flow inhalation chambers. The animals were exposed to the conditioner (9.9 mg/m^3 of air) 5 days a week (4 h/day) for 14 consecutive weeks. Gravimetric analysis was used to determine the aerosol concentration in the chamber atmosphere; the desired concentration was maintained by adjusting the rate of airflow through each chamber. Chamber air flow rates ranged from 18 to 29 cubic feet/min. The body weights of all animals were recorded weekly. Hematologic and serum chemistry studies were conducted with blood samples obtained from rats after the 6th and 13th weeks of exposure. Gross and microscopic examinations of tissues from rats and hamsters were also conducted. There were no significant differences in weight gain, hematologic values, and serum chemistry data between experimental and control groups. There were no exposure-related deaths, and neither gross nor microscopic changes were attributed to Benzalkonium Chloride inhalation.[33]

Acute Oral Toxicity

The oral toxicity of Benzalkonium Chloride (composition: 60.0% C_{14}, 30.0% C_{16}, 5.0% C_{12}, and 5.0% C_{18}) was assessed in white rats (procedure, weights, and strain not stated). The LD_{50} was 525 mg/kg.[34] In another study, the mean acute oral LD_{50} in rats (number and strain not stated) was 400 mg/kg (range, 342–469 mg/kg). The experimental procedure was not stated (Table 5).[35]

The oral toxicity of a moisturizing cream containing 0.13% Benzalkonium Chloride was evaluated using 10 young adult Sprague Dawley rats (5 males, 5 females). Each animal was fasted for 18 h and then given a single dose (5 ml/kg) of the cream via oral gavage. The animals were observed for a period of 14 days postadministration. No toxic effects were observed, and none of the animals died.[36]

In another study, the oral toxicity of a cream containing 0.1% Benzalkonium Chloride was evaluated using 10 rats (weight range 130–180 g) of the Fischer 344 strain. A single dose of 7.0 ml/kg was administered to each animal via oral gavage. None of the animals died during the 2-week observation period (Table 5).[37]

Subchronic Oral Toxicity

Benzalkonium Chloride solutions were administered via stomach tube to 40 male albino rats (Sprague-Dawley, 130 g) once daily for 12 weeks at dosages of 50.0 mg/kg (2 groups of 10 rats) and 100.0 mg/kg (2 groups of 10). The 50.0 mg/kg and 100.0 mg/kg dosages were 1:20 and 1:10 dilutions of 10.0% Benzalkonium Chloride, respectively. Ten animals in both dose groups received Benzalkonium Chloride diluted with distilled water, while the remaining animals received Benzalkonium Chloride diluted with milk. Two control groups (10 rats/group) were given distilled water and milk, respectively. Hematologic studies were conducted at 4 and 12 weeks with rats receiving 100 mg/kg doses. Two rats receiving the 100.0 mg/kg dose died on days 62 and 69, respectively. Animals surviving the 12-week period were killed and examined macroscopically and microscopically. The growth rates of rats given 50.0 mg/kg (in water), 50.0 mg/kg (in milk), and 100 mg/kg (in milk) were similar to those of control groups throughout the experiment. Growth rates of rats given 100.0 mg/kg (in water) were depressed throughout the experiment; the average body weight at 12 weeks was 29.0% less than that of the control (water) group. In hematologic studies, there were no significant changes in any of the following values (all treatment groups): total number of erythrocytes and leukocytes, differential count, hematocrit, and hemoglobin values. Neither gross nor microscopic tissue changes related to Benzalkonium Chloride administration were noted in any of the treatment groups (Table 5).[38]

Chronic Oral Toxicity

The chronic oral toxicity of 10.0% Benzalkonium Chloride was evaluated in 18 beagle dogs (weight range 6.6–9.3 kg). Dosages of 12.5, 25.0, and 50.0 mg/kg (6 animals/dose) were administered via stomach tube once daily for

TABLE 5. Oral Toxicity of Benzalkonium Chloride

Type of study	*Animals tested*	*Test substance*	*Methodology*	*Results*	*References*
Short-term oral toxicity	White rats (no., weights, strain not stated)	Benzalkonium Chloride	—	LD_{50} = 525 mg/kg	34
Short-term oral toxicity	Rats (no., strain, and weights not stated)	Benzalkonium Chloride	—	LD_{50} = 400 mg/kg (range, 342–469 mg/kg)	35
Short-term oral toxicity	10 rats (Fisher 344 strain, 130–180 g)	Cream containing 0.1% Benzalkonium Chloride	Single oral dose of 7.0 ml/kg	No deaths or signs of toxicity	37
Subchronic oral toxicity	20 male albino rats (Sprague-Dawley strain, 130 g)	1:10 and 1:20 dilutions of 10.0% Benzalkonium Chloride	1:10 and 1:20 dilutions administered via stomach tube in doses of 100 mg/kg (10 rats) and 50 mg/kg (10 rats) once daily for 12 weeks	Two rats receiving 100 mg/kg died. No treatment-related gross or microscopic changes	38
Chronic oral toxicity	18 beagle dogs (three groups of six: 6.6–9.3 kg)	10.0% Benzalkonium Chloride (in water)	Doses of 12.5, 25.0, and 50.0 mg/kg (1 dose/group) administered via stomach tube once daily for 52 weeks	Four deaths: 1 dog (50 mg/kg dose), 3 dogs (25 mg/kg dose)	38

52 weeks. The solution was administered in milk to half of the animals, and in water to the remaining half. Two control groups (two animals/group) were given milk and water, respectively. Hematologic studies were conducted with all animals at 6-week intervals throughout the experiment. Urine specimens were also obtained at 6-week intervals. Necropsies were performed on all animals, and tissues were examined macroscopically and microscopically. Significant fluctuations in body weight were not noted in any of the treatment groups. The only deaths reported were one of the three dogs receiving 50.0 mg/kg doses in water and the three dogs receiving 25.0 mg/kg doses in water. In all treatment groups, there were no significant changes in the blood or urine attributable to Benzalkonium Chloride administration. Slight to moderate hyperemia of the small intestine and pyloric portion of the stomach was observed in dogs receiving 50 mg/kg doses in milk. No significant macroscopic lesions were observed in dogs receiving 12.5 or 25.0 mg/kg doses in milk. Moderate to severe irritation of the small intestine was observed in all dogs receiving 50.0 and 25.0 mg/kg doses in water and in two of three dogs receiving 12.5 mg/kg doses in water. The dogs receiving 50.0 and 25.0 mg/kg doses in water also had moderate to severe irritation and congestion of the stomach and intestines. No microscopic changes due to doses of Benzalkonium Chloride in milk were noted. However, congestion and subacute inflammation of the intestines were noted in animals dosed with 12.5 mg/kg of Benzalkonium Chloride in water. These observations were regarded as minor microscopic changes (Table 5).[38]

Ototoxicity

The ototoxicity of Benzalkonium Chloride was evaluated using 13 pigmented guinea pigs. Applications of a 0.1% Benzalkonium Chloride solution in distilled water (5 animals) and a 0.1% Benzalkonium Chloride solution in 70.0% alcohol (8 animals) were made onto the round window membrane of the middle ear via glass pipettes; the entire cavity of the bulla was filled. Exposure periods were of 10, 30, and 60 min duration, after which the bulla cavity was emptied by careful suction and repeatedly washed with physiological saline. Ten of the animals were killed after 2 weeks. Three of the animals exposed to Benzalkonium Chloride in alcohol for 6 min were killed after 9 weeks to evaluate the effect of prolonging the length of postoperative survival of the inner ear sensory epithelia. Microscopic examinations revealed fibrosis in tissues from the tympanic cavity (13 animals), cochlea (10 animals), and vestibulum (5 animals). Inner hair cell loss from the organ of Corti (13 animals) and destruction of vestibular neuroepithelia (8 animals) were also noted. The extent of damage to all tissues examined was more pronounced after 60 min exposures than after 10 and 30 min exposures and was increased by extending the postoperative survival time from 2 to 9 weeks. The application of different Benzalkonium Chloride solutions did not affect differences in the extent of damage to the tympanic cavity, cochlea, organ of Corti, and vestibular sensory epithelia. However, the extent of fibrous tissue formation in the vestibulum was less extensive after exposures (10 and 30 min) to Benzalkonium Chloride in alcohol. Differences were not noted after 60 min exposures.[39]

Dermal Toxicity

The dermal toxicity of Benzalkonium Chloride was evaluated in RFM_f/UnWg and BALB/c mice (4 and 10 weeks old). A disinfectant containing 50% Benzalkonium Chloride, 44–45% water, and 5–6% isopropanol was diluted with water to form 0.8, 3, and 13% Benzalkonium Chloride solutions (effective Benzalkonium Chloride concentrations of 0.1, 1.5, and 6.5%, respectively). The 50% Benzalkonium Chloride solution and its dilutions (0.1, 1.5, and 6.5% Benzalkonium Chloride) were each applied to 8 animals (total of 32 animals, 16 per strain). The test solutions (0.05 ml) were placed on the hair of each animal at the midline of the neck (between the base of the skull and the scapulae) and then rubbed in. Animals were observed for changes in appearance of the application site and body weight for approximately 1 month. Forty-eight animals served as controls for body weight. Six identical experiments were conducted (total of 192 experimental animals). The application sites in mice given the 0.1 and 1.5% solutions had a slightly unkempt appearance for only 3 or 4 days. Sites in animals treated with 6.5 and 50% solutions remained unkempt for several days. Also, small areas on the ears of some of these animals (number not stated) became hyperemic and then necrotic. The edges of necrotic areas on the ears eventually healed. Each animal treated with 6.5 and 50% solutions (number not stated) also had a 5 mm bald spot within the application site 4 weeks after application. A 10% reduction in body weight (2 days postapplication) was noted in animals receiving the 50% solution. After day 5, the rate of weight gain was the same as in the other groups. Animals receiving the 6.5% solution had a slight weight reduction and a growth rate, after day 5, similar to that of the 50% group. Weight reductions were not noted in the 0.1 and 1.5% dose groups. A total of 29 deaths (6 experiments) were reported, all having occurred within 72 h after application. Deaths were confined to animals treated with 6.5 and 50% Benzalkonium Chloride solutions. Results from necropsies of animals that died were as follows: absence of feed and feces in the alimentary tract, hyperemia of the nose and footpads (extravasated blood under the claws), and discoloration on the undersurface of the skin opposite the application site. Additional lesions were not found when visceral tissues from two mice were examined microscopically. The cause of death was not apparent.[40]

Cytotoxicity

The effect of Benzalkonium Chloride on the growth of epithelial cells and fibroblasts was evaluated in tissues from the prostate gland and heart, respectively. Prostatic tissue was excised from adult rats (ages not stated), and cardiac tissue from young rats (1–10 days old). Tissue fragments were treated with the following aqueous solutions of Benzalkonium Chloride: 0.01, 0.02, 0.033, and 0.10%, and cultured with adult cockerel plasma and human serum. The cultures were incubated for 10 days at 37.5°C. Cellular growth (change in size) rates were recorded by planimetric calculation of tissue fragment areas each day, as described by Bengmark et al.[41] All concentrations of Benzalkonium Chloride retarded the growth of epithelial tissue. The greatest degree of

retardation was achieved at a Benzalkonium Chloride concentration of 0.1%. Cellular growth rates were significantly different from controls at all dosages tested. The growth rates of cardiac fibroblasts from newborn animals were lower and more irregular than those of adult epithelial cells (prostate gland). Benzalkonium Chloride concentrations of 0.01 and 0.02% did not result in growth retardation of cardiac fibroblasts that was significantly different from controls. However, concentrations of 0.033 and 0.10% resulted in significant retardation. The greatest degree of growth retardation of cardiac fibroblasts was achieved with a Benzalkonium Chloride concentration of 0.1% (Table 6).[42]

The cytotoxicity of 0.007% Benzalkonium Chloride was evaluated using human conjunctival cell cultures. The cells remained in culture for 48 h (37°C). Each culture was then exposed to 0.5 ml of the test solution at temperatures of 4°C, 15°C, and 37°C during a 16 min period. Control cultures were exposed to a phosphate buffer solution. The exposure time causing 50.0% cell damage in cell culture (CDT_{50}) served as the cytotoxic parameter. The CDT_{50s} for 0.007% Benzalkonium Chloride were 91.0 ± 13.0 s at 4°C, 94.2 ± 11.9 s at 15°C, and 98.9 ± 12.1 s at 37°C. Cellular growth in control cultures was not affected by different experimental temperatures (Table 6).[43]

In another cytotoxicity study, suspension cultures of the murine P815 tumor cell line were exposed to 1.5 M Benzalkonium Chloride during 30 min, 2 h, and 4 h periods. The dosage resulting in 50.0% cytotoxicity (CD_{50}) was determined for each exposure period. CD_{50} values for one experimental trial were as follows: 7.2 ppm (0.5 h), 3.5 ppm (2 h) and 0.6 ppm (4 h). Similar data were obtained for a mouse lymphoma cell line tested with the same concentrations of Benzalkonium Chloride (Table 6).[44]

The hemolytic activity of Benzalkonium Chloride (4.2×10^{-5} M, 3.3×10^{-5} M, and 2.2×10^{-5} M) was assessed using defibrinated blood from rabbits. Mixtures of defibrinated blood (0.05 ml) and aqueous Benzalkonium Chloride (5.0 ml) were incubated in a water bath for 45 min at 37°C, after which the unhemolyzed cells were settled by centrifugation and absorbance readings of the hemolysate were determined with a photoelectric colorimeter. Each absorbance reading was compared with a total hemolysis reading, obtained by laking red cells in distilled water. In determining the hemolytic activity of each of the test solutions, 0.6% sodium chloride was added as an extracellular agent to protect the erythrocytes from simple osmotic hemolysis. The degree of hemolysis occurring in each test solution was expressed as the percentage of total hemolysis. Benzalkonium Chloride concentrations of 2.2×10^{-5} M, 3.3×10^{-5} M, and 4.2×10^{-5} M resulted in 10.0, 50.0, and 100.0% hemolysis, respectively. These data represented the average of a minimum of two, but usually four, similar experiments (Table 6).[45]

Ocular Irritation

The ocular irritation potential of Benzalkonium Chloride was evaluated using 108 rabbits (strain not stated). Aqueous solutions of the test substance (2.0, 1.0, 0.5, 0.1, and 0.01%) were each instilled (one drop) into both eyes twice a day for 7 days; globes were examined grossly and photographed at different time intervals. Animals were killed after the seventh instillation and

TABLE 6. Cytotoxicity of Benzalkonium Chloride

Cells tested	*Test substance*	*Methodology*	*Results*	*References*
Epithelial cells (prostate gland) and fibroblasts (heart) from rats	0.10, 0.033, 0.02 and 0.01% Benzalkonium Chloride	Cell cultures incubated with Benzalkonium Chloride for 10 days	0.10 and 0.033% Benzalkonium Chloride caused significant growth retardation	42
Human conjunctival cells	0.007% Benzalkonium Chloride	Cell cultures exposed to 0.5 ml of test solution for 16 min	Exposure period resulting in 50% cellular damage was 98.9 $\pm$ 12.1 s at 37°C	43
Murine suspension cultures of P815 tumor cell line	1.5 M Benzalkonium Chloride	0.5, 2, and 4-h exposures	Dosages resulting in 50% cytotoxicity were: 7.2 ppm (at 0.5 h), 3.5 ppm (at 2 h), and 0.6 ppm (at 4 h)	44
Defibrinated blood from rabbits	4.2×10^{-5} M, 3.3×10^{-5} M, and 2.2×10^{-5} M Benzalkonium Chloride	Mixtures of blood (0.05 ml) and Benzalkonium Chloride (5.0 ml) were incubated for 45 min	2.2×10^{-5} M, 3.3×10^{-5} M, and 4.2×10^{-5} M concentrations caused 10.0%, 50.0%, and 100.0% hemolysis, respectively	45

ocular tissues were examined microscopically. The 2.0% solution caused conjunctival necrosis, ulceration and haziness of the cornea, and severe iritis, all observed initially on the first day of instillation. By the seventh day, the cornea was vascularized and cloudy; significant tissue damage was also noted. The 1.0% solution caused cloudiness of the cornea and severe injection of the bulbar and palpebral conjunctivae, with some areas of necrosis and large amounts of mucous material. With the 0.5% solution, most of the damage was limited to the bulbar and palpebral conjunctivae, with only occasional corneal damage. No gross damage was noted after instillation of the 0.01 and 0.1% solutions. At microscopic examination, almost total destruction of the corneal epithelium and endothelium of eyes treated with 1.0 and 2.0% solutions was noted. Severe damage to the corneal epithelium was also noted after 7 days of treatment with the 0.5% solution. When eyes were treated with 0.1% Benzalkonium Chloride solution 5 times daily (2 h intervals) for 1 week, damage to corneal endothelial cells was evident (Table 7).[46]

In another ocular irritation study, 50.0% Benzalkonium Chloride was tested at a concentration of 0.65% in water (effective Benzalkonium Chloride concentration = 0.3%). The test solution (0.1 ml) was instilled once into 1 eye of each of 6 rabbits. The eyes were not rinsed. Ocular irritation was scored on days 1, 2, and 3 postinstillation according to the scale used by Draize[47]: 0–110. The total ocular irritation score was 2 on day 1. Irritation had cleared by day 3. The test solution had the potential to induce minimal ocular irritation (Table 7).[48]

The ocular irritation potential of a moisturizing cream containing 0.13% Benzalkonium Chloride was evaluated according to the procedure of Draize.[47] The cream (0.1 ml) was instilled once into one eye of each of six female New Zealand White rabbits. Untreated eyes served as controls. Ocular reactions were scored 1, 2, 3, 4, and 7 days after instillation according to the Draize[47] scale. The moisturizing cream did not induce ocular irritation in any of the animals tested.[49]

In another study, the ocular irritation potential of 0.1% Benzalkonium Chloride was evaluated using 6 young adult New Zealand albino rabbits (male and female). The test substance was instilled into one eye (directly on the cornea) of each animal in volumes of 0.01, 0.03, and 0.10 ml; untreated eyes served as controls. Eyes were examined and scored according to the scale by Draize et al.[50] at 1, 3, 7, 14, and 21 days after instillation. The test substance was not an ocular irritant (Table 7).[51]

Intraocular Toxicity

Benzalkonium Chloride was instilled into the eyes of rabbits and guinea pigs (number not stated) at concentrations of 0.1, 0.5, 1.0, and 10.0%. Corneas were examined microscopically at 4 h and 1 and 7 days postinstillation. At 4 h and day 1, desquamation and necrosis of epithelial cells and leukocytic infiltration were observed in rabbit and guinea pig corneas treated with 0.5, 1.0, and 10.0% Benzalkonium Chloride. Desquamation and necrosis were noted in rabbit corneas 4 h after instillation of 0.1% Benzalkonium Chloride; no microscopic changes were noted in guinea pig corneas. At day 1, desqua-

TABLE 7. Ocular Irritation of Benzalkonium Chloride

Animals tested	*Test substance*	*Methodology*	*Results*	*References*
108 rabbits (strain not stated)	2.0, 1.0, 0.5, 0.1, and 0.01% Benzalkonium Chloride	All five solutions instilled into both eyes twice daily for 7 days. A 0.1% solution instilled into eyes five times daily for 1 week	2.0% solution caused conjunctival necrosis, ulceration, and haziness of the cornea and massive iritis. The 1.0 and 0.5% solutions caused damage to the cornea and bulbar and palpebral conjunctivae. The 0.1 and 0.01% solutions (instilled twice daily) did not cause ocular damage. 0.1% solution (instilled five times daily) damaged corneal endothelium	46
6 rabbits (strain not stated)	0.3% Benzalkonium Chloride	Aqueous solution instilled once into one eye (no rinsing). Ocular irritation scored according to Draize (1959) scale	Minimal ocular irritation Draize score, 2 (max = 110)	48
6 New Zealand albino rabbits	0.1% Benzalkonium Chloride	Instilled into one eye	No ocular irritation	51

mation and necrosis of epithelial cells and leukocytic infiltration were noted in rabbit corneas treated with 0.1% Benzalkonium Chloride; microscopic changes were not noted in guinea pig corneas. Desquamation, necrosis, and leukocytic infiltration were also observed 7 days after the instillation of 0.5, 1.0, and 10.0% Benzalkonium Chloride (rabbits) and 1.0 and 10.0% Benzalkonium Chloride (guinea pigs) (Table 8).[52]

The toxic effects of Benzalkonium Chloride on the corneal epithelium were evaluated using four to six New Zealand albino rabbits. Drops of 0.01% Benzalkonium Chloride in 0.9% saline (neutral pH) were instilled into both eyes. An additional four to six rabbits received instillations of 0.9% saline (negative control). Corneal specimens were examined using scanning electron microscopy (SEM) at 5, 15, and 30 min, and 1, 3, and 6 h after instillation. Specimens were prepared for SEM according to the procedure of Pfister.[53] Results were as follows: peeling of cells at borders, exposing cells beneath (5 min postinstillation); many adjacent cells separated from one another (15 min); most of the surface epithelial cells lying loosely on corneal surface with extensive disruption of plasma membranes (30 min); most of the surface cells prematurely desquamated, exposing prominent nuclear bulges in second cell layer (1 h); second layer of cells desquamating, extensive plasma membrane damage (3 h); newly exposed third cell layer had nearly normal epithelial cell appearance (6 h). Damage to the corneal epithelium was not noted in the negative control group (Table 8).[54]

The effect of Benzalkonium Chloride on the corneal endothelium was evaluated via in vivo and in vitro methods in another study involving New Zealand White rabbits (number not stated). In the in vitro study, corneas were excised from freshly enucleated eyes. The endothelial surface of each was flooded with 0.5 ml of 0.01% Benzalkonium Chloride (pH 6.47); corneas remained in solution for 2 min. Specimens were then examined via SEM and transmission electron microscopy (TEM). Ruptured endothelial cells with exposed nuclei were observed in SEMs. The disorganization of cellular structures, due to cellular edema, was observed in TEMs. In the in vivo study, aqueous humor (0.1 ml) was removed from each globe and replaced with 0.1 ml 0.01% Benzalkonium Chloride. Animals were killed 1.5 h, 3 h, or 2 days after injection and the corneas were excised. Specimens were then prepared for SEM and TEM. Edema of the corneal endothelium was noted 1.5 h, 3 h, and 2 days after injection. Endothelial cells were fusiform in shape and the normal hexagonal pattern was gone. Large areas of bare Descemet's membrane had overlying cellular debris that may have represented remains of the endothelial cells. Other ultrastructural damage induced by Benzalkonium Chloride included mitochondrial swelling, dilation of endoplasmic reticulum, intracytoplasmic vacuole formation, and ruptured plasma membranes (Table 8).[55]

The ocular toxicity of 0.007 and 0.01% Benzalkonium Chloride was evaluated in four albino and four pigmented rabbits. In one group of animals (two pigmented, two albino), 0.2 ml of 0.007% Benzalkonium Chloride (in water and sodium phosphate, pH 7.37) was introduced into the eyes via subconjunctival injection. Injections were repeated once daily for 2 weeks. In the other group (two pigmented, two albino), 0.01% Benzalkonium Chloride (in water and sodium phosphate, pH 6.77) was introduced according to the same

TABLE 8. Intraocular Toxicity of Benzalkonium Chloride

Animals tested	*Test substance*	*Methodology*	*Results*	*References*
Rabbits (no. not stated)	10.0, 1.0, 0.5, and 0.1% Benzalkonium Chloride	Single instillation. Corneas examined at 4 h and 1 and 7 days postinstillation	Desquamation and necrosis of corneal epithelium (10.0, 1.0, 0.5, and 0.1% concentrations)	52
New Zealand albino rabbits (no. not stated)	0.01% Benzalkonium Chloride	Drops instilled into both eyes. Corneas examined at 5, 15, and 30 min and 1, 3, and 6 h after instillation	Desquamation of epithelial cells and extensive disruption of plasma membranes	54
New Zealand White rabbits (no. not stated)	0.01% Benzalkonium Chloride	Excised corneas flooded with solution for 2 min	Ruptured endothelial cells with exposed nuclei. Severe cellular edema	55
New Zealand White rabbits (no. not stated)	0.01% Benzalkonium Chloride	Aqueous humor removed from each eye and replaced with 0.01 ml of test substance	Swelling of corneal endothelium, ruptured plasma membranes, dilation of endoplasmic reticulum, and mitochondrial swelling	55
8 Albino and pigmented rabbits (two albinos and two pigmented/group)	0.01 and 0.007% Benzalkonium Chloride	Subconjunctival injection of both solutions (0.1 ml) once daily for 2 wks. Eyes examined via ophthalmoscopy (daily) and electron microscopy	Extensive elevation of retina and retinal detachment in both treatment groups	56
New Zealand White rabbits (no. not stated)	0.001, 0.0004, and 0.0001% Benzalkonium Chloride	Each concentration in contact with corneal epithelium 30 to 110 min. Corneas examined via scanning electron microscopy	No discernible modification of surface morphology	57
Albino rabbits (no. not stated)	6.5×10^{-6} to 6.5×10^{-3}% Benzalkonium Chloride	Corneal endothelium perfused for 3 h	Severe endothelial cellular damage	58
Guinea pigs (no. not stated)	10.0, 1.0, 0.5, and 0.1% Benzalkonium Chloride	Single instillation. Corneas examined at 4 h and 1 and 7 days postinstillation	Desquamation and necrosis of corneal epithelium (10.0, 1.0, and 0.5% concentrations)	52

procedure. Ophthalmoscopy was performed every day to determine changes in the fundus. After the 2-week administration period, animals were killed and the eyes enucleated. Specimens were then prepared for electron microscopy. No abnormalities of the fundus were observed during the initial 3 days of administration. After the first week of administration, extensive elevation of the retina was noted in two of the four animals receiving the 0.007% solution. Three of the four animals receiving the 0.01% solution also had extensive elevation of the retina after the first week. After 2 weeks, retinal detachment was observed only in the globes of pigmented rabbits in both treatment groups. In electron micrographs, both early and prolonged stages of retinal detachment were observed. The inner retinal layers, especially the nerve fiber layer and inner plexiform layer, had marked edema during the early stage; outer segments of photoreceptors were relatively preserved. In the prolonged state, photoreceptors were atrophic, the outer segments and/or inner segments had disappeared, and, on some occasions, the number of nuclei in the outer nuclear layer had decreased (Table 8).[56]

The effect of Benzalkonium Chloride on corneal morphology was evaluated in New Zealand White rabbits (number not stated). Corneas were removed and bathed in aerated solutions (34°C, pH 7.4) of the following composition: 103.4 mM NaCl, 15.3 mM Na_2SO_4, 10 mM $NaHCO_3$, 2.2 mM K_2HPO_4, 0.5 mM KH_2PO_4, 5.24 mM H_3PO_4, 0.61 mM $MgSO_4$, 0.7 mM calcium gluconate, 26 mM glucose, and 20 mM tris-(hydroxymethyl) aminomethane (Tris). Benzalkonium Chloride was added to solutions at concentrations of 0.001, 0.0004, and 0.0001%; solutions remained in contact with the epithelial surface for 30–110 min. Corneas were then fixed and examined via SEM. One hour after exposure to 0.001% Benzalkonium Chloride, corneal surface cells were loosened or removed, exposing second- and third-layer cells. Also, the plicate appearance of surface cells was lost and some deeper cells had abnormally long microvilli. An increase in the number of cells with peripheral loss of microvilli and microplicae was noted 1 h after exposure to 0.0004% Benzalkonium Chloride. No discernible modification of surface morphology was noted after 2 h of exposure to 0.0001% Benzalkonium Chloride (Table 8).[57]

The effect of Benzalkonium Chloride on the corneal endothelium was investigated using albino rabbits (number not stated). The eyes were enucleated (complete with conjunctival sac and eyelids) and corneas were prepared and mounted in a specular microscope. After a 1 h stabilization period, the corneal endothelium was perfused for 3 h with Benzalkonium Chloride at concentrations ranging from 6.5×10^{-6} to 6.5×10^{-3}% in Ringer's solution. Observations of the corneal endothelium and sequential measurements of corneal thickness were made during the 3 h period. A change in corneal thickness was determined by a computer-fit linear regression line (minimum of five rabbit corneas). Corneas were removed from the specular microscope after perfusion and then fixed and submitted for SEM and TEM. No swelling was noted in corneas perfused with 6.5×10^{-6}% Benzalkonium Chloride. Minimal swelling was noted in those perfused with 6.5×10^{-5}% Benzalkonium Chloride (9.3 μm/h). The corneal swelling rate increased as a function of increasing Benzalkonium Chloride concentration. In SEMs of corneas perfused

6.5×10^{-3}% Benzalkonium Chloride, severe endothelial cell damage was noted. The following changes were observed in TEMs: disorganization of nuclear chromatin, severe damage to the endoplasmic reticulum and mitochondria, and discontinuity of the posterior plasma membrane (Table 8).[58]

Skin Irritation

The skin irritation potentials of 50, 10, 1, 0.1, and 0.01% Benzalkonium Chloride solutions were evaluated in rabbits (number and strain not stated). The solutions were applied in 0.5 ml volumes (duration of exposure not stated). Solutions of 1.0% Benzalkonium Chloride or greater induced skin reactions ranging from erythema (1% of animals tested) to necrosis (50% of the animals tested) (Table 9).[59]

Solutions of 0.1, 1, 5, and 10% Benzalkonium Chloride in water were applied to the clipped skins of five rabbits (strain not stated) via Finn chambers containing occlusive patches. The chambers remained in place for 24 h. Visual examinations were used to identify papules, vesicles, pustules, induration, necrosis, scaling, and scarring. Reactions were assessed daily for 4–5 days. Severe induration and a light yellow staining of test sites were observed in all animals treated with the four concentrations of Benzalkonium Chloride (Table 9).[60]

Aqueous solutions of 0.1, 1.0, and 5.0% Benzalkonium Chloride were applied to the epilated flanks of female albino guinea pigs (approximate weights = 300 g) via patches made of filter paper. Each patch was covered with impermeable tape and fastened with an elastic bandage. Two or three test patches were applied to one flank of each animal. Control patches saturated with water were applied to the contralateral flank. Patches were removed after 24 h and skin specimens were excised and examined microscopically. Twenty-four hour exposures to 0.1% Benzalkonium Chloride did not cause microscopic changes. Exposure to the 1.0% solution resulted in spotted areas of necrosis with nuclear pyknosis of the epidermal cells in the upper part of the stratum Malpighii (beneath the stratum corneum). Exposure to the 5.0% solution resulted in total necrolysis of the epidermis (Table 9).[63]

In another study, a 2.0% Benzalkonium Chloride solution was applied to abraded and intact skins of rabbits (number and strain not stated) via a synthetic cloth worn by each animal for 2 days. Severe erythema, edema, and rawness were observed in abraded and intact skin after 2 days. Slight erythema and skin sloughing were noted 7 days after application. No toxic signs were noted in rabbits (number and strain not stated) in a study in which a cloth impregnated with 2% Benzalkonium Chloride was worn continuously during a 3-week period (Table 9).[59]

The skin irritation potentials of 0.1 and 1.0% Benzalkonium Chloride were evaluated in 40 white rats (strain not stated). The test solutions were applied over a period of 3 months. After 1.5–2 months, the 1% solution induced hyperemia and necrotic changes. An intense epithelialization skin defect was noted after scabs had been shed. Benzalkonium Chloride was also applied to

TABLE 9. Skin Irritation of Benzalkonium Chloride

Animals tested	*Test substance*	*Methodology*	*Results*	*References*
Rabbits (no. and strain not stated)	50.0, 10.0, 1.0, and 0.1% Benzalkonium Chloride	Solutions applied to skin in volumes of 0.5 ml	Concentrations of 1.0%, or greater, induced reactions ranging from erythema to necrosis	59
5 Rabbits (strain not stated)	10.0, 5.0, 1.0 and 0.1% aqueous Benzalkonium Chloride solutions	Solutions applied to clipped skin via Finn chambers containing occlusive patches (24-h exposure)	All concentrations caused severe induration of test sites in five rabbits	60
Rabbits (no. and strain not stated)	2.0% Benzalkonium Chloride	Applied to abraded and intact skin via synthetic cloth worn for 2 days	Severe erythema, edema, and rawness after 2 days (abraded and intact skin). Slight erythema and skin sloughing after 7 days	59
Rabbits (no. and strain not stated)	2.0% Benzalkonium Chloride	Cloth, impregnated with solution, worn continuously during 3-wk period	No signs of toxicity	59
Rabbits (no. and strain not stated)	0.5% Benzalkonium Chloride	Single 24-h application	Severe erythema, eschar formation, and moderate edema	59
9 Albino rabbits	0.5% Benzalkonium Chloride	Single 24-h application (occlusive patch) to clipped skin of back	Practically no skin irritation potential. Primary irritation index = 0.17 (max = 8)	61
9 Rabbits (strain not stated)	0.3% Benzalkonium Chloride	Single 24-h application (occlusive patch) to clipped skin of back	No skin irritation	62
Rabbits (no. and strain not stated)	0.1% Benzalkonium Chloride	Applied to skin for 5 days. Sites covered with plastic wrap	Slight erythema and necrosis persisted 3 wks after treatment period	59
Albino guinea pigs (no. and strain not stated)	5.0, 1.0, and 0.1% Benzalkonium Chloride	Applied to epilated flanks via patches (filter paper). Patches removed after 24 h and skin specimens excised and examined microscopically	5.0% solution caused total necrolysis of epidermis. 1.0% solution caused spotted areas of necrosis in epidermis	63
30 Guinea pigs (strain not stated)	0.5 and 0.1% Benzalkonium Chloride	Two and 5 applications of 0.5 and 0.1% solutions, respectively	Hyperemia observed in all animals	64
40 White rats (strain not stated)	1.0% Benzalkonium Chloride	Solutions applied over 3-month period (method not stated)	1.0% solution induced hyperemia and necrotic changes	64

the skins of 30 guinea pigs (strain not stated) at concentrations of 0.5 and 0.1%. Two applications of the 0.5% solution and five applications of the 0.1% solution were made. Hyperemia was observed in all animals (Table 9).[64]

The skin irritation potential of 0.5% Benzalkonium Chloride was evaluated in rabbits (number and strain not stated). Following a single 24-h application of the test substance (0.5 ml), severe erythema, eschar formation, and moderate edema were noted (Table 9).[59]

The skin irritation potential of Benzalkonium Chloride was evaluated using nine female albino rabbits. A 50.0% Benzalkonium Chloride solution was tested at a concentration of 1.0% in water (effective Benzalkonium Chloride concentration = 0.5%). The test solution (0.5 ml) was applied once to the back (clipped skin) of each animal via an occlusive patch. Patches remained for 24 h. Each site was scored 2 and 24 h after patch removal according to the scale 0 (no irritation or edema) to 4 (deep red erythema with vesiculation or weeping, with or without edema). Barely perceptible erythema was noted in one rabbit at 2 and 24 h postapplication. There were no other observations of skin irritation. The primary irritation index was 0.17 (maximum = 8). The test solution had practically no potential for inducing skin irritation.[61] In another skin irritation study, a 50.0% Benzalkonium Chloride solution was tested at a concentration of 0.65% in water (effective Benzalkonium Chloride concentration = 0.3%) according to the same protocol. Skin irritation was not observed in any of the 9 rabbits tested (Table 9).[62]

In another study, the skin irritation potential of a moisturizing cream containing 0.13% Benzalkonium Chloride was evaluated using six female New Zealand White rabbits (2.6–3.1 kg). The cream (0.5 ml) was applied via a topical, dry patch to dorsal skin that had been clipped free of hair. A total of three 24-h applications were made. Sites were scored 30 min after patch removal according to the scales 0 (no erythema) to 4 (severe erythema to slight eschar formation); 0 (no edema) to 4 (severe edema). No positive reactions were observed.[65]

A 0.1% aqueous solution of Benzalkonium Chloride was applied to the skins of rabbits (number and strain not stated); sites were covered with plastic wrap. The test substance remained in contact with the skin for 5 days. At the end of the treatment period, necrosis and varying degrees of erythema, with diffuse areas of eschar and bleeding, were noted. Slight erythema and necrosis persisted for 3 weeks posttreatment. No systemic toxic signs or hematologic changes were observed (Table 9).[59]

Teratogenicity

Single doses (0, 25, 50, 100, and 200 mg/kg) of aqueous Benzalkonium Chloride solutions were instilled (1 ml/kg) into the vaginas of adult, nulliparous female Wistar rats (169–203 g; groups of 6–8 rats). Dams were killed via CO_2 inhalation on day 21 of gestation. The fetuses were removed by cesarean section and all were examined for viability and external malformations. Two-thirds of the live fetuses from each litter were examined stereomicroscopically for skeletal abnormalities after staining with Alizarin red S. The remainder were fixed in Bouin's fluid and examined for visceral anomalies, using the

freehand razor blade sectioning method of Wilson.[66] A significant reduction in maternal body weight gain was noted on day 6 of pregnancy in dams receiving the 200 mg/kg dose. On days 15 and 20, maternal body weights, when compared to controls, were markedly reduced in groups receiving 100 and 200 mg/kg doses. Reductions in body weight were attributed to small litter sizes and decreased litter weights, since postcesarean body weights, without uterine contents, were similar to those of the control group. Also, vaginas of all necropsied rats given the 100 and 200 mg/kg doses were inflamed. Statistically significant, dosage-related reductions in the mean numbers of live fetuses and litter weights were noted in groups dosed with 50, 100, or 200 mg/kg solutions. No visceral anomalies were observed in Benzalkonium Chloride-exposed fetuses; however, sternal defects (absent, or nonaligned sternebrae, or retarded ossification) were more frequent in the fetuses of 100 and 200 mg/kg-treated dams. There was also a reduction in the number of implantations in treated animals. The mean number of implantations in dams treated with the 200 mg/kg solution was significantly reduced in comparison with the control group (5.4 ± 1.1 vs. 10.8 ± 0.5; $p < 0.05$).[67]

Mutagenicity

The mutagenic potential of Benzalkonium Chloride was evaluated in microbial test systems using the rec-assay in combination with reverse mutation systems.[68] The rec-assay is a simple method capable of detecting DNA-damaging capacity by analyzing differences in growth sensitivities of Rec^+ and Rec^- mutant cells of *Bacillus subtilis*. The bacterial strains used in the reverse mutation systems were TA 1535, 1536, 1537, and 1538 (*Salmonella typhimurium*), and two tryptophan-requiring mutants of *E. coli* (B/r WP2 hcr^+ and WP2 hcr^-). Benzalkonium Chloride was not mutagenic in this test system (Table 10).[69]

Both reversion and rec-assays were used to evaluate the mutagenic potential of Benzalkonium Chloride in another study. In the reversion assays, two tryptophan-requiring strains of *Escherichia coli* (B/r try WP2 and WP2 try hcr) and four strains of *Salmonella typhimurium* requiring histidine and biotin (TA 1535, TA 1536, TA 1537, and TA 1538) were used. A 0.1 ml sample of each bacterial culture was incubated on agar with 0.02 ml of the test solution for 2 days at 37°C. *Bacillus subtilis* strains H17 rec^+ and M45 rec^- were used for the rec-assay. The rec assay was essentially based on the procedure by Kada.[68] Two cultures were incubated on agar for 24 h with 0.02 ml of the test substance at 37°C. Both assays were done in the absence of metabolic activation. Benzalkonium Chloride was not found to be mutagenic (Table 10).[70]

The mutagenic potential of Benzalkonium Chloride was evaluated in the standard plate incorporation assay and the Rosenkranz *E. coli* DNA polymerase A^- assay. *Salmonella typhimurium* strains TA 98, TA 1538, TA 1537, and TA 100 were tested in the plate incorporation assay.[72] Each strain was incubated with the test substance for 48 h (37°C) in the presence or absence of metabolic activation. Incubation was carried out either in the dark or in the presence of a combination of fluorescent (15 W) and incandescent (40 W)

TABLE 10. Mutagenicity of Benzalkonium Chloride

Bacterial strains	*Methodology*	*Results*	*References*
Salmonella typhimurium strains: TA 1535, TA 1536, TA 1537, and TA 1538	Rec-Assay in combination with reverse mutation systems (Kada, 1972)	Not mutagenic	69
E. coli strains: B/r WP2 hcr^+ and WP2 hcr	Rec-Assay in combination with reverse mutation systems (Kada, 1972)	Not mutagenic	69
E. coli strains: B/r try WP2 and WP2 try hcr	Reversion Assay: 0.1 ml sample of each culture incubated on agar with 0.2 ml of Benzalkonium Chloride for 2 days (37°C)	Not mutagenic	70
Salmonella typhimurium strains: TA 1535, TA 1536, TA 1537, and TA 1538	Reversion Assay: 0.1 ml sample of each culture incubated on agar with 0.2 ml of Benzalkonium Chloride for 2 days (37°C)	Not mutagenic	70
Bacillus subtilis strains: H17 rec^+ and M45 rec^-	Rec-Assay (Kada, 1972)	Not mutagenic	70
Salmonella typhimurium strains: TA 98, TA 1538, TA 1537, and TA 100	Plate incorporation assay (Ames et al., 1975)	Not mutagenic	71
E. coli strains: W3110 ($polA^+$) and p3478 ($polA^-$)	*E. coli* DNA polymerase assay (Rosenkranz et al., 1976)	Genetic toxicity	71

lights (350–750 nm emission at 24 inches). The plates were then scored for His^+ revertant colonies. Benzalkonium Chloride (10–100 μg/plate) did not induce mutagenicity in any of the strains in the presence or absence of metabolic activation. The *E. coli* DNA polymerase assay[73] was used because it detects repairable DNA damage and complements the Ames assay. Strains W3110 (pol A^+) (wild-type) and p 3478 (pol A^-) of *E. coli* were each incubated with 20 μl of Benzalkonium Chloride for 24 h (37°C), either in the dark, or illuminated according to the procedure described above for the *Salmonella* assay. For a given treatment, a zone of inhibition on a plate containing the polymerase-deficient strain that was larger than that on plates containing the wild-type strain was an indication of genetic toxicity. Benzalkonium Chloride induced repairable DNA damage. Its genetic toxicity was also enhanced in the presence of visible light (Table 10).[71]

Tumorigenicity

The tumorigenicity of Benzalkonium Chloride was evaluated in a dermal study involving 100 Swiss mice (female) and 10 New Zealand rabbits (8 weeks old, both sexes). Half of the mice and rabbits were treated with 8.5% Benzalkonium Chloride, and the remaining half with 17.0% Benzalkonium Chloride. The solvent for both solutions of Benzalkonium Chloride was either acetone or methanol. The solutions were applied (volume = 0.2 ml) to the backs of mice and to the left ear of each rabbit twice per week. None of the animals survived 80 weeks (mice) and 90 weeks (rabbits) of treatment. The untreated control groups consisted of 100 mice and 19 rabbits. Positive control groups were treated with 0.1% (40 mice) and 1.0% 9,10-dimethylbenz(a)anthracene (15 rabbits). Tumors and lesions were recorded weekly and a complete necropsy was performed on each animal. Skin samples, grossly observed tumors, the lungs, liver, kidneys, and other organs were studied microscopically. A significant decrease in the survival rates of mice and rabbits that was directly attributable to Benzalkonium Chloride was not observed. Benzalkonium Chloride induced ulceration and inflammation in mice and rabbits, but no tumors.[74]

CLINICAL ASSESSMENT OF SAFETY

Ocular Irritation and Intraocular Toxicity

The ocular irritation potential of 0.02% Benzalkonium Chloride (in 0.9% saline) was evaluated in 51 subjects. Benzalkonium Chloride was instilled into one eye and the control solution (0.9% saline) into the other. Following the instillation of test and control solutions (volumes not stated), subjects were asked how their eyes felt. Fourteen subjects experienced irritation in the eye treated with Benzalkonium Chloride solution. Ten of the 14 subjects also experienced irritation in the control eye. The only clinical evidence of ocular irritation was slight conjunctival hyperemia in the eye of one subject treated with Benzalkonium Chloride solution (Table 11).[75]

TABLE 11. Clinical Assessment of Safety

Type of study	*No. of subjects*	*Test substance*	*Methodology*	*Results*	*References*
Ocular irritation	51	0.02% Benzalkonium Chloride	Instilled into 1 eye	Slight conjunctival hyperemia in 1 subject	75
Intraocular toxicity	10	Ophthalmic solution containing Benzalkonium Chloride (.01 mg/ml)	One drop instilled into 1 eye twice daily for 2 wks. Corneal endothelium of each eye photographed with specular microscope before and after treatment	Qualitative analysis of photomicrographs revealed no damage to corneal endothelium	76
Skin irritation	399	Benzalkonium Chloride	American Contact Dermatitis Group and International Contact Dermatitis Group Procedures	Cutaneous reactions in 2 patients	77
Skin irritation	13	10% Benzalkonium Chloride	Patches (type not stated) placed on forearm and removed after 24 h	Primary irritant dermatitis in all patients	78
Skin irritation	12	10% Benzalkonium Chloride	Patches (type not stated) placed on abdominal skin and removed after 24 h	Primary irritant dermatitis in all patients	79
Skin irritation	70	2.5% Benzalkonium Chloride	Patches (type not stated) applied to each patient. Sites evaluated 24 and 48 h after application	Skin irritation in 33 patients	80
Skin irritation	55	2.0, 1.0, 0.5, and 0.1% Benzalkonium Chloride	Simultaneously applied to upper back. Patches sealed in place with tape and removed after 48 h	26 patients had pustular and/or bullous reactions to 0.5, 1.0, and 2.0% Benzalkonium Chloride	81
Skin irritation	21	17% Benzalkonium Chloride	Applied (no patches) to forearm and labia majora. Sites graded 24 and 48 h after treatment	Fourteen subjects with reactions ranging from barely perceptible erythema to erythema with infiltration (labial site). Six subjects with reactions ranging from barely perceptible erythema to erythema (forearm site). At most, a mild irritant	82
Skin irritation	5	5.0, 2.5, and 0.5% Benzalkonium Chloride	Solutions applied to foam-filled plastic wells taped to abdominal skin. Wells removed after 12 h	5.0 and 2.5% solutions induced skin irritation in all subjects	83
Skin irritation	—	5.0 and 1.0% Benzalkonium Chloride	Applied to upper back (6-h exposure) daily for 4 days	Skin irritation	84
Skin irritation	200	0.5% Benzalkonium Chloride	Patches (type not stated) applied to upper arm and removed after 48 h	Mean erythema score = 3 (erythema, homogeneous)	85

TABLE 11. *Continued*

Skin irritation	10	0.1% Benzalkonium Chloride	21-day cumulative irritation test. Closed patches remained for 23 h daily	No evidence of cumulative irritation	86
Skin irritation and sensitization	101	Cream containing 0.1% Benzalkonium Chloride	Applied via semiocclusive patches to back or arm during 6-wk period. Patches removed 24 h after application	Cream was not irritant or contact sensitizer	87
Skin sensitization	100	0.07, 0.05, 0.025, 0.01, and 0.005% Benzalkonium Chloride	Patients treated for conjunctivitis with different preparations containing Benzalkonium Chloride (conc. not stated) for 3 mos or longer. Patients patch-tested with 0.07% Benzalkonium Chloride after treatment. Patients with positive reactions to 0.07% Benzalkonium Chloride patch tested with 0.05, 0.025, 0.01, and 0.005% Benzalkonium Chloride	Positive reactions to 0.07, 0.05, 0.025, and 0.1% Benzalkonium Chloride (6 patients). Two of the 6 had positive reactions to 0.005% Benzalkonium Chloride	88
Skin sensitization	2806	0.1% Benzalkonium Chloride	Patch tested according to Standard International Contact Dermatitis Research Group procedure	66 of the 2806 eczema patients were sensitive to Benzalkonium Chloride	89
Skin sensitization	142	0.1% Benzalkonium Chloride	Patch-tested according to chamber test procedure (Pirila, 1975)	Nine of the 142 patients with external otitis had allergic reactions to Benzalkonium Chloride	90
Skin sensitization	8	Contact lens solution containing Benzalkonium Chloride (conc. not stated) and 0.1% Benzalkonium Chloride	Patch-tested (types of patches not stated) with 0.1% Benzalkonium Chloride and contact lens solution	Allergic conjunctivitis in 3 patients using contact lens solution. Positive reactions in 3 patients patch tested with lens solution and 0.1% Benzalkonium Chloride	91
Skin sensitization	5	0.1 and 0.01% Benzalkonium Chloride	Patch tests (procedure not stated)	All patients had + or ++ allergic reactions to 0.1% Benzalkonium Chloride. Two patients had + reaction to 0.01% Benzalkonium Chloride	92
Skin sensitization	110	0.1% Benzalkonium Chloride	Patch tests (procedure not stated)	One of 110 patients with dermatitis had positive reactions	92
Skin sensitization	130	0.1% Benzalkonium Chloride	Patch tests (procedure not stated)	No positive reactions	92

The effect of Benzalkonium Chloride on the corneal endothelium was evaluated in 10 subjects (male and female; mean age 24.5 years). One drop of an ophthalmic solution containing Benzalkonium Chloride (0.1 mg/ml) was instilled into one eye of each subject twice daily for 2 weeks. A control group of 10 subjects received the same ophthalmic solution without Benzalkonium Chloride. The central corneal endothelium of each eye was photographed twice, once before and once after the treatment period, with a modified contact specular microscope; specular photomicrographs were enlarged to paper prints for computerized image analysis.[93] No abnormalities on the cellular mosaics were observed before or after the treatment period (Table 11).[76]

Skin Irritation

Patients with dermatitis (399) were patch tested with various cosmetic ingredients over a period of 64 months. Patch tests were conducted according to American Contact Dermatitis Group and International Contact Dermatitis Group procedures. Cutaneous reactions to Benzalkonium Chloride were observed in two patients (Table 11).[77]

Primary irritant dermatitis was observed in 13 patients patch tested with 10% aqueous Benzalkonium Chloride. None of the subjects had previously been exposed to the test substance and all had local noninflammatory skin conditions (arms not affected). Patches were placed on the flexor aspect of the forearm and removed after 24 h (Table 11).[78]

In another study, primary irritant dermatitis was observed on the abdominal skin of each of 12 patients (53–75 years old) patch tested with 10.0% aqueous Benzalkonium Chloride (24-h exposure). The patients had venous leg ulcers, but no other skin diseases, prior to exposure. None of the patients had previously been exposed to Benzalkonium Chloride. Reactions of erythema, infiltration, and vesicle formation were graded according to the scale: none (−), weak (+), medium strong (++), and strong (+++). Nine patients had medium strong to strong erythema, while vesiculation was noted in four patients (Table 11).[79]

The skin irritation potential of 2.5% aqueous Benzalkonium Chloride was evaluated in 70 hospital patients with lepromatous leprosy. The patients were classified into two groups according to the clinical leproma pattern: 32 patients without active clinical signs, but with various dystrophic sequelae; and 38 patients with active lesions that were positive for acid-fast bacilli; patients either had or had not been treated for disease symptoms. Fifty patients served as controls. Patch tests were placed on each subject and sites were evaluated 24 and 48 h after application. Observations in the 32 patients without active clinical signs were as follows: no reaction (17 patients), erythema (6 patients), erythema and exudation (7 patients), erythema and bullae with a serous or purulent content (2 patients). Observations in the group with active lesions (38 patients) included: no reaction (20 patients), erythema (12 patients), and erythema and exudation (6 patients). Of the 50 control subjects, 17 did not have reactions, 9 had erythema and exudation, and 24 had erythema and bullae with a serous or purulent content (Table 11).[80]

Four concentrations of Benzalkonium Chloride (0.1, 0.5, 1.0, and 2.0% in distilled water) were simultaneously applied to the upper back of each of 55 hospital patients. The patients had various types of skin diseases. Patches (type not stated) containing each solution were sealed in place with tape and removed after 48 h. Twenty-six cases of severe pustular and/or bullous reactions to 0.5, 1.0 and 2.0% Benzalkonium Chloride were reported (Table 11).[81]

Benzalkonium Chloride 10 μl (17% in water and ethanol) was applied to the forearm and labia majora of 21 female subjects (22–51 years old). Sites (uncovered) were allowed to dry and then graded 24 h (21 subjects) and 48 h (10 subjects) after treatment according to the scale: 0 (no reaction), ± (barely perceptible erythema and/or pigmentation), 1 (erythema, covering the test site), 2 (erythema, infiltration), and 3 (erythema, infiltration, vesicles). The scores for labial and forearm skin at 24 h posttreatment were as follows: 0 (7 subjects, labial skin), ± (2 subjects, labial), 1 (4 subjects, labial), 2 (8 subjects, labial), 0 (15 subjects, forearm skin), ± (4 subjects, forearm), and 1 (2 subjects, forearm). The scores for labial and forearm skin at 48 h were: 0 (6 subjects, labial skin), ± (1 subject, labial), 1 (1 subject, labial), 3 (2 subjects, labial) and 0 (10 subjects, forearm skin) (Table 11).[82]

Confluent erythema and edema with papules were observed in 5 subjects (mean age = 41) tested with 5.0 and 2.5% aqueous Benzalkonium Chloride solutions. However, no reactions were noted after the application of 0.5% aqueous Benzalkonium Chloride. The solutions were each applied to foam-filled plastic wells that were taped to clinically normal abdominal skin. Control wells contained either water or foam inserts. All wells were removed after 12 h of contact (Table 11).[83]

The effect of Benzalkonium Chloride (1.0 and 5.0%) on human epidermal mitosis was evaluated in healthy adult male subjects (number not stated). Both concentrations were applied (occlusive patches, upper back) over a period of 4 days, each being renewed at 1-day intervals. Each subject served as his own control. Patches were removed 6 h prior to the end of the exposure period, at which time excess compound was removed and 0.5% colcemid cream applied (occlusive patches). Patches remained for 6 h, after which 3 mm punch biopsy specimens taken. Specimens were subjected to the Feulgen test and 12 sections of each were then scanned for mitoses to obtain a mitotic index (mitoses per thousand viable cells). One percent Benzalkonium Chloride had no effect on mitosis. However, 5% Benzalkonium Chloride induced a 10-fold increase in the mitotic index, peaking at about 72 h after the initial application. The increase in the mitotic index was accompanied by intense erythema and occasional blistering (Table 11).[84]

Two-hundred subjects (16–29 years old) were patch tested with 0.5% Benzalkonium Chloride in water. Each patch was applied to the outer aspect of the right upper arm and removed after 48 h. Reactions were scored 24 h after removal according to the scale: 0 (no reaction) to 6 (infiltrated erythema with vesicles, pustules and/or erosion). A mean irritation score of 3 (erythema, homogeneous) for the 200 subjects was reported (Table 11).[85]

The skin irritation potential of a cream containing 0.1% Benzalkonium Chloride was evaluated in 10 subjects (18–59 years old). A closed patch

containing 0.2 ml of the cream was applied to the back of each subject. Patches were removed 23 h after application and sites were washed immediately. Reactions were scored 1 h after patch removal. The cream was applied to the same site on each subject for 21 consecutive days. The grading scale for cumulative irritation ranged from 0 (no irritation) to 630 (primary irritation). The total irritation score (all panelists) for the 21 applications was 20, which was interpreted as essentially no evidence of cumulative irritation (Table 11).[86]

Skin Irritation and Sensitization

A skin irritation and sensitization study of a cream containing 0.1% Benzalkonium Chloride was conducted with 101 men and women (18–65 years old). The cream (approx. 0.1 ml) was applied via a semiocclusive patch to the back or arm of each subject during a 6-week period. During the first 3 weeks of testing, patches were applied on Mondays, Wednesdays, and Fridays and removed 24 h after application. Reactions were scored after patch removal according to the following scale: 0 (negative), 1 + (erythema), 2 + (erythema and edema or induration), 3 + (erythema, edema/induration and vesiculation), and 4 + (erythema, edema/induration, bulla, with or without ulceration). The last induction patches were applied on Monday of week 4 and removed 24 h later. Reactions were scored 48 h after patch removal. On Monday of week 6, a challenge patch was placed on each subject (new site) and removed 48 h later. Reactions were scored 48 and 72 h after application. No significant reactions were observed during induction or challenge phases. The cream was neither an irritant nor an allergic contact sensitizer (Table 11).[87]

Skin Sensitization

A sensitization study was conducted with 100 patients who had been treated for conjunctivitis (3 months or longer) with different preparations containing Benzalkonium Chloride. All subjects were patch tested with 0.07% aqueous Benzalkonium Chloride at the conclusion of treatment. Six of the patients had positive reactions at 48 and 72 h. They were then tested with 0.05, 0.025, 0.01, and 0.005% Benzalkonium Chloride. All had positive reactions to 0.05, 0.025, and 0.01% Benzalkonium Chloride; two had positive reactions to 0.005% Benzalkonium Chloride (Table 11).[88]

An epidemiologic study was conducted with 2,806 patients (male and female) with eczema. The patients were patch tested with 0.1% Benzalkonium Chloride in petrolatum according to the standard International Contact Dermatitis Research Group procedure. Reactions were scored 48 and 96 h postapplication. For some patients, another scoring was done 8 days after patch removal. Sixty-six (2.13%) of the 2,806 patients were sensitive to Benzalkonium Chloride (Table 11).[89]

Patients with chronic external otitis (142; at least 3 months' duration) were patch tested with 0.1% Benzalkonium Chloride according to the chamber test procedure.[94] The test substance was applied to the back of each subject and removed after 24 h. Results were interpreted at 2, 4, and sometimes 7 days after application. Only edematous, infiltrative, or vesicular reactions, noted

after the 2nd day of application, were considered to be allergic reactions. Reactions of erythema only, or those that had disappeared by the 2nd day, were not considered to be positive responses. Benzalkonium Chloride induced allergic reactions in 9 (6.3%) of the 142 patients (Table 11).[90]

In another study, three of eight patients experienced allergic conjunctivitis after wearing contact lenses that had been soaked in a lens solution containing Benzalkonium Chloride. All three had positive patch test reactions to the solution and to 0.1% Benzalkonium Chloride at 48 h postapplication (Table 11).[91]

Five patients were patch tested with 0.1 and 0.01% Benzalkonium Chloride. The experimental procedure was not stated. All patients had a + or + + allergic reaction to 0.1% Benzalkonium Chloride. Two patients had a + reaction to 0.01% Benzalkonium Chloride. To assess the incidence of Benzalkonium Chloride sensitivity in the general population, 130 normal subjects were patch tested with 0.1% Benzalkonium Chloride (procedure not stated). At a contact dermatitis clinic 110 patients were also patch tested with 0.1% Benzalkonium Chloride. Sensitivity to Benzalkonium Chloride was not detected in any of the normal subjects. However, a positive reaction was observed in 1 of the 110 patients. Prior to the test, this patient was diagnosed as having eczema secondary to proven contact sensitivity to cetrimide (Table 11).[92]

The skin sensitization potential of a moisturizing cream containing 0.13% Benzalkonium Chloride was evaluated using 150 subjects (18–65 years old). The cream was applied via occlusive patches to the upper back of each subject on Mondays, Wednesdays, and Fridays for 3 consecutive weeks. Each patch remained for 24 h and sites were scored prior to the next patch application. After a 2-week nontreatment period, two challenge patches were applied consecutively to sites adjacent to the original induction sites; patches remained for 48 h. Reactions were scored 48 and 96 h after patch application according to the scale: 0 (no reaction) to 4 (bullae or extensive erosions). No positive reactions to the moisturizing cream were observed.[95] In a similar study (same procedure), a moisturizing cream containing 0.13% Benzalkonium Chloride and a local antiseptic did not induce positive reactions when applied to 155 subjects (18–65 years old).[96]

SUMMARY

Benzalkonium Chloride is a mixture of alkylbenzyldimethylammonium chlorides. One method of production entails treatment of a solution of *N*-alkyl-*N*-methylbenzylamine in a suitable organic solvent with methyl chloride; Benzalkonium Chloride precipitates as it is formed. As of 1986, Benzalkonium Chloride was present in 83 cosmetic formulations at concentrations ranging from $\leq 0.1\%$ to 5%. Its cosmetic uses include foaming and cleansing agent, conditioner, and bactericide. Noncosmetic uses of Benzalkonium Chloride include preservative in ophthalmic solutions, spermicide, and sanitizer for chemically clean surfaces.

Benzalkonium Chloride was not detected in either venous blood or breast milk from women using tampons containing Benzalkonium Chloride (60 mg). Following the instillation of [^{14}C]Benzalkonium Chloride solution onto the corneal surface of rabbits, radioactivity was detected in the corneal epithelium, endothelium, and stroma, and in the bulbar and palpebral conjunctivae. At no time was radioactive material found in the aqueous humor or in any tissues.

No adverse effects were noted when rats and hamsters inhaled a conditioner containing 0.1% Benzalkonium Chloride over a period of 13 consecutive weeks (4 h/day).

Acute oral LD_{50}s for rats dosed with Benzalkonium Chloride ranged from 342 to 525 mg/kg.

In a subchronic toxicity study, Benzalkonium Chloride solutions were administered via stomach tube to 40 albino rats for 12 weeks (once/day) at dosages of 50.0 mg/kg (1:20 dilution) and 100.0 mg/kg (1:10 dilution). Two of 20 animals receiving the 100.0 mg/kg dosage died.

In a chronic toxicity study, Benzalkonium Chloride (10.0%) was administered via stomach tube to 18 beagle dogs at dosages of 12.5, 25.0, and 50.0 mg/kg for 52 weeks (once daily). One of six dogs receiving 50 mg/kg dosages and three of six dogs receiving 25 mg/kg dosages died.

The application of 0.1% Benzalkonium Chloride to the round window membrane (middle ear) in guinea pigs resulted in fibrosis of the tympanic cavity, cochlea, and vestibulum, and destruction of vestibular neuroepithelia. Of the 3 exposure periods, 10, 30, and 60 min, the most damage was noted after 60 min.

Of 96 mice receiving dermal applications of 6.5 and 50% Benzalkonium Chloride, 29 died within 72 h after application.

At concentrations of 0.033 and 0.10%, Benzalkonium Chloride caused significant growth retardation of cardiac fibroblasts (rat). Benzalkonium Chloride (1.5 M) was toxic to murine suspension cultures of the P815 tumor cell line. Benzalkonium Chloride concentrations ranging from 0.000022 to 0.000042 M induced hemolysis in defibrinated blood from rabbits.

Benzalkonium Chloride 1% and 2.0% aqueous induced severe iritis and severe conjunctival injection, respectively, when instilled into the conjunctival sac of rabbits twice daily for 7 days. Benzalkonium Chloride (0.3%) induced minimal ocular irritation when instilled once into the eyes of rabbits. Single instillations of 0.1% Benzalkonium Chloride into the conjunctival sac of albino rabbits did not cause ocular irritation. The instillation of 0.1% Benzalkonium Chloride into the conjunctival sacs of rabbits 5 times daily for 1 week resulted in corneal damage. The instillation of 0.01% Benzalkonium Chloride into the conjunctival sacs of rabbits (5 min–6-h period) resulted in corneal damage.

In *in vivo* intraocular toxicity studies, Benzalkonium Chloride concentrations ranging from 0.007 to 10.0% were tested. Four hours after the instillation of 0.5, 1.0, and 10% Benzalkonium Chloride, corneal damage was noted in rabbits and guinea pigs. The ocular administration of 0.5, 1.0, and 2.0% solutions twice daily for 7 days caused conjunctival damage in rabbits. Following the daily administration of 0.007 and 0.1% Benzalkonium Chloride for 2 weeks, retinal detachment was observed in pigmented but not albino rabbits.

In *in vitro* intraocular toxicity studies, the exposure of rabbit corneas to Benzalkonium Chloride concentrations ranging from 0.0001 to 0.01% resulted in corneal damage. Exposure periods ranged from 2 min (0.01%) to 110 min (0.0001%). The longest exposure was 180 min (0.0065% Benzalkonium Chloride).

Benzalkonium Chloride concentrations of 1.0–50% induced reactions ranging from erythema to necrosis when applied (duration not stated) to the skins of rabbits. In another study, 24-h applications of 1.0 to 10.0% Benzalkonium Chloride to the skins of rabbits resulted in severe induration. Benzalkonium Chloride concentrations of 1.0 and 5.0% induced epidermal necrosis when applied (24-h exposure) to the skins of albino guinea pigs. Applications of 2.0% Benzalkonium Chloride to the skins (abraded and intact) of rabbits resulted in severe erythema (2-day application period). Slight erythema was noted 7 days after application. Applications of 1.0% Benzalkonium Chloride to the skins of white rats during a 2-month period caused hyperemia and necrosis. Following applications of 0.5% Benzalkonium Chloride to the skins of rabbits (24 h exposure), severe erythema, moderate edema, and eschar formation were observed. Benzalkonium Chloride (0.5%) resulted in practically no skin irritation when applied to the skins of albino rabbits (24-h exposure). When 0.1% Benzalkonium Chloride was applied to the skins of rabbits (5-day contact period), slight erythema and necrosis were observed. These reactions were observed for 3 weeks posttreatment.

The instillation of 100 or 200 mg/kg of aqueous Benzalkonium Chloride into the vaginas of pregnant rats resulted in sternal defects in the offspring.

Benzalkonium Chloride was not mutagenic to *Salmonella typhimurium* strains TA 1535, TA 1536, TA 1537, and TA 1538 and *E. coli* strains B/r WP2 hcr^+ and WP2 hcr^- in microbial test systems making up the rec-assay in combination with reverse mutation systems. Mutagenic activity also was not demonstrated in reversion assays involving strains TA 1535, TA 1536, TA 1537, and TA 1538 of *Salmonella typhimurium*, and, in the rec-assay, with *Bacillus subtilis* strains H17 Rec^+ and M45 Rec^-. In the plate incorporation assay, Benzalkonium Chloride was not mutagenic to *Salmonella typhimurium* strains TA 98, TA 1538, TA 1537, and TA 100. In the *E. coli* DNA polymerase assay Benzalkonium Chloride induced repairable DNA damage in strains W3110 (pol A^+) and p3478 (pol A^-).

The dermal application of 8.5 and 17% Benzalkonium Chloride to rabbits and mice did not result in tumor formation or systemic toxic effects, but did produce ulceration and inflammation at the application sites.

Slight conjunctival hyperemia was observed in 1 of 51 human subjects who had received ocular instillations of 0.02% Benzalkonium Chloride.

Cutaneous reactions were observed in 2 of 399 dermatitis patients patch tested with Benzalkonium Chloride over a period of 64 months. In separate studies, primary irritant dermatitis was observed in 13 patients and 12 patients patch tested with 10.0% Benzalkonium Chloride (24-h exposure). In another study, erythema was observed in 33 of 70 leprosy patients patch tested with 2.5% Benzalkonium Chloride. Benzalkonium Chloride concentrations of 0.5, 1.0, and 2.0% induced several pustular and/or bullous reactions in 26 of 55 patients (48-h exposures).

The application of 17.0% Benzalkonium Chloride (24-hour period) to the skin of each of 21 subjects resulted in well-defined erythema (13 subjects). Confluent erythema and edema were noted in the skin of subjects tested with 5.0 and 2.5% Benzalkonium Chloride (12-h exposure). Results from a 21-day skin irritation study of a cream containing 0.1% Benzalkonium Chloride indicated essentially no cumulative irritation.

A cream containing 0.1% Benzalkonium Chloride did not induce skin irritation or sensitization reactions in 101 subjects patch tested during a 6-week period (24-h exposures).

Sensitization reactions were observed in 6 of 100 patients patch-tested with 0.07% Benzalkonium Chloride. The 6 patients also had positive reactions to 0.05, 0.025, and 0.01% Benzalkonium Chloride. Sixty-six of 2,806 patients were sensitive to 0.1% Benzalkonium Chloride. In another study, allergic reactions were observed in 9 of 142 patients patch tested with 0.1% Benzalkonium Chloride. Sensitization reactions were not observed in normal subjects patch-tested with 0.1% Benzalkonium Chloride.

DISCUSSION

Skin irritation studies in humans involved patients and normal subjects. Patients were tested with Benzalkonium Chloride concentrations ranging from 0.1 to 10.0%, and normal subjects with concentrations of 0.1 to 17.0%. Skin irritation was noted in both populations after applications of Benzalkonium Chloride concentrations greater than 0.1%. Skin irritation and ocular irritation were usually noted in animals when Benzalkonium Chloride was tested at concentrations greater than 0.1%.

Skin sensitization was noted in patients tested with Benzalkonium Chloride concentrations ranging from 0.01 to 0.7%. However, there was no incidence of skin sensitization in a population of normal subjects tested with 0.1% Benzalkonium Chloride. Individuals with diseased skin may be at risk for sensitization to Benzalkonium Chloride.

The Expert Panel recognizes that some of the products tested contained concentrations of Benzalkonium Chloride greater than 0.1%. If these products contain proteins or other agents that bind Benzalkonium Chloride, Benzalkonium Chloride concentrations greater than 0.1% would have to be added to yield 0.1% free Benzalkonium Chloride. It is important to note that only free Benzalkonium Chloride is effective as an antimicrobial agent and, also, that the free agent induces dermal toxicity.

CONCLUSION

On the basis of the data presented in this report, the CIR Expert Panel concludes that Benzalkonium Chloride, at concentrations up to 0.1% free, active ingredient, is safe as a cosmetic ingredient as presently used.

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RE-REVIEW DOCUMENT ON BENZALKONIUM CHLORIDE

Benzalkonium Chloride (mixture of alkylbenzyldimethylammonium chlorides) is listed in the *International Cosmetic Ingredient Dictionary and Handbook* **(Gottschalck and McEwen, 2006)**. A CIR Final Safety Assessment on these ingredients was published with the following conclusion: On the basis of the data presented in this report, the CIR Expert Panel concludes that Benzalkonium Chloride, at concentrations up to 0.1% free, active ingredient, is safe as a cosmetic ingredient as presently used. **(Elder, 1989)**.

An updated search of the literature was performed to identify studies on Benzalkonium Chloride that have been published since the Panel's Final Safety Assessment was issued or were omitted from that report. These studies are summarized in this re-review document.

CHEMISTRY

DEFINITION

Benzalkonium Chloride (CAS No. 8001-54-5) is a mixture of alkylbenzyldimethylammonium chlorides **(Gottschalck and McEwen, 2006)**.

CHEMICAL AND PHYSICAL PROPERTIES

According to **Smith et al. (2002)**, Benzalkonium Chloride can have different alkyl chain lengths ranging from C_8H_{17} to $C_{18}H_{37}$, with chain length greatly affecting its chemical properties. The Benzalkonium Chloride materials investigated were mixtures of $C_{12}H_{25}$ and $C_{14}H_{29}$ as well as $C_{14}H_{29}$ on its own. The diaphragm diffusion technique was investigated for its applicability to the measurement of diffusion coefficients of molecules with surfactant properties and the ability to form micelles. The average value of the membrane cell integral diffusion coefficient for Benzalkonium Chloride was 7.78×10^{-6} cm^2s^{-1} at 25°C, and there was no significant effect of alkyl chain length on the measured value of the membrane cell integral diffusion coefficient.

UV ABSORPTION

In a UV spectral analysis of Benzalkonium Chloride by **Withrow et al. (1989)**, the absorption peak was well below 300 nm. There was no measurable absorption above 310 nm.

Table 1. Historical and current cosmetic product uses and concentrations for Benzalkonium Chloride

Product Category	1986 uses (Elder, 1989)	2005 uses (FDA, 2005)	1986 concentrations (Elder, 1989) (%)	2006 concentrations (CTFA, 2006) (%)
Baby products				
Lotions, oils, powders, and creams	4*	2	≤0.1 to 1*	
Other		2		
Eye makeup				
Eye lotion	-	1	-	
Eye makeup remover	-	10	-	
Other	6	-	≤0.1 to 1	
Fragrance Products				
Colognes and toilet waters	-	1	-	
Non-coloring hair products				
Conditioners	45*	12	≤0.1 to 5*	
Rinses		2		
Tonics, dressings, etc.		7		
Shampoos		5		
Other		5		
Hair coloring products				
Bleaches	-	1	-	
Nail care products				
Creams and lotions	-	1		
Other	-	1		
Personal hygiene products				
Underarm deodorants	11*	1	≤0.1 to 1*	
Other		1		
Skin care products				
Skin cleansing creams, lotions, liquids, and pads	6	17	≤0.1 to 1	
Face and neck creams, lotions, powder and sprays	-	3	-	
Body and hand creams, lotions, powder and sprays		3		
Foot powders and sprays	-	1	-	
Moisturizers	4	1	≤0.1 to 1	
Night creams, lotions, powders and sprays	22	1	≤0.1 to 1	
Paste masks/mud packs	-	3	-	
Skin fresheners	-	5	-	
Other	7	2	≤0.1 to 1	
Suntan products				
Suntan gels, creams, and liquids	-	1	-	
Total uses/ranges for Benzalkonium Chloride	83	89	≤0.1 to 5	

* This category was combined when the original safety assessment was performed and is now two or more separate categories.

Benzalkonium Chloride is an ingredient of quasi drugs (in products to be used directly on the body) that are marketed in Japan. Quasi-drugs, by definition, must have a mild effect on the body, but are neither intended for the diagnosis, prevention, or treatment of disease, nor to affect the structure or function of the body **(MHLW, 2000)**.

NONCOSMETIC USE

According to **Keser et al. (2005)**, alcohols, chlorhexidine gluconate (CHG) (Hibiscrub), and

BIOLOGICAL PROPERTIES

DISTRIBUTION

In a study by **Xue et al. (2004)**, the distribution and disposition of Benzalkonium Chloride after the following routes of administration were evaluated: oral, intravascular (jugular vein, JV), femoral artery (FA), femoral vein (FV)and jugular artery (JA). Seventy-four male Sprague-Dawley rats were used in the study.

Twelve rats (weights = 384 to 440 g) and 18 rats (weights = 310 to 470 g) were used to determine the elimination kinetics and distribution of Benzalkonium Chloride, respectively. Each rat in the kinetic study was implanted with two catheters for drug administration and blood sampling. The rats in the distribution study were implanted with a single catheter for administration. A dose of 15 mg/kg of 1% Benzalkonium Chloride solution was infused over 1 minute via JV or FA. In the kinetic study, the blood samples of 0.1, 0.3, 0.5, 0.5, 0.8, 1.0, and 1.5 ml were collected from the femoral vein at 2, 5, 10, 30, 60, 120, and 180 minutes, respectively.

The fatal effects of Benzalkonium Chloride appeared soon in JV- FV- or JA-rats, but took hours in PO or FA rats. No rat receiving Benzalkonium Chloride via FA survived longer than one day. The PO-rats that aspirated Benzalkonium Chloride into their lungs had some systemic symptoms and higher blood and tissue concentrations of Benzalkonium Chloride. The blood Benzalkonium Chloride levels and kinetics were similar among the different routes of intravascular administration, but the lung and kidney levels were higher in JV-rats. Pathological examinations confirmed severe congestion and edema in the lungs and kidneys. These results suggest the following: (1) the toxic effects of Benzalkonium Chloride varied depending on the route of administration. (2) The degree of toxicity correlated with peak blood and tissue concentrations in orally dosed rats. (3) Different toxicological progressions and manifestations were observed in FA- and JV-dosed rats, even though these groups had similar blood concentration profiles. (4) the lung and kidney are reservoirs for Benzalkonium Chloride and considered to be the target organs of Benzalkonium Chloride **(Xue et al., 2004)**.

SKIN PENETRATION ENHANCEMENT

van der Bijl et al. (2002) showed that the permeation of cyclosporin A through intact and de-epithelialized human vaginal mucosa can be enhanced by 0.01% Benzalkonium Chloride. Specimens

were obtained from excess tissue removed from eight postmenopausal patients (mean age: 57 ± 16 years) following vaginal hysterectomies.

EFFECT ON CYTOKINES

Wilmer et al. (1994) examined qualitative and quantitative changes in selected intracellular and secreted cytokines in human keratinocyte cultures (normal human epidermal keratinocytes from breast skin of adult females) in response to the ulcerative agent Benzalkonium Chloride (dissolved in 0.1% aqueous CrO_3). The keratinocytes were exposed to Benzalkonium Chloride anywhere from 3 to 48 hours. At a non-cytotoxic concentration (0.1 µg/ml), Benzalkonium Chloride stimulated the production and intracellular accumulation of IL-1α. It appears that the autocrine stimulation of IL-1α by TNFα cannot explain the apparent intracellular increase of IL-1α that was stimulated by Benzalkonium Chloride, which did not induce TNFα production.

Benzalkonium Chloride (concentrations up to 30% in distilled water) was also applied to mouse (specific-pathogen-free BALB/c ANCr female mice; 10 to 16 weeks old) skin to assess whether the chemical-specific pattern of inflammation correlated with the *in vitro* production of keratinocyte-derived cytokines. Specifically, the test substance was applied to the dorsal surface of both ears. Ear thickness was measured using a micrometer, and one ear per mouse was examined microscopically. Although Benzalkonium Chloride elicited neutrophils to the site of chemical application, time-dependent and chemical-specific patterns of inflammation could not be detected **(Wilmer et al., 1994)**.

Corsini et al. (1998) investigated the effects of five relevant skin allergens (dinitrochlorobenzene, oxazolone, nickel sulfate, penicillin G, and eugenol), two skin irritants (aqueous Benzalkonium Chloride and methylsalicilate) and two compounds with no sensitizing activity (glycerol, and ethanol) on interleukin-1α (IL-1) production in HEL30 cells (C3H mouse-derived keratinocyte cell line). Twenty-fours following treatment, both IL-1 release in conditioned media and cell-associated IL-1 were measured by a specific sandwich ELISA. Under experimental conditions, only contact sensitizers were able to increase in a dose-dependent fashion cell-associated IL-1. Both skin irritants and allergens induced the release of IL-1, because of the irritative properties of both chemicals, while ethanol and glycerol failed to induce changes in IL-1 production. Taken together, these data indicate

that it may be realistic to consider potential skin allergens those chemicals that are able to increase cell-associated IL-1, to consider skin irritants those chemicals that induce only IL-1 release, and to exclude as potential allergens or irritants those chemicals that fail to induce changes in IL-1 production.

INHIBITION OF HISTAMINE RELEASE

In a study by **Niitsuma et al. (1996)**, Benzalkonium Chloride inhibited histamine release from rat peritoneal macrophages induced by plasma bradykinin (BK), oligomer-specific lectin *Datura stramonium* agglutinin, (DSA) synthetic compound 48/80 (48/80), and a polyethylenimine with a molecular weight of 600 (PEI_6), resulting in 50% inhibition (IC50) values of 0.63 ± 0.13 µg/ml (n = 6), 3.04 ± 0.25 µg/ml (n = 8), 1.56 ± 0.12 µg/ml (n = 8), and 0.48 ± 0.01 µg/ml, respectively. At concentrations of 3 and 6 µg/ml, Benzalkonium Chloride almost completely inhibited the histamine release induced by BK and 48/80. Histamine release induced by DSA was also almost completely inhibited by 6 µg/ml Benzalkonium Chloride.

SCLERAL PERMEABILITY ENHANCEMENT

Okabe et al. (2005) investigated the effect and safety of Benzalkonium Chloride on transscleral drug delivery in the rabbit after continuous intrascleral administration using *in vitro* and *in vivo* tests. Twenty-five eyes of 30 albino rabbits were used in the *in vitro* scleral permeability experiment. In the experiment investigating the *in vivo* ocular permeability of large molecules, 24 eyes of 24 albino rabbits were used.

Betamethasone 21-phosphate (BP) aqueous solutions, with or without Benzalkonium Chloride (0.01% or 0.05%), were continuously administered to albino rat sclera with an osmotic pump for 1 week. To investigate the effect of Benzalkonium Chloride on the scleral permeability of Benzalkonium Chloride *in vitro*, the penetration of BP aqueous solution with or without Benzalkonium Chloride across the rabbit sclera was evaluated using a two-chamber Ussing apparatus. To determine the effect of Benzalkonium Chloride on transscleral delivery of large molecules , 20- and 70-k-Da fluorescein isothiocyanate aqueous solutions , with or without Benzalkonium Chloride, were continuously administered to the sclera by an osmotic pump.

Benzalkonium Chloride increased concentrations of BP in the vitreous and retina-choroid

compared with the control (physiological saline). BP was hot detected in the aqueous humor. In the *in vitro* study, Benzalkonium Chloride did not increase the scleral permeability of BP. In the retina-choroid, Benzalkonium Chloride significantly increased concentrations of FD-20, but did not increase concentrations of FD-20 or -70 in the vitreous. No substantial toxic reactions were observed in the retina in electrophysiological or histologic examinations after the addition of Benzalkonium Chloride.

The results of this study demonstrate that Benzalkonium Chloride may improve the ocular penetration of a drug in a transscleral drug delivery system without producing toxic reactions **(Okabe et al., 2005)**.

EFFECT ON ENZYME ACTIVITY

Wenzel et al. (1990) conducted a systematic study comprising 28 synthetic ionic and cationic surfactants in order to examine their effect on the activity of elastase and cathepsin G from human leukocytes against 4-nitroanilide substrates. The whole spectrum, ranging from a complete loss to a pronounced rise in enzymatic activity, was observed at a 0.1% (w/v) surfactant concentration. Most significantly, Benzalkonium Chloride led to a five-fold increase in elastase activity.

Jaganathan and Boopathy (2000) studied the effect of Benzalkonium Chloride on the esterase and aryl acylamidase activities of butyrylcholinesterase (BChe). Acetylcholinesterase (AchE) and BchE from vertebrates display aryl acylamidase activity (AAA) that is capable of hydrolyzing the synthetic substrate o-nitroacetanilide to o-nitroaniline. This AAA activity is strongly inhibited by classical cholinesterase (ChE) inhibitors. Benzalkonium ($\leq$4 to 16 µM) inhibited the esterase activity of human serum and horse serum BChEs and AchEs from electric eel and human erythrocytes in a concentration-dependent manner. The remarkable property of Benzalkonium Chloride was its ability to profoundly activate the AAA activity of human serum and horse serum BchEs, but not the AAA activity of AchEs. Thus, Benzalkonium Chloride seems to preferentially activate the AAA activity of BchEs alone. The authors noted that this is the first report of a compound that inhibits the esterase activity, while simultaneously activating the AAA activity, of BChEs.

EFFECT ON DNA, RNA, AND PROTEIN SYNTHESIS

In a study by **Kajino (1987)**, The exposure of cultured Chinese hamster lung fibroblast cells to Benzalkonium Chloride at doses of 3 to 30 µg/ml for 2 hours elicited inhibition of DNA-, RNA-, and

protein-syntheses, in a dose-related manner. The cytotoxicity of Benzalkonium Chloride was also studied, and the results of the experiment are included in the section on Cytotoxicity later in the report text.

TOXICOLOGY

A toxicity profile on Benzalkonium Chloride by the British Industrial Biological Research Association (BIBRA) is available, and has been ordered from British Columbia **(BIBRA, 1989)**.

KINETIC CHARACTERISTICS AND TOXIC EFFECTS

Xue et al. (2004) investigated the kinetic characteristics and toxic effects of Benzalkonium Chloride following injection via the jugular vein (JV), femoral artery (FA) and oral administration (PO) using male Sprague-Dawley rats (body weights = 310 to 470 g). Both the FA and JV infusion experiments involved 15 rats. The oral dosing experiment involved 34 rats.

In the JV experiment, the elimination kinetics and distribution of Benzalkonium Chloride were studied. The elimination kinetics experiment involved six rats and the distribution experiment involved nine rats. Both groups were infused with a dose of 15 mg/kg, after which blood samples were collected. In the FA experiment (15 rats), the study design was identical to that of the JV experiment, except the sites for a dose (left FA) and blood sampling (right femoral vein). The Benzalkonium Chloride concentrations in blood and tissues (lung, liver, and kidney) were determined by high-performance liquid chromatography with solid phase extraction.

The fatal effects appeared soon after dose administration in JV rats, while the effects were delayed in FA- or PO-rats. After JV administration, most of the rats stopped breathing immediately after the dose, but recovered in 30 to 40 seconds. However, three rats died within 11 minutes, without recovery. After FA administration, all rats appeared to be normal in the initial period, but did not urinate after the dose and gradually became cyanotic. No FA rat died within 4 hours, but all three rats assigned for the distribution study over 24 hours died at 7, 10, and 13 hours (no sample at 24 hours). The blood Benzalkonium Chloride concentrations and the elimination half-lives were similar between JV- and FA-rats, while the distribution of Benzalkonium Chloride in tissues was slightly different.

In the oral dosing experiment (34 rats), fasted rats received a dose of 250 mg/kg

Benzalkonium Chloride by stomach tube. Blood samples were collected for up to 24 hours and the animals were killed. Approximately half of the rats appeared to be normal during the experimental period, while the other half coughed or vomited some of the dose, followed by sneezing or difficulty in breathing. Four rats died at 1, 6, 7, and 24 hours by respiratory irritation. The rats with some symptoms had significantly higher concentrations in the blood and tissues than the rats that appeared normal. The concentrations of Benzalkonium Chloride in the rats that died before their due times were higher than the others, except for the one rat that died at 24 hours. The Benzalkonium Chloride concentrations in blood and tissues were significantly higher in the aspirated PO-rats. The toxic degree of Benzalkonium Chloride was correlated with the Benzalkonium Chloride concentration in orally dosed rats. The lung and kidney had higher Benzalkonium Chloride concentrations, compared to the blood or liver, and they could be target organs of Benzalkonium Chloride **(Xue et al., 2004)**.

ACUTE ORAL TOXICITY

In the oral administration study by **Xue et al. (2004)**, 30 fasted rats (weights = 330 to 368 g) were used. A dose of 250 mg/kg was given by stomach tube. Fifteen of the 30 rats dosed orally had symptoms such as sneezing, diarrhea, or difficulty in breathing. Erosion and petechial hemorrhages were observed in the gastrointestinal tract of all rats that were killed after 4 hours. Four rats died at 1, 6, 7, and 24 hours of respiratory irritation. These results were reported in a distribution and disposition study on Benzalkonium Chloride that is included earlier in the report text (See section on Biological Properties).

SHORT-TERM TOXICITY

Kligman and Kligman (1998) evaluated the short-term toxicity of Benzalkonium Chloride and other chemicals using groups of three hairless albino female mice (Skh:Hairless-1; weights not stated). The mice were treated daily, on five weekdays for 8 weeks, with 1.5% Benzalkonium Chloride. At the end of the treatment period, full thickness excisions were made across the mid-back, horizontal to the cephalad-caudal axis. The specimens were fixed in formalin and processed for light microscopy. An intense inflammatory infiltrate in the dermis resulted in a migration of neutrophils through the epidermis to form a huge exudate within a parakeratotic horny layer. The keratinocytes showed marked cytotoxic changes. There was an increased number of fine elastic fibers in the

dermis.

OCULAR IRRITATION/TOXICITY

Maurer et al. (1998) studied the ocular irritation potential of Benzalkonium Chloride using 5 adult male Sprague-Dawley rats. The right eye of each animal was treated by placing 10 µl directly on the cornea. Untreated left eyes served as controls. At 3 hours and days 1, 2, 3, 4, 7, 14, 21, 28, and 35 post-treatment, the eyes were macroscopically examined for irritation and reactions were scored according to the Draize scale (0 to 110). The eyes and eyelids were collected for microscopic examination. The total mean Draize score for Benzalkonium Chloride (highest mean score ≈40 at 3 hours and day 1; < 10 on days 14-35) was higher at the very early time points and decreased over time. At microscopic examination, changes in the conjunctiva (erosion/attenuation, denudation, and necrosis), cornea (epithelial cell loss), and iris/ciliary body (inflammation) were evident. Collectively, the macroscopic and microscopic changes suggest that Benzalkonium Chloride causes mild ocular irritation.

In another test **(Stern et al., 1998)**, a mean Draize score of 6 (at 24 hours) was reported for 50% Benzalkonium Chloride.

In a study by **Krysiak et al. (1998)**, following the administration of 0.01% and 0.05% aqueous Benzalkonium Chloride into the eyes of rabbits, a weak irritating and rapidly reversible effect was revealed. This text is taken from the English abstract in a Polish publication.

In a study by **Furrer et al. (2000)**, 0.5% aqueous Benzalkonium Chloride was instilled into the eyes of six NMRI albino mice (1 µl instilled) and three male New Zealand white rabbits (10 µl instilled). The corneas were evaluated *in vivo* for ocular tolerance using a confocal laser scanning ophthalmoscope. Mean values for % of the corneal surface that was damaged were: 50.55 ± 1.66 (mice) and 57.0 ± 2.0 (rabbits). Values for 0.9% saline were: 7.28 ± 0.24 (mice) and 1.15 ± 0.23 (rabbits).

The influence of the concentration of Benzalkonium Chloride on ocular irritation potential in mice and rabbits was also studied. Over the concentration range of 0.01% to 0.5%, the test substance was applied to the corneas of mice (1 µl) and rabbits (10 µl) four times per day during three days. The irritation index of Benzalkonium Chloride increased with the test concentration. At the

lowest concentration (0.01%), the concentration at which Benzalkonium Chloride is commonly used as a preservative for aqueous eye drops, Benzalkonium Chloride was not more irritating to the mouse eye than the saline solution. In the case of the rabbit, 0.01% Benzalkonium Chloride was only slightly more irritating than the saline reference (5.78% of corneal surface fluorescent versus 1.15%) **(Furrer et al., 2000)**.

de Saint Jean et al. (2000) studied the toxicity of preserved and unpreserved beta-blocker eye drops in an *in vitro* model fo human conjunctival cells. Chang's conjunctival cell line (ATCC CCL 20.2) was treated for 15 minutes with 0.1%, 0.25% or 0.4% timolol with or without Benzalkonium Chloride and examined immediately or 24 hours later. Cell viability, chromatin condensation, mitochondrial mass and activity, and free radicals production were studied by microplate cold light cytometry. Relative cell number was evaluated using a crystal violet colorimetric test. Additionally, cell size and the expression of an apoptotic marker Apo2.7 were studied by flow cytometry.

Timolol with Benzalkonium Chloride induced a rapid decrease in cell viability, ranging from 40% immediately after treatment to 85% 24 hours later. A small, significantly less important decrease in cell viability was also observed with all tested concentrations of timolol without Benzalkonium Chloride. At 24 hours post-treatment with 0.25% timolol with Benzalkonium Chloride, the relative cell number was reduced by 55%, whereas it did not vary after 0.25% timolol without Benzalkonium Chloride treatment. Only timolol with Benzalkonium Chloride induced chromatin condensation, a decrease in mitochondrial membrane potential, and cell size reduction. Also, reactive oxygen species (ROS) production was significantly more important after cell exposure to timolol with Benzalkonium Chloride.

In this model of conjunctival cells *in vitro*, timolol with Benzalkonium Chloride induced irreversible cytotoxic damage with some characteristics of apoptosis. The authors noted that the active compound of timolol without Benzalkonium Chloride could be responsible for ROS production and for cell viability variations. Oxidative stress could also play a role in timolol with Benzalkonium Chloride-induced toxicity. The authors also noted that *in vitro* toxic effects of antiglaucoma drugs could, in part, explain some ocular surface disorders in long-term treated patients **(de Saint Jean et al., 2000)**.

Noecker et al. (2004) studied the ocular toxicity of the following gluacoma medications containing Benzalkonium Chloride as the preservative: Bimatoprost 0.03% (contains Benzalkonium Chloride; pH 6.7 to 7.8), Dorzolamide 2% (0.008% Benzalkonium Chloride; pH 5.6), Timolol maleate 0.5% (0.01% Benzalkonium Chloride; pH 6.5 to 7.5), and Latanoprost 0.005% (0.02% Benzalkonium Chloride; pH 6.7). Fifteen New Zealand white male rabbits with normal findings on slit-lamp biomicroscopy were used. The upper and lower eyelids of each rabbit were separated, and a single drop was instilled and allowed to moisten the entire cornea. The lids were manually closed, and the rabbit was then allowed to blink normally. Once-daily drops were administered between 7 and 8 a.m. Twice-daily regimens were administered at 7 to 8 a.m. and at 7 to 8 p.m. for 30 days. At the end of treatment, the eyes were enucleated and photomicrographs of the cornea were taken. Corneal damage was graded according to the following scale: 0 (no damage) to 5 (severe damage). Conjunctival specimens were stained and mounted on slides.

Brimatoprost, which contains the lowest Benzalkonium Chloride concentrations of the evaluated medications (0.005%), was associated with significantly less damage than latanoprost, timolol, or dorzolamide ($P = 0.002$). Interestingly, dorzolamide, with a Benzalkonium Chloride concentration of 0.0075%, induced more corneal epithelial damage than either latanoprost or timolol, both of which contain higher levels of Benzalkonium Chloride. These findings suggest that dorzolamide-induced corneal damage may be secondary to low pH (5.6) and not solely to the preservative. Bimatoprost produced significantly less lymphocytic infiltration than latanoprost ($P = 0.033$). Taken together, the findings indicate that 1-month treatment with glaucoma medications containing higher levels of Benzalkonium Chloride resulted in more corneal damage and conjunctival lymphocytic infiltration than lower levels of Benzalkonium Chloride **(Noecker et al., 2004)**.

Denoyer et al. (2006) conducted an *in vivo* assessment of the corneal epithelial toxicity of timolol with Benzalkonium Chloride using very-high-frequency ultrasound imaging. A solution of timolol with 0.01% Benzalkonium Chloride was applied twice daily in the test eyes of ten rabbits, and a Benzalkonium Chloride-free solution of timolol in the control eyes, for 56 days. A 60-MHz ultrasound device was used to evaluate the epithelial damage in Benzalkonium Chloride-exposed eyes, compared to control eyes.

The clinical findings were conjunctival redness, corneal staining and instability of the tear film. *In vivo* VHF ultrasound revealed a thinning of the epithelium of test eyes (from 40.9 ± 1.6 µm at day 0 to 31.8 ± 3.4 µm at day 56; p = 0.0006 for day 0 versus day 56), while the epithelium of control eyes remained unchanged. Ultrasound epithelial thickness was correlated with corneal staining (at day 34 and day 56; p = 0.0025 and 0.0377, respectively) and histological epithelial pachymetry (p = 0.0176 for control and 0.0505 for tested epithelium). Qualitative VHF ultrasound imaging of early epithelial damage was also reported **(Denoyer et al., 2006).**

EFFECT ON MUCOUS MEMBRANES

In a study by **Collin and Carroll (1986)**, ten normal and 20 keratectomized corneas received hourly drops of vehicle with or without Benzalkonium Chloride (0.02% Benzalkonium Chloride or 0.01% Benzalkonium Chloride plus 0.1% disodium ethylenediamine tetraacetate) over the daylight hours of 2 days (18 applications). When applied to the intact cornea, Benzalkonium Chloride with or without Na_2EDTA caused only slight clarification of the endothelial cytoplasm, while a few mitochondrial cristae were displaced. In the keratectomized corneas receiving Benzalkonium Chloride, the majority of the mitochondria of the corneal endothelial cells were pale and swollen, or even disrupted. Many contained aggregations of membranous material either within the mitochondrion or at its outer membrane. The peripheral endothelium was much less affected than the central area of endothelium behind the keratectomy. These results suggest that Benzalkonium Chloride should not be administered to corneas in which the anterior epithelial barrier is incomplete.

Patton et al. (1999) studied the effects of multiple applications of Benzalkonium Chloride and nonoxynol-9 on the vaginal epithelium in the pigtailed macaque (*Macaca nemestrina*). Fourteen sexually mature female pig-tailed macaques were used in the experiments. All were assigned to 1 of 3 treatment groups. A commercially available spermicide containing 4% nonoxynol-9 and one containing 1.2% Benzalkonium Chloride were tested, and each product individually as well as a combination of the products were administered intravaginally. Five macaques received 1.5 ml dose volumes of the Benzalkonium Chloride product (daily for 4 days); 4 received 1.5 ml dose volumes of the nonoxynol-9 product; and 5 received dose volumes of Benzalkonium Chloride product (0.75 ml)

mixed with the nonoxynol-9 product (0.75 ml).

Cervical erythema and vaginal erythema were observed in all three treatment groups. Vaginal epithelial disruption was noted in both the Benzalkonium Chloride and nonoxynol-9 + Benzalkonium Chloride groups. Cervical biopsy specimens from each group revealed acute inflammatory infiltrates with occasional plasma cells and lymphoid follicles. Detection of most microorganisms, including viridans streptococci, decreased in the Benzalkonium Chloride and the nonoxynol-9 + Benzalkonium Chloride groups. It was concluded that Benzalkonium Chloride not only may damage epithelial tissues, but also appears to reduce the population of potentially protective Lactobacillus species in the vagina **(Patton et al., 1999)**.

Cho et al. (2000) evaluated the effect of long-term use of Benzalkonium Chloride on rat nasal respiratory mucosa using 40 Sprague-Dawley rats (4 to 5 weeks old; weights = 120 to 150 g). The following two Benzalkonium Chloride solutions was administered to 2 groups of 9 rats, respectively, and 4 rats comprised the saline-treated control group: Two test solutions, a 0.01 w/v% Benzalkonium Chloride solution that is commonly used as a preservative for nasal drops and a 0.1 w/v% Benzalkonium Chloride solution that could induce dermatitis in humans were made from Benzalkonium Chloride. Each solution (7 µl) was administered into the nasal cavities through both nostrils. Specimens of the head (including nasal cavity) were prepared for microscopic examination. Nasal wheezing was observed in groups that received 0.1 w/v% Benzalkonium Chloride for 2 and 4 weeks. No specific symptoms were observed in the control group.

The control group showed normal respiratory mucosa consisting of pseudostratified columnar epithelium in the nasal septum. In Benzalkonium Chloride treatment groups, inflammatory cell infiltration and the proliferation of intraepithelial glands increased with one week of administration. These histological changes appeared in severe degrees of proliferation of intraepithelial glands, inflammatory cell infiltration, and vascular hyperplasia **(Cho et al., 2000)**.

Lebe et al. (2004) studied the effects of Benzalkonium Chloride in nasal formulations on the mucosal integrity. Benzalkonium (0.01%) was administered to the nostrils of male rats (two groups of 5; weights = 350 to 450 g). One of the groups received 0.01% w/v Benzalkonium Chloride for one

week and the other group received 0.01% Benzalkonium Chloride for four weeks. Clinical symptoms were recorded during treatment and light and electron microscopic examinations were carried out on samples taken from one third central and lower regions of the noses at the end of the treatment periods. Symptomatic changes such as sneezing and nasal rubbing were observed in almost all of the groups, starting from the 6th day of administration. Light and electron microscopy showed histological changes and nasal lesions. The symptomatic and histological changes were more pronounced with prolonged duration of administration. Therefore, it has been concluded that *in vivo* administration of Benzalkonium Chloride may be irritating to the respiratory epithelium of rats.

SKIN IRRITATION/SENSITIZATION

Woolhiser et al. (1998) combined the parameters of a murine local lymph node assay and a mouse ear swelling irritancy assay in an effort to establish a single, rapid screening procedure for the sensitization and irritancy potential of chemicals. In order to validate this assay, a range of chemical irritants and sensitizers was evaluated for their ability to elicit responses in B6C3F1 female mice. The chemicals were administered for four consecutive days to the dorsal and ventral surfaces of each ear. Benzalkonium Chloride was administered at concentrations up to 5.0% in acetone. Ear thickness served to predict irritancy, whereas [^{3}H]thymidine uptake by cervical draining lymph nodes suggested sensitization.

All chemicals known to be potent chemical sensitizers (oxazolone, 2,4-dinitrofluorobenzene, and toluene diisocyanate) produced a marked lymph node cell proliferation in this assay. Animals exposed to irritating agents (sodium lauryl sulfate, croton oil, tetradecane, nonanoic acid, and Benzalkonium Chloride) experienced a significant increase in ear swelling. In addition, these irritating agents elicited low-level lymphocyte proliferation. The authors noted that the combined LLNA/ear swelling assay appears to be a reliable predictor of sensitization and irritancy potential, since it identified the activity of all 8 chemicals tested **(Woolhiser et al., 1998).**

Herouet et al. (1999) studied the capacity of sensitizers, irritants, and neutral chemicals to modulate the surface marker expression and morphology of pure mature Langerhans cells *in vitro*. Contact with four sensitizers (2,4-dinitrobenzenesulfate, 4-ethoxymethylene2-phenyl-2-oxazolin-5-one,

p-Phenylenediamine, mercaptobenzo-thiazole) resulted in a rapid, specific, marked fall in 33D1 expression, a murine specific dendritic cell marker. No effect was observed with two neutral chemicals (sodium chloride, methyl nicotinate) or two irritants (dimethyl sulfoxide, Benzalkonium Chloride). Benzalkonium Chloride and dimethyl sulfoxide were tested at a concentration of 0.2 mg/ml. Collectively, the sensitizers were tested at concentrations up to 5 mg/ml. Sodium lauryl sulfate (0.05 mg/ml), a very irritant detergent, altered morphology and down-regulated all membrane markers **(Herouet et al., 1999)**.

These preliminary data suggest that *in vitro* modulation of 33D1 expression by strong sensitizers may be an approach to the development of an *in vitro* model for the identification of chemicals that have the potential to cause skin sensitization and to distinguish them as far as possible from irritants.

SKIN SENSITIZATION

Goh (1989) evaluated the sensitization potential of Benzalkonium Chloride using a modified Beuhler's technique involving groups of 10 guinea pigs. The induction phase consisted of three weekly 6-hour applications of 10% Benzalkonium Chloride in petrolatum (with Hill Top chambers, under occlusion) to the left shoulder (shaved; same site for all applications). Both 10% Benzalkonium Chloride and 10% Benzalkonium Chloride in petrolatum were tested. The animals remained immobilized for 6 hours and the chambers were removed. This procedure was repeated once per week for the next two weeks, and the induction phase was followed by a two-week non-treatment period.

During the challenge phase, performed at week 5, the test substance (0.5% in petrolatum) was applied to a new site (shaved) on the right flank. The site remained under occlusion for 6 hours. Reactions were scored at 24 hours and 48 hours according to the following scale: 0 (no reaction) to 3 (strong erythema, gross edema). Grades of 1 or greater indicated sensitization. Benzalkonium Chloride induced sensitization in 2 of 10 guinea pigs. None of the 10 guinea pigs in the petrolatum control group had sensitization reactions. The sensitization rate in the dinitrochlorobenzene control group was 9 of 10 guinea pigs **(Goh, 1989)**.

Krysiak et al. (1998) studied the sensitization potential of Benzalkonium Chloride in guinea

pigs. A sensitizing effect of 0.5%, 0.1%, and 0.05% Benzalkonium Chloride aqueous solutions was indicated. The effect differed depending on the concentration that was used. The evaluation covered the proportion of sensitized animals, the nature and intensity of dermal reaction, as well as peripheral blood eosinophil and basophil tests. The results showed that 0.1% aqueous Benzalkonium Chloride solution is an optimum concentration for monitoring the sensitizing effect in humans that are in contact with this substance.

Pichowski et al. (2001) studied allergen-induced changes in interleukin 1β (IL-1β) mRNA expression by human blood-derived dendritic cells. Twelve healthy male and female volunteers (mean age: 48 years, range = 39 to 60) participated in the study. Following five days of culture, the dendritic cells were treated for 30 minutes with 13.89 µM Benzalkonium Chloride (in 0.01% DMSO in medium). Cultures were also treated with known sensitizers such as dinitrofluorobenzene. Data from this study confirm that, under conditions where contact allergens induce increased IL-1β mRNA expression by dendritic cells derived from responsive donors, non-sensitizing skin irritants (in this case, Benzalkonium Chloride) are without effect.

In a study by **Hirota and Moro (2005)**, in order to seek a novel biomarker for predicting skin sensitization, changes in the gene expression profile of THP-1 cells (leukemic cell line, of monocytic origin) on exposure to 2,4-dinitrochlorobenzene (DNCB), p-Phenylenediamine (*p*PD) and nickel sulfate (Ni) were assayed using oligo-DNA microarrays. While the change in gene expression varied depending on the sensitizers, up-regulation of MIP-1β mRNA expression was detected in both DNCB-treated and Ni-treated THP-1 cells. This finding was validated by RT-PCR and confirmed at the protein level by ELISA. Secretion of microphage inflammatory protein 1β from THP-1 was detected after 24 hours of treatment with sensitizers such as DNCB, Ni, 2-mercaptobenzothiazole and cobalt sulfate, while *p*PD and nonsensitizers such as sodium dodecyl sulfate and Benzalkonium Chloride (at 1 µg/ml) had no effect.

CYTOTOXICITY

In a study by **Neville et al. (1986)**, cultured human and rat corneal epithelial cells were used as a model to test cytolytic action of four common preservatives according to the method of Brunner et al. 1968). Benzalkonium Chloride (0.0075 to 0.15%) was found to lyse > 40% of the cells (human and rat) when incubated for 15 minutes at concentrations that are in clinical use in topical ophthalmic

medication. This was not true for 0.0015% Benzalkonium Chloride.

In a study by **Kolde and Knop (1987)**, 15% Benzalkonium Chloride in acetone was painted on to the ears of female BaLB/c mice (8 to 10 weeks old; number of animals not stated). Specimens were examined using electron microscopy. The application of Benzalkonium Chloride induced injury to Langerhans cells that was detectable as early as 1-hour post-application. The Langerhans cells were characterized by mitochondrial swelling and irregular cytoplasmic vacuolization, followed by membrane disruption and disorganization of cellular components.

Kajino (1987) studied the effect of Benzalkonium Chloride on cultured Chinese hamster lung fibroblast V79 cells. The growth of V79 cells was completely inhibited by treatment with Benzalkonium Chloride (30 µg/ml) for 24 to 48 hours. When V79 cells were treated with Benzalkonium Chloride (3 µg/ml) for 2 to 24 hours, the cell survivals were over 75% of untreated cells. The survival of cells exposed at 10 µg/ml for 2 hours, 6 hours, or 12 hours was approximately 65, 30, or 0% of untreated cells, respectively. Treatment of cells with Benzalkonium Chloride at a dose of 30 µg/ml for 2 hours resulted in 100% inhibition of cell survival. The effect of Benzalkonium Chloride on DNA-, RNA-, and protein-syntheses is included in the section on Effects on DNA, RNA, and Protein Synthesis earlier in the report text.

Eun et al. (1994) conducted a comparative study of the cytotoxicity of skin irritants on cultured human oral and skin keratinocytes. Keratinocytes were exposed to sodium lauryl sulfate (SLS) and Benzalkonium Chloride at concentrations of 10^{-4} to 10^{-7} M for 24 hours. Cytotoxicity was evaluated by changes in mitochondrial metabolic activity (MTT) and plasma membrane integrity (lactate dehydrogenase leakage). The decrease in the number of viable cells was statistically significant at a concentration of 1×10^{-5} M ($P < 0.05$), and there was almost total loss of viability at 1×10^{-4} M ($P < 0.05$). There was also a significant decrease in viable cells at 1×10^{-6} M ($P < 0.05$) and at a concentration of 1×10^{-5} M. Benzalkonium Chloride was nearly 100% cytotoxic to both cell types. At concentrations of 1×10^{-4} M SLS and 1×10^{-5} M Benzalkonium Chloride, both irritants increased LDH leakage significantly in both cell types.

Debbasch et al. (1999) studied the cytotoxicity of quaternary ammonium compounds. Cytotoxicity tests were done on a continuous human conjunctival cell line using microplate cold light cytofluorimetry. Membrane integrity (neutral red test), DNA condensation, and reactive oxygen

species (ROS) production were evaluated using living cells treated with different concentrations of Benzalkonium Chloride, benzododecinium bromide, and cetrimide (0.00001 to 0.01%) after 15 minutes of treatment or 15 minutes and 24 hours of cell recovery.

All of the compounds tested showed similar *in vitro* effects. Using the neutral red test, a decrease in membrane integrity was observed, even at concentrations of 0.005% and 0.01% ($p < 0.001$) and after a short time (15 minutes). A stimulation of ROS production (H_2O_2 and O_2) was observed at concentrations of 0.00001% and above ($p < 0.001$), associated with chromatin condensation due to an apoptotic phenomenon. It was concluded that a necrotic phenomenon is suggested at high concentrations of quaternary ammonium preservatives, whereas an apoptotic mechanism exists for lower concentrations. The authors also stated that this toxicity observed in vitro can explain some of the ocular surface damage that is caused by long-term use of preserved eye-drops **(Debbash et al., 1999)**.

In a study by **Moreno (2000)**, the cytotoxicity of Benzalkonium Chloride and other surfactants was evaluated *in vitro* using murine Swiss albino 3T6 fibroblasts. Cell viability was evaluated by measuring the cellular retention of neutral red. Controls and cells incubated with surfactant were loaded for 3 hours with medium containing 0.05% neutral red. After cell lysis, the absorbance of neutral red trapped in cells was read at 535 nm. In the lactate dehydrogenase (LDH) assay, cytotoxicity was measured using the index of lactate dehydrogenase leakage from damaged cells, and was expressed as a percentage of total cellular activity. Arachidonic Acid (AA) release and prostaglandin E_2 (PGE_2) synthesis were also evaluated using 3T6 fibroblast cultures.

Benzalkonium Chloride elicited a concentration-dependent inhibition of neutral red uptake (data not shown), and the concentrations that produced a 10 and 50% decrease (NR_{90} and NR_{50} values, respectively) relative to control cultures were 1.1 ± 0.2 µg/ml and 5.5 ± 0.2 µg/ml, respectively. In the lactate dehydrogenase leakage assay, the cytotoxic effect of Benzalkonium Chloride was dose-dependent, and the lowest concentration that was able to cause a significant increase in LDH was 1 µg/ml. The dose-response curves clearly demonstrated that 3T6 fibroblasts responded to Benzalkonium Chloride treatment with a concentration dependent increase in [^{3}H]AA release, [^{3}H]AA metabolism, and PGE_2 formation. Benzalkonium Chloride induced significant [^{3}H]AA mobilization and metabolism and PGE_2 at the dose of 1 µg/ml. Interestingly, [^{3}H]AA and PGE_2 release occurred at

concentrations of Benzalkonium Chloride that did not cause significant cytotoxicity **(Moreno, 2000)**.

De Saint Jean et al. (2002) studied the *in vitro* toxicity of Benzalkonium Chloride using a human conjunctival cell line. Benzalkonium Chloride was dissolved in serum-free medium at the following concentrations: 0.1% (1 mg/ml), 0.05% (500 µg/ml), 0.01% (100 µg/ml), 0.005% (50 µg/ml), 0.001% (10 µg/ml), and 0.0001% (1 µg/ml). Cells were treated for 10 minutes. Cells were examined prior to Benzalkonium Chloride treatment and at 24, 48, and 72 hours after re-exposure to normal cell culture conditions.

Cell viability decreased significantly ($p < 0.001$), in a dose-dependent manner, after 10 minutes of treatment with Benzalkonium Chloride at concentrations of 0.0001%, 0.0005%, 0.001%, 0.005%, and 0.01%. Only 0.01% Benzalkonium Chloride induced a significant chromatin condensation immediately after treatment. Free radical production was observed after treatment with Benzalkonium Chloride at 0.001% and 0.0001%. After prolonged treatment with Benzalkonium Chloride (15 to 30 minutes), reactive oxygen species production leveled off, or even diminished, which was probably due to the significant cytotoxicity of drugs utilized during long-term exposure (greater than 10 minutes).

Cells were examined 24 hours after treatment with Benzalkonium Chloride. As demonstrated by anticytokeratin and phalloidin labelling, about 75% of cells treated with 0.01% Benzalkonium Chloride presented shrunken cytoskeleton, compared to nontreated cells. With DAPI staining, these cells presented chromatin condensation and fragmentation and reduced nuclear size when compared to the control. Cells treated with 0.001% Benzalkonium Chloride showed mildly diminished cell and nuclear sizes. Chromatin condensation was less frequent, and many cells died without the usual indications of apoptosis. There was neither cell size reduction nor chromatin condensation in 0.0001% Benzalkonium Chloride-treated cells **(De Saint Jean et al., 2002)**.

Boston et al. (2002) examined the morphological changes, cell viability, and lactate dehydrogenase (LDH) activity of human neutrophils exposed to either nasal saline spray (NSS - contains 0.01% Benzalkonium Chloride) or buffered saline without preservatives. Heparinized venous samples were obtained from healthy adult volunteers. The blood was separated, resulting in a cell preparation that was >95% neutrophils. Neutrophils were exposed to one of two commercially available NSS containing Benzalkonium Chloride, one of two control solutions containing phosphate

buffered saline (PBS) without Benzalkonium Chloride or a "homemade" saline solution (HSS) without Benzalkonium Chloride. The final concentration of Benzalkonium Chloride in the experimental conditions was 0.0001% to 0.002%.

In one of the *in vitro* experiments, morphological changes were studied by exposing neutrophils to NSS or PBS at concentrations of 1% to 20% for 3 minutes. Because the nasal spray contains 0.01% Benzalkonium Chloride, the neutrophils were actually exposed to Benzalkonium Chloride at concentrations ranging from 0.0001% to 0.002%. Cells were analyzed for morphological changes by light microscopy.

In a second *in vitro* experiment, a quantitative determination of neutrophil cell lysis was performed using an optimized bioassay to detect LDH that was released from lysed cells. Neutrophils were exposed to the same concentrations of NSS or PBS. LDH released by disrupted neutrophils into solution was measured by spectrophotometry.

Neutrophil morphology was dramatically altered by exposure to NSS. Cells exposed to 20% PBS solution for three minutes exhibited normal cellular architecture with sharp, distinct cell membrane borders, characteristic staining patterns, and typical multi-lobulated nuclei. Neutrophils exposed to 20% NSS for three minutes demonstrated severe disruption of cell membranes and intracellular structures. Similar effects were seen at lower concentrations of NSS, although more intact cells survived. Neutrophils exposed to 20% PBS remained viable and did not stain with trypan blue, while cells exposed to 20% NSS stained dark blue, indicating disruption of their membranes. Similar effects were seen with lower concentrations of NSS, but there was less extensive staining at lower concentrations.

Average LDH activity resulting from neutrophil lysis was graphed as a function of increasing concentration of NSS and PBS, with a constant incubation time of 3 minutes. Neutrophils exposed to NSS at increasing concentrations demonstrated a significant increase in LDH activity at concentrations above 5% ($p < 0.05$). The cells exposed to PBS showed small amounts of LDH activity and little increase in activity with increasing concentrations of PBS.

This study demonstrates that NSS containing Benzalkonium Chloride, even at concentrations far lower than those in commercially available preparations, alters neutrophil morphology, decreases cell viability, and increases neutrophil LDH activity in a concentration- and time-dependent manner. It

was concluded that multi-use preparations of NSS are toxic to human neutrophils, and that substances used to preserve NSS, particularly Benzalkonium Chloride, are responsible for the toxicity **(Boston et al., 2002)**.

In a study by **Goto et al. (2003)**, cells from a human lens epithelial cell line (SrA01/04) were cultured in Dulbecco minimum essential medium supplemented with 5% fetal bovine serum and Benzalkonium Chloride (200 mg/ml). After 7 days of culture, cell morphological changes were assessed using phase-contrast microscopy, and cell-free supernatants were collected for prostaglandin E2 (PGE_2), interleukin 1α (IL-1α), and interleukin 6 (IL-6) iodine 125 radioimmunoassay, enzyme-linked immunosorbent assay, and chemiluninescent enzyme immunoassay, respectively. All cells that were cultured with Benzalkonium Chloride detached from the culture dish and died within 3 days. Secretions of PGE_2 , IL-1α, and IL-6 were 190 to 305 times higher at a x180 dilution of Benzalkonium Chloride compared to controls.

OTOTOXICITY

Barlow et al. (1994) compared the cochlear and middle ear toxicity of the following topical otomicrobial agents: cortisporin otic solution (COS), 0.3% gentamicin ophthalmic solution (GOS), Benzalkonium Chloride (0.026% and 0.05%), and 1% Ofloxacin, a new quinoline antibiotic. Saline (0.9%) was used as the control. The agent was instilled daily for 7 days into the bulla of juvenile guinea pigs (male and female juvenile Hartley strain guinea pigs; starting weight = 250 to 400 g). The animals were killed on the 14th day. The organ of Corti was examined using light microscopy and scanning electron microscopy. The tympanic membrane (TM) and adjacent middle ear mucosa were examined with light microscopy.

The average cochlear hair cell damage was 66% for COS, 6.5% for GOS, and 1% for Ofloxacin, Benzalkonium Chloride, and saline. COS and Benzalkonium Chloride produced moderate mucosal thickening and inflammation. However, this was not statistically different from the mild mucosal thickening produced by saline, GOS, Ofloxacin, and 0.026% Benzalkonium Chloride. There was statistically significant mild to moderate thickening of the TM for all agents, compared to the saline control **(Barlow et al., 1994)**.

TOXICITY TO BONE/SOFT TISSUES

In a study by **Tarbox et al. (1998)**, sixteen rats were separated randomly into two groups:

Benzalkonium Chloride and normal saline. All of the rats had undergone surgical and irritation procedures. A 4-cm incision had been made over the lower lumbar region and the incision was then irrigated with either 0.1% Benzalkonium Chloride or saline. The wound was then closed. Two rats from each group were killed on postoperative days 1, 3, 5, and 7. The surgical wounds were excised and prepared for microscopic examination. Microscopic examination of tissues from the surgical wounds of animals irrigated with 0.1% Benzalkonium Chloride or with normal saline revealed no significant difference in wound inflammation or injury between the Benzalkonium Chloride group and the normal saline group. The bone and cartilage that were present in the sections appeared normal in both groups. Changes were observed in the soft tissues between the various times; however, these changes were similar in both groups and were considered evidence of the normal wound healing process.

NEUROTOXICITY

Goto and Grosfeld (1989) studied the effect of Benzalkonium Chloride on the lower esophagus. Following midline laparotomy, topical Benzalkonium Chloride (0.5%) was applied to the muscularis of the lower 1.0 cm of the esophagus (30-minute application) in 38 Sprague-Dawley rats (weights = 200 g). Thirty-eight additional rats served as controls (unoperated, n = 19; sham laparotomy, n = 19). At three months, the esophagus was evaluated for histologic study and acetylcholinesterase staining. Aganglionosis with positive cholinesterase staining fibers was observed at microscopic examination of the esophagus from rats treated with Benzalkonium Chloride. Topical Benzalkonium Chloride resulted in distal esophageal aganglionosis, characterized by distal narrowing, proximal dilatation, decreased food intake, and limited weight gain, when compared to controls.

In a study by **Zucoloto et al. (1991)**, the jejunum of rats (87 male Wistar rats used in study; weights ≈100g) was treated by serosal application of a 0.2% solution of Benzalkonium Chloride for 30 minutes. Control animals were treated with saline. The animals were allocated to 8 groups of 10 rats each and killed 15, 30, 45, and 60 days after treatment. Segments were removed from the jejunum for neuronal counting, measurement of the smooth muscle area and morphokinetic study of the epithelium. There was a significant reduction in neuron number in the myenteric plexus at 30 days post-treatment. Thickening of smooth muscle was noted at 15 to 60 days post-treatment. No change in epithelial cell proliferation in the jejunum was noted at day 30 or days 15 to 60 post-treatment.

Holle (1998) denervated a region of the small intestine by treating the prepared segment (serosal surface) with a 0.062% Benzalkonium Chloride solution. Male Wistar rats (mean body weight = 350 g) were used. The intestine was then washed with saline and peritoneal and abdominal cavities were closed by sutures. The abdominal cavity was opened again at 20 to 27 days, and, in some animals, seven days after Benzalkonium Chloride treatment.

Following Benzalkonium Chloride, in three cases of the duodenum, jejunum, and ileum, respectively, the myenteric neurons and nerve strands were completely eliminated in the Benzalkonium Chloride segment of the 21-day-treated animals and, in one case, a seven-day-treated animal. In all other cases, including the seven-day-treated animals, neurons and nerves were markedly reduced, damaged, or degenerating in the myenteric plexus, longitudinal muscle layer, and, to a lesser extent, in the circular muscle layer. The most frequent sign of degeneration was vacuolization of the cytoplasm, and, less frequently, of the nucleus. In addition, loss of mitochondrial cristae, damaged or greatly altered organelles, lysis of cell membrane or nuclear membrane, edematous swelling, collagen invasion, and the presence of cellular debris were observed. Fibroblasts as well as macrophages and other phagocytosing cells (e.g. white blood cells) could be observed between the damaged neurons and smooth muscle **(Holle, 1998).**

Kaya et al. (2005) studied the effects of Benzalkonium Chloride on the rat bladder using 15 adult female Wistar rats (weights = 250 to 280 g). The rats were divided into two groups (10 test, 5 control), and the bladders were treated with a local application of 0.3% Benzalkonium Chloride for 30 minutes in the denervation group and saline in the control group. Before and at the 8^{th} week after treatment, cystometry was performed in all rats, and results were recorded. The bladders were removed and whole-mount sections were prepared ad evaluated by immunocytochemistry. Rats treated (denervated) with Benzalkonium Chloride showed a significant increase in bladder capacity ($p < 0.05$), but no significant change was observed in other cystometric parameters ($p > 0.05$) when compared with controls. Immunochemistry results for rats treated with Benzalkonium Chloride indicated none or very few intramural nerve plexuses within the dome of the bladder. The results of this study show that denervation caused by Benzalkonium Chloride is useful for augmentation of the rat bladder. Bladder capacity was increased by the local application of Benzalkonium Chloride without impairing bladder functions.

MUTAGENICITY/GENOTOXICITY

Fukuda (1987) assessed the carcinogenic hazard of 6 substances used in dental practices using Syrian hamster embryo (SHE) cells in cluture. Morphological transformation (MT), unscheduled DNA synthesis (UDS) and sister chromatid exchanges (SCEs) induced in the cells following treatment were used as markers for the risk assessment. MT, which shows a good correlation with carcinogenicity in animals, was induced by treatment with carbol camphor (CC, 0.001 ~0.003 v/v%), eugenol (0.0001~0.001 v/v%) or thymol (3 ~30 µg/ml) for 48 hours. Treatment with EDTA (0.03 µg/ml), Benzalkonium Chloride (0.3 ~3 µg/ml) or benzethonium chloride (0.3 ~ 3 µg/ml) failed to induce MT. All of the three MT-positive substances resulted in UDS, which shows DNA synthesis for repair of DNA damage, indicating that MT by the three substances was initiated by DNA damage.

Exposure of SHE cells to six substances tested, including both MT-positive and -negative substances, for 18 ~ 20 hours elicited a significant level of SCEs, but the inducibility did not exceed twice that of the control level **(Fukuda, 1987)**.

Sakagami et al. (1988) evaluated the genotoxicity of Benzalkonium Chloride using the *umu* test system (Nakamura et al., 1987). Doses up to 5 µg/ml were tested with and without metabolic activation. This method is based on the ability of toxic chemicals to induce *umu* gene expression in *Salmonella typhimurium* TA1535/pSK 1002 in which a plasmid psK1002 carrying a fused gene *umuC*'-'*lacZ* has been introduced. The results for Benzalkonium Chloride were negative with and without metabolic activation. Glutaraldehyde induced potent genotoxic activity, independent of the metabolic activation system, and the activity was increased within the concentration range of 10 to 50 µg/ml.

Sakagami et al. (1988) studied the DNA-damaging capacity and mutagenicity of 10% Benzalkonium Chloride using the liquid rec-assay and Ames test (Ames, et al., 1975) , respectively. Bacillus subtilis suspensions were used in the rec-assay and Salmonella typhimurium strains TA 98 and TA 100 were used in the Ames test. Minimum inhibitory concentrations were determined according to the method of Kosaki et al. (1974), by adding the test substance to the brain-heart infusion agar plates and growing the bacteria on them. For the rec-assay, DNA-damaging capacity was estimated by the growth of tester bacteria, measuring either the ratio of growth rate (Matsui et al., 1980) or the time lag of the growth (Sakagami et al., 1981). The liquid rec-assay revealed the DNA-damaging capacity of Benzalkonium Chloride, which was not positively detected by the minimum

inhibitory concentration method and the Ames test.

Withrow et al. (1989) evaluated the cytotoxicity and mutagenicity of ophthalmic solution preservatives and UVA radiation in L5178Y cells. The following four preservatives commonly used in ophthalmic solutions were tested for their toxic and mutagenic potential in mouse lymphoma cells with and without exposure of the cells to UVA radiation: Benzalkonium Chloride, chlorhexidine, thimerosal, and EDTA. Cell survival and mutagenesis were measured using the L5178Y mouse lymphoma ($TK^{+/-}$) system. Cells were exposed to varying amounts of preservatives for 1 hour, and aliquots were then irradiated with UVA radiation (during exposure to the preservative). Cells were then assayed for survival, and for mutagenesis at the thymidine kinase (TK) locus.

The concentration required to kill 50% of the cells as a result of a 1-hour exposure was approximately 20 µg/ml for chlorhexidine, >3 µg/ml for thimerosal, and 15 µg/ml for Benzalkonium Chloride. Chlorhexidine alone had little effect on the mutation rate, but when combined with UVA exposure, there was approximately a three-fold increase over background in the number of mutants. Thimerosal by itself appears to be weakly mutagenic. When cells were exposed to thimerosal plus UVA treatment, there was approximately a 6-fold increase in the number of mutants over the control value. Treatment of cells with Benzalkonium Chloride alone or in combination with UVA did not induce any mutations. Similarly, EDTA with or without UVA exposure did not change the mutation rate

At concentrations commonly found in ophthalmic solutions, Benzalkonium Chloride, chlorhexidine, and thimerosal were toxic to cells, and thimerosal was slightly mutagenic. When cells were exposed to preservative and UVA irradiation, chlorhexidine was mutagenic and the mutagenic activity of thimerosal was enhanced **(Withrow et al., 1998)**.

Hikiba et al. (2005) assessed the genotoxicity of 14 chemical agents that are used in dental practice using the chromosome aberrations assay involving Syrian hamster embryo (SHE) cells. Statistically significant increases in the frequencies of chromosome aberrations were induced in SHE cells treated with the following chemicals (without metabolic activation): carbol camphor, *m*-cresol, eugenol, guaiacol, zinc oxide, hydrogen peroxide, formaldehyde, and iodine. The results were negative for the following chemicals: thymol, glutaraldehyde, iodoform, Benzalkonuium Chloride (test concentrations up to 30 µM), benzethonium chloride, and chlorhexidine. Only thymol induced chromosome aberrations in the presence of exogenous metabolic activation.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In a study by **Momma et al. (1987)**, relatively high doses of Benzalkonium Chloride (3, 10, and 30 mg/kg) were given by gavage daily at an early stage of pregnancy (0 to 6 days). Animals were killed on the 13th day. Significant changes were not found in body weight, food consumption and general appearance in dams. Also, the number of implantations, number of live fetuses, sex ratio, and body weight in fetuses were not significantly changed. However, there was a tendency toward a slight decrease in pregnancy rate of dams in the groups treated with 10 and 30 mg/kg and a slight increase in the number of dead or resorbed fetuses in the group treated with 30 mg/kg.

In the second experiment, groups of animals were treated with Benzalkonium Chloride at lower doses (1, 50, and 100 µg/kg) during pregnancy (0 to 6 days), while other groups were treated with 1 and 50 µ/kg throughout the entire pregnant period (0 to 18 days). Significant changes were not observed in both treated groups when compared to controls for dams and fetuses.

These results indicate that Benzalkonium Chloride can cause implantation disturbance and/or abortion at a relatively high dose (30 mg/kg), but that it has no reproductive functions at lower dose levels (less than 100 µg/kg) **(Momma et al., 1987)**.

CLINICAL ASSESSMENT OF SAFETY

OCULAR IRRITATION/TOXICITY

Liu et al. (2001) observed a cluster of 12 eyes out of 19 routine cataract surgeries (by phaco-emulsification), performed on a single day, that developed permanent corneal decompensation postoperatively. No eye was identified as having preexisting corneal endothelial disease. An investigation revealed that the cause was inadvertant intracameral use of balanced salt solution preserved with 0.013% Benzalkonium Chloride. In the initial 48 hours after surgery, although only 2 patients reported blurring of vision, corneal edema was present and persisted beyond the 2-week follow-up in 12 of 19 patients. The postoperative appearance of the cornea in the other 7 patients was unremarkable. It was concluded that Benzalkonium Chloride in concentrations that are commonly used extraocularly is highly toxic to the corneal endothelium.

Pisella et al. (2003) conducted a retrospective study using glaucoma patients treated with 0.5% timolol (with [15 patients] or without 0.01% Benzalkonium Chloride [17 patients]). The two

groups were comparable for age and duration of treatment, lasting at least one year. Specimens wee analyzed by flow cytometry for inflammatory profile (using antibodies directed against human leukocyte antigen [HLA] DR and intercellular adhesion molecule-1[ICAM-1] and mucin detection [anti-M1/MUC5AC antibody]). Impression cytology analyses showed a significant increase in the expression of the two inflammatory markers, HLA DR and ICAM-1 in the timolol + Benzalkonium Chloride group and also a significant decrease in goblet cell density in the same group, as compared to the timolol - Benzalkonium Chloride group. It was concluded that the use of long-term preserved beta-blocker in glaucoma patients is associated with a direct subclinical toxicity in the conjunctiva.

MUCOUS MEMBRANE IRRITATION

Hallén and Graf (1995) studied the effect of an oxymetazoline nasal spray (decongestive spray) with and without Benzalkonium Chloride on the nasal mucosa. Twenty healthy volunteers participated in the study. They used the nasal sprays (one dose in each nostril) three times a day for 10 days. Ten of the subjects used a nasal spray composed of oxymetazoline (0.5 mg/ml) and Benzalkonium Chloride (0.1 mg/ml), and the remaining 10 used oxymetazoline nasal spray (0.5 mg/ml) alone. The present trial was performed 3 months after the subjects had used the nasal sprays three times per day during 4 weeks.

The group treated with oxymetazoline nasal spray containing Benzalkonium Chloride developed nasal mucosa swelling during the period of 10 days. The authors stated that the development of nasal mucosa swelling may have been due to vasodilation or to interstitial edema. The group treated with oxymetazoline nasal spray also developed no significant nasal mucosa swelling. Based on the results of this study, the authors sated that Benzalkonium Chloride in decongestant sprays seems to have a long-term effect on the nasal mucosa, at least for three months. Furthermore, they recommended that long-term treatment with nasal decongestive sprays, especially when they contain Benzalkonium Chloride, should be avoided **(Hallén and Graf, 1987)**.

In a study by **Graf et al. (1999)**, 18 patients received oxymetazoline hydrochloride (0.5 mg/ml) nasal spray containing the preservative Benzalkonium Chloride (0.5 mg/ml), and the other 17 were treated with oxymetazoline nasal spray without Benzalkonium Chloride. Before and after the treatment, recordings of the nasal mucosa and minimal cross sectional area were made with rhinostereometry and acoustic rhinometry, followed by histamine hydrochloride challenge tests.

No rebound swelling was found after the 10-day treatment in the two groups with either of the methods or as estimated by symptom scores. In the group receiving oxymetazoline containing Benzalkonium Chloride, but not in the other group, the histamine sensitivity was significantly reduced after treatment ($p < 0.001$). Specifically, in this group, the mean mucosal swelling after histamine hydrochloride challenge before treatment was 1.4 mm with a dose of 1 mg/ml, 1.8 mm with 2 mg/ml, and 2.2 mm with 4 mg/ml. After 10 days of treatment, the corresponding values for mucosal swelling were 0.5, 0.8, and 1.1 mm. The reduction in mucosal swelling after histamine challenge was significant at all 3 histamine provocation levels (analysis of variance, $p < 0.001$). The mean mucosal swelling after 10 days was 0.21 mm, compared with the reference value before starting the medication.

The authors noted that it is safe to use topical nasal oxymetazoline with or without Benzalkonium Chloride for 10 days in patients with vasomotor rhinitis; however, this study indicates that Benzalkonium Chloride in nasal decongestant sprays affects the nasal mucosa also after short-term use **(Graf et al., 1999)**.

SKIN IRRITATION

In a study by **Willis et al. (1986)**, 17 non-atopic, healthy adult patients (ages not stated) were patch-tested (occlusive Finn chambers) with 0.5% Benzalkonium Chloride for a maximum of 48 hours. The Finn chambers were applied to the left forearm. Irritant contact dermatitis was induced in the patients.

Agner and Serup (1988) studied the skin irritation potential of Benzalkonium Chloride using 16 healthy volunteers. Closed patch tests containing 3% Benzalkonium Chloride (in distilled water) were applied to both arms (antero-lateral surface of upper arm) using Finn chambers on Scanpor tape. Patches were removed at 24 hours post-application. Reactions were scored at 24 and 96 hours post-application. Reactions were scored according to the following scale: 0 (no reaction) to 3 (strong, positive reaction - advanced erythema, infiltration, possibly vesicles, bullae, pustules and/or pronounced crustation). Median visual scores of 1.5 and 0 were reported at 24 and 96 hours post-application. Scores of 1 and 2 are defined as weak positive reaction and moderate positive reaction, respectively.

Willis et al. (1988a) evaluated the skin irritation potential of Benzalkonium Chloride and other

mononuclear cells. Occasional foci of necrotic damage were evident in the upper stratum spinosum. Ultrastructurally, the affected keratinocytes were characterized by shrunken pyknotic nuclei, disrupted organelles, and membranes and considerable intracytoplasmic vacuolation. Lipid accumulation within keratinocytes was rarely observed **(Willis et al., 1989)**.

Willis et al. (1990) studied the behavior of epidermal CD1+ cells in experimentally induced irritant contact dermatitis. Ten healthy non-atopic male volunteers were patch tested (on the volar aspect of the forearm using Finn chambers) with 0.5% (w/v) aqueous Benzalkonium Chloride. After 48 hours of contact, the patches were removed and reactions were scored 1 hour later according to the following scale 0 (negative) to 3 (erythema, edema, and vesiculation). Half of the volunteers received vehicle control patch tests of yellow soft paraffin and propan-1-ol , with the remaining half receiving patch tests of distilled water and occlusion alone. Punch biopsies were obtained from the test and control sites and examined microscopically. The majority of the irritation reactions were mild to severe. All controls were negative during visual assessment. Benzalkonium Chloride (0.5% aqueous) induced irritant contact dermatitis.

For Benzalkonium Chloride-treated sites, the numbers of CD1+ cells within the epidermis were not significantly different form those of their appropriate control group. Regarding the dendrite cell length of CD1+ cells, the dendrites present in the reaction with Benzalkonium Chloride and, also, in the vehicle and occlusion controls were not significantly different from those of normal skin. Qualitative assessment of the distribution of CD1+ cells within the epidermis suggested that there was little or no difference between Benzalkonium Chloride-treated and normal skin. That is, the majority of CD1+ cells were located in the mid or basal epidermis. Ultrastructural examination of Benzalkonium Chloride patch test sites indicated that the majority of epidermal cells with Langerhans cell-like characteristics were found to possess Birbeck granules. Some of these cells resembled those in normal skin, but many exhibited morphologic features that were indicative of metabolic activation, or, less commonly, of cellular damage **(Willis et al., 1990)**.

In a study by **Krysiak et al. (1998)**, the threshold irritating effect of Benzalkonium Chloride on rabbit skin was determined. As a threshold concentration of Benzalkonium Chloride evoking slight inflammatory reaction on the skin in a single, closed exposure and in multiple, open exposure, the following values were adopted for its aqueous solutions: >0.5 to 1% and 0.05%, respectively. This

text is taken from the English abstract in a Polish publication.

Wallengren (2000) applied 1% aqueous Benzalkonium Chloride to the dorsal skin of 21 subjects using Al-tests®. The study group consisted of 25 female patients with nickel contact allergy (mean age = 47 ± 10 years) and 8 patients (3 men, 5 women; mean age = 39 ± 14 years) with birch-pollen-induced allergic rhinitis. Details regarding the composition of the group of 21 patients were not included. The test substance was applied for 48 hours and the reaction was examined 24 hours after patch removal. The response area was estimated planimetrically. Benzalkonium Chloride induced a sharply demarcated homogenous erythema, sometimes with edema. Further details were not provided.

Santucci et al. (2003) evaluated cutaneous responses to irritants. From January to March of 2001, 520 subjects (40 healthy volunteers; 480 affected by active skin diseases) were tested. All subjects were simultaneously patch tested with 15 µL of 1% aqueous Benzalkonium Chloride. The following groups were tested: Group 1 (40 healthy nonatopic volunteers), Group 2 (40 atopic subjects with active dermatitis), Group 3 (57 patients with active psoriasis), Group 4 (124 eczematous subjects with positive patch test responses), Group 5 (140 eczematous subjects with no patch test responses), Group 6 (79 patients with active urticaria), and Group 7 (40 subjects with generalized pruritus). Patch tests were applied to both sides of the upper back for two days using Al-test on Fixomul. Readings were performed one hour after removal of the strips. Results were scored according to the following scale: - (no visible reaction) to ++++ (erythema with vesicles or bullae).

Isolated positive reactions to 1% aqueous Benzalkonium Chloride occurred in: 42% of the healthy subjects, 40% of eczema patients with positive patch tests, 38% of patients with both psoriasis and eczema with negative patch tests, 33% of patients with urticaria, 30% of patients with atopic dermatitis, and 27% of patients with generalized pruritus **(Santucci et al., 2003)**.

Marriott et al. (2005) conducted two 23-hour patch tests on Benzalkonium Chloride (0.1% and 0.2%) to determine irritant susceptibility. Fifty-eight healthy adults (19 males, 39 females; ages = 22 to 58, mean age of 39 years) participated in the study. Benzalkonium Chloride (50 µl) was applied to patches consisting of Finn chambers on Fixomull tape. Each panelist had two patches (containing 0.1% and 0.2% Benzalkonium Chloride, respectively) applied, one on each upper outer arm. The patch with the lower concentration also held the negative control, ultrapure water. The patches were

applied for 23 hours, after which they were removed. The second patches were then applied to the same sites for a further period of 23 hours. Erythema, dryness, and other visual parameters were assessed by trained skin assessors using the grading scheme by Basketter et al. (1997).

Panelists with an erythema grade of <6 were as follows: 18 subjects (0.1% Benzalkonium Chloride) and 14 subjects (0.2% Benzalkonium Chloride). The following panelists had an erythema grade of >6: 39 subjects (0.1% Benzalkonium Chloride) and 43 (0.2% Benzalkonium Chloride). There were also 7 panelists who also developed an erythema grade of ≥6 to ultrapure water **(Marriott et al., 2005)**.

REDUCTION OF SKIN IRRITATION POTENTIAL

McFadden et al. (2000) conducted a study to determine whether or not Benzalkonium Chloride reduces the skin irritation potential of sodium dodecyl sulfate. Fifty-four non-atopic adult volunteers (16 males, mean age = 34.7; 38 females, mean age = 34.6). Sodium dodecyl sulfate (20%) was applied for 2 hours under occlusion (Finn chamber on Scanpor). Benzalkonium Chloride (1%) was then applied to the same site. Various controls, including sodium dodecyl sulfate application followed by water for 2 hours, were included. The irritant reaction was assessed at 24 hours and 48 hours. Forty of the 54 subjects had some reaction when Sodium dodecyl sulfate was applied for 2 hours, followed by either Benzalkonium Chloride or the water control under occlusion. In comparison to the water control, where Benzalkonium Chloride was applied after sodium dodecyl sulfate, 20 of the 40 responders had a weaker reaction, but only 4 had a stronger response. This study shows that Benzalkonium Chloride applied to skin exposed to sodium dodecyl sulfate attentuates the resulting irritant reaction.

SKIN SENSITIZATION

De Groot et al. (1986) studied the prevalence of contact allergies to commonly used preservatives, including Benzalkonium Chloride. During the period from January 1 to April 30, 1985, 627 consecutive patients were patch-tested with 0.1% aqueous Benzalkonium Chloride according to ICDRG recommendations. Irritant or dubious (?+) reactions were observed frequently (27 patients). The frequency of positive reactions to 0.1% aqueous Benzalkonium Chloride was 8/627 or 1.3%.

Kokelj and Cantarutti (1986) evaluated the incidence of sensitization reactions to Benzalkonium Chloride and numerous other ingredients in a vehicle test series and the more common

allergens that are contained in creams and ointments. Twenty-five patients with leg ulcers (10 males, 15 females; average age: 69 years; range: 45 to 87 years). Patch test evaluations were performed according to the procedure of Wilkinson et al., 1970, and test results indicated one positive reaction to Benzalkonium Chloride. The greatest number of positive reactions was induced by balsam of Peru (7 reactions) and neomycin (7 reactions).

In a study by **Klein et al. (1991)**, five positive reactions to 0.1% Benzalkonium Chloride in petrolatum were observed in a population of 32 patients that were tested with an eye or preservatives series. All reactions were ++ or +++ and considered to be allergic, by virtue of erythema, infiltration and sometimes vesicles, spreading over the margins of the test area, and a crescendo from days 2 to 3. In one patient (elderly), the reaction was clearly relevant, because he did not tolerate Benzalkonium Chloride-containing-eyedrops, showed no reaction to all other compounds that he used, and his conjunctivitis and eyelid eczema cleared after removal of the eyedrops. The other 4 patients were young nurses who suffered from recurrent hand eczema and had contact with Benzalkonium Chloride-containing surface disinfectants.

Three nurses were willing to undergo additional tests. Two had an additional patch test and showed positive reactions to higher dilutions and all 3 performed a use test by applying an aqueous solution of Benzalkonium Chloride on a small circumscribed area of the volar aspect of the forearm 5 x/day for 4 days. The use test was positive only in one of the patients, whose eczema improved after removal of the Benzalkonium Chloride-containing disinfectant from her working environment. In contrast, the other two neither reacted to a 0.05% (which is the working concentrations in surface-disinfectant solutions) nor to a 0.1% solution. Despite their positive patch test reactions, the two nurses with negative use tests were therefore considered not to be allergic to Benzalkonium Chloride.

Benzalkonium Chloride may thus cause sensitization in hospital personnel in rare instances, but positive patch test reactions must be interpreted with great caution, even if they occur in higher dilutions than recommended in the literature and show the typical characteristics of allergic reactions. Patients should be encouraged to perform use tests, which can conveniently be performed on the forearms **(Klein et al. 1991)**.

Fuchs et al. (1993) conducted a multicenter study to determine whether Benzalkonium Chloride is a relevant contact allergen or irritant. At eight dermatological clinics, a total of 2146

patients was tested with 0.1% Benzalkonium Chloride between May of 1990 and December of 1991. In 225 cases, an allergic reaction was proven, with 258 irritant reactions in addition. Only 12 cases were clinically relevant. Therefore, Benzalkonium Chloride is considered a weak allergen.

In a study by **Perrenoud et al. (1994)**, from February 1989 to January 1990, the Swiss Contact Dermatitis Research Group conducted a 1-year study to examine the frequency of sensitization to a series of 13 common preservatives. A group of 2295 consecutive outpatients with suspected allergic contact dermatitis (age range: 7 to 90 years; mean age: 42; 911 males, 1384 females) was tested. The percentages of positive reactions to the preservatives studied were as follows, in descending order: formaldehyde (5.7%), 0.1% aqueous Benzalkonium Chloride (5.5%), Kathon CG (5.5%), thimerosal (4.2%), chlorhexidine digluconate (2.0%), dmdm hydantoin (1.7%), paraben mix (1.7%), chloroacetamide (1.5%), bronopol (1.2%), imidazolidinyl urea (1.0%), quaternium 15 (1.0%), triclosan (0.8%), and 2,4-dichlorobenzyl alcohol (0.4%).

In a study by **Stables et al. (1996)**, 92 children (45 girls, 47 boys; ages: 3 to 14, mean age of 9.3 years) were referred for patch testing at the Regional Contact Dermatitis Investigation unit in Glasgow, UK during the period from 1979 to 1993. The European standard series of allergens was applied to all children, together with any additional series as indicated from the history. Patch testing was performed on the unaffected skin of the upper part of the back using Finn chambers on Scanpor tape. Readings wee graded according to the ICDRG recommendations (2- and 4-day readings). Relevance of each positive reaction was determined by reviewing the patient's history.

At the time of referral for patch testing, 45 of the 92 children were diagnosed as having atopic dermatitis. Twenty-six had localized dermatitis affecting the face or perineum or hands and/or feet only (with no associated personal or family features of atopy). Fifteen had juvenile plantar dermatosis (JPD), and there were 2 each with orofacial granulomatosis, vaccination reaction, and atypical psoriasis. Patch test results indicated that nickel was the most common allergen (10 of 92 patients or 10.9%). A single positive reaction to Benzalkonium Chloride was reported **(Stables et al., 1996)**.

Aoki (1997) studied the clinical manifestations and causative agents in cases with allergic contact dermatitis due to eye drops in patients who consulted the Department of Dermatology, Nippon Medical School during the period of 9 years between January of 1987 and December of 1995. Among the 66,165 cases who visited the department, 3,903 were suspected of having contact dermatitis or

drug eruption and underwent patch testing. Of these, 141 (3.6%) were patch tested with eye drops and 49 cases (34.8%) reacted positively and were diagnosed as having allergic contact dermatitis.

The 49 cases diagnosed as having allergic contact dermatitis from eye drops included 17 males and 32 females. 81.6% of the patients were over 40 years of age. The most frequent ages were between 60 and 69 years. The period from onset to the first visit to the dermatology department was less than 2 weeks in 51.4% of the cases. However, three patients consulted the department after more than six months. The determined allergens were noted in the order of frequency as follows: fradiomycin sulfate (14 cases), ketotifen fumarate (6 cases), dibekacin sulfate (3 cases), befunolol hydrochloride (3 cases), phenylephrine hydrochloride (3 cases), and Benzalkonium Chloride, preservative in eye drops, (3 cases) **(Aoki, 1997)**.

Schnuch et al. (1998) collected patch test data and data from the patients' history from 24 departments participating in the Information Network of Departments of Dermatology from January 1, 1990 to December 31, 1994. Patch test data from 28,349 patients tested with preservatives of the standard series (SS), from 11,485 patients tested additionally with a preservative series (PS), and from 1787 patients tested with an industrial baccata tray (IB) were evaluated. 11,308 patients were patch-tested with Benzalkonium Chloride. Patch tests were performed according to recommendations of the International Contact Dermatitis Research Group and the German Contact Dermatitis Group. Finn chambers on Scanpor were used in 19 departments and other systems were used in five departments. The patch test concentrations were as follows: Benzalkonium Chloride (0.1% in petrolatum), alkylaminobenzoate (0.3% in petrolatum), MDBGN/PE (0.5% in petrolatum), chloroacetamide (0.2% in petrolatum), diazolidinyl urea (2% in petrolatum), octylgallate (0.3% in petrolatum), and BronopolTM (0.5% in petrolatum).

Nine of the 24 centers applied patch tests for 24 hours, and, the remainder (15 of 24) for 48 hours. Readings were done until at least 72 hours after application of the test chambers. For this study, only readings at 72 hours were considered.

The most important allergens of the PS were, in women, alkylaminobenzoate (contained in milking fat) (2.5%), Methyldibromoglutaronitrile/phenoxyethanol (MDBGN/PE, 2.2%), Benzalkonium Chloride (1.8% - 207 patients with positive reactions), chloroacetamide (1.4%), diazolidinyl urea (1.3%), octylgallate (1.2%), and 2-bromo-2-nitropropane-1,3-diol (Bronopol^{TM} ,1.1%). In men, rates

differed only with regard to alkylaminobenzoate (0.9%) **(Schnuch et al., 1998)**.

Nettis et al. (2002) studied occupational irritant and allergic contact dermatitis among healthcare workers. The authors reviewed their database for data from 1994 to 1998 and selected 360 consecutive patients working in healthcare environments and experiencing contact dermatitis of the hands, wrists, and forearms. The authors found that allergic contact dermatitis and irritant contact dermatitis were considered work-related in 16.5% (72/436) and 44.4% (194/436) of diagnoses, respectively. In this study, the major relevant etiological agents that induced occupational allergic contact dermatitis were: nickel sulfate (41 patch positivities), glutaraldehyde (5), Benzalkonium Chloride (7), thiuram mix (15), carba mix (9), and tetramethylthiuram monosulfide (6).

Herbst et al. (2004) evaluated patch test results of the Information Network of the Departments of Dermatology. During a 5-year period (1995-1999), of a total of 49, 256 patch-tested patients, 1053 (2.1%) were eventually diagnosed as allergic periorbital dermatitis (APD) and 588 (1.2%) as non-allergic periorbital dermatitis (NAPD). Patient characteristics between APD, NAPD and other cases (OCs) differed with respect to sex (19.7% male in both periorbital groups versus 36.3% in OCs), atopic dermatitis (10.4% in APD versus 60.2% in NAPD versus 16.9% in OCs) and age, APD being substantially more often (68.2%), ages 40 and above, than NAPD (52.6%). For the APD group, of the 893 patients patch tested with 0.1% Benzalkonium Chloride in petrolatum, 1.9% positive reactions. For the NAPD group, of the 491 patients patch-tested with 0.1% Benzalkonium Chloride in petrolatum, 0.3% had positive reactions. For the OC patients, of the 27,122 patients patch-tested with 0.1% Benzalkonium Chloride in petrolatum, 0.9% had positive reactions.

Saap et al. (2004) patch-tested 54 leg ulcer patients (19 men, 35 women; mean age= 65.24 ± 13.96 years), with the NACDG standard series and a comprehensive supplemental series of 52 allergens. Patch tests (Finn chambers) were applied to hairless skin of the back of each patient. Patch test results were read 48 hours and 96 or 120 hours after application and reactions were scored according to the following scale: 1+ (weak positive) to 3+ (extremely strong positive). Sixty-three percent (n = 34) of the patients had one or multiple positive patch test results, and 37% (n = 20) had no positive patch test result. The most common allergens were balsam of Peru (30% of the patients, [16/54]), bacitracin (24% [13/54]), fragrance mix (20% [11/54]), propylene glycol (14% [7/52]), Benzalkonium Chloride (13% [7/54]), carba mix (11% [6/54]), nickel sulfate (11% [6/54], and control

gel hydrocolloid (11% [6/54]).

According to a study by **Dastychova et al. (2004)**, a group of 514 patients (178 men, 336 women; average age = 42.8 years) suffering from chronic eczema was tested by means of epicutaneous tests for contact hypersensitivity to selected auxiliary substances of dermatological external and cosmetic preparations. The testing took place from April of 2001 to December of 2002. In 194 patients, the principal diagnosis was atopic eczema. Of the preservatives, the most frequently sensitizing agents were as follows: thiomersal (in 13.6% of the patients); phenylmercuric acetate (in 7.8%); formaldehyde (in 5.6%); Bronopol (in 5.1%); chlorhexidine (in 3.3%); dibromodicyanobutane/phenoxyethanol (in 2.9%); chloroacetamide (in 2.1%); Kathon CG and parabens (in 1.9%); imidazolidinyl urea and diazolidinyl urea (in 1.4%); glutaraldehyde (in 1.2%); DMDM Hydantoin (in 1.0%); dichlorophen (in 0.8%); sorbic acid, phenoxyethanol, and triclosan (all in 0.6%); Benzalkonium Chloride, quaternium-15, and chlorocresol (all in 0.4%); and chloroquinaldol (in 0.2% of the group of patients.

Pratt et al. (2004) reported the results of patch testing from January 1, 2001 to December 31, 2002, by the North American Contact Dermatitis Group. For this study period, Benzalkonium Chloride was one of the new additions to the NACDG screening tray. Up to 4,913 patients (2 to 97 years old) were patch-tested with a screening series of 65 allergens. Patch testing was done with a standardized technique using Finn Chambers on Scanpor tape. The patches remained in place for 48 hours, and test sites were evaluated initially at 48 to 72 hours, and again between 72 and 168 hours after placement. A positive reaction was interpreted as +, ++, or +++, manifested by erythematous papules, vesicles and/or a spreading reaction, sometimes with crusting and ulceration. The clinical relevance of the positive patch test reactions was determined by the patient's history and clinical skin examination findings. Present relevance was divided into definite, probable, and possible relevance.

Relevance was considered definite if the reaction to a use test with the putative item containing the suspected allergen was positive or if a positive patch-test reaction to the object or product was obtained. Relevance was considered probable if the substance identified by patch testing could be verified as present in the known skin contactants of the patient, and if the clinical distribution fit. Relevance was considered possible if the patient was exposed to circumstances in which skin contact with materials known to contain the putative allergen likely occurred.

Of the 4,892 patients patch tested with 0.1% aqueous Benzalkonium Chloride, 4.3% had positive reactions, 2.8% had irritant reactions, and 1.5% had reactions that were classified as unknown. Relevance was summarized as follows: definite relevance (0.9%), probable relevance (5.2%), possible relevance (20.8%), and past relevance (8.5%). 2.5% nickel sulfate yielded the highest frequency of positive reactions (16.7%) in 4,901 patients; 0.4% had irritant reactions **(Pratt et al., 2004)**.

A study by **Boyvat et al. (2005)** was designed to evaluate the frequency of contact sensitivity to 14 common preservatives among patients with contact dermatitis in Turkey. From 2000 to 2004, 308 patients with a diagnosis of contact dermatitis were patch tested in the Department of Dermatology of Ankara University School of Medicine. All patients were patch tested with the European standard series. Of the 308 patients suspected of having contact dermatitis, 23 patients were found to have positive reactions to one or more preservatives. The preservatives that were the most frequent cause of positive reactions were as follows: thimerosal (1.6%), Benzalkonium Chloride (5 positive reactions or 1.6%), formaldehyde (1.3%), and MDBGN/PE (0.9%).

CASE REPORTS

In a case report by **Fisher (1987)**, a 36-year-old operating nurse acquired a pruritic eczematous eruption around an abrasion after he had applied a medicated adhesive strip containing Benzalkonium Chloride to the inner aspect of the thigh. A strongly positive patch test result was obtained with a 1:1000 aqueous solution of Benzalkonium Chloride.

Miszkiel et al.(1988) investigated the possibility that Benzalkonium Chloride-induced bronchoconstriction results from the endogenous release of histamine by examining the effect of the selective histamine antagonists terfenadine and astemizole on the airways' response to inhaled Benzalkonium Chloride and histamine in 12 asthmatic subjects (6 men, 6 women; mean age = 29 ± 4 years). All were atopic, demonstrating a greater than 3 mm wheal following skin prick test to at least two common allergens and had a mean baseline forced expiratory volume (FEV_1) of 94 ± 7% predicted values. Airway calibre was measured before and during the inhalation challenge as the FEV_1. On each challenge day, Benzalkonium Chloride was dissolved in 0.9% sodium chloride to produce a range of doubling concentrations of 0.4 to 50 mg/ml. Histamine monophosphate was dissolved in 0.9% sodium chloride to produce a range of doubling concentrations of 0.03 to 64 mg/ml.

Benzalkonium Chloride and histamjne caused concentration-related decreases in FEV_1 in all of the subjects, with Benzalkonium Chloride being 7.4 times less potent as a bronchoconstrictor agonist than histamine. Terfenadine displaced to the right the Benzalkonium Chloride and histamine concentration response curves by 3.7- and 111-fold, respectively. Terfenadine attentuated the initial (5 minutes) bronchoconstrictor response to Benzalkonium Chloride by 40% However, over the whole 45-minute period, the response was reduced by only 13%, compared with 86% inhibition of the response to histamine.

Eight of the 12 subjects undertook a time course study with inhaled Benzalkonium Chloride after pretreatment with the chemically unrelated histamine antagonist astemizole. Astemizole inhibited Benzalkonium Chloride-induced bronchoconstriction to an almost identical degree as that achieved with terfenadine.

It was concluded that the initial bronchoconstrictor effect of Benzalkonium Chloride is due, in part, to histamine release **(Miszkiel et al., 1988)**.

Trevisan et al. (1988) reported a case of a 6-year-old girl affected by an itching erhthematous, vesciculous, desquamative dermatitis of the ears and cheeks. Dermatitis appeared after applications of earrings and a diagnosis of contact dermatitis to metal was made. Patch testing with metals (including gold chloride) gave negative results. On the contrary, positive results were achieved with Lysoform Medical (1:20 and 1:1000), a liquid disinfectant used to clean earring pins, and with Benzalkonium Chloride present in the Lysoform.

In a case report by **Okamoto et al. (1991)**, a 37-year-old female presented with a scaly, erythematous eruption on the second and third fingers of the left hand over a three-month period. She had been working in the ophthalmology department of a hospital for two years, and her routine work there was to instill mydiratic agents into patients with eye diseases, as part of their fundoscopic examination. The eyedrops that were used contained Benzalkonium Chloride and other ingredients. Patch test results for Benzalkonium Chloride were negative.

According to **Boucher et al. (1992)**, a 64-year-old male was admitted to the Ottawa General Hospital because of an exacerbation of chronic obstructive airway disease. His presenting symptoms were increasing shortness of breath and productive cough. The patient was started on intermittent albuterol, being exposed to 600 µg of Benzalkonium Chloride per treatment. The drug combination

was administered every 1 to 2 hours initially, but was changed to every four hours because the patient was complaining of increased difficulty in breathing after treatments. The patient improved and early discharge was anticipated, however, he began to complain of increased shortness of breath after each nebulized treatment.

Considering the time course of the bronchoconstriction, the amount of preservative present in the nebulized solution, and the lack of reaction to the drugs administered via metered-dose inhalation, the authors noted that this case is suggestive of a preservative-induced severe paradoxical bronchoconstriction. The authors noted that two classes of preservative agents have been mostly involved in bronchoconstriction reactions, sulfite derivatives (sodium metabisulfite and sodium bisulfite) and Benzalkonium Chloride **(Boucher et al., 1992)**.

Ponder and Wray (1993) reported a case of a 58-year-old gynecologic nurse with a long history of allergic rhinitis, chronic sinusitis, and relative airway disease. The patient was scheduled for sinus surgery and, during a preoperative jet nebulization treatment with Proventil (contains Benzalkonium Chloride), flushing and itching of the face and neck were observed. The patient was initially tested (epicutaneous method) with 0.001% and 0.01% Benzalkonium Chloride (diluted with saline), and the results were negative. Subsequently, when tested intradermally with the 0.001% Benzalkonium Chloride solution, a positive response was reported. Within a few minutes of the intradermal test, the patient experienced a systemic reaction consisting of runny nose, throat fulness, and facial flushing (with pitting edema of the face and a severe cough). The results for three healthy control subjects were negative in both epicutaneous and intradermal tests.

In a case report by **Corazzo and Virgili (1993)**, a 48-year old female farm worker (assigned to pig breeding) had a 1-year history of widespread recurrent vesicular eczema of the hands, face, neck, thighs, axillae, groins and abdominal folds. She was exposed to detergents and disinfectants on the job, and was subsequently patch tested with the European standard series and the animicrobials and preservatives series. The following positive reactions were reported: 5% nickel sulfate in petrolatum (++ on day 2; +++ on day 3), 0.1% aqueous Benzalkonium Chloride (- on day 2; ++ on day 3), and 0.01% aqueous Benzalkonium Chloride (?+ on day 2; + on day 3). Patch tests with Benzalkonium Chloride at a concentration of 0.01% aqueous and two products at a concentration of 10% aqueous were negative in five controls. The authors stated that the positive reaction to

Benzalkonium Chloride at the lower test concentration and the close relationship between flares of eczema and use of the products are sufficient evidence for the diagnosis of occupational allergic contact dermatitis from Benzalkonium Chloride.

In a case report by **Cusano and Luciano (1993)**, a 36-year-old nurse was seen for an intensely itchy eczema, which had begun on the hands and forearms about 4 months previously and had worsened in the last month, spreading to the upper arms and face. Patch test results were positive on day 2 (+++) and day 3 (+++). Because of extreme bullous reactions to Benzalkonium Chloride two healthy volunteers and 8 patients with contact dermatitis were patch tested. Positive reactions were not observed.

According to **Corazza and Virgili (1993)**, a 48-year-old female had a one-year history of widespread recurrent vesicular eczema of the hands, face, neck, thighs, axillae, groins, and abdominal folds. She was a farm worker assigned to pig breeding, and used detergents and disinfectants. Patch test results for 0.1% aqueous Benzalkonium Chloride were negative on day 2 and ++ on day 3. Subsequent patch testing with 0.01% Benzalkonium Chloride yielded the following results: ?+ on day 2 and + on day 3.

In a case report by **Cox (1994)**, a 78-year-old female presented with marked periorbital erythema and edema, and erythema of the nail folds of both hands. The clinical differential diagnosis of the facial rash included contact allergy, drug eruption, and dermatomyositis. Patch testing to the European standard series and an eyedrop/contact lens series was carried out, when her general condition permitted, demonstrating a ++ reaction to Benzalkonium Chloride. The patient had used Teoptic eyedrops (cartereol 2%, with Benzalkonium Chloride preservative) for glaucoma. The facial and nailfold eruptions settled rapidly with moderate-potency topical corticosteriod, and the eyedrops were changed to metipranolol 0.3%. No relapse occurred during the following two months.

According to **Stanford and Georgouras (1996)**, a 14-year-old boy presented with severe dermatitis of the right forearm, where it had been in contact with a plaster cast over the previous month. Upon removal of the cast, the underlying skin was erythematous and edematous with vesicles and bullae. There were no systemic symptoms, no past or family history of atopy and no previous exposure to plaster casts. Antiseptic solution had not been used prior to application of the cast. On examination, a severe dermatitis was evident around the right forearm. A milder eczematous area on

the abdomen corresponded to the area where the cast had rested while in the sling. The patient developed a strong allergic reaction to the Gypsona® plaster, as well as to both 0.1% aqueous and 0.01% aqueous Benzalkonium Chloride.

In a case report by **Gonzalo Garijo et al. (1996)**, a 37-year-old male with a history of allergic rhinitis from sensitization to dust mites experienced urticaria, dyspnea, wheezing, rhinorrhea, and dysphonia 20 minutes after intraarticular administration of mepivacaine hydrochloride and paramethasone acetate (includes the excipients Benzalkonium Chloride, polysorbate 80, and povidone) in his right knee. After treatment, symptoms resolved uneventfully over the next 24 hours. Two months after discharge from the hospital, the patient was seen in the allergy department.

Because of suspected anaphylactic shock, the patient was admitted for controlled provocation tests. Prior *in vitro* studies with these substances (histamine release test, basophil degranulation test, and lymphoblast transformation test - test details not provided) were negative. Results of provocation testing polysorbate 80 and Benzalkonium Chloride were negative, but were positive with povidone. The reaction to povidone appeared 5 minutes after intramuscular administration of the pure preparation (1 mL), and included aqueous rhinorrhea, conjunctival reddening, dysphagia, dyspnea, rash on the back, and discrete edema of the uvula and anterior pillars **(Gonzalo Garijo et al., 1996)**.

In a case report by **Ortiz-Frutos (1996)**, a 41-year-old clerk, with no prior diseases, presented with itchy erthematous lesions, with vesicles and exudation, on the neck. Two days prior to onset, she had begun to apply a scarf soaked in commercial ethanol (96%) to treat pharyngitis. She had previously used commercial ethanol as a disinfectant, with no problems. After the first outbreak had been cured, she began to spray a perfume on her neck, which caused fresh eczema to break out. The perfume company confirmed the presence of Benzalkonium Chloride as a denaturant of the ethanol that was used in the manufacture of her perfume. The following positive patch reactions to Benzalkonium Chloride were reported: 0.1% Benzalkonium Chloride (++ on days 2 and 4) and 0.01% aqueous Benzalkonium Chloride (+ on day 2 and ++ on day 4)

According to **Krogsrud and Larsen (1997)**, a laboratory technician, without previous skin diseases and non-atopic, developed urticaria on her face and neck 4 to 6 hours after washing a laminar air flow (LAF) bench with Benzalkonium Chloride solution. Itching began on the same evening. The skin became red and vesicles appeared on the chin and on the front of the neck. The

the abdomen corresponded to the area where the cast had rested while in the sling. The patient developed a strong allergic reaction to the Gypsona® plaster, as well as to both 0.1% aqueous and 0.01% aqueous Benzalkonium Chloride.

In a case report by **Gonzalo Garijo et al. (1996)**, a 37-year-old male with a history of allergic rhinitis from sensitization to dust mites experienced urticaria, dyspnea, wheezing, rhinorrhea, and dysphonia 20 minutes after intraarticular administration of mepivacaine hydrochloride and paramethasone acetate (includes the excipients Benzalkonium Chloride, polysorbate 80, and povidone) in his right knee. After treatment, symptoms resolved uneventfully over the next 24 hours. Two months after discharge from the hospital, the patient was seen in the allergy department.

Because of suspected anaphylactic shock, the patient was admitted for controlled provocation tests. Prior *in vitro* studies with these substances (histamine release test, basophil degranulation test, and lymphoblast transformation test - test details not provided) were negative. Results of provocation testing polysorbate 80 and Benzalkonium Chloride were negative, but were positive with povidone. The reaction to povidone appeared 5 minutes after intramuscular administration of the pure preparation (1 mL), and included aqueous rhinorrhea, conjunctival reddening, dysphagia, dyspnea, rash on the back, and discrete edema of the uvula and anterior pillars **(Gonzalo Garijo et al., 1996)**.

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symptoms lasted for 2 days. No symptoms were recognized on the hands or the arms, and she showed no symptoms of the airways and mucosa. Patch test (*Finn chambers on Scanpor tape) results for 0.1% aqueous Benzalkonium Chloride were negative. Patches were applied for 2 days and readings were done at days 2 and 3.

According to case reports by **Purohit et al. (2000)**, three female nurses manifested asthma symptoms upon handling disinfectant solutions containing Benzalkonium Chloride. Two of the nurses handled solutions containing 10% and 40% Benzalkonium Chloride, respectively. The concentration of Benzalkonium Chloride in the solution handled by the third nurse was not stated. A work-related decrease in peak expiratory flow rates was observed in all three nurses. The diagnosis was confirmed by challenge tests where the patients were exposed, in a closed chamber, to the suspect disinfectant contained in a tray. All of the nurses developed early or delayed symptoms upon exposure. Similar challenge tests to placebo or other disinfectants devoid of quaternary ammonium compound were negative.

According to Park et al. (2000), a 35-year-old female presented with an 8-month history of intensely pruritic eczema, beginning on the feet and spreading to the lower legs. The lesions were erythematous to dark-brownish, edematous, lichenified, and exudative. There were no systemic symptoms and no personal or family history of atopy. She had been treated intermittently with Triaxin® , an anitfungal solution. On patch testing with the ingredients of Triaxin®, Benzalkonium Chloride (0.1% aqueous, 0.01% aqueous) elicited ++ reactions on days 2 and 4. Patch tests with Benzalkonium Chloride (0.1% aqueous and 0.01% aqueous) in 5 control subjects were negative. A focal flare of skin lesions was noted during patch testing. Distilled water produced negative results.

In a case report by **Kanerva et al. (2000)**, a 32-year-old non-atopic cook was referred for investigation of a papular eruption on th backs of her hands and lower arms and a facial dermatitis, after cleaning the oven at work with a disinfecting detergent for sanitary and foodstuff areas. She had worked in the same occupation for 10 years and had twice earlier had symptoms from a similar product for cleaning ovens. Neomycin sulfate, nickel sulfate, and Benzalkonium Chloride (Epikon, 0.1% aqueous) provoked +++ reactions.

In a second case, a 42-year-old patient, cleaner with hand dermatitis, had a ++ patch test reaction to Benzalkonium Chloride (0.1% aqueous) and a +++ reaction to formaldehyde (1%

aqueous). Both reactions were considered relevant and caused by detergents that she had used at work **(Kanerva et al., 2000)**.

In a case report by **Wong and Watson (2001)**, an 81-year-old female developed multiple large, tense, hemorrhagic bullae on the palm, an acute vesicular eczematous eruption on the forearm, after the application of a Plaster of Paris splint. Subsequent patch testing revealed positive reactions to both the Plaster of Paris bandage and to Benzalkonium Chloride. The patient was patch tested with Benzalkonium Chloride (0.1% in petrolatum), and readings were performed at 48 and 96 hours, and a 1+ reaction was reported.

In a case report by **Chowdhury and Statham (2002),** a 65-year-old man presented with an acute erythematous rash after using Timodine® cream. There was no personal or family history of atopy. Patch test (Finn chambers) results indicated a ++ reaction to Timodine® cream and a + reaction to 0.1% aqueous Benzalkonium Chloride.

Walker et al. (2004) reported a case of a 47-year-old varnish maker with a bilateral hyperkeratotic palmar dermatitis for 8 months. In addition to a standard series, the patient was patch-tested with his own medicaments, gloves, and components of face, eye, and coolant series. The patient had ++ reactions to 0.05% 1,2-benzisothiazolin-3-one in petrolatum and 0.1% Benzalkonium Chloride in petrolatum at 2 and 4 days.

Kim and Ahn (2004) reported a case of anaphylactic shock in a 23-year-old asthmatic female, following an intradermal skin test with a salbutamol solution containing Benzalkonium Chloride. Since the patient complained of cough and dyspnea after inhalation therapy with a nebulizer solution, an intradermal skin test was conducted using the same solution (serial dilution), which contained Benzalkonium Chloride. A positive reaction was obtained with a 1:10 dilution of the solution (wheal: 4 x 5 mm in diameter, flare: 18 x 20 mm). Approximately 10 minutes later, the patient reported dizziness, palpitations, and dyspnea. On examination, tachycardia, tachypnea, and hypotension were found. She was resuscitated with a subcutaneous injection of epinephrine and an infusion of saline. One month later, a bronchial provocation test with Benzalkonium Chloride was conducted and the patient showed a positive response. In the bronchial provocation test, the patient inhaled 3 ml of 0.9% NaCl solution containing 600 µg of Benzalkonium Chloride.

In a case report by **Oiso et al. (2005)**, a 62-year-old female was treated for itchy

erythematous macules on the face, especially on the forehead and the neck, after using a shampoo containing Benzalkonium Chloride. Patch tests involving the cosmetics, shampoo, and rinse that were used by the patient were performed. The patient was also patch tested with the ingredients of the shampoo. A positive reaction to the shampoo (at 100 times dilution, + at day 2 and day 3) or 0.1% aqueous Benzalkonium Chloride (+ at day 2 and day 3) was observed. However, no reaction to the shampoo (at 1000 times dilution) or to 0.01% aqueous Benzalkonium Chloride was observed on days 2, 3, and 4.

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DISCUSSION

Arachidyl Propionate was used in 31 cosmetic products in 1981, based on voluntary reports provided to FDA by industry, with concentrations of use ranging from 3% to 10% (Elder 1990). In 2005, Arachidyl Propionate was reportedly used in 47 cosmetic products (FDA 2006). Data from an industry survey in 2006 indicated that Arachidyl Propionate was used at concentrations ranging from 0.001% to 7% (CTFA 2006).

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Benzalkonium Chloride

CONCLUSION

In a safety assessment of Benzalkonium Chloride (Elder 1989), the Cosmetic Ingredient Review (CIR) Expert Panel stated that Benzalkonium Chloride, at concentrations up to 0.1% free, active ingredient, is safe as a cosmetic ingredient as presently used. The Expert Panel reviewed newly available studies since that assessment, along with updated information regarding types and concentration of use, noting that these studies were similar to those already included in the original safety assessment and, therefore, raised no new safety issues. The Panel confirmed the safety of Benzalkonium Chloride at concentrations up to 0.1% free, active ingredient and did not reopen the safety assessment.

DISCUSSION

Benzalkonium Chloride was used in 83 products in 1986, based on voluntary reports provided to FDA by industry, at concentrations of ≤0.1% to 5% (Elder 1989). Data provided to FDA in 2006 indicated that Benzalkonium Chloride was used in 89 products (FDA 2006). Current use concentration data from a cosmetics industry survey indicated that Benzalkonium Chloride is being used in cosmetics at concentrations ranging from 0.01% to 0.5% (CTFA 2006). The available usage and use concentration data are given in Table 4 as a function of product category.

It appears that the maximum reported use concentration of 0.5% (i.e., 0.5% Benzalkonium Chloride in a liquid towelette [personal hygiene product]) exceeds the Panel's 0.1% concentration limit for Benzalkonium Chloride, which is based on skin irritation and sensitization potential. However, it was determined that this is not a concern because Benzalkonium Chloride is bound in the liquid towelette product, and, therefore, the concentration that comes in contact with the skin would be expected to be <0.1%.

The Panel recognizes that there are data gaps regarding use and concentration of this ingredient. However, the overall information available on the types of products in which this ingredient is used and at what concentration indicate a pattern of use, which was considered by the Expert Panel in assessing safety.

The Panel noted that Benzalkonium Chloride can increase the dermal penetration of other chemicals (e.g., betamethasone phosphate). The CIR Expert Panel advised formulators to consider this if the other ingredients in a formulation include those found safe by CIR on the basis that they did not penetrate the skin.

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TABLE 4
Historical and current cosmetic product uses and concentrations for Benzalkonium Chloride

Product category	1986 uses (Elder 1989)	2006 uses (FDA 2006)	1986 concentrations (Elder 1989) (%)	2006 concentrations (CTFA 2006) (%)
Baby products	4[a]		≤ 0.1 – 1[a]	
Shampoos		—		0.03
Lotions, oils, powders, and creams		2		0.03–0.1
Other		2		0.03
Bath products				
Soaps and detergents	—	—	—	0.1
Eye makeup	6[a]		≤ 0.1 – 1[a]	
Eyebrow pencils		—		0.02
Eyeliners		—		0.02
Eye shadow		—		0.02
Eye lotion		1		0.02
Eye makeup remover		10		0.01–0.05
Mascaras		—		0.02–0.1
Other		—		0.02
Fragrance Products				
Colognes and toilet waters	—	1	—	0.1
Perfumes	—	—	—	0.1
Powders	—	—	—	0.08–0.1
Sachets	—	—	—	0.1
Other	—	—	—	0.1
Noncoloring hair products	45[a]		≤ 0.1 – 5[a]	
Conditioners		12		0.05
Straighteners		—		0.1
Permanent waves		—		0.1
Rinses		2		0.1
Shampoos		—		0.1
Tonics, dressings, etc.		7		0.02–0.05
Hair-coloring products				
Dyes and colors	—	—	—	0.02
Tints	—	—	—	0.02
Rinses	—	—	—	0.02
Color sprays	—	—	—	0.02
Lighteners with color	—	—	—	0.02
Bleaches	—	1	—	0.02
Makeup				
Blushers	—	—	—	0.1
Face powders	—	—	—	0.1
Foundations	—	—	—	0.1
Makeup bases	—	—	—	0.1
Makeup fixatives	—	—	—	0.1
Other	—	—	—	0.1
Nail care products				
Cuticle softeners	—	—	—	0.1
Creams and lotions	—	1	—	—
Other	—	1	-	0.01–0.1

(Continued on next page)

TABLE 4
Historical and current cosmetic product uses and concentrations for Benzalkonium Chloride *(Continued)*

Product category	1986 uses (Elder 1989)	2006 uses (FDA 2006)	1986 concentrations (Elder 1989) (%)	2006 concentrations (CTFA 2006) (%)
Oral hygiene products				
Mouthwashes and breath fresheners	—	—	—	0.03
Personal hygiene products	11[a]		≤ 0.1 − 1[a]	
Underarm deodorants		1		0.1
Douches		—		0.1
Feminine deodorants		—		0.1
Other		1		0.1—0.5[b]
Shaving products				
Aftershave lotions	—	—	—	0.1
Shaving cream	—	—	—	0.1
Shaving soap	—	—	—	0.1
Skin care products				
Skin cleansing creams, lotions, liquids, and pads	6	17	≤ 0.1 − 1	0.05–0.1
Depilatories	—	—	—	0.1
Face and neck creams, lotions, powder, and sprays	—	3	—	0.06–0.1
Body and hand creams, lotions, powder, and sprays	—	3	—	0.09–0.1
Foot powders and sprays	—	1	—	0.08–0.1
Moisturizers	4	1	≤ 0.1 − 1	0.1
Night creams, lotions, powders, and sprays	—	1	≤ 0.1 − 1	0.1
Paste masks/mud packs	—	3	—	0.1
Skin fresheners	—	5	—	0.1
Other	7	2	≤ 0.1 − 1	0.1
Suntan products				
Suntan gels, creams, and liquids	—	1	—	—
Total uses/ranges for Benzalkonium Chloride	83	89	≤ 0.1 − 5	0.01–0.5

[a]These categories were combined when the original safety assessment was performed and are now two or more separate categories.
[b]0.5% in a towelette product.

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Cetearyl Alcohol, Cetyl Alcohol, Isostearyl Alcohol, Myristyl Alcohol, and Behenyl Alcohol

~~CONCLUSION~~

~~In a safety assessment of Cetearyl Alcohol, Cetyl Alcohol, Isostearyl Alcohol, Myristyl Alcohol, and Behenyl Alcohol (Elder, 1988), the Cosmetic Ingredient review (CIR) Expert Panel stated these cosmetic ingredients were safe in the present practices of use. The Expert Panel reviewed newly available studies since that assessment, along with updated information regarding types and concentrations of use. The Panel confirmed the safety of Cetearyl Alcohol, Cetyl Alcohol, Isostearyl Alcohol, Myristyl Alcohol, and Behenyl Alcohol in the practices of use and concentrations as given in Table 5, and did not reopen the safety assessment.~~

~~DISCUSSION~~

~~Cetearyl Alcohol was used in 56 cosmetic products in 1982, based on voluntary reports provided to FDA by industry, with use concentrations ranging from > 1% to 25% (Elder 1988). In 2006, Cetearyl Alcohol was reportedly used in 1435 cosmetic products (FDA 2006). Data from an industry survey in 2005 indicated~~

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: September 18, 2025

SUBJECT: Benzalkonium Chloride

Purity specifications for Benzalkonium Chloride in the Japanese Standards for Quasi-drug Ingredients (JSQI)

純度試験

（1）アンモニウム塩　本品の表示量に従い，塩化ベンザルコニウム 0.1g に対応する量をとり，水 5 mL を加えて溶かし，水酸化ナトリウム試液 3 mL を加えて加熱するとき，発生するガスは，潤したリトマス紙を青変しない．

（2）重金属　本品 1.0g をとり，第 2 法により操作し，試験を行うとき，その限度は，20ppm 以下である．ただし，比較液には，鉛標準液 2.0mL をとる．

（3）ヒ素　本品 1.0g をとり，第 3 法により試料溶液を調製し，試験を行うとき，その限度は，2 ppm 以下である．

Rough translation:

Purity test
(1) Ammonium salt: Take the amount of this product corresponding to 0.1g of benzalkonium chloride according to the label, add 5mL of water to dissolve, add 3mL of sodium hydroxide solution and heat. The gas generated does not turn moistened litmus paper blue.
(2) Heavy metals: When 1.0 g of this product is taken and tested according to Method 2, the limit is 20 ppm or less. However, 2.0 mL of lead standard solution is used as the comparison solution.
(3) Arsenic: When 1.0 g of this product is taken, a sample solution is prepared using Method 3, and the test is performed, the limit is 2 ppm or less.

Permitted Uses and Concentrations of Benzalkonium Chloride in Japan

医薬部外品添加物リスト 22/133

連番	添加物の名称	成分コード	規格コード	英　名	外原規2021における成分名	旧外原規における成分名	粗原基における成分名	粗配規における成分名	医薬部外品の種類							その他
									(1)薬用石けん・シャンプー・リンス等、除毛剤	(2)育毛剤	(3)その他の薬用化粧品、殺菌防止剤、忌避剤	備　考	(4)薬用口唇類	(5)薬用歯みがき類	(6)浴用剤	
373	塩化ベンザルコニウム液	500072	51	Benzalkonium Chloride Solution	塩化ベンザルコニウム液		塩化ベンザルコニウム液		3.0	0.050	0.050		0.050	0.010	0.050	塩化ベンザルコニウム及び塩化ベンザルコニウム液を塩化ベンザルコニウムに換算して、塩化ベンザルコニウムとして合計。

Explanation / Rough translation:
The right-hand side columns show the permitted uses, concentrations and conditions.
- (1) Medicated soaps, shampoos, rinses, etc., depilatories: 3.0%
- (2) Hair growth agents: 0.050%
- (3) Other medicated cosmetics, deodorants, repellents: 0.050%
- (4) Medicated lip products: 0.050%
- (5) Medicated toothpastes: 0.010%
- (6) Bath agents: 0.050%
- Other: Benzalkonium chloride and benzalkonium chloride solution were converted to benzalkonium chloride and the total was calculated as benzalkonium chloride.

October 27, 2025

To whom it may concern,

This letter is in response to your recent inquiry regarding the presence of impurity in Stearalkonium Chloride.

Based on the best available information and a review of raw materials, manufacturing processes, and product storage, the following substances may be present as impurities:

- Free Amine + Free Amine HCl (Mixture) < 4.25%
- Toluene (108-88-3) < 0.025%

This information is provided for general informational purposes only and does not constitute a representation, warranty, or guarantee of product composition, performance, or fitness for any particular purpose. Our company makes no express or implied warranties regarding the accuracy or completeness of this information.

The presence, concentration, and impact of impurities may vary depending on production conditions, handling, and storage. These statements do not apply to any product subjected to unintended contamination, misuse, neglect, accident, improper installation, or use inconsistent with instructions furnished by our company.

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 27, 2025

SUBJECT: Benzalkonium and Stearalkonium Chlorides

Anonymous. 2025. Summary Information Alkonium Chlorides – Information provided by a cosmetic ingredient supplier.

October 2025

Summary Information Alkonium Chlorides
Provided by a Cosmetic Ingredient Supplier

The supplier recommends use concentrations of 0.05-0.1% Benzalkonium Chloride in all product types.

The supplier recommends use concentrations of 0.43-1.7% Stearalkonium Chloride in all product types.

Impurities: Benzalkonium Chloride (50% aqueous)

<200 ppm Benzyl Chloride

<2000 ppm Benzyl Alcohol

<100 ppm Benzal Chloride

Heavy Metals: <2ppm Mercury

Dermal irritation data for Stearalkonium Chloride (tested as 85% Stearalkonium Chloride in Glycerin)

1. VitroDerm reconstructed Epidermis
   Completed in 2019
   Mixture tested at 3.5% diluted in water
   Exposure time: 20 hours
   Results: Non-irritant
2. VitroDerm reconstructed Epidermis
   Completed in 2011
   Cosmetic product containing 0.58% of the mixture
   Exposure time: 20 hours
   Results: Non-irritant

Ocular irritation data for Stearalkonium Chloride (tested as 85% Stearalkonium Chloride in Glycerin)

1. OECD 492: Reconstructed human Cornea-like Epithelium (RhCE) test

Completed in 2017
Mixture tested undiluted
Results: Irritating

2. SkinEthic™ reconstructed Human Epithelial Corneal Model
   Completed in 2017
   Mixture tested at 3.52% (diluted in water)
   Exposure time: 1, 3 and 24 hours
   Results: Irritating

Concentration of Use by FDA Product Category[1] - Alkonium Chlorides and Bromides*

Behenalkonium Chloride
Benzalkonium Bromide
Benzalkonium Chloride
Lauralkonium Bromide
Lauralkonium Chloride
Myristalkonium Chloride
Caprylylalkonium Chloride
Cetalkonium Chloride
Cetearalkonium Bromide
Stearalkonium Chloride

Ingredient	Product Category	Maximum Concentration of Use
Behenalkonium Chloride	Eye makeup removers	0.011%
Behenalkonium Chloride	Hair conditioners Leave-on Rinse-off	 0.48% 1.9%
Behenalkonium Chloride	Hair sprays Pump spray	 0.48%
Benzalkonium Chloride	Baby lotions, oils and creams	0.053%
Benzalkonium Chloride	Eye makeup removers	0.015%
Benzalkonium Chloride	Hair sprays Pump sprays	 0.009%
Benzalkonium Chloride	Makeup bases Traditional	 0.11%
Benzalkonium Chloride	Dentifrices	0.1%
Benzalkonium Chloride	Bath soaps and body washes	0.13%
Benzalkonium Chloride	Deodorants Not spray	 0.025-0.11%
Benzalkonium Chloride	Disposable wipes	0.35%
Benzalkonium Chloride	Other personal cleanliness products Rinse-off hand wash	 0.13%
Benzalkonium Chloride	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.015-0.11%
Benzalkonium Chloride	Face and neck products (not spray) Leave-on	 0.005-0.054%
Benzalkonium Chloride	Body and hand products (not spray) Leave-on Rinse-off	 0.04% 0.13%
Benzalkonium Chloride	Body and hand spray	0.1%
Benzalkonium Chloride	Moisturizing products (not spray)	0.11%
Benzalkonium Chloride	Paste masks and mud packs	0.097%
Benzalkonium Chloride	Skin fresheners	0.11%
Stearalkonium Chloride	Hair conditioners Leave-on Rinse-off	 1.1-1.8% 1.3-2.6%

[1] The FDA cosmetic product categories under MoCRA were used for this survey.

Stearalkonium Chloride	Tonics, dressings and other hair grooming aids	0.1-0.14%
Stearalkonium Chloride	Other hair preparation (noncoloring) Leave-on	 2%
Stearalkonium Chloride	Hair dyes and colors	1.5%
Stearalkonium Chloride	Hair tints	1.5%
Stearalkonium Chloride	Hair rinses (coloring) Rinse-off	 2.5%

*Ingredients included in the title of the table but not found in the table were included in the concentration of use survey, but no uses were reported.

Information collected in 2025

Table prepared: October 20, 2025