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

Status:	Tentative Amended Report for Public Comment
Last Panel Review:	June 15-16, 2026
Release Date:	July 2, 2026

*All interested persons are provided 60 days from the above release date (i.e., **by August 31, 2026**) to comment on this safety assessment, and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Executive Director, Dr. Bart Heldreth.*

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Bruce A. Brod, M.D., M.H.C.I., F.A.A.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
WoE	weight-of-evidence
ZO-1	tight junction zonula occludens-1

ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) reassessed the safety of Benzalkonium Chloride and Stearalkonium Chloride, and also reviewed 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 with the conclusion that these 6 ingredients are safe in cosmetics in the present practices of use and concentration described in this safety assessment when formulated to be non-irritating.

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 *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 in May 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]

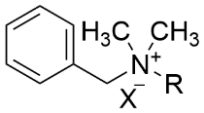


Figure 1. Alkonium halides, wherein R is an even numbered alkyl chain ($C_{10} – C_{22}$) and X is bromide or chloride.

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).*

Nitrosation

In the presence of nitrites, nitrogen oxides, or other nitrosating agents, alkylbenzyldimethylammonium chlorides will give rise to traces of N-nitrosamines.[2] *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.

Following the passage of MoCRA, the FDA created a number of new product categories, including "(17) Other preparations (i.e., those preparations that do not fit another category)" (Category 17). CIR and the Panel utilize these product categories to inform, in part, the weight-of-evidence (WoE) in exposure and risk analyses. However, citation of Category 17, alone, as the product use category is uninformative. When a product is co-categorized with both Category 17 and one or more common product use categories (e.g., eye makeup preparations), or other information in the RLD such as the product name are indicative of use type (e.g., "lip balm" is part of the product name), then those uses are accounted for and may be considered to be part of the "the present practices of use" for those products; as such, the Panel's safety conclusion may be inclusive thereon. Conversely, for instances wherein the use of an ingredient in a cosmetic product is only categorized as Category 17 and other information in the RLD is not indicative of use type, the present practices of use for these products remain unknown and the Panel's safety assessment conclusion, therefore, does not apply thereon.

Use of the ingredients included in this report 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%).

For some ingredients, reported uses in baby products are identified in the RLD; however, concentration of use data are not available for every reported category of baby product. In cases where a baby product category is reported without an associated concentration, and an analogous adult product category with reported concentration data is available, the adult product use level may be considered informative for exposure characterization. In such cases, concentrations in the corresponding baby product category are not expected to exceed those reported for the analogous adult product category. For example, Benzalkonium Chloride has no reported concentration of use in baby wipes; however, it is reported in disposable wipes at a maximum concentration of 0.35%, and concentrations in baby wipes are not expected to exceed this value. The category "other baby products" represents a miscellaneous grouping of reported uses not assigned to specific baby product subcategories. Given its broad and non-specific nature, it does not correspond to a single analogous adult product category; therefore, the adult-product concentration bridging approach is not applied to this category.

Ingredients reviewed in this report 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. An oral median lethal dose (LD_{50}) of 525 mg/kg was established in an acute 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

Some genotoxicity assays are no longer considered appropriate as standard methods for evaluating genotoxic potential under current OECD guidance. (See https://www.cir-safety.org/cir-findings for the identification of such study types.) Although these assays (as identified in the guidance) may be reported in the published literature, unpublished reports, or data submissions, the results from these assays are of limited interpretability and therefore the studies are not summarized herein.[4,13]

Benzalkonium Chloride (concentration not stated) was not mutagenic to in bacterial reverse mutation assay using Salmonella typhimurium strains TA1535, TA1536, TA1537, and TA1538 and Escherichia coli strains B/r WP2 hcr$^+$ and WP2 hcr strains.[4] *Benzalkonium Chloride at 10% also did not induce DNA-damaging activity in S. typhimurium strains 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. 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.[72] 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.[73] 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 study-dependent concentrations ranging from 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%).[54,56,71,74-87]

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.[88] 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)).[83]

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).[75] 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.[89] 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.[90]

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.[91] 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.[92] 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.[93] 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.[94]

The inhibitory effects of Benzalkonium Chloride homologs with varying alkyl chain lengths (C1 – C18) on human and rat 11β-HSD2 were evalauted.[95] 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.[96] 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).[97] 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).[98] 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%.[99] 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.[8,100] 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.[101] 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).[97] 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).[102] 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).[103]

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).[104] 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.[87] 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.[105] 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.[106] 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.[107] 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.[108,109] 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).[110] 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.[8,111] 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.[112-129]

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.[130,131] Other effects such as asthma, as well as stomach, respiratory, and ocular irritation have also been reported in the occupational setting.[132,133]

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).[8,98,131,134-149] 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.[150,151]

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).[152] 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).[153]

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).[154] 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.[155]

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.[156] 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.[157] 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. 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 patients that were 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/d 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.

DISCUSSION

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), and this conclusion was re-affirmed in a re-review published in 2008. However, after evaluation of previous and new data (including 2025 RLD), and in accordance with the product categories and concentrations of use identified in the Use section and Use table, the Panel issued a revised conclusion stating that these 6 alkonium chlorides and bromides are safe in cosmetics in the present practices of use and concentration described in this safety assessment when formulated to be non-irritating.

This conclusion is supported by FDA-permitted uses of Benzalkonium Chloride and Stearalkonium Chloride in topical drug products at concentrations up to 0.1 and 3.15%, respectively. Additional support comes from the use of Benzalkonium Chloride in OTC and prescription drug products applied to the eyes, ears, and nasal mucosa.

Use in baby products has been reported, but concentration of use data were not available for certain baby product categories. When use in a baby product category is reported without an associated concentration, and an analogous adult product category with reported use concentrations is available, the adult product concentration is considered informative for exposure characterization. Accordingly, concentrations in the corresponding baby product categories are not expected to exceed those reported for the analogous adult product categories. Thus, although no concentration of use is reported for Benzalkonium Chloride in baby wipes, the ingredient is reported in disposable wipes at a maximum concentration of 0.35%, and concentrations in baby wipes are not expected to exceed this value. The category “other baby products” represents a miscellaneous grouping of reported uses not assigned to specific baby product subcategories; given its broad and non-specific nature, an analogous adult product category could not be identified, and the adult-product bridging is not applied.

Impurity data were not available for each ingredient reviewed in this report; however, this need was mitigated by available manufacturing process information and existing impurity data for Benzalkonium Chloride and Stearalkonium Chloride. The Panel also expressed concern regarding heavy metal impurities that may be present in these ingredients and stated 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 Benzalkonium Chloride demonstrated positive results in several in vitro genotoxicity assays. However, these the lack of corroborating findings in in vivo genotoxicity assays and a dermal carcinogenicity assay allayed any concerns.

An HRIPT for Benzalkonium Chloride at the current maximum use concentration was not available. However, based on the totality of available data, including NACDG patch test data, the Panel concluded that Benzalkonium Chloride is not likely to be a sensitizer under conditions of use, although it may elicit irritation. Reported positive reactions, particularly among atopic individuals, were of limited or uncertain clinical relevance and were considered more consistent with irritant responses rather than allergic sensitization. Accordingly, the Panel emphasized that products formulated with 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. Therefore, 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.

The Panel noted that these ingredients may enhance dermal and mucosal 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.

In addition, the Panel noted that these ingredients may theoretically form trace *N*-nitrosamines in the presence of nitrosating agents. Accordingly, the Panel cautioned that these ingredients should not be used in formulations containing *N*-nitrosating agents.

The Panel also considered data suggesting potential endocrine activity of Benzalkonium Chloride in in vitro studies; 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 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, potentially necessitating higher total concentrations to achieve 0.1% free active substance.

At least 1 use for the ingredients assessed in this assessment is reported in the RLD under Category 17, "other preparations." For instances wherein the use of an ingredient in a cosmetic product is only categorized as Category 17, and other information in the RLD is not indicative of use type, the present practices of use for such products remain unknown and the Panel's safety assessment conclusion, therefore, does not apply thereon.

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 frequency and concentration of use data are now available (and in some cases mandated) for ingredients marketed for use with airbrush delivery systems in certain product categories, 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 that if these ingredients are used in airbrush formulations, the data are insufficient to support safe use when applied with such delivery system.

CONCLUSION

The Expert Panel for Cosmetic Ingredient Safety concluded that the following 6 alkonium chlorides and bromides are safe in cosmetics in the present practices of use and concentration described in this safety assessment when formulated to be non-irritating:

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

* *Not reported to be in current use. Were this ingredient not in current use to be used in the future, the expectation is that it would be used in product categories and at concentrations comparable to others in this group.*

TABLES

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

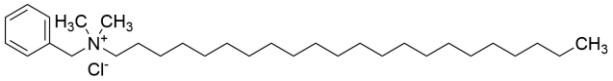
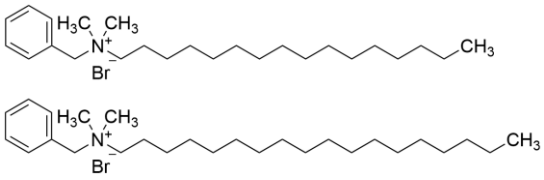
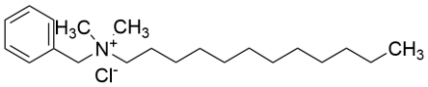
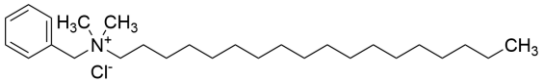
Ingredient/CAS No.	Definition	Function(s)
Behenalkonium Chloride 16841-14-8	Behenalkonium Chloride is the quaternary ammonium salt that conforms to the structure: 	Antistatic Agents
Benzalkonium Bromide 91080-29-4	Benzalkonium Bromide is the mixture of alkylbenzyldimethylammonium bromides that conforms 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	158
Vapor pressure (mmHg @ 25ºC)	0 (estimated; modified grain method)	159
Melting Point (ºC)	284 (estimated)	159
Boiling Point (ºC)	654 (estimated)	159
Water Solubility (mg/l @ 25ºC)	0.0002 (estimated; WSKOW v1.42)	159
log K_{ow} (@ 25ºC)	7.74 (estimated; WSKOW v1.42)	159
Benzalkonium Bromide		
Formula Weight (g/mol; median (range))	440.0 (355.4 – 524.7)	160
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	160
Lauralkonium Chloride		
Physical Form	solid	11
Color	white	11
Formula Weight (g/mol)	340	161
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	160
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	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)
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		

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			
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

Table 7. Repeated dose toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
				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
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)	97
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	**98**

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	99
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%)	162
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	97
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.	**101**

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%)	134
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%)	134
0.1%	water	30 patients using inhaled or intranasal corticoid steroids for allergic conditions	2 patients (6.7%)	135
0.1%	water	91 dermatitis patients	5 patients (5.5%)	136
0.1%	water	131 cheilitis patients	17 patients (13%)	137
0.1%	water	149 dermatitis patients	5 patients (3.4%)	138
0.1%	water	215 periorbital dermatitis patients	10 patients (4.7%)	139
0.1%	water	429 dermatitis patients (evaluating for late delayed positive reactions)	2 patients (0.5%)	140
0.1%	water	584 dermatitis patients	71 patients (12.2%)	98
0.1%	water	615 dermatitis patients	198 patients (32.2%)	131
0.1%	petroleum	1389 dermatitis patients	48 patients (3.5%)	141
0.1%	NR	2295 dermatitis patients	126 patients (5.5%)	8
0.1%	water	2546 dermatitis patients	176 patients (6.9%)	142
NR	NR	2611 dermatitis patients	47 patients (1.8%)	143
0.1%	water	2694 dermatitis patients	164 patients (6.1%)	144
0.1%	water	3043 dermatitis patients	37 patients (1.2%)	145
0.1%	petroleum	4116 dermatitis patients	21 patients (0.5%)	146
0.1%	water	4892 dermatitis patients	210 people (4.3%)	147
0.1%	water	7390 dermatitis patients	108 patients (1.5%)	148
0.1%	petroleum	42,898 dermatitis patients	361 patients (0.8%)	149

NR = not reported

REFERENCES

1. Personal Care Products Council. 2026. *International Cosmetic Ingredient Dictionary*. https://incipedia.personalcarecouncil.org/winci/. Date Accessed: January 9, 2026.

2. Andersen FA (ed),. Final report on the safety assessment of stearalkonium chloride. *J Am Coll Toxicol*. 1982;1(2):57–69.

3. Andersen FA (ed). Annual review of cosmetic ingredient safety assessments - 2001/2002. *Int J Toxicol*. 2003;22(Suppl 1):1–35.

4. Johnson WJ. Final report on the safety assessment of benzalkonium chloride. *J Am Coll Toxicol*. 1989;8(4):589–625.

5. Andersen FA (ed),. Annual review of cosmetic ingredient safety assessments: 2005/2006. *Int J Toxicol*. 2008;27(Suppl 1):77–142.

6. European Chemicals Agency. 2025. Benzododecinium chloride. https://chem.echa.europa.eu/100.004.865/overview?searchText=139-07-1. Date Accessed: July 8, 2025.

7. European Chemicals Agency. 2025. Benzyldimethyl(octadecyl)ammonium chloride. https://chem.echa.europa.eu/100.004.117/dossier-view/c778b2d7-19f4-47a4-9411-03d1a0653fd2/1d822676-9cd9-45e2-8f31-dd74e8cd8a4e_1d822676-9cd9-45e2-8f31-dd74e8cd8a4e?searchText=122-19-0. Date Accessed: July 8, .

8. Australian Industrial Chemicals Introduction Scheme. 2022. Benzalkonium halides evaluation statement. https://chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.industrialchemicals.gov.au/sites/default/files/2022-06/EVA00071%20-%20Evaluation%20Statement%20-%2030%20June%202022.pdf. Date Accessed: July 8, 2025.

9. Kera H, Fuke C, Usumoto Y, et al. Kinetics and distribution of benzalkonium compounds with different alkyl chain length following intravenous administration in rats. *Leg Med (Tokyo)*. 2021;48:101821.

10. Merchel Piovesan Pereira B, Tagkopoulos I. Benzalkonium chlorides: Uses, regulatory status, and microbial resistance. *Appl Environ Microbiol*. 2019;85(13):377.

11. European Chemicals Agency. 2025. Benzododecinium chloride. https://chem.echa.europa.eu/100.004.865/dossier-view/01fa4790-2920-4461-8a6f-2d15c8c94038/9e5ade28-a0a5-4fb5-94a9-2cc47e88acca_9e5ade28-a0a5-4fb5-94a9-2cc47e88acca?searchText=139-07-1. Date Accessed: July 16, 2025.

12. European Chemicals Agency. 2025. Benzyldimethyl(octadecyl)ammonium chloride. https://chem.echa.europa.eu/100.004.117/dossier-view/c778b2d7-19f4-47a4-9411-03d1a0653fd2/1d822676-9cd9-45e2-8f31-dd74e8cd8a4e_1d822676-9cd9-45e2-8f31-dd74e8cd8a4e?searchText=stearalkonium%20chloride. Date Accessed: July 15, 2025.

13. Johnson W. J. 2006. Re-review document on Benzalkonium Chloride. [Unpublished re-review document reviewed by the Panel at the June 2006 Panel meeting].

14. Kuca K, Marek J, Stodulka P, et al. Preparation of benzalkonium salts differing in the length of a side alkyl chain. *Molecules*. 2007;12(10):2341–2347.

15. Anonymous. 2025. Summary information: alkonium chlorides - information provided by supplier. [Unpublished data submitted by Personal Care Products Council on October 27, 2025].

16. Anonymous. 2025. Purity and concentrations of use of Benzalkonium Chloride in Japan. [Unpublished data submitted by Personal Care Products Council on September 18, 2025].

17. Anonymous. 2025. Letter regarding impurities that may be found in Stearalkonium Chloride. [Unpublished data submitted by Personal Care Products Council on October 27, 2025].

18. United States Food and Drug Administration. Federal Food, Drug, and Cosmetic Act Section 612 Title 21.

19. Hicks J., Eisenmann C., Nikitakis J., Kim D., Flores W. 2025. Personal Care Products Council (PCPC) RLD Mapping Project Report. Washington, DC. [Analysis results provided as a courtesy to CIR].

20. U.S. Food and Drug Administration Office of Colors and Cosmetics (OCAC). 2025. Data from: Registration and Listing of Cosmetic Product Facilities and Products. College Park, MD. [Obtained under the Freedom of Information Act].

21. Personal Care Products Council. 2025. Concentration of Use by FDA Product Category: Alkonium Chlorides and Bromides. [Unpublished data submitted to Cosmetic Ingredient Review on October 21, 2025].

22. EUR-Lex. 2025. Access to European Union Law. https://eur-lex.europa.eu/homepage.html. Date Accessed: April 21, 2025.

23. US Food and Drug Administration. 2023. Over-the-Counter Monograph M003: First Aid Antiseptic Drug Products for Over-the-Counter Human Use. https://www.accessdata.fda.gov/scripts/cder/omuf/index.cfm?event=OrderDetail&orderid=OTC000030. Date Accessed: April 30, 2026.

24. US Food and Drug Administration. 2025. Ingredient Search for Approved Drug Products. https://www.accessdata.fda.gov/scripts/cder/iig/index.cfm. Date Accessed: July 8, 2025.

25. US Food and Drug Administration. 2025. Over-the-Counter (OTC) Related Federal Register Notices, Ingredient References, and other Regulatory Information. https://www.fda.gov/about-fda/center-drug-evaluation-and-research-cder/over-counter-otc-related-federal-register-notices-ingredient-references-and-other-regulatory. Date Accessed: July 8, 2025.

26. Thacker M, Sahoo A, Reddy AA, et al. Benzalkonium chloride-induced dry eye disease animal models: Current understanding and potential for translational research. *Indian J Ophthalmol*. 2023;71(4):1256–1262.

27. Faria NVLd, Sampaio MOBd, Viapiana GN, et al. Effects of benzalkonium chloride and cyclosporine applied topically to rabbit conjunctiva: A histomorphometric study. *Arq Bras Oftalmol*. 2019;82(4):310–316.

28. Fukuda M, Takeda N, Ishida H, et al. Benzalkonium chloride-induced corneal epithelial injury in rabbit reduced by rebamipide. *J Ocul Pharmacol Ther*. 2022;38(1):85–91.

29. Vereertbrugghen A, Pizzano M, Sabbione F, et al. Hyaluronate protects from benzalkonium chloride-induced ocular surface toxicity. *Transl Vis Sci Technol*. 2024;13(10):31.

30. Moon J, Ko JH, Yoon CH, Kim MK, Oh JY. Effects of 20% human serum on corneal epithelial toxicity induced by benzalkonium chloride: In vitro and clinical studies. *Cornea*. 2018;37(5):617–623.

31. Seguin RP, Herron JM, Lopez VA, Dempsey JL, Xu L. Metabolism of benzalkonium chlorides by human hepatic cytochromes P450. *Chem Res Toxicol*. 2019;32(12):2466–2478.

32. Morse CN, Hite CC, Wamer NC, Gadient JN, Baki G, Prestwich EG. MALDI-TOF imaging analysis of benzalkonium chloride penetration in ex vivo human skin. *PLoS One*. 2024;19(2):e0297992.

33. Scientific Committee on Cosmetology. 2000. Reports of the Scientific Committee on Cosmetology (9th series). https://health.ec.europa.eu/system/files/2016-11/scc_o_9_0.pdf. Date Accessed: July 28, 2025.

34. DeLeo PC, Tu V, Fuls J. Systemic absorption of benzalkonium chloride after maximal use of a consumer antiseptic wash product. *Regul Toxicol Pharmacol*. 2021;124:104978.

35. Nokhodchi A, Shokri J, Dashbolaghi A, Hassan-Zadeh D, Ghafourian T, Barzegar-Jalali M. The enhancement effect of surfactants on the penetration of lorazepam through rat skin. *Int J Pharm*. 2003;250(2):359–369.

36. Lopez VA, Lim JJ, Seguin RP, et al. Oral exposure to benzalkonium chlorides in male and female mice reveals alteration of the gut microbiome and bile acid profile. *Toxicol Sci*. 2024;202(2):265–277.

37. Kim G, Jeong J, Park JE, et al. Adrenal glands as secondary target organs of inhaled benzalkonium chlorides. *Ecotoxicol Environ Saf*. 2025;305:119258.

38. Kim G, Jeong J, Park JE, et al. Benzalkonium chloride accumulates and causes toxicity in the heart and pancreas following the repeated inhalation exposures. *Sci Total Environ*. 2025;1009:181067.

39. Xue Y, Zhang S, Tang M, et al. Comparative study on toxic effects induced by oral or intravascular administration of commonly used disinfectants and surfactants in rats. *J Appl Toxicol*. 2012;32(7):480–487.

40. Lee H, Park K. Acute toxicity of benzalkonium chloride in balb/c mice following intratracheal instillation and oral administration. *Environ Anal Health Toxicol*. 2019;34(3):e2019009.

41. Larsen ST, Verder H, Nielsen GD. Airway effects of inhaled quaternary ammonium compounds in mice. *Basic Clin Pharmacol Toxicol*. 2012;110(6):537–543.

42. Swiercz R, Hałatek T, Wasowicz W, Kur B, Grzelińska Z, Majcherek W. Pulmonary irritation after inhalation exposure to benzalkonium chloride in rats. *Int J Occup Med Environ Health*. 2008;21(2):157–163.

43. Choi SM, Roh TH, Lim DS, Kacew S, Kim HS, Lee B. Risk assessment of benzalkonium chloride in cosmetic products. *J Toxicol Environ Health B Crit Rev*. 2018;21(1):8–23.

44. Dolan LC, Wheeler JA, Burdock GA. Safety studies conducted on a sanitizing agent containing benzalkonium chloride. *J Food Sci*. 2013;78(1):119.

45. Swiercz R, Hałatek T, Stetkiewicz J, et al. Toxic effect in the lungs of rats after inhalation exposure to benzalkonium chloride. *Int J Occup Med Environ Health*. 2013;26(4):647–656.

46. Choi H, Lee Y, Lim C, et al. Assessment of respiratory and systemic toxicity of benzalkonium chloride following a 14-day inhalation study in rats. *Part Fibre Toxicol*. 2020;17(1):5.

47. Baptista M, Publicover SJ, Ramalho-Santos J. In vitro effects of cationic compounds on functional human sperm parameters. *Fertil Steril*. 2013;99(3):705–712.

48. Alfaiate MI, António Santos R, Silva AF, et al. Comparative in vitro study on the local tolerance and efficacy of benzalkonium chloride, myristalkonium chloride and nonoxynol-9 as active principles in vaginal contraceptives. *Eur J Contracept Reprod Health Care*. 2021;26(4):334–342.

49. Hostetler KA, Fisher LC, Burruss BL. Prenatal developmental toxicity of alkyl dimethyl benzyl ammonium chloride and didecyl dimethyl ammonium chloride in CD rats and new zealand white rabbits. *Birth Defects Res*. 2021;113(12):925–944.

50. DeSesso JM, Harris SB, Scialli AR, Williams AL. Systematic assessment of quaternary ammonium compounds for the potential to elicit developmental and reproductive effects. *Birth Defects Res*. 2021;113(20):1484–1511.

51. Ferk F, Misík M, Hoelzl C, et al. Benzalkonium chloride (BAC) and dimethyldioctadecyl-ammonium bromide (DDAB), two common quaternary ammonium compounds, cause genotoxic effects in mammalian and plant cells at environmentally relevant concentrations. *Mutagenesis*. 2007;22(6):363–370.

52. Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. Salmonella mutagenicity tests: V. results from the testing of 311 chemicals. *Environ Mol Mutagen*. 1992;19 Suppl 21:2–141.

53. Hori I, Higo Y, Ohno M, Tsutsui TW, Tsutsui T. Assessment using human dental pulp cells of clastogenicity of antiseptics used in dental practice and agents for root canal enlargement and cleaning. *Odontology*. 2007;95(1):30–37.

54. Ye J, Wu H, Zhang H, et al. Role of benzalkonium chloride in DNA strand breaks in human corneal epithelial cells. *Graefes Arch Clin Exp Ophthalmol*. 2011;249(11):1681–1687.

55. Zhang H, Wu H, Yang J, Ye J. Sodium perbarate and benzalkonium chloride induce DNA damage in chang conjunctival epithelial cells. *Cutan Ocul Toxicol*. 2017;36(4):336–342.

56. Deutschle T, Porkert U, Reiter R, Keck T, Riechelmann H. In vitro genotoxicity and cytotoxicity of benzalkonium chloride. *Toxicol In Vitro*. 2006;20(8):1472–1477.

57. Lim C, Shin K, Seo D. Genotoxicity study of 2-methoxyethanol and benzalkonium chloride through comet assay using 3D cultured HepG2 cells. *Environ Anal Health Toxicol*. 2022;37(4):2022031.

58. Sarkar J, Chaudhary S, Namavari A, et al. Corneal neurotoxicity due to topical benzalkonium chloride. *Invest Ophthalmol Vis Sci*. 2012;53(4):1792–1802.

59. Zhang R, Park M, Richardson A, et al. Dose-dependent benzalkonium chloride toxicity imparts ocular surface epithelial changes with features of dry eye disease. *Ocul Surf*. 2020;18(1):158–169.

60. Ivakhnitskaia E, Souboch V, Dallacasagrande V, et al. Benzalkonium chloride, a common ophthalmic preservative, compromises rat corneal cold sensitive nerve activity. *Ocul Surf*. 2022;26:88–96.

61. Chen W, Li Z, Hu J, et al. Corneal alternations induced by topical application of benzalkonium chloride in rabbit. *PLoS One*. 2011;6(10):e26103.

62. Okahara A, Kawazu K. Local toxicity of benzalkonium chloride in ophthalmic solutions following repeated applications. *J Toxicol Sci*. 2013;38(4):531–537.

63. Kim JR, Oh TH, Kim HS. Effects of benzalkonium chloride on the ocular surface of the rabbit. *Jpn J Ophthalmol*. 2011;55(3):283–293.

64. Okahara A, Kawazu K. Local toxicity of benzalkonium chloride in ophthalmic solutions following repeated applications. *J Toxicol Sci*. 2013;38(4):531–537.

65. Rasmussen CA, Kaufman PL, Kiland JA. Benzalkonium chloride and glaucoma. *J Ocul Pharmacol Ther*. 2014;30(2-3):163–169.

66. McCarey B, Edelhauser H. In vivo corneal epithelial permeability following treatment with prostaglandin analogs [correction of analoges] with or without benzalkonium chloride. *J Ocul Pharmacol Ther*. 2007;23(5):445–451.

67. Yang Q, Zhang Y, Liu X, Wang N, Song Z, Wu K. A comparison of the effects of benzalkonium chloride on ocular surfaces between C57BL/6 and BALB/c mice. *Int J Mol Sci*. 2017;18(3):509.

68. Moghadam SH, Saliaj E, Wettig SD, et al. Effect of chemical permeation enhancers on stratum corneum barrier lipid organizational structure and interferon alpha permeability. *Mol Pharm*. 2013;10(6):2248–2260.

69. Uematsu M, Kumagami T, Kusano M, et al. Acute corneal epithelial change after instillation of benzalkonium chloride evaluated using a newly developed in vivo corneal transepithelial electric resistance measurement method. *Ophthalmic Res*. 2007;39(6):308–314.

70. Nakagawa S, Usui T, Yokoo S, et al. Toxicity evaluation of antiglaucoma drugs using stratified human cultivated corneal epithelial sheets. *Invest Ophthalmol Vis Sci*. 2012;53(9):5154–5160.

71. Kusano M, Uematsu M, Kumagami T, Sasaki H, Kitaoka T. Evaluation of acute corneal barrier change induced by topically applied preservatives using corneal transepithelial electric resistance in vivo. *Cornea*. 2010;29(1):80–85.

72. Zhang Z, Huang Y, Xie H, et al. Benzalkonium chloride suppresses rabbit corneal endothelium intercellular gap junction communication. *PLoS One*. 2014;9(10):e109708.

73. Fukuda M, Sasaki Y, Kiyoi T, et al. Investigation of corneal epithelial tight junction disruption and barrier function impairment induced by benzalkonium chloride. *J Ocul Pharmacol Ther*. 2026:10807683261441060.

74. Kwon D, Lim Y, Kwon J, et al. Evaluation of pulmonary toxicity of benzalkonium chloride and triethylene glycol mixtures using in vitro and in vivo systems. *Environ Toxicol*. 2019;34(5):561–572.

75. Poumay Y, Dupont F, Marcoux S, Leclercq-Smekens M, Hérin M, Coquette A. A simple reconstructed human epidermis: Preparation of the culture model and utilization in in vitro studies. *Arch Dermatol Res*. 2004;296(5):203–211.

76. Vitoux M, Kessal K, Melik Parsadaniantz S, et al. Benzalkonium chloride-induced direct and indirect toxicity on corneal epithelial and trigeminal neuronal cells: Proinflammatory and apoptotic responses in vitro. *Toxicol Lett*. 2020;319:74–84.

77. Ammar DA, Kahook MY. Effects of benzalkonium chloride- or polyquad-preserved fixed combination glaucoma medications on human trabecular meshwork cells. *Mol Vis*. 2011;17:1806–1813.

78. Umetsu A, Ida Y, Sato T, Furuhashi M, Ohguro H, Watanabe M. Benzalkonium chloride greatly deteriorates the biological activities of human corneal stroma fibroblasts in a concentration-dependent manner. *Graefes Arch Clin Exp Ophthalmol*. 2024;262(6):1847–1855.

79. Rath A, Eichhorn M, Träger K, Paulsen F, Hampel U. In vitro effects of benzalkonium chloride and prostaglandins on human meibomian gland epithelial cells. *Ann Anat*. 2019;222:129–138.

80. Takahashi H, Tajima K, Hattori T, Yamakawa N, Ito N, Goto H. Novel primary epithelial cell toxicity assay using porcine corneal explants. *Cornea*. 2015;34(5):567–575.

81. Ho C, Wu M, Lan M, Tan C, Yang A. In vitro effects of preservatives in nasal sprays on human nasal epithelial cells. *Am J Rhinol*. 2008;22(2):125–129.

82. Pozarowska D, Pozarowski P. Benzalkonium chloride (BAK) induces apoptosis or necrosis, but has no major influence on the cell cycle of jurkat cells. *Folia Histochem Cytobiol*. 2011;49(2):225–230.

83. Ryu O, Park BK, Bang M, et al. Effects of several cosmetic preservatives on ROS-dependent apoptosis of rat neural progenitor cells. *Biomol Ther (Seoul)*. 2018;26(6):608–615.

84. Chen X, Sullivan DA, Sullivan AG, Kam WR, Liu Y. Toxicity of cosmetic preservatives on human ocular surface and adnexal cells. *Exp Eye Res*. 2018;170:188–197.

85. Seibold LK, Ammar DA, Kahook MY. Acute effects of glaucoma medications and benzalkonium chloride on pre-adipocyte proliferation and adipocyte cytotoxicity in vitro. *Curr Eye Res*. 2013;38(1):70–74.

86. Yokonishi T, McKey J, Ide S, Capel B. Sertoli cell ablation and replacement of the spermatogonial niche in mouse. *Nat Commun*. 2020;11(1):40.

87. Fichorova RN, Bajpai M, Chandra N, et al. Interleukin (IL)-1, IL-6, and IL-8 predict mucosal toxicity of vaginal microbicidal contraceptives. *Biol Reprod*. 2004;71(3):761–769.

88. Jeon H, Kim D, Yoo J, Kwon S. Effects of benzalkonium chloride on cell viability, inflammatory response, and oxidative stress of human alveolar epithelial cells cultured in a dynamic culture condition. *Toxicol In Vitro*. 2019;59:221–227.

89. Varani J, Perone P, Spahlinger DM, et al. Human skin in organ culture and human skin cells (keratinocytes and fibroblasts) in monolayer culture for assessment of chemically induced skin damage. *Toxicol Pathol*. 2007;35(5):693–701.

90. Epstein SP, Chen D, Asbell PA. Evaluation of biomarkers of inflammation in response to benzalkonium chloride on corneal and conjunctival epithelial cells. *J Ocul Pharmacol Ther*. 2009;25(5):415–424.

91. Marple B, Roland P, Benninger M. Safety review of benzalkonium chloride used as a preservative in intranasal solutions: An overview of conflicting data and opinions. *Otolaryngol Head Neck Surg*. 2004;130(1):131–141.

92. Oh H, Park S, Lee S, Chun H, Shin W, Kim W. In vitro neurotoxicity evaluation of biocidal disinfectants in a human neuron-astrocyte co-culture model. *Toxicol In Vitro*. 2022;84:105449.

93. Wei S, Hu X, Hu X, Wan Y, Fan G, Wang J. In vitro evaluation for estrogenic mechanisms of the disinfectant benzalkonium chloride as an emerging contaminant. *Braz J Med Biol Res*. 2023;56:e12784.

94. Datta S, He G, Tomilov A, Sahdeo S, Denison MS, Cortopassi G. In vitro evaluation of mitochondrial function and estrogen signaling in cell lines exposed to the antiseptic cetylpyridinium chloride. *Environ Health Perspect*. 2017;125(8):087015.

95. Wang Q, Zhang H, Wang F, et al. Benzalkonium disinfectants as emerging endocrine disruptors: Inhibition of 11β-hydroxysteroid dehydrogenase 2 in human and rat models via steric and hydrophobic mechanisms: SPR and docking and enzymatic analysis. *Bioorg Chem*. 2026;173:109594.

96. Sadakane K, Ichinose T. Effect of the hand antiseptic agents benzalkonium chloride, povidone-iodine, ethanol, and chlorhexidine gluconate on atopic dermatitis in NC/nga mice. *Int J Med Sci*. 2015;12(2):116–125.

97. Rohm and Haas. 1992. Initial Submission: Letter from Rohm and Haas Company to US EPA Submitting Enclosed Information on an Acute Skin and Eye Irritation Study in Rabbits with Four ComponentsJuly 29, 2025, 2025.

98. Lee SS, Hong DK, Jeong NJ, et al. Multicenter study of preservative sensitivity in patients with suspected cosmetic contact dermatitis in korea. *J Dermatol*. 2012;39(8):677–681.

99. Zhang H, Shi Y, Wang C, et al. An improvement of LLNA:DA to assess the skin sensitization potential of chemicals. *J Toxicol Sci*. 2017;42(2):129–136.

100. Osimitz TG, Droege W. Quaternary ammonium compounds: Perspectives on benefits, hazards, and risk. *Toxicology Research and Application*. 2021;5:23978473211049085.

101. Xu M, Sivak JG, McCanna DJ. Ocular toxicology: Synergism of UV radiation and benzalkonium chloride. *Cutan Ocul Toxicol*. 2020;39(4):370–379.

102. Mathias NR, Moench P, Heran C, Hussain MA, Smith RL. Rat nasal lavage biomarkers to assess preclinical irritation potential of nasal drug formulations and excipients. *J Pharm Sci*. 2009;98(2):495–502.

103. D'Cruz OJ, Uckun FM. Preclinical evaluation of a dual-acting microbicidal prodrug WHI-07 in combination with vanadocene dithiocarbamate in the female reproductive tract of rabbit, pig, and cat. *Toxicol Pathol*. 2007;35(7):910–927.

104. Vincent KL, Bell BA, Johnston RK, et al. Benzalkonium chloride causes colposcopic changes and increased susceptibility to genital herpes infection in mice. *Sex Transm Dis*. 2010;37(9):579–584.

105. Vincent KL, Vargas G, Bourne N, et al. Image-based noninvasive evaluation of colorectal mucosal injury in sheep after topical application of microbicides. *Sex Transm Dis*. 2013;40(11):854–859.

106. Trocme S, Hwang L, Bean GW, Sultan MB. The role of benzalkonium chloride in the occurrence of punctate keratitis: A meta-analysis of randomized, controlled clinical trials. *Ann Pharmacother*. 2010;44(12):1914–1921.

107. Rizzo JA, Medeiros D, Silva AR, Sarinho E. Benzalkonium chloride and nasal mucociliary clearance: A randomized, placebo-controlled, crossover, double-blind trial. *Am J Rhinol*. 2006;20(3):243–247.

108. Goldstein MH, Silva FQ, Blender N, Tran T, Vantipalli S. Ocular benzalkonium chloride exposure: Problems and solutions. *Eye (Lond)*. 2022;36(2):361–368.

109. Baudouin C, Labbé A, Liang H, Pauly A, Brignole-Baudouin F. Preservatives in eyedrops: The good, the bad and the ugly. *Prog Retin Eye Res*. 2010;29(4):312–334.

110. Stevens AM, Kestelyn PA, De Bacquer D, Kestelyn PG. Benzalkonium chloride induces anterior chamber inflammation in previously untreated patients with ocular hypertension as measured by flare meter: A randomized clinical trial. *Acta Ophthalmol*. 2012;90(3):221.

111. Nițescu V, Lescaie A, Boghițoiu D, Ulmeanu C. Benzalkonium chloride poisoning in pediatric patients: Report of case with a severe clinical course and literature review. *Toxics*. 2024;12(2):139.

112. Okeke CAV, Khanna R, Ehrlich A. Quaternary ammonium compounds and contact dermatitis: A review and considerations during the COVID-19 pandemic. *Clin Cosmet Investig Dermatol*. 2023;16:1721–1728.

113. Satoji Y, Tobu S, Matsushita K, Udo K, Noguchi M. Benzalkonium chloride induced corrosive balanoposthitis in a man requiring clean intermittent catheterization. *IJU Case Rep*. 2021;4(2):101–103.

114. George M, Joshi SV, Concepcion E, Lee H. Paradoxical bronchospasm from benzalkonium chloride (BAC) preservative in albuterol nebulizer solution in a patient with acute severe asthma. A case report and literature review of airway effects of BAC. *Respir Med Case Rep*. 2017;21:39–41.

115. Robinson AJ, Foster RS, Halbert AR, King E, Orchard D. Granular parakeratosis induced by benzalkonium chloride exposure from laundry rinse aids. *Australas J Dermatol*. 2017;58(3):e138–e140.

116. Dear K, Gan D, Stavrakoglou A, Ronaldson C, Nixon RL. Hyperkeratotic flexural erythema (more commonly known as granular parakeratosis) with use of laundry sanitizers containing benzalkonium chloride. *Clin Exp Dermatol*. 2022;47(12):2196–2200.

117. Sun G, Li T, Zhang J, Liu H, Xu Y, Zhang B. Systemic contact dermatitis in a family: Case report. *Medicine (Baltimore)*. 2024;103(33):e39272.

118. Mezger E, Wendler O, Mayr S, Bozzato A. Anaphylactic reaction following administration of nose drops containing benzalkonium chloride. *Head Face Med*. 2012;8:29.

119. Anderson D, Faltay B, Haller NA. Anaphylaxis with use of eye-drops containing benzalkonium chloride preservative. *Clin Exp Optom*. 2009;92(5):444–446.

120. Chen J, Yin M, Wu R, Dou X. Allergic contact dermatitis caused by benzalkonium chloride from laundry disinfectant. *Int J Dermatol*. 2024;63(1):119–120.

121. Li X, Gao T, Zhi L. Granular parakeratosis induced by benzalkonium chloride masquerading as allergic contact dermatitis after circumcision. *Dermatitis*. 2025.

122. Galadari A, Darrigade A, Boralevi F, Milpied B. A child polysensitized to all constituents of biseptine. *Contact Dermatitis*. 2020;82(1):65–66.

123. Tartari F, Vincenzi C, Di Altobrando A, Bruni F, Neri I. Allergic contact dermatitis to benzalkonium chloride with erythema multiforme-like reaction in a child. *Contact Dermatitis*. 2020;82(6):397–399.

124. Zhang AJ, Boyd AH, Schlarbaum JP, Warshaw EM. Allergic contact dermatitis secondary to the use of a bandage impregnated with benzalkonium chloride. *Contact Dermatitis*. 2018;79(6):387–388.

125. Darrigade AS, Léauté-Labrèze C, Boralevi F, Taïeb A, Milpied B. Allergic contact reaction to antiseptics in very young children. *J Eur Acad Dermatol Venereol*. 2018;32(12):2284–2287.

126. Kefala K, Ponvert C. Allergic contact dermatitis to chlorhexidine-containing antiseptics and their excipients in children: A series of six cases. *Pediatr Dermatol*. 2023;40(1):151–153.

127. Darrigade A, Dendooven E, Mangodt E, Vermander E, Hagendorens M, Aerts O. A peculiar case of sensitization to candelilla cera and sucrose (di)stearate in a toddler. *Contact Dermatitis*. 2020;82(1):54–55.

128. Hann S, Hughes TM, Stone NM. Flexural allergic contact dermatitis to benzalkonium chloride in antiseptic bath oil. *Br J Dermatol*. 2007;157(4):795–798.

129. Yu M, Meng F, Tian S. Systemic contact dermatitis triggered by benzalkonium chloride in laundry detergent A case initially misdiagnosed as eczema. *Clin Cosmet Investig Dermatol*. 2026;19:577154.

130. Suneja T, Belsito DV. Occupational dermatoses in health care workers evaluated for suspected allergic contact dermatitis. *Contact Dermatitis*. 2008;58(5):285–290.

131. Isaac J, Scheinman PL. Benzalkonium chloride: An irritant and sensitizer. *Dermatitis*. 2017;28(6):346–352.

132. Purohit A, Kopferschmitt-Kubler M-, Moreau C, Popin E, Blaumeiser M, Pauli G. Quaternary ammonium compounds and occupational asthma. *Int Arch Occup Environ Health*. 2000;73(6):423–427.

133. Kumbhar VR, Geddugol SB. Prevalence of health effects due to disinfectant exposure and its impact on selected physiological parameters among class D workers: A descriptive cross-sectional study. *Cureus*. 2025;17(3).

134. Bohaty B, Fricker C, González S, Gill. M, Nedorost S. Visual and confocal microscopic interpretation of patch tests to benzethonium chloride and benzalkonium chloride. *Skin Res Technol*. 2012;18(3):272–277.

135. Bennett ML, Fountain JM, McCarty MA, Sherertz EF. Contact allergy to corticosteroids in patients using inhaled or intranasal corticosteroids for allergic rhinitis or asthma. *Am J Contact Dermat*. 2001;12(4):193–196.

136. Wang KL, Rainosek EM, Yang YW, et al. Pediatric patch testing at mayo clinic between 2016 and 2020. *Dermatitis*. 2024;35(4):355–360.

137. Kanokrungsee S, Likittanasombat S, Chaweekulrat P, Kumpangsin T, Boonchai W. Prevalence and causative allergens of contact cheilitis in thailand. *Contact Dermatitis*. 2023;89(5):345–351.

138. Ajayi A, Hall M, Yiannias JA, et al. Trends in patch testing of black patients: The mayo clinic decade experience (january 1, 2011, to december 31, 2020). *Dermatitis*. 2023;34(2):113–119.

139. Huang CX, Yiannias JA, Killian JM, Shen JF. Seven common allergen groups causing eyelid dermatitis: Education and avoidance strategies. *Clin Ophthalmol*. 2021;15:1477–1490.

140. Viggiano T, Yiannias JA, Yang YW. A retrospective review of late delayed positive patch testing greater than day 8 at mayo clinic from 2001 to 2020. *Dermatitis*. 2022;33(6):411–416.

141. Arora P, Brumley C, Arrington K, Hylwa S. Allergic contact dermatitis in skin of color: A retrospective study from a comprehensive patch testing center. *Dermatitis*. 2025;36(2):141–146.

142. Veverka KK, Hall MR, Yiannias JA, et al. Trends in patch testing with the mayo clinic standard series, 2011-2015. *Dermatitis*. 2018;29(6):310–315.

143. Kadivar S, Belsito DV. Occupational dermatitis in health care workers evaluated for suspected allergic contact dermatitis. *Dermatitis*. 2015;26(4):177–183.

144. Zawawi S, Yang YW, Cantwell HM, et al. Trends in patch testing with the mayo clinic standard series, 2017-2021. *Dermatitis*. 2023;34(5):405–412.

145. Houle M, DeKoven JG, Atwater AR, Reeder MJ, Warshaw EM. North american contact dermatitis group patch test results: 2021–2022. *Dermatitis*. 2025.

146. DeKoven JG, Warshaw EM, Reeder MJ, et al. North american contact dermatitis group patch test results: 2019-2020. *Dermatitis*. 2023;34(2):90–104.

147. Pratt MD, Belsito DV, DeLeo VA, Fowler JF. North american contact dermatitis group patch test results, 2001-2002 study period. *Dermatitis*. 2004;15(4):176–183.

148. Dear K, Palmer A, Nixon R. Contact allergy and allergic contact dermatitis from benzalkonium chloride in a tertiary dermatology center in melbourne, australia. *Contact Dermatitis*. 2021.

149. Uter W, Lessmann H, Geier J, Schnuch A. Is the irritant benzalkonium chloride a contact allergen? A contribution to the ongoing debate from a clinical perspective. *Contact Dermatitis*. 2008;58(6):359–363.

150. Basketter DA, Marriott M, Gilmour NJ, White IR. Strong irritants masquerading as skin allergens: The case of benzalkonium chloride. *Contact Dermatitis*. 2004;50(4):213–217.

151. Rundle CW, Hu S, Presley CL, Dunnick CA. Triclosen and its alternatives in antibacterial soaps. *Dermatitis*. 2019;30(6):352–357.

152. Dao H, Fricker C, Nedorost ST. Sensitization prevalence for benzalkonium chloride and benzethonium chloride. *Dermatitis*. 2012;23(4):162–166.

153. Scheman A, Hipolito R, Severson D, Youkhanis N. Contact allergy cross-reactions: Retrospective clinical data and review of the literature. *Dermatitis*. 2017;28(2):128–140.

154. Lee BH, Kim S. Benzalkonium chloride induced bronchoconstriction in patients with stable bronchial asthma. *Korean J Intern Med*. 2007;22(4):244–248.

155. Hodder R, Pavia D, Dewberry H, et al. Low incidence of paradoxical bronchoconstriction in asthma and COPD patients during chronic use of respimat soft mist inhaler. *Respir Med*. 2005;99(9):1087–1095.

156. Canavez ADPM, de Oliveira Prado Corrêa G, Isaac VLB, Schuck DC, Lorencini M. Integrated approaches to testing and assessment as a tool for the hazard assessment and risk characterization of cosmetic preservatives. *J Appl Toxicol*. 2021;41(10):1687–1699.

157. National Institute for Public Health and the Environment (RIVM) of the Netherlands. Cosmetics fact sheet default parameters for estimating consumer exposure - updated version 2025. https://Www.rivm.nl/bibliotheek/rapporten/2025-0099.pdf. 2025.

158. PubChem. 2025. Behenalkonium Chloride. https://pubchem.ncbi.nlm.nih.gov/compound/Behenalkonium-chloride#section=Other-Identifiers. Date Accessed: July 30, 2025.

159. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11, Washington, DC. 2012. United States Environmental Protection Agency..

160. PerkinElmer Informatics. 2026. ChemDraw Professional (Software)..

161. PubChem. 2025. Benzododecinium Chloride. https://pubchem.ncbi.nlm.nih.gov/compound/8753. Date Accessed: July 30, 2025.

162. Stern M, Klausner M, Alvarado R, Renskers K, Dickens M. Evaluation of the EpiOcular((TM)) tissue model as an alternative to the draize eye irritation test. *Toxicol In Vitro*. 1998;12(4):455–461.