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

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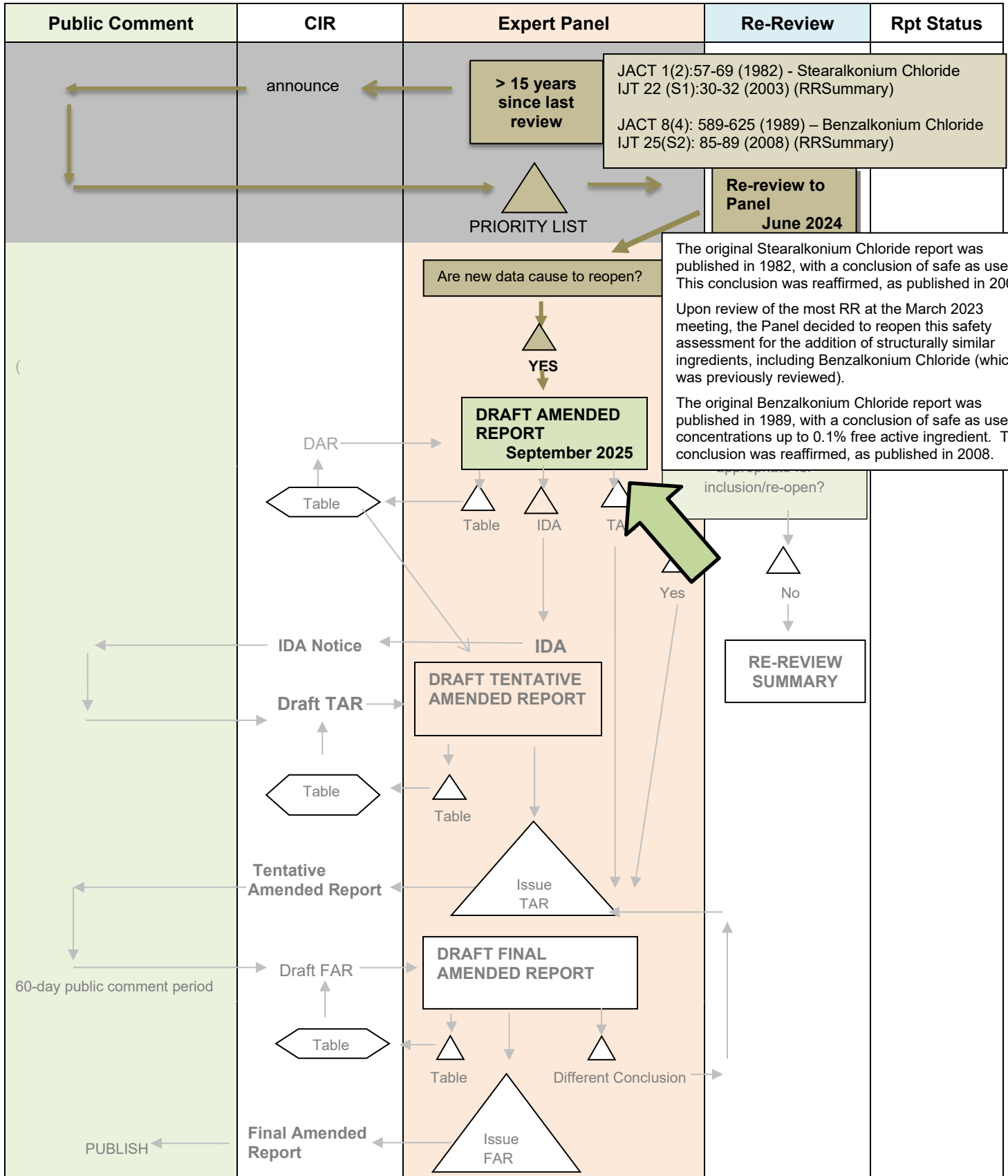
Status: Draft Amended Report for Panel Review  
Release Date: August 15, 2025  
Panel Meeting Date: September 8 – 9, 2025

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Samuel 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. 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., Senior Scientific Analyst/Writer, CIR.

# RE-REVIEW FLOW CHART

INGREDIENT/FAMILY Alkonium Chlorides and Bromides

MEETING September 2025





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### Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons  
From: Priya Ferguson, M.S.  
Senior Scientific Analyst/Writer, CIR  
Date: August 15, 2025  
Subject: Draft Amended Report on the Safety Assessment of the Alkonium Chlorides and Bromides

The Expert Panel for Cosmetic Ingredient Safety (Panel) first published a review of the safety of Stearalkonium Chloride in 1982 (identified as *originalreportSAC\_AlkoniumChlorides\_092025* in the pdf). The Panel concluded that Stearalkonium Chloride is “safe when incorporated in cosmetic products similar to those presently marketed.” The Panel considered a re-review of this report and re-affirmed the 1982 conclusion, as published in 2003 (*rereviewSAC\_AlkoniumChlorides\_092025*).

Because it had been at least 15 years since the re-review was published, in accordance with Cosmetic Ingredient Review (CIR) Procedures, the Panel again re-reviewed this ingredient in March 2023. At this meeting, the report was re-opened for the addition of structurally similar ingredients. One of the additions, Benzalkonium Chloride, was also previously reviewed by the Panel in a safety assessment published in 1989 (*originalreportBAC\_AlkoniumChlorides\_092025*) with the conclusion that “Benzalkonium Chloride, at concentrations up to 0.1% free, active ingredient, is safe as a cosmetic ingredient as presently used.” The Panel considered a re-review of this ingredient (*RRdataBAC\_AlkoniumChlorides\_092025*), and re-affirmed the 1989 conclusion, as published in 2008 (*rereviewBAC\_AlkoniumChlorides\_092025*). Accordingly, a Draft Amended Report on these ingredients, as well as 4 previously unreviewed ingredients (Behenalkonium Chloride, Benzalkonium Bromide, Cetearalkonium Bromide, and Lauralkonium Bromide) has been prepared (*report\_AlkoniumChlorides\_092025*) and submitted for your review.

Benzalkonium Chloride and Stearalkonium Chloride were previously reported to be used in 79 (as of 2006) and 151 total formulations (as of 2001), respectively. According to 2023 VCRP data, these ingredients were used in 69 and 88 formulations, respectively. The maximum reported concentration of use for Benzalkonium Chloride has remained consistent, with usage up to 0.5% reported in both 2006 and 2023. The maximum concentration of use for Stearalkonium Chloride has decreased from a reported 7% in 2001. RLD data (2024) indicate that Stearalkonium Chloride is currently used in 885 formulations, while Benzalkonium Chloride appears in 565 formulations.

Numerous studies on Benzalkonium Chloride were identified in the literature, many of which are cited in the *Other Relevant Studies* section of this report. Since the relevance of these studies to cosmetic safety is uncertain, the Panel is asked to advise whether any can be removed to help clarify the report.

Supporting documents for this report package include a flow chart (*flow\_AlkoniumChlorides\_092025*), report history (*history\_AlkoniumChlorides\_092025*), a search strategy (*search\_AlkoniumChlorides\_092025*), a data profile (*datapofile\_AlkoniumChlorides\_092025*), minutes from the meetings at which the original reports were discussed (*originalminutes\_AlkoniumChlorides\_092025*), and transcripts from the recent meeting at which reopening this report was discussed (*transcripts\_AlkoniumChlorides\_092025*).

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

## **Alkonium Chlorides and Bromides History**

**1982**

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

**1989**

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

**2003**

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

**2008**

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

**March 2023**

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

**September 2025**

Panel reviews Draft Amended Report on alkonium chlorides and bromides

**Alkonium Chlorides and Bromides Data Profile\* - September 2025 - Writer, Priya Ferguson**

				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization					Ocular Irritation		Clinical Studies		
	Reported Use	Method of Mfg	Impurities	log P/log K <sub>ow</sub>	Dermal Penetration	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/Multicenter	Case Reports		
Behenalkonium Chloride	X	X		X																											
Benzalkonium Bromide	X	X																													
Benzalkonium Chloride	XO	XO	XO		X	XO	X O	X O	X	o	xo	xo		xo	xo	x	x			XO	XO					O	XO	X	XO		
Cetearalkonium Bromide	X	X																													
Lauralkonium Chloride	X	X		X		X																									
Stearalkonium Chloride	XO	X	XO	X				O							x				O	XO				O		O	XO				

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

**Alkonium Chlorides and Bromides**

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

X = relevant hits found

**Search Strategy**

INCI names, CAS numbers, and technical names searched along with “typical search terms” listed below

**Typical Search Terms (this is informational – not for inclusion for search strategy that goes to the Panel)**

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

**MARCH 2023 MEETING – FIRST REVIEW/SECOND RE-REVIEW****Belsito Team – March 6, 2025**

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

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

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

**DR. BELSITO:** You're on Page 9?

**DR. GREMILLION:** Yeah.

**DR. BELSITO:** That's the old report.

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

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

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

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

**DR. HELDRETH:** That's Page 6.

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

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

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

**DR. GREMILLION:** Okay.

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

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

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

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

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

**DR. GREMILLION:** Okay.

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

**DR. GREMILLION:** Yeah, that was the teratogenicity.

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

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

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

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

**DR. KLAASSEN:** That's what I thought.

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

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

**DR. HELDRETH:** We have not --

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

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

**DR. RETTIE:** Yeah.

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

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

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

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

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

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

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

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

**DR. BELSITO:** Okay.

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

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

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

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

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

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

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

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

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

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

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

**DR. BELSITO:** What pages are we on here?

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

**DR. ANSELL:** That goes back to --

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

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

**MS. CHERIAN:** Yes.

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

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

**DR. BELSITO:** I don't know.

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

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

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

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

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

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

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

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

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

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

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

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

**DR. BELSITO:** Yeah, I know.

**DR. GREMILLION:** Yeah.

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

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

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

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

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

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

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

### Cohen Team – March 6, 2025

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

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

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

**DR. ROSS:** I thought it was a reopen.

**DR. BERGFELD:** I did, too.

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

**DR. COHEN:** Oh, did I reverse it?

**DR. ROSS:** Yeah.

**DR. TILTON:** Yeah.

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

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

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

**MS. CHERIAN:** In the memo.

**DR. COHEN:** Okay.

**MS. CHERIAN:** It should've been 3.

**DR. COHEN:** Okay.

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

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

**MS. CHERIAN:** Three.

**DR. ROSS:** No, it's three.

**DR. COHEN:** It's three.

**DR. SLAGA:** Three.

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

**DR. SLAGA:** Yeah.

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

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

**DR. SLAGA:** Yeah.

**DR. BERGFELD:** Me too.

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

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

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

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

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

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

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

**DR. COHEN:** EU.

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

**MS. CHERIAN:** Three percent.

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

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

**MS. EISENMANN:** Correct.

**MS. FIUME:** Okay, thank you.

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

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

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

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

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

## Propylene Carbonate

**DR. COHEN:** We'll move on to propylene carbonate. Okay, get rid of that. Okay, so propylene carbonate was first published in a review in 1987, with the conclusion of safe. And the 1987 conclusion was reaffirmed in 2006. We've got some new toxicologic data. We know that propylene carbonate is used at up to 5 percent as an inactive ingredient in a FDA-approved topical drug formulation. We have frequency of use that has increased quite a bit from 178 uses, in 2002, to 911 uses in 2022.

And in 2003 its use was up to five percent. And according to 2022 concentration of use, propylene carbonates used up to 17.9 percent. It's used in baby products as well. And from the old report, the max use is close to sensitization studies which have appeared to have some positives, at least that's what I could see in the old report, but any comments, thoughts?

**DR. TILTON:** So, I was recommending to reopen primarily based on the new ocular irritation data and increased use in eye makeup preparations.

**DR. ROSS:** I would second that. I mean, I think also the increased uses, increase concentration, the new data with ocular, yeah, reopen.

**DR. COHEN:** Tom?

**DR. SLAGA:** I agree.

**DR. COHEN:** Yeah. I had it as a reopen as well. All right, any --

**DR. ROSS:** We had a propylene glycol in there as well, we had probably caught that.

**DR. COHEN:** What do you mean?

**DR. ROSS:** It was just a typo. Propylene Glycol -- was misidentified as propylene glycol on page four. I'm sure it's --

**DR. COHEN:** Okay. And we're sure that it was meant to be propylene carbonate and not glycol?

**DR. ROSS:** Yeah.

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

### Full Panel – March 7, 2025

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

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

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

**DR. BERGFELD:** Is that a motion?

**DR. COHEN:** That's a motion.

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

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

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

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

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

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

## Expert Panel for Cosmetic Ingredient Safety Meeting Transcripts

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

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

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

**DR. COHEN:** Right, right.

**DR. RETTIE:** My understanding is that the BAKs are a family that includes the C<sub>18</sub> chainline, which is -- I can't say this word -- stearalkonium chloride, which is the single thing we're talking about here.

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

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

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

**DR. COHEN:** Yes.

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

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

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

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

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

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

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

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

**DR. BELSITO:** Yes.

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

## STEARALKONIUM CHLORIDE

### **Draft Report Review (November 1979)**

The Report contained the following Conclusion: It is the opinion of the Expert Panel based on the evidence at hand, which it believes to be relevant and accumulated in a reasonable manner, that the cosmetic ingredient, Stearalkonium Chloride, is safe when incorporated in concentrations similar to those presently marketed.

Dr. Beyer instructed that, in view of the suggested revisions, the document be revised and mailed to the panel for further review. Upon adoption, the document will be issued as a Tentative Report for a 90-day comment period.

### **Draft Tentative Report Review (March 1980)**

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

### **Draft Final Report Review**

Not available

### **First Re-Review (November 2001)**

Dr. Belsito stated that a CIR Final Report with the following conclusion on this ingredient was published in 1982: On the basis of the evidence at hand, the Expert Panel concludes that the cosmetic ingredient, Stearalkonium Chloride, is safe when incorporated in cosmetic products in concentrations similar to those presently marketed.

Dr. Belsito noted that very little new information on Stearalkonium Chloride has entered the published literature since the Final Report was published, and that his Team concluded that the Final Safety Assessment on this ingredient does not need to be reopened.

The Panel unanimously concluded that the CIR Final Safety Assessment on Stearalkonium Chloride does not need to be reopened.

## BENZALKONIUM CHLORIDE

### **Draft Report Review**

Not available

### **Draft Tentative Report Review (April 1988)**

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

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

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

### **Draft Final Report Review**

Not available

### **First Re-Review (June 2006)**

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

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

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Status: Draft Amended Report for Panel Review  
Release Date: August 15, 2025  
Panel Meeting Date: September 8 – 9, 2025

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Samuel 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. 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., 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
<i>Dictionary</i>	<i>International Cosmetic Ingredient Dictionary and Handbook</i>
DNA	deoxyribonucleic acid
DMSO	dimethyl sulfoxide
EC	European Commission
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 <sub>50</sub>	exposure time required to reduce tissue viability to 50%
EU	European Union
FDA	Food and Drug Administration
FEV <sub>1</sub>	forced expiratory volume in 1 sec
GD	gestation days
GP1R	G-protein coupled estrogen receptor 1
HRIPT	human repeated-insult patch test
IC <sub>50</sub>	half-maximal inhibitory concentration
IgE	immunoglobulin E
IL	interleukin
IL-6	interleukin-6
IL-1 $\alpha$	interleukin-1alpha
IFN $\alpha$	interferon alpha-2b
LC <sub>50</sub>	median lethal concentration
LD <sub>50</sub>	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
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
QSAR	quantitative structure-activity relationship
RLD	Registration and Listing Data
RNA	ribonucleic acid
r.o.	rinse-off
ROS	reactive oxygen species
SCC	Scientific Committee on Cosmetology
SCCS	Scientific Committee on Consumer Safety
SED	systemic exposure dose
SDS	sodium dodecyl sulfate

SLS	sodium lauryl sulfate
TG	test guidelines
US	United States
UV	ultraviolet
ZO-1	tight junction zonula occludens-1

## INTRODUCTION

This assessment reviews the safety of the following 6 ingredients as used in cosmetic formulations:

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

\*previously reviewed

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

Stearalkonium Chloride was previously reviewed by the Expert Panel for Cosmetic Ingredient Safety (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).<sup>2</sup> The Panel also considered a re-review of this report and re-affirmed the 1982 conclusion, as published in 2003.<sup>3</sup> 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).<sup>4</sup> The Panel considered a re-review of this ingredient and re-affirmed the 1989 conclusion, as published in 2008.<sup>5</sup>

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 July 2025. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the 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)<sup>6,7</sup> and Australian Industrial Chemicals Introduction Scheme (AICIS) websites.<sup>8</sup> 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.

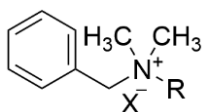
It should be noted that Benzalkonium Chloride consists of mixtures of alkylbenzyltrimethylammonium chlorides, and may therefore have varying chain lengths.<sup>9</sup> They are typically commercialized as compounds ranging from C<sub>8</sub> – C<sub>18</sub>, with higher biocide activity for C<sub>12</sub> and C<sub>14</sub> derivatives.<sup>10</sup> Some review sources (e.g., ECHA and AICIS) list test substances in studies by chain length (e.g., C<sub>12</sub> - C<sub>16</sub> alkylbenzyltrimethylammonium chloride).<sup>8,11,12</sup> 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<sub>12</sub> – C<sub>16</sub>)). 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<sup>13</sup> 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.<sup>1</sup> These ingredients are quaternary ammonium halides, specifically, alkylbenzyltrimethylammonium salts with chloride or bromide counterions, as shown in Figure 1.<sup>8</sup>



**Figure 1.** Alkylbenzyltrimethylammonium halides, wherein R is an even numbered alkyl chain (C<sub>10</sub> – C<sub>22</sub>) and X is bromide or chloride.

These cationic surfactants have varying carbon chain lengths of the hydrophobic alkyl moieties (C<sub>10</sub> – C<sub>22</sub>; e.g., Stearalkonium Chloride (CAS No. 122-19-0) has a chain length of C<sub>18</sub>). 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 alkylbenzyltrimethylammonium chlorides.<sup>9</sup>

### 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.<sup>4</sup> Chemical properties of Benzalkonium Chloride and the other ingredients reviewed in this report may be found in Table 2.

### Method of Manufacture

*Benzalkonium Chloride is prepared by treating a solution of N-alkyl-N-methylbenzylamine in an organic solvent with methyl chloride (the appropriate solvent results in quaternary compound precipitates).<sup>4</sup> This ingredient may be formulated by preparing a primary amine from a fatty acid or a blend of fatty acids, followed by methylation of the primary amine to form dimethyl alkylamine. This tertiary amine is then quaternized with benzyl chloride.*

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.<sup>14</sup> 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<sub>22</sub>, cetearyl C<sub>16</sub> - C<sub>18</sub>, lauryl C<sub>12</sub>, stearyl C<sub>18</sub>, 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.<sup>4</sup> It may also be sold in a mixture of water, ethyl alcohol, and isopropyl alcohol. The total alkylbenzyltrimethylammonium chloride content of Benzalkonium Chloride is not less than 97%.*

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

### Benzalkonium Chloride

Several impurities may be present in raw Benzalkonium Chloride.<sup>15</sup> These impurities include benzyl alcohol, benzaldehyde, and benzyl chloride.

### Reactivity

*These cationic can be used to precipitate sulfated hydrocolloids at critical temperature and pH values. These substances may lower surface tension, making possible many chemical reactions (e.g., hydrolysis of carboxylic acid esters of polyvinyl alcohol). In the presence of nitrites, nitrogen oxides, or other nitrosating agents, alkylbenzyltrimethylammonium chlorides will give rise to traces of N-nitrosamines. The significant impurities, alkyl dimethylamines, 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.<sup>13</sup> There was no measurable absorption above 310 nm. The maximum UV absorption was observed to be 262 nm for this ingredient.<sup>4</sup>*

### 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 these ingredients in cosmetics. Data included herein were obtained from the FDA and the Personal Care Products Council (Council), and it is these values that define the present practices of use and concentration that are assessed by the Panel. Responses to a survey conducted by the Council indicate maximum reported concentrations of use. Frequency of use were obtained from the FDA Voluntary Cosmetic Registration Program (VCRP) database and from Registration and Listing Data (RLD). Since 2024, as a result of the Modernization of Cosmetics Regulation Act (MoCRA) of 2022, 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.<sup>16</sup> Please note, at this time, it is not appropriate to contrast data from the VCRP and RLD to determine a trend in frequency of use because there are numerous differences in the ways the data for the VCRP and the RLD were collected and processed, and because reporting frequency of use is now mandatory (as opposed to the past practice of voluntary reporting). Although the VCRP program is now defunct, trends in frequency of use from the RLD alone are not yet possible in that a baseline is currently not available.

According to 2024 RLD, Stearalkonium Chloride has the highest number of uses (it is used in 885 total formulations; Table 3 and Table 4).<sup>17</sup> This ingredient is also reported to have the highest concentration of use. It is used at up to 3.8% in tonics, dressings, and other hair grooming aids.<sup>18</sup>

These ingredients may result in incidental ingestion, incidental ocular exposure, and mucous membrane exposure as they are reported to be used in formulations used in the mouth (e.g., Benzalkonium Chloride is used in mouthwashes and breath fresheners at 0.3%), near the eye (e.g., Benzalkonium Chloride is used in other eye makeup preparations at up to 0.01%) and other mucus membranes (Benzalkonium Chloride is used in bath soaps and body washes at up to 0.14%).<sup>19</sup> In addition, these ingredients are reported to be used in baby products (e.g., Benzalkonium Chloride is used in other baby products (concentration of use not stated)).

Benzalkonium Chloride and Stearalkonium Chloride were previously reported to be used in 79 (as reported in 2006) and 151 (as reported in 2001) formulations, respectively.<sup>3,5</sup> According to 2023 VCRP, these ingredients were used in 69 and 88 formulations, respectively.<sup>17,20</sup> Maximum concentrations of use for Benzalkonium Chloride have remained unchanged since 2006 (Benzalkonium Chloride was reported to be used at up to 0.5% in both 2006 and 2023).<sup>19</sup> The concentration of use for Stearalkonium Chloride has decreased (in 2001, Stearalkonium Chloride was reported to be used at up to 7%).

These ingredients may be used in cosmetic sprays and powders, and could possibly be inhaled (e.g., Benzalkonium Chloride is used in perfumes (concentration 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.

Some products containing these ingredients may be marketed for use with airbrush delivery systems. With the advent of MoCRA and the current product categories outlined by the FDA, it is now mandatory that cosmetic products used in airbrush delivery systems be reported as such for some, but not all, product categories in the RLD. In other words, a reliable source of frequency of use data regarding the use of cosmetic ingredients in conjunction with airbrush delivery systems is now available, in some instances. Some of the reported product categories for these ingredients as listed in the RLD do require designation if airbrush application is used (e.g., indoor tanning preparations), but no airbrush use was indicated. Additionally, the Council currently surveys the cosmetic industry for maximum reported use concentrations of ingredients in products which may be used in conjunction with an airbrush delivery system; thus, this type of data may also be available, when submitted. Please note that no concentration of use data were provided indicating airbrush application. Nevertheless, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety. Without information regarding the consumer habits and practices data or product particle size data (or other relevant particle data, e.g., diameter) related to this use technology, the data profile is incomplete, and the Panel is not able to determine safety for use in airbrush formulations. Accordingly, the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

Benzalkonium Bromide and Benzalkonium Chloride are listed in the European Union (EU) Cosmetic Regulation 1223/2009 Annex V (list of preservatives allowed in cosmetic products) with a maximum concentration of 0.1% in cosmetic formulations.<sup>21</sup> 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<sub>14</sub> 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.<sup>4</sup> 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.<sup>8</sup> These uses include excipients in pharmaceutical formulations and in hospital or household/commercial grade disinfectants. Stearalkonium Chloride is reported to be used as an inactive ingredient in an FDA-approved topical drug at 3.15%.<sup>22</sup> Benzalkonium Chloride is reported to be used in both OTC and prescription formulations (e.g., glaucoma medications, eye/ear drops, nasal sprays).<sup>8,23</sup> 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.<sup>24-28</sup> 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 5.

### **TOXICOKINETIC STUDIES**

*The average value of the membrane cell integral diffusion coefficient for Benzalkonium Chloride was 0.000008 cm<sup>2</sup>/s at 25° C, indicating low diffusivity across lipid membranes.<sup>13</sup> 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<sub>10</sub>–C<sub>16</sub>) by human hepatic cytochrome P450 enzymes, metabolic stability in human liver microsomes increased with alkyl chain length.<sup>29</sup> The study demonstrated that Benzalkonium Chloride undergoes 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<sub>10</sub> < C<sub>12</sub> < C<sub>14</sub> < C<sub>16</sub>.

#### **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.<sup>30</sup> 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.<sup>31</sup> The absorbed dose was determined to be approximately 0.05% in an assay in which Benzalkonium Chloride (80.5% active ingredient; radiolabeled and non-radiolabeled) was applied to human skin samples at 0.03%.<sup>11</sup> Systemic exposure to Benzalkonium Chloride (0.13%) via hand soap was determined to be very low in a study performed in 32 subjects.<sup>32</sup>

#### **Penetration Enhancement**

*Permeation of cyclosporin A through intact and de-epithelialized human vaginal mucosa was enhanced by 0.01% Benzalkonium Chloride.<sup>13</sup> 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.<sup>33</sup> 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).<sup>13</sup> 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 alkylbenzyltrimethylammonium chlorides (predominantly C<sub>12</sub>, C<sub>14</sub>, and C<sub>16</sub>) was administered orally, rectally, or intramuscularly to rabbits, dogs, and cats, at 10 times the lethal dose.<sup>2</sup> 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).<sup>4</sup>*

*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 [<sup>14</sup>C]-Benzalkonium Chloride.<sup>4</sup> 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 6. 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.<sup>12</sup> 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).<sup>11</sup> 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).<sup>12</sup> 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.<sup>34</sup>

## **TOXICOLOGICAL STUDIES**

### **Acute Toxicity Studies**

*Of 96 mice receiving dermal applications of 6.5 and 50% Benzalkonium Chloride, 29 died within 72 h after application. Other effects at these concentrations included permanent bald spots, ear necrosis, and weight loss, while lower concentrations (0.1 – 1.5%) produced mild, temporary effects. A dermal median lethal dose (LD<sub>50</sub>) of 525 mg/kg was established in an acute oral toxicity assay in which Benzalkonium Chloride was given to white rats.<sup>4</sup> 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.<sup>13</sup>*

*LD<sub>50</sub>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.<sup>2</sup> An aqueous solution containing 20% Stearalkonium Chloride yielded an LD<sub>50</sub> 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<sub>50</sub> greater than 0.0625 g/kg but less than 1.25 g/kg. An oral LD<sub>50</sub> 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 7. LD<sub>50</sub>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.<sup>8,11</sup> The lowest oral LD<sub>50</sub> for Benzalkonium Chloride was reported to be 234 mg/kg following administration of 10% Benzalkonium Chloride to rats.<sup>11,12,35,36</sup> A no-observed-effect-level (NOEL) of < 0.049 mg/m<sup>3</sup> was determined in an acute inhalation toxicity assay in which female mice were administered aerosolized Benzalkonium Chloride.<sup>37</sup> In a different acute inhalation toxicity assay performed in rats, a median LC<sub>50</sub> of 52.84 mg/m<sup>3</sup> Benzalkonium Chloride was determined.<sup>8,38</sup>

### **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.<sup>13</sup> 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.<sup>4</sup> 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<sup>3</sup> 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.<sup>4</sup> 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 8. A no-observed-adverse-effect-level (NOAEL) of 5000 mg/kg was determined in a 91-d assay in which Sprague-Dawley rats (10/sex/group) were given a sanitizing agent containing 0.01% Benzalkonium Chloride (up to 5000 mg/kg) via gavage.<sup>39</sup> An 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).<sup>11</sup> 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).<sup>12</sup> 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<sup>3</sup> (6 h/d; 3 d).<sup>38</sup> 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<sup>3</sup>; 6/d exposure; 5 d).<sup>40</sup> A no-observed-adverse-effect concentration (NOAEC) of < 0.08 mg/m<sup>3</sup> 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<sup>3</sup> (6 h/d exposure).<sup>41</sup>

## **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.<sup>13</sup> 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.<sup>4</sup> 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 9. 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.<sup>42</sup> Similar effects were observed when human sperm was treated with 6.81 mM Benzalkonium Chloride.<sup>43</sup> 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.<sup>44</sup> However, isolated fetal abnormalities were observed across dosed groups. A reproductive NOAEL of 2000 ppm for F<sub>0</sub>, F<sub>1</sub>, and F<sub>2</sub> 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).<sup>11</sup> 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.<sup>12</sup> 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.<sup>44</sup> 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.<sup>8,45</sup>

### **GENOTOXICITY STUDIES**

*Benzalkonium Chloride (concentration not stated) was not mutagenic to Salmonella typhimurium strains TA1535, TA1536, TA1537, and TA1538 and Escherichia coli strains B/r WP2 hcr<sup>+</sup> and WP2 hcr in rec-assays in combination with reverse mutation systems.<sup>4</sup> The mutagenic activity of Benzalkonium Chloride (concentration not stated) was not demonstrated in reversion assays involving strains S. typhimurium TA1535, TA1536, TA1537, and TA1538, and in a rec-assay, with Bacillus subtilis strains H17 rec<sup>+</sup> and M45 rec<sup>-</sup>. Similarly, the deoxyribonucleic acid (DNA)-damaging capacity of Benzalkonium Chloride (10%) was not positively detected when evaluated in a rec-assay using Bacillus subtilis and S. typhimurium TA98 and TA100.<sup>13</sup> In a plate incorporation assay, Benzalkonium Chloride (concentration not stated) was not mutagenic to S. typhimurium strains TA98, TA1538, TA1537, and TA100. In an E. coli DNA polymerase assay, Benzalkonium Chloride induced repairable DNA damage in strains W3110 (polA<sup>+</sup>) and p3478 (polA<sup>-</sup>). No genotoxicity was observed (with and without metabolic activation) when the genotoxicity potential of Benzalkonium Chloride (up to 5 µg/ml) was evaluated in an umu assay using S. typhimurium TA1535/pSK. Benzalkonium Chloride (0.3 – 30 µg/ml) elicited a significant level of sister chromatid exchanges when evaluated in Syrian hamster embryo cells; however, the inducibility did not exceed twice that of the control level. 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 10. 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%).<sup>46,47</sup> 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).<sup>48,49</sup> 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.<sup>50</sup> 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).<sup>11,39,49</sup> 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.<sup>12,51</sup> 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.<sup>11,12</sup> 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.<sup>11,12,52</sup> Statistically significant increases in micronucleus formation (compared to controls) were observed at 1 mg/l in a micronucleus assay (performed without metabolic activation; peripheral human lymphocytes) using Benzalkonium Chloride.<sup>49</sup> Negative results were obtained in vivo assays performed using rats given either 0.01% Benzalkonium Chloride (up to 20 ml/kg) or 400 mg/kg Benzalkonium Chloride.<sup>8,39</sup>

## **CARCINOGENICITY STUDIES**

*The tumorigenicity of Benzalkonium Chloride was evaluated in a dermal assay using 100 female mice and 10 rabbits (male and female).<sup>4</sup> 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.<sup>4</sup> 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* agglutinin, synthetic compound 48/80, and polyethyleneimine, resulting in half-maximal inhibitory concentrations (IC<sub>50</sub>s) of 0.63, 3.04, 1.56, and 0.48, respectively.<sup>13</sup>*

### **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).<sup>13</sup> 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.<sup>4</sup> 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.<sup>4</sup> 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 treated with 0.01% Benzalkonium Chloride (0.1 mg/ml) 2x/d for 2 wk.*

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).<sup>53</sup> 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.<sup>54</sup> 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.<sup>55</sup>

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).<sup>56</sup> 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<sub>12</sub> – C<sub>16</sub>).<sup>57</sup> 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.<sup>58</sup> 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).<sup>59</sup> Applications occurred multiple times per day (6 – 8 x/d) for several weeks (3 – 52 wk).

### **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.<sup>60</sup> 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.

## Cytotoxicity

*Cytotoxicity was observed in multiple cell types (rat prostatic epithelial cells, rat cardiac fibroblasts, human conjunctival cells, corneal epithelial cells, human neutrophils) in in vitro assays performed using Benzalkonium Chloride at varying concentrations (0.00001% - 0.15%).<sup>4,13</sup> 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  $\geq 1$  µg/ml), human and rabbit corneal epithelial cells (at  $\geq 0.001\%$ ), human trabecular meshwork cells (at  $\geq 0.001\%$ ), human respiratory epithelial cells (at  $\geq 0.005\%$ ), reconstructed human epidermis (at  $\geq 0.5$  mg/ml), human corneal stromal fibroblasts (at  $\geq 0.00000001\%$ ), human meibomian gland epithelial cells (at  $\geq 50$  µg/ml in one assay and at  $\geq 0.005$  µg/ml in a different assay), human conjunctival cells (at  $\geq 0.005$  µg/ml), human vaginal cells (at  $\geq 0.05$  mM), porcine corneal explants (at 0.01%), in human nasal epithelial cells (at  $\geq 0.01\%$ ), T-lymphocytic Jurkat cells (at  $\geq 0.0001\%$ ), human limbal epithelial stem cells (at  $\geq 0.00002\%$ ), rat neural progenitor cells (at 1 µM), human primary subcutaneous pre-adipocytes (at  $\geq 0.005\%$ ), human gingival fibroblasts (at  $\geq 0.05$  mM), and Sertoli cells (at 0.02%).<sup>46,48,61-75</sup>

### Transport and Interaction of Cosmetic Product Material on the Ocular Surface

The transfer of cosmetic product material to any area of the ocular surface precedes the onset of adverse effects—such as discomfort and tear film instability—associated with eye cosmetics containing Benzalkonium Chloride.<sup>76</sup> The inter-transport of cosmetic product material across pre-corneal tear film occurs due to diffusion and drift processes. Diffusion is driven by concentration gradients while the drift of cationic Benzalkonium Chloride is influenced by electric field. The inherent electric field is determined by the distribution of ions secreted into the aqueous layer, and the negative glycocalyx charge at the mucin layer. Because of this, those with mucin deficiency may experience an increased risk of adverse effects following the use of eye cosmetics containing Benzalkonium Chloride.

### Reactive Oxygen Species (ROS) Generation

*A stimulation of ROS (dioxide 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.<sup>13</sup> Arachidonic acid release was evaluated in murine Swiss albino 3T6 fibroblasts treated with Benzalkonium Chloride. Benzalkonium Chloride induced significant [<sup>3</sup>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.<sup>77</sup> Treatment with the test substance did not induce production of extracellular ROS as low concentrations ( $\leq 5$  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  $\geq 20$  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)).<sup>71</sup>

### 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.<sup>13</sup> 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.*

### Accumulation Following Ocular Administration

Ocularly applied Benzalkonium Chloride is reported to accumulate in the cornea, lens, conjunctiva, iris, ciliary muscle epithelium, and trabecular meshwork.<sup>78</sup> 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).<sup>13</sup> 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.<sup>79,80</sup>

### Barrier Disruption

The effect of Benzalkonium Chloride (0.05% in water) on stratum corneum organization and permeability of the protein drug interferon alpha-2b (IFN $\alpha$ ) was evaluated in human skin samples (full-thickness breast skin) *in vitro*.<sup>81</sup> 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, IFN $\alpha$  permeation remained low (total IFN $\alpha$  permeation approximately 1 ng/cm<sup>2</sup> of skin; PBS resulted in permeation of approximately 1.5 ng/cm<sup>2</sup> 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).<sup>56,64,82,83</sup>

### Effect on Cytokines

*In an assay performed in human keratinocytes, Benzalkonium Chloride (0.1  $\mu$ g/ml) stimulated the production and intracellular accumulation of interleukin-1 $\alpha$  (IL-1 $\alpha$ ).<sup>13</sup> The release of IL-1 $\alpha$  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-1 $\alpha$  and interleukin (IL)-8 was evaluated in reconstructed human epidermis (20 h incubation).<sup>62</sup> Reconstructed human epidermis treated with the test substance released large amounts of IL-1 $\alpha$  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  $\mu$ M Benzalkonium Chloride.<sup>84</sup> 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.<sup>85</sup>

### 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.<sup>86</sup> 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 (Ribonucleic Acid), and Protein Synthesis

*Inhibition of DNA, RNA, and protein synthesis was observed in a dose-dependent manner in Chinese hamster lung fibroblast cells treated with Benzalkonium Chloride (3 – 30  $\mu$ g/ml).<sup>13</sup> 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.<sup>13</sup> 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  $\mu$ g/ml) on human-derived SH-SY5Y astrocyte cultures was evaluated via a neurite outgrowth assay.<sup>87</sup> 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  $\mu$ g/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.<sup>88</sup> 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, 3 $\beta$ -HSD2, 17 $\beta$ -HSD-1, 17 $\beta$ -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 ER $\alpha$  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 ER $\alpha$  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  $\mu\text{M}$  for 24 h.<sup>89</sup>

#### **Effect of Lauralkonium Chloride on Gut Microbiome and Bile Acid Profile**

The effect of orally-ingested Lauralkonium Chloride (120  $\mu\text{g/g/d}$ ; 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.<sup>34</sup> 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.<sup>90</sup> 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).

#### **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.<sup>91</sup> 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  $\gamma$ -scintigraphy with technetium<sup>99m</sup>-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).

### **DERMAL IRRITATION AND SENSITIZATION STUDIES**

*Multiple studies report concentration-dependent dermal irritation associated with Benzalkonium Chloride.<sup>4,13</sup> In rats and rabbits, Benzalkonium Chloride concentrations  $\geq 0.1\%$  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  $\geq 1.25\%$  in rabbits, with primary irritation indices up to 6.0 at 25%.<sup>2</sup> 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 11. Stearalkonium Chloride (undiluted) was determined to be a non-irritant in a reconstructed human epidermis assay.<sup>12</sup> Benzalkonium Chloride (50 – 80% active ingredient) was irritating/corrosive in a dermal irritation assay performed in rabbits (n = 6; occlusive conditions; tested neat).<sup>11</sup> 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).<sup>92</sup> 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).<sup>93</sup> 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%.<sup>94</sup> 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).<sup>12</sup>

#### **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.<sup>8</sup> Alerts for skin sensitization by quaternary ammonium cations (haptens acting through 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.<sup>8,95</sup> Due to their chemical structures, it is unlikely that the chemicals in this group would bind to proteins in this manner.

### Phototoxicity

Details regarding the following phototoxicity assay may be found in Table 11. The combined toxic effect of UV radiation (0.17 J/cm<sup>2</sup>) and Benzalkonium Chloride (0.001 - 0.004%) was evaluated in cultured human corneal epithelial cells.<sup>96</sup> 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 administered with Benzalkonium Chloride at all concentrations.

### Ocular Irritation Studies

*Benzalkonium Chloride exhibited concentration-dependent ocular irritation in animal studies.*<sup>4,13</sup> 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  $\leq$  1.25%.<sup>2</sup> 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 12. 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 (tested at 2%) was determined to be moderately irritating in an EpiOcular™ assay.<sup>57</sup> Ocular irritation was observed at concentrations  $\geq$  0.01% Benzalkonium Chloride when evaluated in male rabbits (3/group).<sup>57</sup> A mixture containing 40% Stearalkonium Chloride was observed to be corrosive to the eyes in an assay performed in rabbits (n = 2).<sup>92</sup> Mild conjunctival hyperemia was observed in a Draize assay performed in rabbits (n = 7) treated with 0.002% Stearalkonium Chloride.<sup>8</sup>

### 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).*<sup>13</sup> 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).*<sup>13</sup> 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). Nasal 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.

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  $\mu$ l).<sup>97</sup> 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-1 $\alpha$  (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).<sup>98</sup>

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).<sup>99</sup> 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.<sup>75</sup> 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) to cause mucosal disruption was evaluated in an assay in which yearling female sheep (4/group) were treated rectally.<sup>100</sup> 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 hyporeflexive 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 treatment latanoprost or timolol.<sup>101</sup> 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.

### **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).<sup>13</sup> 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.<sup>102,103</sup> 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).<sup>104</sup> 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.<sup>13</sup> 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.<sup>8,105</sup> 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.<sup>106-122</sup>

### **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.<sup>13</sup> 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.<sup>123,124</sup> Other effects such as asthma, as well as stomach, respiratory, and ocular irritation have also been reported in the occupational setting.<sup>125,126</sup>

### **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.<sup>4,13</sup> 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 13).<sup>8,93,124,127-142</sup> 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.<sup>143,144</sup>

### **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).<sup>145</sup> 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).<sup>146</sup>

### **Benzalkonium Chloride Administration to Asthma Patients**

Airway response to Benzalkonium Chloride and histamine was evaluated in 12 asthmatic patients.<sup>13</sup> Airway caliber was measured before and during the inhalation challenge as the forced expiratory volume in 1 second (FEV<sub>1</sub>). 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<sub>1</sub> 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<sub>1</sub> decreased by 15% or more, or 3 doses were administered) were evaluated in 30 subjects with bronchial asthma (10 healthy patients used as control).<sup>147</sup> FEV<sub>1</sub> was measured at baseline and 15 min after each dose. FEV<sub>1</sub> reductions following inhalation of the test substance was significantly higher in asthmatics than normal subjects (p < 0.05; mean percent fall in FEV<sub>1</sub> 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<sub>1</sub> 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.<sup>148</sup>

## **RISK ASSESSMENT**

Margin of exposure (MOE) is a quantitative factor calculated for cosmetic ingredients by dividing the NOAEL 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 MOE 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)).<sup>149</sup> 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 treated with topical 1% Benzalkonium Chloride). 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.<sup>31</sup> 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.<sup>150</sup> 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).

### 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 2024 RLD and 2022 concentration of use data, Stearalkonium Chloride has the highest number and concentration of use (it is used in 885 formulations at up to 3.8%). Benzalkonium Chloride and Stearalkonium Chloride were reported to be used in 79 and 151 formulations, respectively, in their previous reviews. According to 2023 VCRP data, these ingredients were used in 69 and 88 formulations, respectively. The maximum concentration of use for Benzalkonium Chloride has remained unchanged since 2006 (up to 0.5%); however, the concentration of Stearalkonium Chloride has decreased since 2003 (previously reported to be used at up to 7%).

Benzalkonium Chloride homologues are metabolized by human liver enzymes via NADPH-dependent  $\omega$ - and ( $\omega-1$ ) 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. The absorbed dose was determined to be 0.05% in an assay in which Benzalkonium Chloride (80.5% active ingredient) was applied to human skin samples at 0.03%. 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  $\mu$ g/g/d) resulted in maximal blood and liver levels of 1000 and 550 nm, respectively.

Acute dermal LD<sub>50</sub> 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<sub>50</sub> was 234 mg/kg in rats given 10% Benzalkonium Chloride. Inhalation studies found a NOEL of < 0.049 mg/m<sup>3</sup> in female mice exposed to Benzalkonium Chloride and an LC<sub>50</sub> of 52.84 mg/m<sup>3</sup> in rats.

In repeated-dose toxicity studies, a NOAEL of 5000 mg/kg was determined in a 91-d gavage study in rats given a sanitizing agent containing 0.01% 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<sup>3</sup> Benzalkonium Chloride. A NOAEC of < 0.08 mg/m<sup>3</sup> was identified in a 14-d inhalation study in rats exposed to up to 20 mg/m<sup>3</sup> 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.

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 other assays. Benzalkonium Chloride tested negative in Ames assays up to 110 µg/plate and mammalian gene mutation assays at up to 100 µg/ml. Stearalkonium Chloride was negative in Ames assays up to 5000 µg/plate. Chromosomal aberration assays with Benzalkonium Chloride (24 – 98 µg/ml and 0.0003%) were negative, though micronucleus formation increased at 1 mg/l. In vivo rat studies with doses up to 20 ml/kg (using Benzalkonium Chloride at 0.01%) and 400 mg/kg. Negative results were obtained in in vivo assays performed using rats given either 0.01% Benzalkonium Chloride (up to 20 ml/kg) or 400 mg/kg Benzalkonium Chloride

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

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.

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 as low as 0.00000001%.

Adverse effects from eye cosmetics containing Benzalkonium Chloride occur after the product spreads across the ocular surface via diffusion and electric field-driven drift. Individuals with mucin deficiency may be at higher risk for toxicity following use of eye cosmetics containing Benzalkonium Chloride.

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.

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 enhance 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%.

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.

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.

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 ( $p < 0.01$ ).

Stearalkonium Chloride (undiluted) was non-irritating in a reconstructed human epidermis assay, 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. 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  $\geq 0.01\%$  caused ocular irritation in rabbits. Stearalkonium Chloride was moderately irritating at 2%, corrosive at 40%, and caused mild conjunctival hyperemia at 0.002% in rabbit studies.

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 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  $\mu\text{g}$  in the latanoprost solution did not raise keratitis risk compared to the 6.2  $\mu\text{g}$  in timolol solution-treated patients.

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

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

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

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

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

In a study of 30 asthmatic subjects, inhalation of nebulized Benzalkonium Chloride (600  $\mu\text{g}$  doses) caused significant reductions in FEV<sub>1</sub> 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  $\mu\text{g}$  per actuation) found no bronchoconstriction.

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

## **PREVIOUS DISCUSSION**

### **Discussion from Original Report on Benzalkonium Chloride Published in 1989**

*Skin irritation studies in humans involved patients and normal subjects. Patients were tested with Benzalkonium Chloride concentrations ranging from 0.1 to 10.0%, and normal subjects with concentrations of 0.1 to 17.0%. Skin irritation was noted in both populations after applications of Benzalkonium Chloride concentrations greater than 0.1%. Skin irritation and ocular irritation were usually noted in animals when Benzalkonium Chloride was tested at concentrations greater than 0.1%.*

*Skin sensitization was noted in patients tested with Benzalkonium Chloride concentrations ranging from 0.01 to 0.7%. However, there was no incidence of skin sensitization in a population of normal subjects tested with 0.1% Benzalkonium Chloride. Individuals with diseased skin may be at risk for sensitization to Benzalkonium Chloride.*

*The Expert Panel recognizes that some of the products tested contained concentrations of Benzalkonium Chloride greater than 0.1%. If these products contain proteins or other agents that bind Benzalkonium Chloride, Benzalkonium Chloride concentrations greater than 0.1% would have to be added to yield 0.1% free Benzalkonium Chloride. It is important to note that free Benzalkonium Chloride is effective as an antimicrobial agent, and, also, that the free agent induces dermal toxicity.*

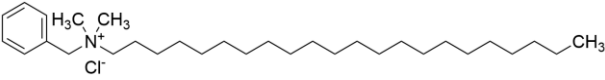
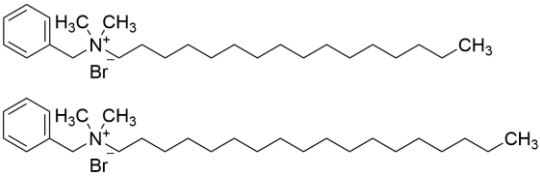
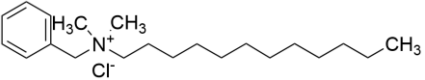
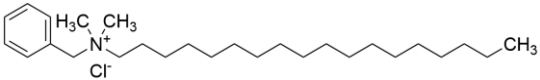
#### **DISCUSSION**

To be developed.

#### **CONCLUSION**

To be determined.

**TABLES****Table 1. Definitions, idealized structures, and reported functions<sup>1, CIR Staff</sup>**

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 alkylbenzyltrimethylammonium 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 alkylbenzyltrimethylammonium 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
<b>Behenalkonium Chloride</b>		
Formula Weight (g/mol)	480.2	151
Vapor pressure (mmHg @ 25°C)	0 (estimated; modified grain method)	152
Melting Point (°C)	284 (estimated)	152
Boiling Point (°C)	654 (estimated)	152
Water Solubility (mg/l @ 25°C)	0.0002 (estimated; WSKOW v1.42)	152
log K <sub>ow</sub> (@ 25°C)	7.74 (estimated; WSKOW v1.42)	152
<b>Benzalkonium Bromide</b>		
Formula Weight (g/mol; median (range))	440.0 (355.4 – 524.7)	153
<b>Benzalkonium Chloride</b>		
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

**Table 2. Chemical properties**

<b>Property</b>	<b>Value</b>	<b>Reference</b>
UV Absorption ( $\lambda$ ) (nm)	262	4
<b>Cetearalkonium Bromide</b>		
Formula Weight (g/mol; range)	440.6 – 468.6	153
<b>Lauralkonium Chloride</b>		
Physical Form	solid	11
Color	white	11
Formula Weight (g/mol)	340	154
Density (g/ml @ 20°C)	1.03	11
Vapor pressure (mmHg @ 20°C)	0	11
Melting Point (°C)	45.2	11
Boiling Point (°C)	162.7	11
Water Solubility (g/l)	65 - 175	11
log $K_{ow}$ (@ 24°C)	1.1	11
<b>Stearalkonium Chloride</b>		
Physical Form	solid	12
Color	white	12
Formula Weight (g/mol)	424.2	153
Odor	sweet	12
Specific Gravity (@ 20°C)	1.02	12
Melting Point (°C)	63.4	12
Water Solubility (mg/l @ 20°C)	20.8	12
log $K_{ow}$ (@ 20°C)	3.89 (estimated)	12

**Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category**

	BENZALKONIUM CHLORIDE					STEARALKONIUM CHLORIDE				
	# of Uses			Max Conc of Use		# of Uses			Max Conc of Use	
	RLD (2024) <sup>17</sup>	VCRP (2023) <sup>20</sup>	VCRP (2006) <sup>5</sup>	% (2023) <sup>19</sup>	% (2006) <sup>5</sup>	RLD (2024) <sup>17</sup>	VCRP (2023) <sup>17,20</sup>	VCRP (2001) <sup>3</sup>	% (2022) <sup>18</sup>	% (2001) <sup>3</sup>
<b>Totals*</b>	<b>564</b>	<b>69</b>	<b>79</b>	<b>0.001 – 0.5</b>	<b>0.01 – 0.5</b>	<b>885</b>	<b>88</b>	<b>151</b>	<b>0.0055 – 3.8</b>	<b>0.3 – 7</b>
<b>summarized by likely duration and exposure**</b>										
<b>Duration of Use</b>										
Leave-On	***	38	33	0.002 – 0.47	0.01 – 0.1	***	22	25	0.0055 – 3.8	0.3 – 3
Rinse-Off	***	31	46	0.001 – 0.5	0.01 – 0.5	***	66	121	0.25 – 3	0.4 – 7
Diluted for (Bath) Use	***	NR	NR	0.13	NR	***	NR	5	NR	NR
<b>Exposure Type</b>										
Eye Area	***	9	11	0.0066 – 0.01	0.01 – 0.1	***	NR	NR	NR	NR
Incidental Ingestion	***	NR	NR	0.3	0.03	***	NR	NR	NR	NR
Incidental Inhalation-Spray	***	14 <sup>a</sup> ; 5 <sup>b</sup>	1; 15 <sup>a</sup> ; 7 <sup>b</sup>	0.055 – 0.13; 0.035 – 0.3 <sup>a</sup>	0.02 – 0.1; 0.02 – 1 <sup>a</sup> ; 0.06 – 0.1 <sup>b</sup>	***	1; 12 <sup>a</sup> ; 2 <sup>b</sup>	3; 15 <sup>a</sup> ; 2 <sup>b</sup>	0.0055; 0.09 – 3.8 <sup>a</sup>	0.3 – 3 <sup>a</sup>
Incidental Inhalation-Powder	***	1; 5 <sup>b</sup>	7 <sup>b</sup> ; 2 <sup>c</sup>	0.01; 0.01 – 0.1 <sup>c</sup>	0.08 – 0.1; 0.06 – 0.1 <sup>b</sup> ; 0.03 – 0.1 <sup>c</sup>	***	2 <sup>b</sup>	2 <sup>b</sup>	NR	NR
Dermal Contact	***	67	55	0.002 – 0.25	0.01 – 0.5	***	3	10	NR	0.3
Deodorant (underarm)	***	NR	1 <sup>a</sup>	0.25	0.1	***	NR	NR	NR	NR
Hair - Non-Coloring	***	2	21	0.002 – 0.5	0.02 – 0.1	***	79	140	0.0055 – 3.8	0.7 - 7
Hair-Coloring	***	NR	1	0.001	0.02	***	6	NR	0.38 – 1.3	0.4 – 2
Nail	***	NR	2	0.1	0.01 – 0.1	***	NR	1	NR	NR
Mucous Membrane	***	20	1	0.098 – 0.3	0.03 – 0.5	***	NR	NR	NR	NR
Baby Products	***	3	4	NR	0.03 – 0.1	***	NR	NR	NR	NR
<b>as reported by product category</b>										
<b>Baby Products</b>	<b>26</b>									
Baby Lotions/Oils/Powders/Creams	2	NR	2	NR	0.03 – 0.1					
Baby Wipes	23	NA	NA	NR	NA		NA	NA		NA
Other Baby Products	1 (r.o.)	3	2	NR	0.03					
<b>Bath Preparations</b>	<b>1</b>					<b>3</b>				
Bubble Baths	1	NR	NR	NR	NR	1	NR	5	NR	NR
Other Bath Preparations	NR	NR	NR	0.13	NR	2	NR	NR	NR	NR
<b>Eye Makeup Preparations (not children's)</b>	<b>50</b>									
Eyebrow Pencil	3	NR	NR	NR	0.02					
Eyeliner	NR	NR	NR	NR	0.02					
Eye Shadow	NR	NR	NR	NR	0.02					
Eye Lotion	NR	1	1	NR	0.02					
Eye Makeup Remover	22	1	10	NR	0.01 – 0.05					
Mascara	2	NR	NR	NR	0.02 – 0.1					
Eyelash and Eyebrow Adhesives, Glues, and Sealants	12	NA	NA	NR	NA		NA	NA		NA
Eyelash and Eyebrow Preparations (primers, conditioners, serums, fortifiers)	9	NA	NA	NR	NA		NA	NA		NA
Other Eye Makeup Preparations	2	7	NR	0.0066 – 0.01	0.02					
<b>Fragrance Preparations</b>	<b>1</b>									
Cologne and Toilet Water	NR	NR	1	NR	0.1					
Perfumes	1	NR	NR	NR	0.1					

**Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category**

	BENZALKONIUM CHLORIDE					STEARALKONIUM CHLORIDE				
	# of Uses			Max Conc of Use		# of Uses			Max Conc of Use	
	RLD (2024) <sup>17</sup>	VCRP (2023) <sup>20</sup>	VCRP (2006) <sup>5</sup>	% (2023) <sup>19</sup>	% (2006) <sup>5</sup>	RLD (2024) <sup>17</sup>	VCRP (2023) <sup>17,20</sup>	VCRP (2001) <sup>3</sup>	% (2022) <sup>18</sup>	% (2001) <sup>3</sup>
Powders (dusting/talcum, excl aftershave talc)	NR	NR	NR	NR	0.08 – 0.1					
Sachets	NR	NR	NR	NR	0.1					
Other Fragrance Preparation	1	NR	NR	NR	0.1					
<b><i>Hair Preparations (non-coloring)</i></b>	<b>177</b>					<b>597</b>				
Hair Conditioners	23 (l.o.); 36 (r.o.)	NR	12	0.005 – 0.007	0.05	45 (l.o.); 381 (r.o.)	55	107	0.25 - 3	0.7 – 7
Hair Sprays (aerosol fixatives)	8	NR	NR	NR	NR	1	1	3	0.0055	NR
Hair Straighteners	NR	NR	NR	NR	0.1	7	1	NR	NR	NR
Permanent Waves	4	NR	NR	NR	0.1	NR	NR	2	NR	NR
Rinses (non-coloring)	4	NR	2	NR	0.1	40	2	5	NR	3
Shampoos (non-coloring)	4 (l.o.); 35 (r.o.)	NR	NR	0.2 – 0.5	0.1	1 (l.o.); 19 (r.o.)	2	4	1.5	2
Tonics, Dressings, and Other Hair Grooming Aids	39	1	7	0.035 – 0.05	0.02 – 0.05	30	12	14	0.09 – 3.8	2 – 3
Wave Sets						3	NR	2	NR	NR
Other Hair Preparations	48 (l.o.); 10 (r.o.)	1	NR	0.002 – 0.47	NR	48 (l.o.); 65 (r.o.)	6	3	NR	2
<b><i>Hair Coloring Preparations</i></b>	<b>3</b>					<b>247</b>				
Hair Dyes and Colors (all types requiring caution statements and patch tests)	1	NR	NR	NR	0.02	153	1	NR	NR	0.5 – 2
Hair Tints	NR	NR	NR	NR	0.02	68	NR	NR	NR	NR
Hair Rinses (coloring)	1 (r.o.)	NR	NR	NR	0.02	1 (l.o.); 53 (r.o.)	NR	NR	1.3	NR
Hair Shampoos (coloring)	2 (r.o.)	NR	NR	0.001	NR	3 (r.o.)	4	NR	NR	NR
Hair Color Sprays (aerosol)	NR	NR	NR	NR	0.02					
Hair Lighteners with Color	NR	NR	NR	NR	0.02	3	NR	NR	NR	NR
Hair Bleaches	NR	NR	1	NR	0.02	NR	NR	NR	0.38	0.4
Other Hair Coloring Preparation	1	NR	NR	NR	NR	2 (l.o.); 4 (r.o.)	1	NR	NR	NR
<b><i>Makeup Preparations (not eye; not children's)</i></b>	<b>2</b>									
Blushers and Rouges (all types)	NR	NR	NR	NR	0.1					
Face Powders	1	1	NR	0.01	0.1					
Foundations	NR	NR	NR	0.002	0.1					
Makeup Bases	NR	NR	NR	NR	0.1					
Makeup Fixatives	NR	NR	NR	NR	0.1					
Other Makeup Preparations	1 (l.o.)	3	NR	0.01	0.1					
<b><i>Manicuring Preparations</i></b>	<b>1</b>									
Cuticle Softeners	1	NR	NR	0.1	0.1					
Nail Creams and Lotions	NR	NR	1	NR	NR					
Nail Extenders										
Nail Polish and Enamel						NR	NR	1	NR	NR
Other Manicuring Preparations	NR	NR	1	NR	0.01					
<b><i>Oral Products</i></b>										
Mouthwashes and Breath Fresheners	NR	NR	NR	0.3	0.03					
<b><i>Personal Cleanliness</i></b>	<b>173</b>					<b>4</b>				
Bath Soaps and Body Washes	20	3	NR	0.098 – 0.14	0.1	4	NR	NR	NR	NR
Deodorants (underarm)	1	NR	1	0.25 (not spray)	0.1					

**Table 3. Frequency (RLD/VCRP) and concentration of use according to likely duration and exposure and by product category**

	BENZALKONIUM CHLORIDE					STEARALKONIUM CHLORIDE				
	# of Uses			Max Conc of Use		# of Uses			Max Conc of Use	
	RLD (2024) <sup>17</sup>	VCRP (2023) <sup>20</sup>	VCRP (2006) <sup>5</sup>	% (2023) <sup>19</sup>	% (2006) <sup>5</sup>	RLD (2024) <sup>17</sup>	VCRP (2023) <sup>17,20</sup>	VCRP (2001) <sup>3</sup>	% (2022) <sup>18</sup>	% (2001) <sup>3</sup>
Douches	1	NR	NR	NR	0.1					
Feminine Deodorants	2 (r.o.)	NR	1	NR	0.1					
Disposable Wipes	117	NA	NA	NR	NA		NA	NA		NA
Other Personal Cleanliness Products	20 (l.o.); 20 (r.o.)	16	1	NR	0.1 – 0.5	1 (r.o.)	NR	1	NR	NR
<b>Shaving Preparations</b>	<b>1</b>					<b>8</b>				
Aftershave Lotions	NR	NR	NR	NR	0.1					
Beard Softeners						1	NR	NR	NR	NR
Pre-shave Lotions (all types)	1	NR	NR	NR	NR					
Shaving Creams (aerosol, brushless, lather)	NR	NR	NR	NR	0.1	7	NR	NR	NR	NR
Shaving Soaps (cakes, sticks, etc.)	NR	NR	NR	NR	0.1					
<b>Skin Care Preparations</b>	<b>88</b>					<b>29</b>				
Cleansing	28	10	17	0.13	0.05 – 0.1	8	NR	NR	NR	NR
Depilatories	NR	NR	NR	NR	0.1					
Face and Neck (excluding shaving preparations)	13 (l.o.); 6 (r.o.)	1	3	NR	0.06 – 0.1	2 (l.o.)	NR	NR	NR	NR
Body and Hand (excluding shaving preparations)	15 (l.o.); 1 (r.o.)	3	3	0.055 – 0.13 (spray); 0.01 – 0.1	0.06 – 0.1	7 (l.o.); 2 (r.o.)	2	2	NR	NR
Foot Powders and Sprays	2	NR	1	NR	0.08 – 0.1					
Moisturizing	22	12	1	0.13 (not spray)	0.1	18	NR	1	NR	0.3
Night	NR	NR	1	NR	0.1					
Paste Masks (mud packs)	NR	1	3	NR	0.1					
Skin Fresheners	4	1	5	NR	0.1					
Other Skin Care Preparations	6 (l.o.); 7 (r.o.)	3	2	0.002	0.1	1 (l.o.); 1 (r.o.)	1	1	NR	NR
<b>Suntan Preparations</b>	<b>4</b>									
Suntan Gels, Creams, and Liquids	NR	NR	1	NR	NR					
Indoor Tanning Preparations	4	NR	NR	NR	NR					
<b>Other Preparations (i.e., those preparations that do not fit another category)</b>	<b>71</b>	NA	NA		NA	<b>4</b>	NA	NA		NA

NA – not applicable (this category was not part of the VCRP); NR – not reported

l.o. – leave-on; r.o. – rinse-off

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

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

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

<sup>a</sup> It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

<sup>b</sup> 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

<sup>c</sup> It is possible these products are powders, but it is not specified whether the reported uses are powders.





**Table 4. Frequency and concentration of use according to likely duration and exposure and by product category**

	BEHENALKONIUM CHLORIDE		BENZALKONIUM BROMIDE		CETEARALKONIUM BROMIDE		LAURALKONIUM CHLORIDE	
	# of Uses	Max Conc of Use	# of Uses	Max Conc of Use	# of Uses	Max Conc of Use	# of Uses	Max Conc of Use
	RLD (2024) <sup>17</sup>	% (2023) <sup>19</sup>	RLD (2024) <sup>17</sup>	% (2023) <sup>19</sup>	RLD (2024) <sup>17</sup>	% (2023) <sup>19</sup>	RLD (2024) <sup>17</sup>	% (2023) <sup>19</sup>
Face and Neck (excluding shaving preps)								
Body and Hand (excluding shaving preps)								
Foot Powders and Sprays								
Moisturizing							1	NR
Night			1	NR				
Paste Masks (mud packs)								
Skin Fresheners			1	NR				
Other Skin Care Preparations								
<b>Suntan Preparations</b>								
Suntan Gels, Creams, and Liquids								
Indoor Tanning Preparations								
<b>Other Preparations (i.e., those that do not fit another category)</b>								

NR – not reported

l.o. – leave-on; r.o. – rinse-off

\*The total FOU provided for RLD refers to the ingredient count supplied by FDA, and is not a summation of the number of uses per category because each product may be categorized under multiple *product* categories.

For data supplied by the Council survey, the sum of all exposure types may not equal the sum of total uses because each ingredient may be used in cosmetics with multiple *exposure* types.

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

\*\*\* RLD are presented below by product category (as supplied by FDA), but are not summarized by likely duration and exposure

<sup>a</sup> It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

<sup>b</sup> It is possible these products are powders, but it is not specified whether the reported uses are powders.

<sup>c</sup> 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

Table 5. CFR citations

CFR Citation	Regulation Information	Ingredient(s)
21CFR172.165	These ingredients may be used safely as a food additive under the following conditions: <ol style="list-style-type: none"> <li>1) pH (5% active solution) 7.0 – 8.0; total amines, maximum 1% as combined free amines and amine hydrochlorides</li> <li>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</li> <li>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</li> </ol>	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: <ol style="list-style-type: none"> <li>1) they are applied to the sugar mill grinding, crusher, and/or diffuser systems</li> <li>2) used at <math>0.05 \pm 0.005</math> ppm for Lauralkonium Chloride and <math>0.30 \pm 0.030</math> for Stearalkonium Chloride</li> <li>3) may adhere to proper labeling</li> </ol>	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 6. Dermal absorption/penetration, penetration enhancement, and ADME studies**

Test Article	Vehicle	Test Population	Concentration/Dose	Protocol	Results	Reference
<b>DERMAL ABSORPTION/PENETRATION</b>						
Benzalkonium Chloride (C <sub>12</sub> – C <sub>14</sub> )	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.	30
radiolabeled and non-radiolabeled Benzalkonium Chloride (80.5% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	water	full-thickness human skin samples	0.03 and 0.3%	OECD TG 428; automated flow-through diffusion cell system; 24 h diffusion	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.	31
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 <sub>12</sub> and C <sub>14</sub> homologues of Benzalkonium Chloride	Among the 32 subjects, the C <sub>12</sub> 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 <sub>14</sub> 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.	32
<b>PENETRATION ENHANCEMENT</b>						
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 <sup>2</sup> ; 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 <sup>2</sup> /h, respectively. Control samples were not treated with Benzalkonium Chloride and yielded a steady-state flux of 0.12 µg/cm <sup>2</sup> /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.	33

ADME						
Dermal						
radiolabeled and non-radiolabeled Benzalkonium Chloride (49.9% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	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 <sub>12</sub> – C <sub>16</sub> )	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
radiolabeled and non-radiolabeled Benzalkonium Chloride (49.9% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	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	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	12

				intervals from 0 – 168 h after administration	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.	
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 <sub>6</sub> , C <sub>18</sub> , C <sub>10</sub> , and C <sub>12</sub> ) were quantified in the blood, liver, and feces (C <sub>10</sub> observed in highest amounts; maximum amount reported in male and female feces; approximately 475 µM).	34

EPA = Environmental Protection Agency; 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 7. Acute toxicity studies**

Test Article	Vehicle	Animals/Group	Concentration/Dose	Protocol	LD <sub>50</sub> /LC <sub>50</sub> /Results	Reference
<b>DERMAL</b>						
Benzalkonium Chloride (50 – 80% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	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 <sub>50</sub> = 3.56 ml/kg bw (after correcting for 100% active test substance, the LD <sub>50</sub> was calculated to be 2730 mg/kg bw)	11
Benzalkonium Chloride	NR	NR	82.26%	NR	LD <sub>50</sub> = 930 mg/kg bw	8
<b>ORAL</b>						
Benzalkonium Chloride (50 – 80% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	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 <sub>50</sub> = 0.43 ml/kg bw (after correcting for 100% active test substance, the LD <sub>50</sub> 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 <sub>50</sub> = 234 - 525 mg/kg	35
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 <sub>50</sub> = 241.7 mg/kg	36
Benzalkonium Chloride (50% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	water	Sprague-Dawley rats (5/sex/group)	500, 794, 1260, and 2000 mg/kg bw	OECD TG 401; gavage administration; 14-d observations	LD <sub>50</sub> = 795 mg/kg bw	12

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**INHALATION**


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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 <sup>3</sup>	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 <sup>3</sup>  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 <sup>3</sup> and below.	37
aerosolized Benzalkonium Chloride (95% purity)	air	female Wistar rats (5/group)	37.64 and 52.84 mg/m <sup>3</sup>	head/nose inhalation; 4 h single exposure; 14-d observation	LC <sub>50</sub> = 52.84 mg/m <sup>3</sup> ; out of the 5 rats, 2 died within the first 24 h after exposure to 52.84 mg/m <sup>3</sup>	8,38

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EPA = Environmental Protection Agency; LC<sub>50</sub> = medial lethal concentration; LD<sub>50</sub> = 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 8. Repeated dose toxicity studies**

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
<b>ORAL</b>							
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	OECD TG 408; once daily gavage administration; controls given water	NOAEL = 5000 mg/kg  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.	39
Benzalkonium Chloride (79.7 - 80.5% active ingredient; C <sub>12</sub> - C <sub>16</sub> )	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

**Table 8. Repeated dose toxicity studies**

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
Benzalkonium Chloride (50% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	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
<b>INHALATION</b>							
aerosolized Benzalkonium Chloride (95% purity)	air	female Wistar rats (5/group)	3 d	30 mg/m <sup>3</sup>	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.	38
aerosolized Benzalkonium Chloride (95% purity)	air	female Wistar rats (7 – 10/group)	5 d	0 and 35 mg/m <sup>3</sup>	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.	40

**Table 8. Repeated dose toxicity studies**

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
aerosolized Benzalkonium Chloride	air	Fischer 344 rats (5/sex/dose)	14 d	0.8, 4, 20 mg/m <sup>3</sup>	6 h/d exposure; MMAD of 1.1 – 1.61 µm; no other details regarding treatment provided	NOAEC = < 0.8 mg/m <sup>3</sup>  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.	41

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 9. Developmental and reproductive toxicity studies**

Test Article	Vehicle	Test Population	Dose/Concentration	Procedure	Results	Reference
<b>IN VITRO</b>						
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).	42

**Table 9. Developmental and reproductive toxicity studies**

Test Article	Vehicle	Test Population	Dose/Concentration	Procedure	Results	Reference
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).	43
<b>ANIMAL</b>						
Benzalkonium Chloride (81.09% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	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.	44

**Table 9. Developmental and reproductive toxicity studies**

Test Article	Vehicle	Test Population	Dose/Concentration	Procedure	Results	Reference
Benzalkonium Chloride (81.09% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	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 <sub>0</sub> , F <sub>1</sub> , and F <sub>2</sub> 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
Benzalkonium Chloride (49.9% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	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

**Table 9. Developmental and reproductive toxicity studies**

Test Article	Vehicle	Test Population	Dose/Concentration	Procedure	Results	Reference
Benzalkonium Chloride (81.09% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	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.	44
Benzalkonium Chloride (49.9% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	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.	8,45

EPA = Environmental Protection Agency; GD = gestation days; LD<sub>50</sub> = 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 10. Genotoxicity studies**

Test Article	Vehicle	Concentration/Dose	Test System	Protocol	Results	Reference
<b>IN VITRO</b>						
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 0.001% resulted in 2.56-, 2.74-, 2.95-, and 3.24-fold increases in single-strand breaks compared to the control ( $p < 0.001$ ).	46
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 ( $p < 0.01$ ).	47
Benzalkonium Chloride (C <sub>12</sub> – C <sub>16</sub> )	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 moment 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 moments compared to negative controls ( $p < 0.001$ ). This effect was not observed at 0.05%.	48
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	49
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	50
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	<i>S. typhimurium</i> TA1535, TA 1537, TA98, TA100 and <i>E. coli</i> WP2uvrA	OECD TG 471; Ames assay performed with and without metabolic activation; appropriate positive and negative controls used	non-mutagenic; controls gave expected results	39
Benzalkonium Chloride (50 – 80% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	water	0, 0.15, 0.5, 1.5, 5, 15, and 50 µg/plate	<i>S. typhimurium</i> 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	<i>S. typhimurium</i> TA98, TA100, and TA102	<i>Salmonella</i> /microsome assay; 48 h incubation; number of his <sup>+</sup> colonies manually counted; performed with and without metabolic activation; appropriate positive and negative controls used	non-mutagenic; controls gave expected results	49
Benzalkonium Chloride (C <sub>12</sub> – C <sub>16</sub> )	NR	without metabolic activation: 0, 5, 10, 12.5, 15, 17.5, and 20 n/ml  with metabolic activation: 0, 15, 30, 45, 60, 75, and 90 n/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 <sub>12</sub> – C <sub>16</sub> )		experiment 1 without metabolic activation: 0, 1, 5, 10, 13, 16, 20, 25, 35, 50, and 65 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

**Table 10. Genotoxicity studies**

Test Article	Vehicle	Concentration/Dose	Test System	Protocol	Results	Reference
		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				
Benzalkonium Chloride (50 – 80% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	cell culture medium	experiment 1: 0, 4, 8, 16, 20 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
		experiment 2: 0, 4, 8, 12, 16, 24 µg/ml				
Benzalkonium Chloride (99.24% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	NR	without metabolic activation: 0, 0.36, 1.09, 3.27, and 9.8 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
		with metabolic activation; 0, 6.1, 3, 12.25, 24.5, 49, and 98 µg/ml				
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	52
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 ≤ 0.05); nuclear division indices were similar to control at all test concentrations; controls gave expected results	49
Stealkonium 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	<i>S. typhimurium</i> TA98, TA100, TA1535, and TA1537 and <i>E. coli</i> 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
Stealkonium Chloride (93.9% purity)	NR	0, 1, 3, 10, 33, 100, 166, 333, 666, 1000, 1666, 3333 µg/plate	<i>S. typhimurium</i> 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	51
<b>IN VIVO</b>						
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	39

**Table 10. Genotoxicity studies**

Test Article	Vehicle	Concentration/Dose	Test System	Protocol	Results	Reference
Benzalkonium Chloride (C <sub>12</sub> – C <sub>16</sub> )	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 Cooperation and Development; OPPTS = Office of Prevention, Pesticides, and Toxic Substances; PBS = phosphate-buffered saline; SDS = sodium dodecyl sulfate; TG = test guidelines

**Table 11. Dermal irritation, sensitization, and phototoxicity studies**

Test Article	Vehicle	Concentration/Dose	Test Population/System	Protocol	Results	Reference
<b>IRRITATION</b>						
<b>IN VITRO</b>						
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
<b>ANIMAL</b>						
Benzalkonium Chloride (50 – 80% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	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)	92
<b>HUMAN</b>						
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	93
<b>SENSITIZATION</b>						
<b>ANIMAL</b>						
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%	94
Benzalkonium Chloride (50% active ingredient; C <sub>12</sub> – C <sub>16</sub> )	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

ATP = adenosine triphosphate; 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 12. Ocular irritation studies**

Test Article	Vehicle	Concentration/Dose	Test Population	Protocol	Results	Reference
<b>IN VITRO</b>						
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 ( $\leq 60\%$ ), 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 was 2%)	EpiOcular™ tissues (n = 2)	EpiOcular™ assay and MTT assay; up to 30 min incubation	moderately irritating ( $ET_{50} = 28.3\%$ )	155
<b>ANIMAL</b>						
Benzalkonium Chloride (C <sub>12</sub> – C <sub>16</sub> )	saline	0.001, 0.003, 0.005, 0.01, and 0.03; 50 $\mu$ l	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 $\leq 0.005\%$ ; irritation observed at concentrations $\geq 0.01\%$ , with severity increasing in a dose-dependent manner.	57
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	92
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
<b>PHOTOTOXICITY</b>						
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 <sup>2</sup> ) 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.	96

$ET_{50}$  = 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 Cooperation and Development; SDS = sodium dodecyl sulfate; TG = test guideline

**Table 13. Patch testing in patients using Benzalkonium Chloride**

Patch Test Concentration	Number of Patients/Patient Type	Number of Positive Reactions (% of total)	Reference
0.15%	10 patients (8 patients with history of rash or positive patch test to Benzalkonium Chloride; 2 patients receiving routine patch testing)	2 patients (20%)	127
0.1%	30 patients using inhaled or intranasal corticoid steroids for allergic conditions	2 patients (6.7%)	128
0.1%	91 dermatitis patients	5 patients (5.5%)	129
0.1%	131 cheilitis patients	17 patients (13%)	130
0.1%	149 dermatitis patients	5 patients (3.4%)	131
0.1%	215 periorbital dermatitis patients	10 patients (4.7%)	132
0.1%	429 dermatitis patients (evaluating for late delayed positive reactions)	2 patients (0.5%)	133
0.1%	584 dermatitis patients	71 patients (12.2%)	93
0.1%	615 dermatitis patients	198 patients (32.2%)	124
0.1%	1389 dermatitis patients	48 patients (3.5%)	134
0.1%	2295 dermatitis patients	126 patients (5.5%)	8
0.1%	2546 dermatitis patients	176 patients (6.9%)	135
NR	2611 dermatitis patients	47 patients (1.8%)	136
0.1%	2694 dermatitis patients	164 patients (6.1%)	137
0.1%	3043 dermatitis patients	37 patients (1.2%)	138
0.1%	4116 dermatitis patients	21 patients (0.5%)	139
0.1%	4892 dermatitis patients	210 people (4.3%)	140
0.1%	7390 dermatitis patients	108 patients (1.5%)	141
0.1%	42,898 dermatitis patients	361 patients (0.8%)	142

NR = not reported

## REFERENCES

1. Nikitakis J, Kowcz A. 2025. wINCI: *International Cosmetic Ingredient Dictionary and Handbook*. <https://incipedia.personalcarecouncil.org/winci/>. Date Accessed: July 30, 2025.
2. Andersen FA (ed). Final report on the safety assessment of stealkonium 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. Benzyltrimethyl(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](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-%202030%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](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. Benzyltrimethyl(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=stealkonium%20chloride](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=stealkonium%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. Santos M, Li M, Rustum AM. A single RP-LC method for the determination of benzalkonium chloride and its potential impurities in benzalkonium chloride raw material. *Chroma*. 2010;71(5):499–503.
16. United States Food and Drug Administration. Federal Food, Drug, and Cosmetic Act Section 612 Title 21.
17. U.S. Food and Drug Administration Office of the Chief Scientist. 2024. Registration and Listing Data - Frequency of Use of Cosmetic Products. [Obtained under the Freedom of Information Act from the Division of Freedom of Information; requested as "Frequency of Use Data" July 17, 2024; received July 30, 2024].

18. Personal Care Products Council. 2022. Concentration of use by FDA product category: Stearalkonium Chloride. [Unpublished data submitted by Personal Care Products Council on October 31, 2022; ID: 1].
19. Personal Care Products Council. 2023. Concentration of use by FDA product category - alkonium chlorides and bromides. [Unpublished data submitted by Personal Care products Council on July 5, 2023].
20. US Food and Drug Administration Center for Food Safety & Applied Nutrition. 2023. Voluntary Cosmetic Registration Program - Frequency of Use of Cosmetic Ingredients. College Park, MD [Obtained under the Freedom of Information Act from CFSSAN; requested as "Frequency of Use Data" 2023; received February 2, 2023].
21. EUR-Lex. 2025. Access to European Union Law. <https://eur-lex.europa.eu/homepage.html>. Date Accessed: April 21, 2025.
22. 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.
23. 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.
24. 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.
25. 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.
26. 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.
27. 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.
28. 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.
29. 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.
30. Morse CN, Hite CC, Wamer NC, Gadiant JN, Baki G, Prestwich EG. MALDI-TOF imaging analysis of benzalkonium chloride penetration in ex vivo human skin. *PLoS One*. 2024;19(2):e0297992.
31. 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](https://health.ec.europa.eu/system/files/2016-11/scc_o_9_0.pdf). Date Accessed: July 28, 2025.
32. 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.
33. 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.
34. 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.
35. 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.

36. 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.
37. Larsen ST, Verder H, Nielsen GD. Airway effects of inhaled quaternary ammonium compounds in mice. *Basic Clin Pharmacol Toxicol.* 2012;110(6):537–543.
38. 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.
39. Dolan LC, Wheeler JA, Burdock GA. Safety studies conducted on a sanitizing agent containing benzalkonium chloride. *J Food Sci.* 2013;78(1):119.
40. 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.
41. 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.
42. 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.
43. 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.
44. 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.
45. 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.
46. 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.
47. 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.
48. Deutsche 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.
49. Ferik F, Misik 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.
50. 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.
51. 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.
52. 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.
53. Sarkar J, Chaudhary S, Namavari A, et al. Corneal neurotoxicity due to topical benzalkonium chloride. *Invest Ophthalmol Vis Sci.* 2012;53(4):1792–1802.

54. 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.
55. 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.
56. 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.
57. Okahara A, Kawazu K. Local toxicity of benzalkonium chloride in ophthalmic solutions following repeated applications. *J Toxicol Sci.* 2013;38(4):531–537.
58. 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.
59. Okahara A, Kawazu K. Local toxicity of benzalkonium chloride in ophthalmic solutions following repeated applications. *J Toxicol Sci.* 2013;38(4):531–537.
60. Zhang Z, Huang Y, Xie H, et al. Benzalkonium chloride suppresses rabbit corneal endothelium intercellular gap junction communication. *PLoS One.* 2014;9(10):e109708.
61. 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.
62. 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.
63. 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.
64. 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.
65. 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.
66. 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.
67. 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.
68. 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.
69. 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.
70. 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.
71. 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.

72. 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.
73. 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.
74. 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.
75. Fichorova RN, Bajpai M, Chandra N, et al. Interleukin (IL)-1, IL-6, and IL-8 predict mucosal toxicity of vaginal microbicide contraceptives. *Biol Reprod.* 2004;71(3):761–769.
76. Malik A, Claoué C. Transport and interaction of cosmetic product material within the ocular surface: Beauty and the beastly symptoms of toxic tears. *Cont Lens Anterior Eye.* 2012;35(6):247–259.
77. 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.
78. Rasmussen CA, Kaufman PL, Kiland JA. Benzalkonium chloride and glaucoma. *J Ocul Pharmacol Ther.* 2014;30(2-3):163–169.
79. 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.
80. 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.
81. Moghadam SH, Saliq 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.
82. 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.
83. 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.
84. 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.
85. 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.
86. 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.
87. 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.
88. 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.
89. 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.

90. 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.
91. 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.
92. Rohm and Haas. 1992Initial 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, 20252025.
93. 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.
94. 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.
95. Osimitz TG, Droege W. Quaternary ammonium compounds: Perspectives on benefits, hazards, and risk. *Toxicology Research and Application.* 2021;5:23978473211049085.
96. Xu M, Sivak JG, McCanna DJ. Ocular toxicology: Synergism of UV radiation and benzalkonium chloride. *Cutan Ocul Toxicol.* 2020;39(4):370–379.
97. 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.
98. 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.
99. 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.
100. 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.
101. 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.
102. Goldstein MH, Silva FQ, Blender N, Tran T, Vantipalli S. Ocular benzalkonium chloride exposure: Problems and solutions. *Eye (Lond).* 2022;36(2):361–368.
103. 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.
104. 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.
105. 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.
106. 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.
107. 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.

108. 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.
109. 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.
110. 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.
111. 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.
112. Mezger E, Wendler O, Mayr S, Bozzato A. Anaphylactic reaction following administration of nose drops containing benzalkonium chloride. *Head Face Med*. 2012;8:29.
113. Anderson D, Faltay B, Haller NA. Anaphylaxis with use of eye-drops containing benzalkonium chloride preservative. *Clin Exp Optom*. 2009;92(5):444–446.
114. 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.
115. Li X, Gao T, Zhi L. Granular parakeratosis induced by benzalkonium chloride masquerading as allergic contact dermatitis after circumcision. *Dermatitis*. 2025.
116. Galadari A, Darrigade A, Boralevi F, Milpied B. A child polysensitized to all constituents of biseptine. *Contact Dermatitis*. 2020;82(1):65–66.
117. 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.
118. 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.
119. 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.
120. 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.
121. 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.
122. 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.
123. Suneja T, Belsito DV. Occupational dermatoses in health care workers evaluated for suspected allergic contact dermatitis. *Contact Dermatitis*. 2008;58(5):285–290.
124. Isaac J, Scheinman PL. Benzalkonium chloride: An irritant and sensitizer. *Dermatitis*. 2017;28(6):346–352.
125. 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.
126. 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).

127. Benjamin B, Chris F, Salvador G, Melissa G, Susan N. Visual and confocal microscopic interpretation of patch tests to benzethonium chloride and benzalkonium chloride. *Skin Res Technol*. 2012;18(3):272–277.
128. 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.
129. Wang KL, Rainosek EM, Yang YW, et al. Pediatric patch testing at mayo clinic between 2016 and 2020. *Dermatitis*. 2024;35(4):355–360.
130. Kanokkrungsee 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.
131. 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.
132. 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.
133. 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.
134. 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.
135. 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.
136. Kadivar S, Belsito DV. Occupational dermatitis in health care workers evaluated for suspected allergic contact dermatitis. *Dermatitis*. 2015;26(4):177–183.
137. 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.
138. Houle M, DeKoven JG, Atwater AR, Reeder MJ, Warshaw EM. North american contact dermatitis group patch test results: 2021–2022. *Dermatitis*. 2025.
139. DeKoven JG, Warshaw EM, Reeder MJ, et al. North american contact dermatitis group patch test results: 2019-2020. *Dermatitis*. 2023;34(2):90–104.
140. 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.
141. 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.
142. 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.
143. 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.
144. Rundle CW, Hu S, Presley CL, Dunnick CA. Triclosen and its alternatives in antibacterial soaps. *Dermatitis*. 2019;30(6):352–357.
145. Dao H, Fricker C, Nedorost ST. Sensitization prevalence for benzalkonium chloride and benzethonium chloride. *Dermatitis*. 2012;23(4):162–166.

146. 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.
147. Lee BH, Kim S. Benzalkonium chloride induced bronchoconstriction in patients with stable bronchial asthma. *Korean J Intern Med*. 2007;22(4):244–248.
148. 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.
149. 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.
150. 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.
151. PubChem. 2025. Behenalkonium Chloride. <https://pubchem.ncbi.nlm.nih.gov/compound/Behenalkonium-chloride#section=Other-Identifiers>. Date Accessed: July 30, 2025.
152. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11, Washington, DC. 2012. United States Environmental Protection Agency..
153. PerkinElmer Informatics. 2026. ChemDraw Professional (Software)..
154. PubChem. 2025. Benzododecinium Chloride. <https://pubchem.ncbi.nlm.nih.gov/compound/8753>. Date Accessed: July 30, 2025.
155. 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.

## 4

# Final Report on the Safety Assessment of Stearalkonium Chloride

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

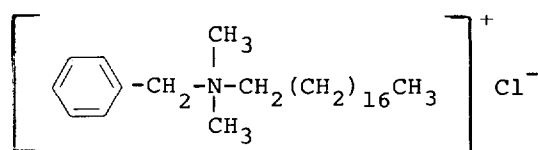
Studies have failed to establish with certainty the oral LD50 in rats of Stearalkonium Chloride, the value falling between 0.5 and 1.25 g/kg. In mice, an LD50 value of 0.760–0.113 g/kg was reported in a seven-day oral study. Single application dermal studies with concentrations of up to 25% have shown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

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

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

## CHEMICAL PROPERTIES

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



The respective structures of Cetalkonium and Myristalkonium Chlorides, two compounds closely related to Stearalkonium Chloride, are the same as above, except that the stearyl ( $-\text{CH}_2(\text{CH}_2)_{16}\text{CH}_3$ ) moiety is replaced by cetyl ( $-\text{CH}_2(\text{CH}_2)_{14}\text{CH}_3$ ) or myristyl ( $-\text{CH}_2(\text{CH}_2)_{12}\text{CH}_3$ ) moieties. The safety of Cetalkonium and Myristalkonium Chlorides is not under review in this report. Information and data pertaining to these two compounds have been included to permit a more complete appraisal of the safety of Stearalkonium Chloride. The three alkonium compounds are prepared by the quaternization of the appropriate alkyldimethylamine (stearyl, cetyl, or myristyl) with benzyl chloride. Each is a free flowing powder normally sold as a dispersion in isopropyl alcohol and/or water.<sup>(3)</sup>

### Physical Properties

The melting points of a homologous series of this class of compounds decrease sharply from chain lengths of  $\text{C}_8$  to  $\text{C}_{11}$  and gradually increase with longer alkyl groups. Those with chain lengths of  $\text{C}_8$  to  $\text{C}_{13}$  are soluble in water. The odd-numbered compounds are more soluble in 95% ethanol than even-numbered ones.<sup>(3)</sup> The pH ranges for 1% and 10% aqueous solutions of Stearalkonium Chloride are 3.5–6.5 and 3–4, respectively.<sup>(4,5)</sup> The ability to lower the surface tension of water increases with increasing chain length ( $\text{C}_8$  to  $\text{C}_{19}$ ) until a minimum of 42–43 dynes/cm is approached.<sup>(3)</sup>

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

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

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

**TABLE 1.** Critical Micelle Concentration Values.<sup>a</sup>

<i>Ingredient</i>	<i>CMC mole/dm<sup>3</sup></i>
Stearalkonium Chloride	Not available
Cetalkonium Chloride	$2.9 \times 10^{-4}$
Myristalkonium Chloride	$1.9 \times 10^{-3}$

<sup>a</sup>Data from Ref. 8.

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

### Reactivity

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

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

### Analytical Methods

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

Cationic surfactants react with thymolsulfonaphthalein dyes to form large cation-anion complexes. Following a series of extractions, photometric determination of the cationic surfactant complex with thymolsulfonaphthalein is made by the colorimetry at 555 nm.<sup>(17)</sup>

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

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

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

### Impurities

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

**TABLE 2.** Impurities.<sup>a</sup>

<i>Chemical names of impurities</i>	<i>Percent present in material</i>
Stearyl Alcohol	3-6
Stearyl Dimethylamine Hydrochloride	1.5-4 (combined)
Stearyl Dimethylamine	

<sup>a</sup>Data from Ref. 3.

### PURPOSE AND FREQUENCY OF USE IN COSMETICS

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

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

**TABLE 3.** Cosmetic Properties of Stearalkonium Chloride.<sup>a</sup>

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

<sup>a</sup>Data from Ref. 1.

**TABLE 4.** Product Formulation Data on Stearalkonium Chloride.<sup>a</sup>

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

<sup>a</sup>Data from Refs. 26, 27.

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

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

### Potential Interactions with other Ingredients

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

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

## BIOLOGICAL PROPERTIES

### General Effects

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

Standard antibacterial and antifungal tests were performed on a series of alkyldimethylammonium chlorides of C<sub>8-19</sub> chain length. The most consistent amount of bactericidal and fungistatic activity was seen in compounds of C<sub>12-16</sub> chain-length. The bactericidal action of a series of these compounds on a myxobacterium, pathogenic to fish, was greatest for the Hexadecyl compound.<sup>(31,32)</sup>

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

### Secondary Effects

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

Concentrations of Stearalkonium Chloride producing 100% and 50% hemolysis of isolated erythrocytes from rabbits and sheep, respectively, have been determined to be  $2.4 \times 10^{-5} M$  and  $3.0 \times 10^{-5} M$ .<sup>(36,37)</sup>

### Absorption, Metabolism, Storage, and Excretion

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

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

## Animal Toxicology

### General Studies

#### *Oral toxicity: acute*

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

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

#### *Eye irritation: acute*

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

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

#### *Dermal irritation: acute*

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

**TABLE 5.** Oral LD50 in Rats.

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

**TABLE 6.** Primary Eye Irritation Scores in Rabbits.<sup>a</sup>

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

<sup>a</sup>Total score possible/animal/observation interval = 110.

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

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

#### *Fish toxicity*

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

#### *Subchronic studies: dermal irritation*

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

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

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

#### *Chronic studies*

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

### **Special Studies**

#### *Teratology*

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

**TABLE 7.** Chronic Feeding of an Undiluted Alkyldimethylbenzylammonium Chloride.<sup>a</sup>

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

<sup>a</sup>Data from Ref. 49.

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

### Clinical Assessment of Safety

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

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

### SUMMARY

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

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

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

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

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

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

### CONCLUSION

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

### REFERENCES

1. COSMETIC, TOILETRY AND FRAGRANCE ASSOCIATION (CTFA). (1978). Submission of data by CTFA. Stearalkonium Chloride group: a summary of unpublished safety data.\*
2. ESTRIN, N.F., (ed.). (1977). *CTFA Cosmetic Ingredient Dictionary*, 2nd ed. Washington, DC: Cosmetic, Toiletry and Fragrance Association.
3. CUTLER, R.A., CIMIGOTTI, E.B., OKOLOWICH, T.J., and WETTERAU, W.F. (1967). Alkyl benzyldimethylammonium chlorides; a comparative study of the odd and even-chain homologs of 12 different quaternary ammonium compound antimicrobial agents. *Soap, Chem. Spec.* **43**(3), 84,88,90,92.
4. CTFA. (1978) Submission of data by CTFA. Cosmetic ingredient descriptions for Stearalkonium Chloride, Olealkonium Chloride group: a summary of unpublished safety data.\*
5. WINDHOLZ, M., (ed.). (1976). *Merck Index*, 9th ed. Rahway, NJ: Merck.
6. COHEN, I. and VASSILIADES, T. (1961). Critical phenomena in aqueous solutions of long-chain quaternary ammonium salts. II. Specificity and light-scattering properties. *J. Phys. Chem.* **65**, 1774-81.
7. CZAPKIEWICZ, J. and SLIWA, B. (1970). Conductometric studies of micellization of long-chain alkyldimethylbenzylammonium chlorides. *Rocz. Chem.* **44**(7/8), 1565-70.
8. BLOOM, H. and PEINSBOROUGH, V.C. (1969). Viscosity and conductance micelle studies in molten pyridinium chloride. *Aust. J. Chem.* **22**(3), 519-25.
9. BLOOM, H. and PEINSBOROUGH, V.C. (1968). Surface tensions and densities of solutions in large organic ions in molten pyridinium chloride. *Aust. J. Chem.* **21**(6), 1525-30.
10. CHANDLER, R.C. and McBAIN, J.W. (1949). Diffusion and osmotic coefficients, conductivity, membrane analyses, and the determination of micellar charge and composition in some colloidal electrolytes. *J. Phys. Colloid Chem.* **53**, 930-44.
11. BLOIS, D.W. and SWARBRICK, J. (1971). Interfacial properties of alkylbenzyldimethylammonium chlorides. *J. Colloid Interface Sci.* **36**(2), 226-33.
12. GRAHAM, H.D. and THOMAS, L.B. (1962). Quantitative aspects of the interaction of carrageenan with cationic substances. II. Precipitation with long-chain quaternary ammonium detergents. *J. Food Sci.* **27**(1), 98-105.
13. KLUGE, A. (1961). The properties of quaternary ammonium salts and their use in cosmetic products for treatment of hair. *Parfuen. Kosmet.* **42**, 341-46.

---

\*Available upon request: Administrator, Cosmetic Ingredient Review, 1110 Vermont Ave. N.W., Suite 810, Washington, DC 20005

14. BLUME, R.C. (Jan. 8, 1952). Heterogeneous, basic hydrolysis of carboxylic acid esters of polyvinyl alcohol with quaternary ammonium bases. U.S. Pat. 2,581,832.
15. BORRMEISTER, B. and SCHIFFNER, R. (1967). Quantitative determination of anionic and cationic surfactants by means of two-phase titration. *Dtsch. Textiltech.* **17**(5), 303-7.
16. GASPARIĆ, J. and HANZLIK, J. (1961). Identification of organic compounds. XLIII. Paper chromatography of quaternary alkylpyridinium and ammonium salts. *Collection Czech. Chem. Commun.* **26**, 2954-56.
17. RUF, E. (1964). Photometric determination of cationic tensides and cation-active amphotensides. *Z. Anal. Chem.* **204**(5), 344-55.
18. SPADA, A., COPPINI, D., and MONTORSI, M. (1957). Determination of quaternary ammonium compounds of antiseptic action. *Farmace Ed. Sci.* **12**, 582-85.
19. TILLSON, A.H., EISENBERG, W.V., and WILSON, J.B. (1952). Identification of certain quaternary ammonium compounds as reineckates. *J. Assoc. Off. Agric. Chem.* **35**, 459-65.
20. WILSON, J.B. (1952). Determination of quaternary ammonium compounds as reineckates. *J. Assoc. Off. Agric. Chem.* **35**, 455-58.
21. GAUTIER, J.A., RENAULT, J., and PELLERIN, F. (1955). Alkalimetric determination of salts or organic bases after precipitation as tetraphenylboron compounds. I. Quaternary ammonium compounds with long chains. *Ann. Pharm. Fr.* **13**, 725-30.
22. KOJIMA, T. and OKA, H. (1968). Application of lithium aluminum hydride to analytical chemistry. II. Gas-liquid chromatography of long-chain quaternary ammonium cationic surfactants. *Kogyo Kagaku Zasshi* **71**(11), 1844-47.
23. KIGER, J.L., and KIGER, J.G. (1967). Procedure for rapid, individual, serial analysis of common quaternary ammonium derivatives and related compounds. *Ann. Pharm. Fr.* **25**(9-10), 601-12.
24. KOENIG, H. (1970). Examination of detergents by nuclear magnetic resonance spectroscopy. *Fresenius' Z. Anal. Chem.* **251**(4), 225-62.
25. PELLERIN, F., GAUTIER, J.A., and DEMAY, D. (1964). Determination of organic bases by semimicrotitrimetry using sodium lauryl sulfate. II. Scope of the method. *Ann. Pharm. Fr.* **22**(8-9), 495-504.
26. FOOD AND DRUG ADMINISTRATION (FDA). (Aug. 31, 1976). Cosmetic product formulation data. FDA Computer Printout.
27. FDA. (Feb. 21, 1980). Personal communication, M. Greif to Director, CIR.
28. POWERS, D.H. (1972). Shampoos. Revised by N.D. Steigelmeyer and E.W. Lang. Vol. 2, In: *Cosmetics: Science and Technology*, 2nd ed., 3 vols. pp. 73-116. M.S. Balsam and E. Sagarin (eds.). New York: Wiley-Interscience.
29. CELLA, J.A., EGGENBERGER, D.N., NOEL, D.R., HARRIMAN, L.A., and HARWOOD, H.J. (1952). The relation of structure and critical concentration to bactericidal activity of quaternary ammonium salts. *J. Am. Chem. Soc.* **74**, 2061-62.
30. LAWRENCE, C.A., KWARTLER, C.E., WILSON, V.L., and KIVELA, E.W. (1947). Germicidal action of some benzyl quaternary ammonium compounds having substituents in the aromatic nucleus. *J. Am. Pharm. Assoc. Sci. Ed.* **36**, 353-58.
31. CUTLER, R.A., CLIMIOTTI, E.B., OKOLOWICH, T.J., and WETTERAU, W.F. (1967). Alkylbenzyl dimethyl-ammonium chlorides. *Soap Chem. Spec.* **43**(4), 74,76,80,92,96.
32. RUCKER, R.R., JOHNSON, H.E., and ORDAL, E.J. (1949). An investigation of the bactericidal action and fish toxicity of two homologous series of quaternary ammonium compounds. *J. Bact.* **57**, 225-34.
33. SCHLOSSMAN, M.L. (1976). Quaternized lanolin in cosmetics. *Soap Cosmet. Chem. Spec.* **52**(10), 33-4,38,40.
34. MAIOROVICI, C. and ARIESAN, V. (1958). Quaternary ammonium disinfectants of the zephiran chloride type. *Farmacia (Bucharest)* **6**, 29-36.
35. GALL, D. (1966). The adjuvant activity of aliphatic nitrogenous bases. *Immunology* **11**(4), 369-86.
36. CADWALLADER, D.E. and ANSEL, H.C. (1965). Hemolysis of erythrocytes by antibacterial preservatives. II. Quaternary ammonium salts. *J. Pharm. Sci.* **54**(7), 1010-12.
37. ROSS, S. and SILVERSTEIN, A.M. (1954). Hemolysis by colloidal electrolytes. *J. Colloid Sci.* **9**, 157-65.
38. BOGS, U. and LOHSE, E. (1971). On the distribution of cationic surface-active agents in the body of mammals. *Arch. Toxikol.* **28**(1), 68-71.
39. WARF INSTITUTE. (1976). Submission of data by CTFA. Acute oral toxicity study in rats: Stearalkonium Chloride.\*
40. CONSUMER PRODUCT TESTING CO. (1977). Submission of data by CTFA. Acute oral toxicity study in rats: Stearalkonium Chloride.\*
41. FINNEGAN, J.K. (1953). Toxicological observations in certain surface-active agents. *Proc. Sci. Sec. Toilet Goods Assoc.* **20**, 16.
42. CONSUMER PRODUCT TESTING CO. (1977). Submission of data by CTFA. Rabbit eye irritation study: Stearalkonium Chloride.\*

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43. WARF INSTITUTE. (1976). Submission of data by CTFA. Rabbit eye irritation study: Stearalkonium Chloride.\*
44. LEBERCO LABORATORIES. (1976). Submission of data by CTFA. Rabbit eye irritation study: Stearalkonium Chloride.\*
45. SCHOENBER, T.G. (1975). New look at cationic surfactants for today's low pH shampoos. *Cosmet. Perfum.* **90**(3), 89-92.
46. CONSUMER PRODUCT TESTING CO. (1977). Submission of data by CTFA. Primary irritation studies with rabbits: Stearalkonium Chloride.\*
47. WARF INSTITUTE. (1976). Submission of data by CTFA. Primary skin irritation study with rabbits: Stearalkonium Chloride.\*
48. PUTTERMAN, G.J., WOLEJSZA, N.F., WOLFRAM, M.A., and LADEN, K. (1977). The effect of detergents on swelling of stratum corneum. *J. Soc. Cosmet. Chem.* **28**, 521-32.
49. WELLS LABORATORIES. (1972). Submission of data by CTFA. Subchronic dermal toxicity study with rabbits: Myristalkonium Chloride.
50. FITZHUGH, O.G. and NELSON, A.A. (1948). Chronic oral toxicities of surface-active agents. *J. Am. Pharm. Assoc. Sci. Ed.* **37**(1), 29-32.
51. FOOD AND DRUG RESEARCH LABORATORIES. (1977). Submission of data by CTFA. Teratology study with rats: Myristalkonium Chloride.\*
52. INDUSTRIAL BIOLOGY RESEARCH AND TESTING LABORATORIES. (1958). Submission of data by CTFA. Human repeated insult patch test: Stearalkonium Chloride.\*
53. NORTH AMERICAN CONTACT DERMATITIS GROUP. (7/1/78 to 6/30/79). Allergic Indices.

**TABLE 29**  
Stearalkonium Chloride use

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

- Dennis, K. J., and T. Shibamoto. 1989. Production of malonaldehyde from squalene, a major skin surface lipid, during UV-irradiation. *Photochem. Photobiol.* 49:711–716.
- Desai, K. N., H. Wei, and C. A. Lamartiniere. 1996. The preventive and therapeutic potential of the squalene-containing compound, Roindex, on tumor promotion and regression. *Cancer Lett.* 10:93–96.
- Doran, T. I., R. Baff, P. Jacobs, and E. Pacia. 1991. Characterization of human sebaceous cells in vitro. *J. Invest. Dermatol.* 96:341–348.
- Elder, R. L., ed. 1982. Final report on the safety assessment of Squalene and Squalane. *J. Am. Coll. Toxicol.* 1:37–56.
- Fan, S. R., I. C. Ho, F. L. F. Yeoh, C. J. Lin, and T. C. Lee. 1996. Squalene inhibits sodium-arsenite-induced sister chromatid exchanges and micronuclei in Chinese hamster ovary-K1 cells. *Mutat. Res.* 368:165–169.
- Food and Drug Administration. 2001. Frequency of use of cosmetic ingredients. FDA database. Washington, DC: FDA.
- Gylling, H., and T. A. Miettinen. 1994. Postabsorptive metabolism of dietary squalene. *Atherosclerosis* 106:169–178.
- Holms, B. C., H. W. Xu, L. Jacobsson, A. Larsson, H. Luthman, and J. C. Lorentzen. 2001. Rats made congenic for Oia3 on chromosome 10 become susceptible to squalene-induced arthritis. *Human Mol. Genet.* 10:565–572.
- Kamimura, H., K. Fuchigami, H. Nioue, and R. Komada. 1991. Studies on distribution and excretion of squalane in dogs administered after 2 weeks. *Fukuoka Acta Med.* 82:300–304.
- Kamimura, H., N. Koga, K. Oguri, and H. Yoshimura. 1989. Studies on distribution, excretion and subacute toxicity of squalane in dogs. *Fukuoka Acta Med.* 80:269–280.
- Katdare, M., H. Singhal, H. Newmark, M. P. Osborne, and N. T. Telang. 1997. Prevention of mammary preneoplastic transformation by naturally-occurring tumor inhibitors. *Cancer Lett.* 111:141–147.
- Kelly, G. S. 1999. Squalene and its potential clinical uses. *Altern. Med. Rev.* 4:29–36.
- Khono, Y., Y. Egawa, S. Itoh, S. Nagaoka, M. Takahashi, and K. Mukai. 1995. Kinetic study of quenching reaction of singlet oxygen and scavenging reaction of free radical by squalene in n-butanol. *Biochim. Biophys. Acta* 1256:52–56.
- Kligman, L. H., and A. M. Kligman. 1979. The effect on rhino mouse skin of agents which influence keratinization and exfoliation. *J. Invest. Dermatol.* 73:354–358.
- Leyden, J. 1998. Pharmacokinetics and pharmacology of terbinafine and itraconazole. *J. Am. Acad. Dermatol.* 38:S42–S47.
- Mills, O. H., M. Porte, and A. M. Kligman. 1978. Enhancement of comedogenic substances by ultraviolet radiation. *Br. J. Dermatol.* 98:145–150.
- Murakoshi, M., H. Nishino, H. Tokuda, A. Iwashima, J. Okuzumi, H. Kitano, and R. Iwasaki. 1992. Inhibition by squalene of the tumor-promoting activity of 12-O-tetradecanoylphorbol-13-acetate in mouse skin carcinogenesis. *Int. J. Cancer* 52:950–952.
- Nakagawa, M., T. Yamaguchi, H. Fukawa, J. Ogata, S. Komiyama, S. Akiyama, and M. Kuwano. 1985. Potentiation by squalene of the cytotoxicity of anticancer agents against cultured mammalian cells and murine tumor. *Jpn. J. Cancer Res.* 76:315–320.
- Pepe, R. C., J. A. Wenninger, and G. N. McEwen, Jr., eds. 2002. *International cosmetic ingredient dictionary and handbook*, 9th ed. Washington, DC: CTFA.
- Picardo, M., C. Zompetta, C. De Luca, A. Amantea, A. Faggioni, M. Nazzaro Porro, and S. Passi. 1991. Squalene peroxides may contribute to ultraviolet light-induced immunological effects. *Photodermatol. Photoimmunol. Photomed.* 8:105–110.
- Relas, H., H. Gylling, and T. A. Miettinen. 2001. Fate of intravenously administered squalene and plant sterols in human subjects. *J. Lipid Res.* 42:988–994.

**TABLE 30**  
~~Wheat Germ Glycerides use~~

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

<sup>a</sup>Originally, Face and Neck and Body and Hand were combined as one category, but now they are separated.

<sup>b</sup>No longer a product category.

<sup>c</sup>Wrinkle smoothing (removers) are now part of the Moisturizing category.

Richter, E., and S. G. Schäfer. 1982. Effect of squalene on hexachlorobenzene (HCB) concentration in tissues of mice. *J. Environ. Sci. Health* B17:195–203.

Saint-Leger, D., A. Bague, E. Cohen, and M. Chivot. 1986. A possible role for squalene in the pathogenesis of acne. I. In vitro study of squalene oxidation. *Br. J. Dermatol.* 114:535–542.

Stewart, M. E. 1992. Sebaceous gland lipids. *Semin. Dermatol.* 11:100–105.

Storm, H. M., S. Y. Oh, B. F. Kimler, and S. Norton. 1993. Radioprotection of mice by dietary squalene. *Lipids* 28:555–559.

Thiele, J. J., C. Schroeter, S. N. Hsieh, M. Podda, and L. Packer. 2001. The antioxidant network of the stratum corneum. *Curr. Prob. Dermatol.* 29:26–42.

Tilvis, R., P. T. Kovanen, and T. A. Miettinen. 1982. Metabolism of squalene in human fat cells. *J. Biol. Chem.* 257:10300–10305.

Yoder, J. A., B. W. Stevens, and K. C. Crouch. 1999. Squalene: A naturally abundant mammalian skin secretion and long distance tick attractant (acarid: ixodidae). *J. Med. Entomol.* 36:526–529.

ilar to those presently marketed” (Elder 1982). New studies, along with the updated information regarding uses and use concentrations, were considered by the CIR Expert Panel. The Panel determined to not reopen this safety assessment.

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

## REFERENCES

Cosmetic, Toiletry, and Fragrance Association (CTFA). 2001. Concentration of use information. Unpublished data submitted by CTFA.<sup>2</sup>

Elder, R. L., ed. 1982. Final report on the safety assessment of stearalkonium chloride. *J. Am. Coll. Toxicol.* 1:57–69.

## STEARALKONIUM CHLORIDE

A safety assessment of Stearalkonium Chloride was published in 1982 with the conclusion that this ingredient is “safe when incorporated in cosmetic products in concentrations sim-

<sup>2</sup>Available from Director, Cosmetic Ingredient Review, 1101 17th Street NW, Suite 310, Washington, DC 20036, USA.

**TABLE 31**  
~~Triticum Vulgare (Wheat) Gluten use~~

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

Food and Drug Administration (FDA). 2001. Frequency of use of cosmetic ingredients. *FDA database*. Washington, DC: FDA.

Gordon, V. C., S. Mirhashemi, and R. Wei. 1998. Evaluation of the CORROSI-TEX method to determine the corrosivity potential of surfactants, surfactant-based formulations, chemicals, and mixtures. In *Advances in Animal Alternative Safety Efficacy Test*, ed. S. Salem and A. Sidney, 309–329. Washington, DC: Taylor & Francis.

Herman, J. R., and P. Bass. 1989. Enteric neuronal ablation: Structure activity relationship in a series of alkyldimethylbenzylammonium chlorides. *Fundam. Appl. Toxicol.* 13:576–584.

Palmer, A. K., A. M. Bottomley, J. A. Edwards, and R. Clark. 1983. Absence of embryotoxic effects in rats with three quaternary ammonium compounds (Cationic Surfactants). *Toxicology* 26:313–315.

Pepe, R. C., J. A. Wenninger, and G. N. McEwen, Jr., eds. 2002. *International cosmetic ingredient dictionary and handbook*, 9th ed. Washington, DC: CTFA.

Rohm & Haas Company. 1992. Initial submission: Letter from Rohm & Haas Company to USEPA submitting enclosed information on an acute skin & eye irritation study in rabbits with four components with attachments. NTIS Report no. OTS0543739.

Stern, M., M. Klausner, R. Alvarado, K. Renskers, and M. Dickens. 1998. Evaluation of the EpiOcular tissue model as an alternative to the Draize eye irritation test. *Toxicol. In Vitro* 12:455–461.

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

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

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

**TABLE 32**  
~~Triticum Vulgare (Wheat) Starch use~~

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

2

# Final Report on the Safety Assessment of Benzalkonium Chloride

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

## CHEMISTRY

**B**enzalkonium Chloride (CAS No. 8001-54-5), is the U.S. Pharmacopeia (USP) name for alkyltrimethylbenzylammonium chloride.<sup>1</sup> It is a mixture of alkylbenzyltrimethylammonium chlorides that conforms generally to the formula<sup>2</sup> in Figure 1.

The R represents a mixture of alkyls, including all or some of the group beginning with n-C<sub>8</sub>H<sub>17</sub> and extending through higher homologs, with n-C<sub>12</sub>H<sub>25</sub>, n-C<sub>14</sub>H<sub>29</sub>, and n-C<sub>16</sub>H<sub>33</sub> making up the major portion.<sup>3</sup> On the anhydrous basis, the content of n-C<sub>12</sub>H<sub>25</sub> is not less than 40.0% and the content of n-C<sub>14</sub>H<sub>29</sub> is not less than 20.0% of the total alkylbenzyltrimethylammonium chloride content. The amounts of n-C<sub>12</sub>H<sub>25</sub> and n-C<sub>14</sub>H<sub>29</sub> together make up not less than 70.0% of the total. The total alkylbenzyltrimethylammonium chloride content of Benzalkonium Chloride is not less than 97%.<sup>3</sup> Properties of Benzalkonium Chloride are listed in Table 1.

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

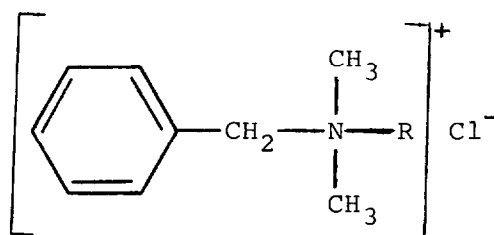


FIG. 1. Benzalkonium Chloride.

TABLE 1. Properties of Benzalkonium Chloride

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

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

## METHODS OF PRODUCTION

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

**TABLE 2.** Properties of 50% and 80% Benzalkonium Chloride<sup>4</sup>

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

## REACTIVITY

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

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

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

## ANALYTICAL METHODS

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

## IMPURITIES

Information concerning impurities in Benzalkonium Chloride is not available.

## USE

## Purpose in Cosmetics

Benzalkonium Chloride has the following cosmetic uses: foaming and cleansing agent, conditioner, and bactericide.<sup>7</sup>

## Scope and Extent of Use in Cosmetics

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

TABLE 3. Product Formulation Data<sup>21</sup>

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

## Surfaces to which Applied

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

## Frequency and Duration of Application

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

## Noncosmetic Use

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

**TABLE 4.** OTC Panel Recommendations for Benzalkonium Chloride

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

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

ANPR, Advanced Notice of Proposed Rulemaking; PR, Proposed Rule; FR, Final Rule; S, Safety; E, Effectiveness.

atoms; the aqueous solutions may contain either ethyl or isopropyl alcohol as an optional ingredient.<sup>22</sup> Benzalkonium Chloride is commonly used as a preservative in ophthalmic medications and solutions for contact lenses, and is also a potent spermicide.<sup>23,24</sup> It appears in the list of inactive ingredients for approved prescription drug products prepared by the FDA.<sup>25</sup> Some over-the-counter drug uses of Benzalkonium Chloride and their respective safety evaluations are listed in Table 4.<sup>26</sup>

## BIOLOGICAL PROPERTIES

### Absorption and Distribution

The absorption of Benzalkonium Chloride by the vaginal mucosa was evaluated in three women (ages 23, 27, and 36 years old) using tampons containing Benzalkonium Chloride (60 mg). Venous blood samples (10 ml) were drawn 15 min prior to tampon application and during the following intervals after application: 15 min, 1 h, 3 h, and 24 h. The content of Benzalkonium Chloride in each blood sample was determined via HPLC (detection sensitivity < 50 ng/ml of blood). Benzalkonium Chloride was not detected in blood samples at any time during the study.<sup>27</sup>

In another study, the recovery of Benzalkonium Chloride from the blood and breast milk of four women using tampons containing Benzalkonium Chloride (60 mg) was evaluated. Venous blood and breast milk samples were taken 15 min prior to tampon application and 3 and 24 h after application. The HPLC method mentioned above was used for determining the content of Benzalkonium Chloride in each sample. Benzalkonium Chloride was not detected in any of the four subjects.<sup>28</sup>

A 50  $\mu$ l drop of [<sup>14</sup>C]-Benzalkonium Chloride solution was placed on the corneal surface (lids held open) of young and adult New Zealand albino or Dutch belted rabbits. Normal blinking resumed after the lower lid was elevated to prevent fluid runoff. At various intervals after administration, the eye was washed with at least 1 ml saline and the following tissues and fluids were removed: bulbar and palpebral conjunctiva, aqueous humor, corneal epithelium, endothelium and stroma, iris-ciliary body, lens, vitreous, retina, and choroid. A plasma sample was obtained by direct cardiac puncture. Multiple drops of the radioactive solution were also applied. After administration of one drop, Benzalkonium Chloride was found in the corneal epithelium, endothelium, and stroma, and in the bulbar and palpebral conjunctivae. At no time was radioactive material found in the aqueous humor or any other tissues. Benzalkonium Chloride loss from ocular tissues was such that about one-third to two-thirds of its concentration (depending on the tissue) at 30 min remained after 24 h; measurable values existed for as long as 120 h. The administration of multiple drops led to continued accumulation of Benzalkonium Chloride.<sup>29</sup>

### Effect on Histamine Release

The effect of Benzalkonium Chloride on histamine release was evaluated using mixed cellular suspensions (11.0% mast cells) from peritoneal cavities of

Wistar rats (200–400 g). Benzalkonium Chloride concentrations of 0.3 and 3.0  $\mu\text{g}/\text{ml}$  caused approximately 70.0 and 14.0% inhibition of histamine release from mast cells, respectively, in the presence of a potent histamine releaser. Concentrations of 10.0 and 30.0  $\mu\text{g}/\text{ml}$  caused approximately 70.0% and 90.0% increases in histamine release, respectively. Benzalkonium Chloride also antagonized histamine release induced by ATP, bradykinin, polylysine, and curare, but not that induced by antigens, enzymes, monoamines, or detergents.<sup>30</sup> In another study, the challenge of rat mast cells (2.0–10.0% purity) with Benzalkonium Chloride concentrations of 1.0 and 5.0  $\mu\text{g}/\text{ml}$  produced a concentration-dependent inhibition of histamine secretion.<sup>31</sup>

### Effect on Enzymatic Activity

The effect of Benzalkonium Chloride on the hydrolysis of *p*-toluene-sulfonyl-L-arginine methyl ester hydrochloride (substrate for trypsin and chymotrypsin) was evaluated. In separate experiments, trypsin and chymotrypsin (0.275 mg/ml) were each incubated with various concentrations of Benzalkonium Chloride (0.005–0.040 M) at 30°C and pH 3.0. Aliquots (10  $\mu\text{l}$ ) of the mixtures were removed over the next 48 h to assay for remaining activity. The substrate concentration in each assay was 0.0015 M. A sigmoid curve was obtained when the percentage inhibition of trypsin was plotted against Benzalkonium Chloride concentration and showed 50.0% inhibition with 0.015 M Benzalkonium Chloride. A similar curve was obtained for chymotrypsin, with 50.0% inhibition occurring at a 10-fold lower Benzalkonium Chloride concentration.<sup>32</sup>

## ANIMAL TOXICOLOGY

### Subchronic Inhalation Toxicity

An inhalation toxicity study of an aerosolized hair conditioner containing 0.2% of a 50.0% Benzalkonium Chloride solution (effective Benzalkonium Chloride concentration = 0.1%) was conducted with 12 female albino rats of the CD strain (mean weight 216 g) and 12 Syrian Golden hamsters (mean weight 88 g). Exposures were carried out in 500 L dynamic flow inhalation chambers. The animals were exposed to the conditioner (9.9 mg/m<sup>3</sup> of air) 5 days a week (4 h/day) for 14 consecutive weeks. Gravimetric analysis was used to determine the aerosol concentration in the chamber atmosphere; the desired concentration was maintained by adjusting the rate of airflow through each chamber. Chamber air flow rates ranged from 18 to 29 cubic feet/min. The body weights of all animals were recorded weekly. Hematologic and serum chemistry studies were conducted with blood samples obtained from rats after the 6th and 13th weeks of exposure. Gross and microscopic examinations of tissues from rats and hamsters were also conducted. There were no significant differences in weight gain, hematologic values, and serum chemistry data between experimental and control groups. There were no exposure-related deaths, and neither gross nor microscopic changes were attributed to Benzalkonium Chloride inhalation.<sup>33</sup>

### Acute Oral Toxicity

The oral toxicity of Benzalkonium Chloride (composition: 60.0% C<sub>14</sub>, 30.0% C<sub>16</sub>, 5.0% C<sub>12</sub>, and 5.0% C<sub>18</sub>) was assessed in white rats (procedure, weights, and strain not stated). The LD<sub>50</sub> was 525 mg/kg.<sup>34</sup> In another study, the mean acute oral LD<sub>50</sub> in rats (number and strain not stated) was 400 mg/kg (range, 342–469 mg/kg). The experimental procedure was not stated (Table 5).<sup>35</sup>

The oral toxicity of a moisturizing cream containing 0.13% Benzalkonium Chloride was evaluated using 10 young adult Sprague Dawley rats (5 males, 5 females). Each animal was fasted for 18 h and then given a single dose (5 ml/kg) of the cream via oral gavage. The animals were observed for a period of 14 days postadministration. No toxic effects were observed, and none of the animals died.<sup>36</sup>

In another study, the oral toxicity of a cream containing 0.1% Benzalkonium Chloride was evaluated using 10 rats (weight range 130–180 g) of the Fischer 344 strain. A single dose of 7.0 ml/kg was administered to each animal via oral gavage. None of the animals died during the 2-week observation period (Table 5).<sup>37</sup>

### Subchronic Oral Toxicity

Benzalkonium Chloride solutions were administered via stomach tube to 40 male albino rats (Sprague-Dawley, 130 g) once daily for 12 weeks at dosages of 50.0 mg/kg (2 groups of 10 rats) and 100.0 mg/kg (2 groups of 10). The 50.0 mg/kg and 100.0 mg/kg dosages were 1:20 and 1:10 dilutions of 10.0% Benzalkonium Chloride, respectively. Ten animals in both dose groups received Benzalkonium Chloride diluted with distilled water, while the remaining animals received Benzalkonium Chloride diluted with milk. Two control groups (10 rats/group) were given distilled water and milk, respectively. Hematologic studies were conducted at 4 and 12 weeks with rats receiving 100 mg/kg doses. Two rats receiving the 100.0 mg/kg dose died on days 62 and 69, respectively. Animals surviving the 12-week period were killed and examined macroscopically and microscopically. The growth rates of rats given 50.0 mg/kg (in water), 50.0 mg/kg (in milk), and 100 mg/kg (in milk) were similar to those of control groups throughout the experiment. Growth rates of rats given 100.0 mg/kg (in water) were depressed throughout the experiment; the average body weight at 12 weeks was 29.0% less than that of the control (water) group. In hematologic studies, there were no significant changes in any of the following values (all treatment groups): total number of erythrocytes and leukocytes, differential count, hematocrit, and hemoglobin values. Neither gross nor microscopic tissue changes related to Benzalkonium Chloride administration were noted in any of the treatment groups (Table 5).<sup>38</sup>

### Chronic Oral Toxicity

The chronic oral toxicity of 10.0% Benzalkonium Chloride was evaluated in 18 beagle dogs (weight range 6.6–9.3 kg). Dosages of 12.5, 25.0, and 50.0 mg/kg (6 animals/dose) were administered via stomach tube once daily for

**TABLE 5.** Oral Toxicity of Benzalkonium Chloride

<i>Type of study</i>	<i>Animals tested</i>	<i>Test substance</i>	<i>Methodology</i>	<i>Results</i>	<i>References</i>
Short-term oral toxicity	White rats (no., weights, strain not stated)	Benzalkonium Chloride	—	LD <sub>50</sub> = 525 mg/kg	34
Short-term oral toxicity	Rats (no., strain, and weights not stated)	Benzalkonium Chloride	—	LD <sub>50</sub> = 400 mg/kg (range, 342–469 mg/kg)	35
Short-term oral toxicity	10 rats (Fisher 344 strain, 130–180 g)	Cream containing 0.1% Benzalkonium Chloride	Single oral dose of 7.0 ml/kg	No deaths or signs of toxicity	37
Subchronic oral toxicity	20 male albino rats (Sprague-Dawley strain, 130 g)	1:10 and 1:20 dilutions of 10.0% Benzalkonium Chloride	1:10 and 1:20 dilutions administered via stomach tube in doses of 100 mg/kg (10 rats) and 50 mg/kg (10 rats) once daily for 12 weeks	Two rats receiving 100 mg/kg died. No treatment-related gross or microscopic changes	38
Chronic oral toxicity	18 beagle dogs (three groups of six: 6.6–9.3 kg)	10.0% Benzalkonium Chloride (in water)	Doses of 12.5, 25.0, and 50.0 mg/kg (1 dose/group) administered via stomach tube once daily for 52 weeks	Four deaths: 1 dog (50 mg/kg dose), 3 dogs (25 mg/kg dose)	38

52 weeks. The solution was administered in milk to half of the animals, and in water to the remaining half. Two control groups (two animals/group) were given milk and water, respectively. Hematologic studies were conducted with all animals at 6-week intervals throughout the experiment. Urine specimens were also obtained at 6-week intervals. Necropsies were performed on all animals, and tissues were examined macroscopically and microscopically. Significant fluctuations in body weight were not noted in any of the treatment groups. The only deaths reported were one of the three dogs receiving 50.0 mg/kg doses in water and the three dogs receiving 25.0 mg/kg doses in water. In all treatment groups, there were no significant changes in the blood or urine attributable to Benzalkonium Chloride administration. Slight to moderate hyperemia of the small intestine and pyloric portion of the stomach was observed in dogs receiving 50 mg/kg doses in milk. No significant macroscopic lesions were observed in dogs receiving 12.5 or 25.0 mg/kg doses in milk. Moderate to severe irritation of the small intestine was observed in all dogs receiving 50.0 and 25.0 mg/kg doses in water and in two of three dogs receiving 12.5 mg/kg doses in water. The dogs receiving 50.0 and 25.0 mg/kg doses in water also had moderate to severe irritation and congestion of the stomach and intestines. No microscopic changes due to doses of Benzalkonium Chloride in milk were noted. However, congestion and subacute inflammation of the intestines were noted in animals dosed with 12.5 mg/kg of Benzalkonium Chloride in water. These observations were regarded as minor microscopic changes (Table 5).<sup>38</sup>

### Ototoxicity

The ototoxicity of Benzalkonium Chloride was evaluated using 13 pigmented guinea pigs. Applications of a 0.1% Benzalkonium Chloride solution in distilled water (5 animals) and a 0.1% Benzalkonium Chloride solution in 70.0% alcohol (8 animals) were made onto the round window membrane of the middle ear via glass pipettes; the entire cavity of the bulla was filled. Exposure periods were of 10, 30, and 60 min duration, after which the bulla cavity was emptied by careful suction and repeatedly washed with physiological saline. Ten of the animals were killed after 2 weeks. Three of the animals exposed to Benzalkonium Chloride in alcohol for 6 min were killed after 9 weeks to evaluate the effect of prolonging the length of postoperative survival of the inner ear sensory epithelia. Microscopic examinations revealed fibrosis in tissues from the tympanic cavity (13 animals), cochlea (10 animals), and vestibulum (5 animals). Inner hair cell loss from the organ of Corti (13 animals) and destruction of vestibular neuroepithelia (8 animals) were also noted. The extent of damage to all tissues examined was more pronounced after 60 min exposures than after 10 and 30 min exposures and was increased by extending the postoperative survival time from 2 to 9 weeks. The application of different Benzalkonium Chloride solutions did not affect differences in the extent of damage to the tympanic cavity, cochlea, organ of Corti, and vestibular sensory epithelia. However, the extent of fibrous tissue formation in the vestibulum was less extensive after exposures (10 and 30 min) to Benzalkonium Chloride in alcohol. Differences were not noted after 60 min exposures.<sup>39</sup>

### Dermal Toxicity

The dermal toxicity of Benzalkonium Chloride was evaluated in RFM<sub>i</sub>/UnWg and BALB/c mice (4 and 10 weeks old). A disinfectant containing 50% Benzalkonium Chloride, 44–45% water, and 5–6% isopropanol was diluted with water to form 0.8, 3, and 13% Benzalkonium Chloride solutions (effective Benzalkonium Chloride concentrations of 0.1, 1.5, and 6.5%, respectively). The 50% Benzalkonium Chloride solution and its dilutions (0.1, 1.5, and 6.5% Benzalkonium Chloride) were each applied to 8 animals (total of 32 animals, 16 per strain). The test solutions (0.05 ml) were placed on the hair of each animal at the midline of the neck (between the base of the skull and the scapulae) and then rubbed in. Animals were observed for changes in appearance of the application site and body weight for approximately 1 month. Forty-eight animals served as controls for body weight. Six identical experiments were conducted (total of 192 experimental animals). The application sites in mice given the 0.1 and 1.5% solutions had a slightly unkempt appearance for only 3 or 4 days. Sites in animals treated with 6.5 and 50% solutions remained unkempt for several days. Also, small areas on the ears of some of these animals (number not stated) became hyperemic and then necrotic. The edges of necrotic areas on the ears eventually healed. Each animal treated with 6.5 and 50% solutions (number not stated) also had a 5 mm bald spot within the application site 4 weeks after application. A 10% reduction in body weight (2 days postapplication) was noted in animals receiving the 50% solution. After day 5, the rate of weight gain was the same as in the other groups. Animals receiving the 6.5% solution had a slight weight reduction and a growth rate, after day 5, similar to that of the 50% group. Weight reductions were not noted in the 0.1 and 1.5% dose groups. A total of 29 deaths (6 experiments) were reported, all having occurred within 72 h after application. Deaths were confined to animals treated with 6.5 and 50% Benzalkonium Chloride solutions. Results from necropsies of animals that died were as follows: absence of feed and feces in the alimentary tract, hyperemia of the nose and footpads (extravasated blood under the claws), and discoloration on the undersurface of the skin opposite the application site. Additional lesions were not found when visceral tissues from two mice were examined microscopically. The cause of death was not apparent.<sup>40</sup>

### Cytotoxicity

The effect of Benzalkonium Chloride on the growth of epithelial cells and fibroblasts was evaluated in tissues from the prostate gland and heart, respectively. Prostatic tissue was excised from adult rats (ages not stated), and cardiac tissue from young rats (1–10 days old). Tissue fragments were treated with the following aqueous solutions of Benzalkonium Chloride: 0.01, 0.02, 0.033, and 0.10%, and cultured with adult cockerel plasma and human serum. The cultures were incubated for 10 days at 37.5°C. Cellular growth (change in size) rates were recorded by planimetric calculation of tissue fragment areas each day, as described by Bengmark et al.<sup>41</sup> All concentrations of Benzalkonium Chloride retarded the growth of epithelial tissue. The greatest degree of

retardation was achieved at a Benzalkonium Chloride concentration of 0.1%. Cellular growth rates were significantly different from controls at all dosages tested. The growth rates of cardiac fibroblasts from newborn animals were lower and more irregular than those of adult epithelial cells (prostate gland). Benzalkonium Chloride concentrations of 0.01 and 0.02% did not result in growth retardation of cardiac fibroblasts that was significantly different from controls. However, concentrations of 0.033 and 0.10% resulted in significant retardation. The greatest degree of growth retardation of cardiac fibroblasts was achieved with a Benzalkonium Chloride concentration of 0.1% (Table 6).<sup>42</sup>

The cytotoxicity of 0.007% Benzalkonium Chloride was evaluated using human conjunctival cell cultures. The cells remained in culture for 48 h (37°C). Each culture was then exposed to 0.5 ml of the test solution at temperatures of 4°C, 15°C, and 37°C during a 16 min period. Control cultures were exposed to a phosphate buffer solution. The exposure time causing 50.0% cell damage in cell culture (CDT<sub>50</sub>) served as the cytotoxic parameter. The CDT<sub>50s</sub> for 0.007% Benzalkonium Chloride were 91.0 ± 13.0 s at 4°C, 94.2 ± 11.9 s at 15°C, and 98.9 ± 12.1 s at 37°C. Cellular growth in control cultures was not affected by different experimental temperatures (Table 6).<sup>43</sup>

In another cytotoxicity study, suspension cultures of the murine P815 tumor cell line were exposed to 1.5 M Benzalkonium Chloride during 30 min, 2 h, and 4 h periods. The dosage resulting in 50.0% cytotoxicity (CD<sub>50</sub>) was determined for each exposure period. CD<sub>50</sub> values for one experimental trial were as follows: 7.2 ppm (0.5 h), 3.5 ppm (2 h) and 0.6 ppm (4 h). Similar data were obtained for a mouse lymphoma cell line tested with the same concentrations of Benzalkonium Chloride (Table 6).<sup>44</sup>

The hemolytic activity of Benzalkonium Chloride ( $4.2 \times 10^{-5}$  M,  $3.3 \times 10^{-5}$  M, and  $2.2 \times 10^{-5}$  M) was assessed using defibrinated blood from rabbits. Mixtures of defibrinated blood (0.05 ml) and aqueous Benzalkonium Chloride (5.0 ml) were incubated in a water bath for 45 min at 37°C, after which the unhemolyzed cells were settled by centrifugation and absorbance readings of the hemolysate were determined with a photoelectric colorimeter. Each absorbance reading was compared with a total hemolysis reading, obtained by laking red cells in distilled water. In determining the hemolytic activity of each of the test solutions, 0.6% sodium chloride was added as an extracellular agent to protect the erythrocytes from simple osmotic hemolysis. The degree of hemolysis occurring in each test solution was expressed as the percentage of total hemolysis. Benzalkonium Chloride concentrations of  $2.2 \times 10^{-5}$  M,  $3.3 \times 10^{-5}$  M, and  $4.2 \times 10^{-5}$  M resulted in 10.0, 50.0, and 100.0% hemolysis, respectively. These data represented the average of a minimum of two, but usually four, similar experiments (Table 6).<sup>45</sup>

### Ocular Irritation

The ocular irritation potential of Benzalkonium Chloride was evaluated using 108 rabbits (strain not stated). Aqueous solutions of the test substance (2.0, 1.0, 0.5, 0.1, and 0.01%) were each instilled (one drop) into both eyes twice a day for 7 days; globes were examined grossly and photographed at different time intervals. Animals were killed after the seventh instillation and

**TABLE 6.** Cytotoxicity of Benzalkonium Chloride

<i>Cells tested</i>	<i>Test substance</i>	<i>Methodology</i>	<i>Results</i>	<i>References</i>
Epithelial cells (prostate gland) and fibroblasts (heart) from rats	0.10, 0.033, 0.02 and 0.01% Benzalkonium Chloride	Cell cultures incubated with Benzalkonium Chloride for 10 days	0.10 and 0.033% Benzalkonium Chloride caused significant growth retardation	42
Human conjunctival cells	0.007% Benzalkonium Chloride	Cell cultures exposed to 0.5 ml of test solution for 16 min	Exposure period resulting in 50% cellular damage was $98.9 \pm 12.1$ s at 37°C	43
Murine suspension cultures of P815 tumor cell line	1.5 M Benzalkonium Chloride	0.5, 2, and 4-h exposures	Dosages resulting in 50% cytotoxicity were: 7.2 ppm (at 0.5 h), 3.5 ppm (at 2 h), and 0.6 ppm (at 4 h)	44
Defibrinated blood from rabbits	$4.2 \times 10^{-5}$ M, $3.3 \times 10^{-5}$ M, and $2.2 \times 10^{-5}$ M Benzalkonium Chloride	Mixtures of blood (0.05 ml) and Benzalkonium Chloride (5.0 ml) were incubated for 45 min	$2.2 \times 10^{-5}$ M, $3.3 \times 10^{-5}$ M, and $4.2 \times 10^{-5}$ M concentrations caused 10.0%, 50.0%, and 100.0% hemolysis, respectively	45

ocular tissues were examined microscopically. The 2.0% solution caused conjunctival necrosis, ulceration and haziness of the cornea, and severe iritis, all observed initially on the first day of instillation. By the seventh day, the cornea was vascularized and cloudy; significant tissue damage was also noted. The 1.0% solution caused cloudiness of the cornea and severe injection of the bulbar and palpebral conjunctivae, with some areas of necrosis and large amounts of mucous material. With the 0.5% solution, most of the damage was limited to the bulbar and palpebral conjunctivae, with only occasional corneal damage. No gross damage was noted after instillation of the 0.01 and 0.1% solutions. At microscopic examination, almost total destruction of the corneal epithelium and endothelium of eyes treated with 1.0 and 2.0% solutions was noted. Severe damage to the corneal epithelium was also noted after 7 days of treatment with the 0.5% solution. When eyes were treated with 0.1% Benzalkonium Chloride solution 5 times daily (2 h intervals) for 1 week, damage to corneal endothelial cells was evident (Table 7).<sup>46</sup>

In another ocular irritation study, 50.0% Benzalkonium Chloride was tested at a concentration of 0.65% in water (effective Benzalkonium Chloride concentration = 0.3%). The test solution (0.1 ml) was instilled once into 1 eye of each of 6 rabbits. The eyes were not rinsed. Ocular irritation was scored on days 1, 2, and 3 postinstillation according to the scale used by Draize<sup>47</sup>: 0–110. The total ocular irritation score was 2 on day 1. Irritation had cleared by day 3. The test solution had the potential to induce minimal ocular irritation (Table 7).<sup>48</sup>

The ocular irritation potential of a moisturizing cream containing 0.13% Benzalkonium Chloride was evaluated according to the procedure of Draize.<sup>47</sup> The cream (0.1 ml) was instilled once into one eye of each of six female New Zealand White rabbits. Untreated eyes served as controls. Ocular reactions were scored 1, 2, 3, 4, and 7 days after instillation according to the Draize<sup>47</sup> scale. The moisturizing cream did not induce ocular irritation in any of the animals tested.<sup>49</sup>

In another study, the ocular irritation potential of 0.1% Benzalkonium Chloride was evaluated using 6 young adult New Zealand albino rabbits (male and female). The test substance was instilled into one eye (directly on the cornea) of each animal in volumes of 0.01, 0.03, and 0.10 ml; untreated eyes served as controls. Eyes were examined and scored according to the scale by Draize et al.<sup>50</sup> at 1, 3, 7, 14, and 21 days after instillation. The test substance was not an ocular irritant (Table 7).<sup>51</sup>

### **Intraocular Toxicity**

Benzalkonium Chloride was instilled into the eyes of rabbits and guinea pigs (number not stated) at concentrations of 0.1, 0.5, 1.0, and 10.0%. Corneas were examined microscopically at 4 h and 1 and 7 days postinstillation. At 4 h and day 1, desquamation and necrosis of epithelial cells and leukocytic infiltration were observed in rabbit and guinea pig corneas treated with 0.5, 1.0, and 10.0% Benzalkonium Chloride. Desquamation and necrosis were noted in rabbit corneas 4 h after instillation of 0.1% Benzalkonium Chloride; no microscopic changes were noted in guinea pig corneas. At day 1, desqua-

**TABLE 7.** Ocular Irritation of Benzalkonium Chloride

<i>Animals tested</i>	<i>Test substance</i>	<i>Methodology</i>	<i>Results</i>	<i>References</i>
108 rabbits (strain not stated)	2.0, 1.0, 0.5, 0.1, and 0.01% Benzalkonium Chloride	All five solutions instilled into both eyes twice daily for 7 days. A 0.1% solution instilled into eyes five times daily for 1 week	2.0% solution caused conjunctival necrosis, ulceration, and haziness of the cornea and massive iritis. The 1.0 and 0.5% solutions caused damage to the cornea and bulbar and palpebral conjunctivae. The 0.1 and 0.01% solutions (instilled twice daily) did not cause ocular damage. 0.1% solution (instilled five times daily) damaged corneal endothelium	46
6 rabbits (strain not stated)	0.3% Benzalkonium Chloride	Aqueous solution instilled once into one eye (no rinsing). Ocular irritation scored according to Draize (1959) scale	Minimal ocular irritation Draize score, 2 (max = 110)	48
6 New Zealand albino rabbits	0.1% Benzalkonium Chloride	Instilled into one eye	No ocular irritation	51

mation and necrosis of epithelial cells and leukocytic infiltration were noted in rabbit corneas treated with 0.1% Benzalkonium Chloride; microscopic changes were not noted in guinea pig corneas. Desquamation, necrosis, and leukocytic infiltration were also observed 7 days after the instillation of 0.5, 1.0, and 10.0% Benzalkonium Chloride (rabbits) and 1.0 and 10.0% Benzalkonium Chloride (guinea pigs) (Table 8).<sup>52</sup>

The toxic effects of Benzalkonium Chloride on the corneal epithelium were evaluated using four to six New Zealand albino rabbits. Drops of 0.01% Benzalkonium Chloride in 0.9% saline (neutral pH) were instilled into both eyes. An additional four to six rabbits received instillations of 0.9% saline (negative control). Corneal specimens were examined using scanning electron microscopy (SEM) at 5, 15, and 30 min, and 1, 3, and 6 h after instillation. Specimens were prepared for SEM according to the procedure of Pfister.<sup>53</sup> Results were as follows: peeling of cells at borders, exposing cells beneath (5 min postinstillation); many adjacent cells separated from one another (15 min); most of the surface epithelial cells lying loosely on corneal surface with extensive disruption of plasma membranes (30 min); most of the surface cells prematurely desquamated, exposing prominent nuclear bulges in second cell layer (1 h); second layer of cells desquamating, extensive plasma membrane damage (3 h); newly exposed third cell layer had nearly normal epithelial cell appearance (6 h). Damage to the corneal epithelium was not noted in the negative control group (Table 8).<sup>54</sup>

The effect of Benzalkonium Chloride on the corneal endothelium was evaluated via *in vivo* and *in vitro* methods in another study involving New Zealand White rabbits (number not stated). In the *in vitro* study, corneas were excised from freshly enucleated eyes. The endothelial surface of each was flooded with 0.5 ml of 0.01% Benzalkonium Chloride (pH 6.47); corneas remained in solution for 2 min. Specimens were then examined via SEM and transmission electron microscopy (TEM). Ruptured endothelial cells with exposed nuclei were observed in SEMs. The disorganization of cellular structures, due to cellular edema, was observed in TEMs. In the *in vivo* study, aqueous humor (0.1 ml) was removed from each globe and replaced with 0.1 ml 0.01% Benzalkonium Chloride. Animals were killed 1.5 h, 3 h, or 2 days after injection and the corneas were excised. Specimens were then prepared for SEM and TEM. Edema of the corneal endothelium was noted 1.5 h, 3 h, and 2 days after injection. Endothelial cells were fusiform in shape and the normal hexagonal pattern was gone. Large areas of bare Descemet's membrane had overlying cellular debris that may have represented remains of the endothelial cells. Other ultrastructural damage induced by Benzalkonium Chloride included mitochondrial swelling, dilation of endoplasmic reticulum, intracytoplasmic vacuole formation, and ruptured plasma membranes (Table 8).<sup>55</sup>

The ocular toxicity of 0.007 and 0.01% Benzalkonium Chloride was evaluated in four albino and four pigmented rabbits. In one group of animals (two pigmented, two albino), 0.2 ml of 0.007% Benzalkonium Chloride (in water and sodium phosphate, pH 7.37) was introduced into the eyes via subconjunctival injection. Injections were repeated once daily for 2 weeks. In the other group (two pigmented, two albino), 0.01% Benzalkonium Chloride (in water and sodium phosphate, pH 6.77) was introduced according to the same

**TABLE 8.** Intraocular Toxicity of Benzalkonium Chloride

<i>Animals tested</i>	<i>Test substance</i>	<i>Methodology</i>	<i>Results</i>	<i>References</i>
Rabbits (no. not stated)	10.0, 1.0, 0.5, and 0.1% Benzalkonium Chloride	Single instillation. Corneas examined at 4 h and 1 and 7 days postinstillation	Desquamation and necrosis of corneal epithelium (10.0, 1.0, 0.5, and 0.1% concentrations)	52
New Zealand albino rabbits (no. not stated)	0.01% Benzalkonium Chloride	Drops instilled into both eyes. Corneas examined at 5, 15, and 30 min and 1, 3, and 6 h after instillation	Desquamation of epithelial cells and extensive disruption of plasma membranes	54
New Zealand White rabbits (no. not stated)	0.01% Benzalkonium Chloride	Excised corneas flooded with solution for 2 min	Ruptured endothelial cells with exposed nuclei. Severe cellular edema	55
New Zealand White rabbits (no. not stated)	0.01% Benzalkonium Chloride	Aqueous humor removed from each eye and replaced with 0.01 ml of test substance	Swelling of corneal endothelium, ruptured plasma membranes, dilation of endoplasmic reticulum, and mitochondrial swelling	55
8 Albino and pigmented rabbits (two albinos and two pigmented/group)	0.01 and 0.007% Benzalkonium Chloride	Subconjunctival injection of both solutions (0.1 ml) once daily for 2 wks. Eyes examined via ophthalmoscopy (daily) and electron microscopy	Extensive elevation of retina and retinal detachment in both treatment groups	56
New Zealand White rabbits (no. not stated)	0.001, 0.0004, and 0.0001% Benzalkonium Chloride	Each concentration in contact with corneal epithelium 30 to 110 min. Corneas examined via scanning electron microscopy	No discernible modification of surface morphology	57
Albino rabbits (no. not stated)	$6.5 \times 10^{-6}$ to $6.5 \times 10^{-3}$ % Benzalkonium Chloride	Corneal endothelium perfused for 3 h	Severe endothelial cellular damage	58
Guinea pigs (no. not stated)	10.0, 1.0, 0.5, and 0.1% Benzalkonium Chloride	Single instillation. Corneas examined at 4 h and 1 and 7 days postinstillation	Desquamation and necrosis of corneal epithelium (10.0, 1.0, and 0.5% concentrations)	52

procedure. Ophthalmoscopy was performed every day to determine changes in the fundus. After the 2-week administration period, animals were killed and the eyes enucleated. Specimens were then prepared for electron microscopy. No abnormalities of the fundus were observed during the initial 3 days of administration. After the first week of administration, extensive elevation of the retina was noted in two of the four animals receiving the 0.007% solution. Three of the four animals receiving the 0.01% solution also had extensive elevation of the retina after the first week. After 2 weeks, retinal detachment was observed only in the globes of pigmented rabbits in both treatment groups. In electron micrographs, both early and prolonged stages of retinal detachment were observed. The inner retinal layers, especially the nerve fiber layer and inner plexiform layer, had marked edema during the early stage; outer segments of photoreceptors were relatively preserved. In the prolonged state, photoreceptors were atrophic, the outer segments and/or inner segments had disappeared, and, on some occasions, the number of nuclei in the outer nuclear layer had decreased (Table 8).<sup>56</sup>

The effect of Benzalkonium Chloride on corneal morphology was evaluated in New Zealand White rabbits (number not stated). Corneas were removed and bathed in aerated solutions (34°C, pH 7.4) of the following composition: 103.4 mM NaCl, 15.3 mM Na<sub>2</sub>SO<sub>4</sub>, 10 mM NaHCO<sub>3</sub>, 2.2 mM K<sub>2</sub>HPO<sub>4</sub>, 0.5 mM KH<sub>2</sub>PO<sub>4</sub>, 5.24 mM H<sub>3</sub>PO<sub>4</sub>, 0.61 mM MgSO<sub>4</sub>, 0.7 mM calcium gluconate, 26 mM glucose, and 20 mM tris-(hydroxymethyl) aminomethane (Tris). Benzalkonium Chloride was added to solutions at concentrations of 0.001, 0.0004, and 0.0001%; solutions remained in contact with the epithelial surface for 30–110 min. Corneas were then fixed and examined via SEM. One hour after exposure to 0.001% Benzalkonium Chloride, corneal surface cells were loosened or removed, exposing second- and third-layer cells. Also, the plicate appearance of surface cells was lost and some deeper cells had abnormally long microvilli. An increase in the number of cells with peripheral loss of microvilli and microplicae was noted 1 h after exposure to 0.0004% Benzalkonium Chloride. No discernible modification of surface morphology was noted after 2 h of exposure to 0.0001% Benzalkonium Chloride (Table 8).<sup>57</sup>

The effect of Benzalkonium Chloride on the corneal endothelium was investigated using albino rabbits (number not stated). The eyes were enucleated (complete with conjunctival sac and eyelids) and corneas were prepared and mounted in a specular microscope. After a 1 h stabilization period, the corneal endothelium was perfused for 3 h with Benzalkonium Chloride at concentrations ranging from  $6.5 \times 10^{-6}$  to  $6.5 \times 10^{-3}$ % in Ringer's solution. Observations of the corneal endothelium and sequential measurements of corneal thickness were made during the 3 h period. A change in corneal thickness was determined by a computer-fit linear regression line (minimum of five rabbit corneas). Corneas were removed from the specular microscope after perfusion and then fixed and submitted for SEM and TEM. No swelling was noted in corneas perfused with  $6.5 \times 10^{-6}$ % Benzalkonium Chloride. Minimal swelling was noted in those perfused with  $6.5 \times 10^{-5}$ % Benzalkonium Chloride (9.3  $\mu\text{m}/\text{h}$ ). The corneal swelling rate increased as a function of increasing Benzalkonium Chloride concentration. In SEMs of corneas perfused

$6.5 \times 10^{-3}\%$  Benzalkonium Chloride, severe endothelial cell damage was noted. The following changes were observed in TEMs: disorganization of nuclear chromatin, severe damage to the endoplasmic reticulum and mitochondria, and discontinuity of the posterior plasma membrane (Table 8).<sup>58</sup>

### Skin Irritation

The skin irritation potentials of 50, 10, 1, 0.1, and 0.01% Benzalkonium Chloride solutions were evaluated in rabbits (number and strain not stated). The solutions were applied in 0.5 ml volumes (duration of exposure not stated). Solutions of 1.0% Benzalkonium Chloride or greater induced skin reactions ranging from erythema (1% of animals tested) to necrosis (50% of the animals tested) (Table 9).<sup>59</sup>

Solutions of 0.1, 1, 5, and 10% Benzalkonium Chloride in water were applied to the clipped skins of five rabbits (strain not stated) via Finn chambers containing occlusive patches. The chambers remained in place for 24 h. Visual examinations were used to identify papules, vesicles, pustules, induration, necrosis, scaling, and scarring. Reactions were assessed daily for 4–5 days. Severe induration and a light yellow staining of test sites were observed in all animals treated with the four concentrations of Benzalkonium Chloride (Table 9).<sup>60</sup>

Aqueous solutions of 0.1, 1.0, and 5.0% Benzalkonium Chloride were applied to the epilated flanks of female albino guinea pigs (approximate weights = 300 g) via patches made of filter paper. Each patch was covered with impermeable tape and fastened with an elastic bandage. Two or three test patches were applied to one flank of each animal. Control patches saturated with water were applied to the contralateral flank. Patches were removed after 24 h and skin specimens were excised and examined microscopically. Twenty-four hour exposures to 0.1% Benzalkonium Chloride did not cause microscopic changes. Exposure to the 1.0% solution resulted in spotted areas of necrosis with nuclear pyknosis of the epidermal cells in the upper part of the stratum Malpighii (beneath the stratum corneum). Exposure to the 5.0% solution resulted in total necrosis of the epidermis (Table 9).<sup>63</sup>

In another study, a 2.0% Benzalkonium Chloride solution was applied to abraded and intact skins of rabbits (number and strain not stated) via a synthetic cloth worn by each animal for 2 days. Severe erythema, edema, and rawness were observed in abraded and intact skin after 2 days. Slight erythema and skin sloughing were noted 7 days after application. No toxic signs were noted in rabbits (number and strain not stated) in a study in which a cloth impregnated with 2% Benzalkonium Chloride was worn continuously during a 3-week period (Table 9).<sup>59</sup>

The skin irritation potentials of 0.1 and 1.0% Benzalkonium Chloride were evaluated in 40 white rats (strain not stated). The test solutions were applied over a period of 3 months. After 1.5–2 months, the 1% solution induced hyperemia and necrotic changes. An intense epithelialization skin defect was noted after scabs had been shed. Benzalkonium Chloride was also applied to

**TABLE 9.** Skin Irritation of Benzalkonium Chloride

<i>Animals tested</i>	<i>Test substance</i>	<i>Methodology</i>	<i>Results</i>	<i>References</i>
Rabbits (no. and strain not stated)	50.0, 10.0, 1.0, and 0.1% Benzalkonium Chloride	Solutions applied to skin in volumes of 0.5 ml	Concentrations of 1.0%, or greater, induced reactions ranging from erythema to necrosis	59
5 Rabbits (strain not stated)	10.0, 5.0, 1.0 and 0.1% aqueous Benzalkonium Chloride solutions	Solutions applied to clipped skin via Finn chambers containing occlusive patches (24-h exposure)	All concentrations caused severe induration of test sites in five rabbits	60
Rabbits (no. and strain not stated)	2.0% Benzalkonium Chloride	Applied to abraded and intact skin via synthetic cloth worn for 2 days	Severe erythema, edema, and rawness after 2 days (abraded and intact skin). Slight erythema and skin sloughing after 7 days	59
Rabbits (no. and strain not stated)	2.0% Benzalkonium Chloride	Cloth, impregnated with solution, worn continuously during 3-wk period	No signs of toxicity	59
Rabbits (no. and strain not stated)	0.5% Benzalkonium Chloride	Single 24-h application	Severe erythema, eschar formation, and moderate edema	59
9 Albino rabbits	0.5% Benzalkonium Chloride	Single 24-h application (occlusive patch) to clipped skin of back	Practically no skin irritation potential. Primary irritation index = 0.17 (max = 8)	61
9 Rabbits (strain not stated)	0.3% Benzalkonium Chloride	Single 24-h application (occlusive patch) to clipped skin of back	No skin irritation	62
Rabbits (no. and strain not stated)	0.1% Benzalkonium Chloride	Applied to skin for 5 days. Sites covered with plastic wrap	Slight erythema and necrosis persisted 3 wks after treatment period	59
Albino guinea pigs (no. and strain not stated)	5.0, 1.0, and 0.1% Benzalkonium Chloride	Applied to epilated flanks via patches (filter paper). Patches removed after 24 h and skin specimens excised and examined microscopically	5.0% solution caused total necrosis of epidermis. 1.0% solution caused spotted areas of necrosis in epidermis	63
30 Guinea pigs (strain not stated)	0.5 and 0.1% Benzalkonium Chloride	Two and 5 applications of 0.5 and 0.1% solutions, respectively	Hyperemia observed in all animals	64
40 White rats (strain not stated)	1.0% Benzalkonium Chloride	Solutions applied over 3-month period (method not stated)	1.0% solution induced hyperemia and necrotic changes	64

the skins of 30 guinea pigs (strain not stated) at concentrations of 0.5 and 0.1%. Two applications of the 0.5% solution and five applications of the 0.1% solution were made. Hyperemia was observed in all animals (Table 9).<sup>64</sup>

The skin irritation potential of 0.5% Benzalkonium Chloride was evaluated in rabbits (number and strain not stated). Following a single 24-h application of the test substance (0.5 ml), severe erythema, eschar formation, and moderate edema were noted (Table 9).<sup>59</sup>

The skin irritation potential of Benzalkonium Chloride was evaluated using nine female albino rabbits. A 50.0% Benzalkonium Chloride solution was tested at a concentration of 1.0% in water (effective Benzalkonium Chloride concentration = 0.5%). The test solution (0.5 ml) was applied once to the back (clipped skin) of each animal via an occlusive patch. Patches remained for 24 h. Each site was scored 2 and 24 h after patch removal according to the scale 0 (no irritation or edema) to 4 (deep red erythema with vesiculation or weeping, with or without edema). Barely perceptible erythema was noted in one rabbit at 2 and 24 h postapplication. There were no other observations of skin irritation. The primary irritation index was 0.17 (maximum = 8). The test solution had practically no potential for inducing skin irritation.<sup>61</sup> In another skin irritation study, a 50.0% Benzalkonium Chloride solution was tested at a concentration of 0.65% in water (effective Benzalkonium Chloride concentration = 0.3%) according to the same protocol. Skin irritation was not observed in any of the 9 rabbits tested (Table 9).<sup>62</sup>

In another study, the skin irritation potential of a moisturizing cream containing 0.13% Benzalkonium Chloride was evaluated using six female New Zealand White rabbits (2.6–3.1 kg). The cream (0.5 ml) was applied via a topical, dry patch to dorsal skin that had been clipped free of hair. A total of three 24-h applications were made. Sites were scored 30 min after patch removal according to the scales 0 (no erythema) to 4 (severe erythema to slight eschar formation); 0 (no edema) to 4 (severe edema). No positive reactions were observed.<sup>65</sup>

A 0.1% aqueous solution of Benzalkonium Chloride was applied to the skins of rabbits (number and strain not stated); sites were covered with plastic wrap. The test substance remained in contact with the skin for 5 days. At the end of the treatment period, necrosis and varying degrees of erythema, with diffuse areas of eschar and bleeding, were noted. Slight erythema and necrosis persisted for 3 weeks posttreatment. No systemic toxic signs or hematologic changes were observed (Table 9).<sup>59</sup>

### Teratogenicity

Single doses (0, 25, 50, 100, and 200 mg/kg) of aqueous Benzalkonium Chloride solutions were instilled (1 ml/kg) into the vaginas of adult, nulliparous female Wistar rats (169–203 g; groups of 6–8 rats). Dams were killed via CO<sub>2</sub> inhalation on day 21 of gestation. The fetuses were removed by cesarean section and all were examined for viability and external malformations. Two-thirds of the live fetuses from each litter were examined stereomicroscopically for skeletal abnormalities after staining with Alizarin red S. The remainder were fixed in Bouin's fluid and examined for visceral anomalies, using the

freehand razor blade sectioning method of Wilson.<sup>66</sup> A significant reduction in maternal body weight gain was noted on day 6 of pregnancy in dams receiving the 200 mg/kg dose. On days 15 and 20, maternal body weights, when compared to controls, were markedly reduced in groups receiving 100 and 200 mg/kg doses. Reductions in body weight were attributed to small litter sizes and decreased litter weights, since postcesarean body weights, without uterine contents, were similar to those of the control group. Also, vaginas of all necropsied rats given the 100 and 200 mg/kg doses were inflamed. Statistically significant, dosage-related reductions in the mean numbers of live fetuses and litter weights were noted in groups dosed with 50, 100, or 200 mg/kg solutions. No visceral anomalies were observed in Benzalkonium Chloride-exposed fetuses; however, sternal defects (absent, or non-aligned sternbrae, or retarded ossification) were more frequent in the fetuses of 100 and 200 mg/kg-treated dams. There was also a reduction in the number of implantations in treated animals. The mean number of implantations in dams treated with the 200 mg/kg solution was significantly reduced in comparison with the control group ( $5.4 \pm 1.1$  vs.  $10.8 \pm 0.5$ ;  $p < 0.05$ ).<sup>67</sup>

### Mutagenicity

The mutagenic potential of Benzalkonium Chloride was evaluated in microbial test systems using the rec-assay in combination with reverse mutation systems.<sup>68</sup> The rec-assay is a simple method capable of detecting DNA-damaging capacity by analyzing differences in growth sensitivities of Rec<sup>+</sup> and Rec<sup>-</sup> mutant cells of *Bacillus subtilis*. The bacterial strains used in the reverse mutation systems were TA 1535, 1536, 1537, and 1538 (*Salmonella typhimurium*), and two tryptophan-requiring mutants of *E. coli* (B/r WP2 hcr<sup>+</sup> and WP2 hcr<sup>-</sup>). Benzalkonium Chloride was not mutagenic in this test system (Table 10).<sup>69</sup>

Both reversion and rec-assays were used to evaluate the mutagenic potential of Benzalkonium Chloride in another study. In the reversion assays, two tryptophan-requiring strains of *Escherichia coli* (B/r try WP2 and WP2 try hcr) and four strains of *Salmonella typhimurium* requiring histidine and biotin (TA 1535, TA 1536, TA 1537, and TA 1538) were used. A 0.1 ml sample of each bacterial culture was incubated on agar with 0.02 ml of the test solution for 2 days at 37°C. *Bacillus subtilis* strains H17 rec<sup>+</sup> and M45 rec<sup>-</sup> were used for the rec-assay. The rec assay was essentially based on the procedure by Kada.<sup>68</sup> Two cultures were incubated on agar for 24 h with 0.02 ml of the test substance at 37°C. Both assays were done in the absence of metabolic activation. Benzalkonium Chloride was not found to be mutagenic (Table 10).<sup>70</sup>

The mutagenic potential of Benzalkonium Chloride was evaluated in the standard plate incorporation assay and the Rosenkranz *E. coli* DNA polymerase A<sup>-</sup> assay. *Salmonella typhimurium* strains TA 98, TA 1538, TA 1537, and TA 100 were tested in the plate incorporation assay.<sup>72</sup> Each strain was incubated with the test substance for 48 h (37°C) in the presence or absence of metabolic activation. Incubation was carried out either in the dark or in the presence of a combination of fluorescent (15 W) and incandescent (40 W)

**TABLE 10.** Mutagenicity of Benzalkonium Chloride

<i>Bacterial strains</i>	<i>Methodology</i>	<i>Results</i>	<i>References</i>
<i>Salmonella typhimurium</i> strains: TA 1535, TA 1536, TA 1537, and TA 1538	Rec-Assay in combination with reverse mutation systems (Kada, 1972)	Not mutagenic	69
<i>E. coli</i> strains: B/r WP2 hcr <sup>+</sup> and WP2 hcr	Rec-Assay in combination with reverse mutation systems (Kada, 1972)	Not mutagenic	69
<i>E. coli</i> strains: B/r try WP2 and WP2 try hcr	Reversion Assay: 0.1 ml sample of each culture incubated on agar with 0.2 ml of Benzalkonium Chloride for 2 days (37°C)	Not mutagenic	70
<i>Salmonella typhimurium</i> strains: TA 1535, TA 1536, TA 1537, and TA 1538	Reversion Assay: 0.1 ml sample of each culture incubated on agar with 0.2 ml of Benzalkonium Chloride for 2 days (37°C)	Not mutagenic	70
<i>Bacillus subtilis</i> strains: H17 rec <sup>+</sup> and M45 rec <sup>-</sup>	Rec-Assay (Kada, 1972)	Not mutagenic	70
<i>Salmonella typhimurium</i> strains: TA 98, TA 1538, TA 1537, and TA 100	Plate incorporation assay (Ames et al., 1975)	Not mutagenic	71
<i>E. coli</i> strains: W3110 (polA <sup>+</sup> ) and p3478 (polA <sup>-</sup> )	<i>E. coli</i> DNA polymerase assay (Rosenkranz et al., 1976)	Genetic toxicity	71

lights (350–750 nm emission at 24 inches). The plates were then scored for His<sup>+</sup> revertant colonies. Benzalkonium Chloride (10–100 µg/plate) did not induce mutagenicity in any of the strains in the presence or absence of metabolic activation. The *E. coli* DNA polymerase assay<sup>73</sup> was used because it detects repairable DNA damage and complements the Ames assay. Strains W3110 (pol A<sup>+</sup>) (wild-type) and p 3478 (pol A<sup>-</sup>) of *E. coli* were each incubated with 20 µl of Benzalkonium Chloride for 24 h (37°C), either in the dark, or illuminated according to the procedure described above for the *Salmonella* assay. For a given treatment, a zone of inhibition on a plate containing the polymerase-deficient strain that was larger than that on plates containing the wild-type strain was an indication of genetic toxicity. Benzalkonium Chloride induced repairable DNA damage. Its genetic toxicity was also enhanced in the presence of visible light (Table 10).<sup>71</sup>

### Tumorigenicity

The tumorigenicity of Benzalkonium Chloride was evaluated in a dermal study involving 100 Swiss mice (female) and 10 New Zealand rabbits (8 weeks old, both sexes). Half of the mice and rabbits were treated with 8.5% Benzalkonium Chloride, and the remaining half with 17.0% Benzalkonium Chloride. The solvent for both solutions of Benzalkonium Chloride was either acetone or methanol. The solutions were applied (volume = 0.2 ml) to the backs of mice and to the left ear of each rabbit twice per week. None of the animals survived 80 weeks (mice) and 90 weeks (rabbits) of treatment. The untreated control groups consisted of 100 mice and 19 rabbits. Positive control groups were treated with 0.1% (40 mice) and 1.0% 9,10-dimethylbenz(a)anthracene (15 rabbits). Tumors and lesions were recorded weekly and a complete necropsy was performed on each animal. Skin samples, grossly observed tumors, the lungs, liver, kidneys, and other organs were studied microscopically. A significant decrease in the survival rates of mice and rabbits that was directly attributable to Benzalkonium Chloride was not observed. Benzalkonium Chloride induced ulceration and inflammation in mice and rabbits, but no tumors.<sup>74</sup>

## CLINICAL ASSESSMENT OF SAFETY

### Ocular Irritation and Intraocular Toxicity

The ocular irritation potential of 0.02% Benzalkonium Chloride (in 0.9% saline) was evaluated in 51 subjects. Benzalkonium Chloride was instilled into one eye and the control solution (0.9% saline) into the other. Following the instillation of test and control solutions (volumes not stated), subjects were asked how their eyes felt. Fourteen subjects experienced irritation in the eye treated with Benzalkonium Chloride solution. Ten of the 14 subjects also experienced irritation in the control eye. The only clinical evidence of ocular irritation was slight conjunctival hyperemia in the eye of one subject treated with Benzalkonium Chloride solution (Table 11).<sup>75</sup>

**TABLE 11.** Clinical Assessment of Safety

<i>Type of study</i>	<i>No. of subjects</i>	<i>Test substance</i>	<i>Methodology</i>	<i>Results</i>	<i>References</i>
Ocular irritation	51	0.02% Benzalkonium Chloride	Instilled into 1 eye	Slight conjunctival hyperemia in 1 subject	75
Intraocular toxicity	10	Ophthalmic solution containing Benzalkonium Chloride (.01 mg/ml)	One drop instilled into 1 eye twice daily for 2 wks. Corneal endothelium of each eye photographed with specular microscope before and after treatment	Qualitative analysis of photomicrographs revealed no damage to corneal endothelium	76
Skin irritation	399	Benzalkonium Chloride	American Contact Dermatitis Group and International Contact Dermatitis Group Procedures	Cutaneous reactions in 2 patients	77
Skin irritation	13	10% Benzalkonium Chloride	Patches (type not stated) placed on forearm and removed after 24 h	Primary irritant dermatitis in all patients	78
Skin irritation	12	10% Benzalkonium Chloride	Patches (type not stated) placed on abdominal skin and removed after 24 h	Primary irritant dermatitis in all patients	79
Skin irritation	70	2.5% Benzalkonium Chloride	Patches (type not stated) applied to each patient. Sites evaluated 24 and 48 h after application	Skin irritation in 33 patients	80
Skin irritation	55	2.0, 1.0, 0.5, and 0.1% Benzalkonium Chloride	Simultaneously applied to upper back. Patches sealed in place with tape and removed after 48 h	26 patients had pustular and/or bullous reactions to 0.5, 1.0, and 2.0% Benzalkonium Chloride	81
Skin irritation	21	17% Benzalkonium Chloride	Applied (no patches) to forearm and labia majora. Sites graded 24 and 48 h after treatment	Fourteen subjects with reactions ranging from barely perceptible erythema to erythema with infiltration (labial site). Six subjects with reactions ranging from barely perceptible erythema to erythema (forearm site). At most, a mild irritant	82
Skin irritation	5	5.0, 2.5, and 0.5% Benzalkonium Chloride	Solutions applied to foam-filled plastic wells taped to abdominal skin. Wells removed after 12 h	5.0 and 2.5% solutions induced skin irritation in all subjects	83
Skin irritation	—	5.0 and 1.0% Benzalkonium Chloride	Applied to upper back (6-h exposure) daily for 4 days	Skin irritation	84
Skin irritation	200	0.5% Benzalkonium Chloride	Patches (type not stated) applied to upper arm and removed after 48 h	Mean erythema score = 3 (erythema, homogeneous)	85

TABLE 11. *Continued*

Skin irritation	10	0.1% Benzalkonium Chloride	21-day cumulative irritation test. Closed patches remained for 23 h daily	No evidence of cumulative irritation	86
Skin irritation and sensitization	101	Cream containing 0.1% Benzalkonium Chloride	Applied via semioclusive patches to back or arm during 6-wk period. Patches removed 24 h after application	Cream was not irritant or contact sensitizer	87
Skin sensitization	100	0.07, 0.05, 0.025, 0.01, and 0.005% Benzalkonium Chloride	Patients treated for conjunctivitis with different preparations containing Benzalkonium Chloride (conc. not stated) for 3 mos or longer. Patients patch-tested with 0.07% Benzalkonium Chloride after treatment. Patients with positive reactions to 0.07% Benzalkonium Chloride patch tested with 0.05, 0.025, 0.01, and 0.005% Benzalkonium Chloride	Positive reactions to 0.07, 0.05, 0.025, and 0.1% Benzalkonium Chloride (6 patients). Two of the 6 had positive reactions to 0.005% Benzalkonium Chloride	88
Skin sensitization	2806	0.1% Benzalkonium Chloride	Patch tested according to Standard International Contact Dermatitis Research Group procedure	66 of the 2806 eczema patients were sensitive to Benzalkonium Chloride	89
Skin sensitization	142	0.1% Benzalkonium Chloride	Patch-tested according to chamber test procedure (Pirila, 1975)	Nine of the 142 patients with external otitis had allergic reactions to Benzalkonium Chloride	90
Skin sensitization	8	Contact lens solution containing Benzalkonium Chloride (conc. not stated) and 0.1% Benzalkonium Chloride	Patch-tested (types of patches not stated) with 0.1% Benzalkonium Chloride and contact lens solution	Allergic conjunctivitis in 3 patients using contact lens solution. Positive reactions in 3 patients patch tested with lens solution and 0.1% Benzalkonium Chloride	91
Skin sensitization	5	0.1 and 0.01% Benzalkonium Chloride	Patch tests (procedure not stated)	All patients had + or ++ allergic reactions to 0.1% Benzalkonium Chloride. Two patients had + reaction to 0.01% Benzalkonium Chloride	92
Skin sensitization	110	0.1% Benzalkonium Chloride	Patch tests (procedure not stated)	One of 110 patients with dermatitis had positive reactions	92
Skin sensitization	130	0.1% Benzalkonium Chloride	Patch tests (procedure not stated)	No positive reactions	92

The effect of Benzalkonium Chloride on the corneal endothelium was evaluated in 10 subjects (male and female; mean age 24.5 years). One drop of an ophthalmic solution containing Benzalkonium Chloride (0.1 mg/ml) was instilled into one eye of each subject twice daily for 2 weeks. A control group of 10 subjects received the same ophthalmic solution without Benzalkonium Chloride. The central corneal endothelium of each eye was photographed twice, once before and once after the treatment period, with a modified contact specular microscope; specular photomicrographs were enlarged to paper prints for computerized image analysis.<sup>93</sup> No abnormalities on the cellular mosaics were observed before or after the treatment period (Table 11).<sup>76</sup>

### Skin Irritation

Patients with dermatitis (399) were patch tested with various cosmetic ingredients over a period of 64 months. Patch tests were conducted according to American Contact Dermatitis Group and International Contact Dermatitis Group procedures. Cutaneous reactions to Benzalkonium Chloride were observed in two patients (Table 11).<sup>77</sup>

Primary irritant dermatitis was observed in 13 patients patch tested with 10% aqueous Benzalkonium Chloride. None of the subjects had previously been exposed to the test substance and all had local noninflammatory skin conditions (arms not affected). Patches were placed on the flexor aspect of the forearm and removed after 24 h (Table 11).<sup>78</sup>

In another study, primary irritant dermatitis was observed on the abdominal skin of each of 12 patients (53–75 years old) patch tested with 10.0% aqueous Benzalkonium Chloride (24-h exposure). The patients had venous leg ulcers, but no other skin diseases, prior to exposure. None of the patients had previously been exposed to Benzalkonium Chloride. Reactions of erythema, infiltration, and vesicle formation were graded according to the scale: none (–), weak (+), medium strong (++), and strong (+++). Nine patients had medium strong to strong erythema, while vesiculation was noted in four patients (Table 11).<sup>79</sup>

The skin irritation potential of 2.5% aqueous Benzalkonium Chloride was evaluated in 70 hospital patients with lepromatous leprosy. The patients were classified into two groups according to the clinical leproma pattern: 32 patients without active clinical signs, but with various dystrophic sequelae; and 38 patients with active lesions that were positive for acid-fast bacilli; patients either had or had not been treated for disease symptoms. Fifty patients served as controls. Patch tests were placed on each subject and sites were evaluated 24 and 48 h after application. Observations in the 32 patients without active clinical signs were as follows: no reaction (17 patients), erythema (6 patients), erythema and exudation (7 patients), erythema and bullae with a serous or purulent content (2 patients). Observations in the group with active lesions (38 patients) included: no reaction (20 patients), erythema (12 patients), and erythema and exudation (6 patients). Of the 50 control subjects, 17 did not have reactions, 9 had erythema and exudation, and 24 had erythema and bullae with a serous or purulent content (Table 11).<sup>80</sup>

Four concentrations of Benzalkonium Chloride (0.1, 0.5, 1.0, and 2.0% in distilled water) were simultaneously applied to the upper back of each of 55 hospital patients. The patients had various types of skin diseases. Patches (type not stated) containing each solution were sealed in place with tape and removed after 48 h. Twenty-six cases of severe pustular and/or bullous reactions to 0.5, 1.0 and 2.0% Benzalkonium Chloride were reported (Table 11).<sup>81</sup>

Benzalkonium Chloride 10  $\mu$ l (17% in water and ethanol) was applied to the forearm and labia majora of 21 female subjects (22–51 years old). Sites (uncovered) were allowed to dry and then graded 24 h (21 subjects) and 48 h (10 subjects) after treatment according to the scale: 0 (no reaction),  $\pm$  (barely perceptible erythema and/or pigmentation), 1 (erythema, covering the test site), 2 (erythema, infiltration), and 3 (erythema, infiltration, vesicles). The scores for labial and forearm skin at 24 h posttreatment were as follows: 0 (7 subjects, labial skin),  $\pm$  (2 subjects, labial), 1 (4 subjects, labial), 2 (8 subjects, labial), 0 (15 subjects, forearm skin),  $\pm$  (4 subjects, forearm), and 1 (2 subjects, forearm). The scores for labial and forearm skin at 48 h were: 0 (6 subjects, labial skin),  $\pm$  (1 subject, labial), 1 (1 subject, labial), 3 (2 subjects, labial) and 0 (10 subjects, forearm skin) (Table 11).<sup>82</sup>

Confluent erythema and edema with papules were observed in 5 subjects (mean age = 41) tested with 5.0 and 2.5% aqueous Benzalkonium Chloride solutions. However, no reactions were noted after the application of 0.5% aqueous Benzalkonium Chloride. The solutions were each applied to foam-filled plastic wells that were taped to clinically normal abdominal skin. Control wells contained either water or foam inserts. All wells were removed after 12 h of contact (Table 11).<sup>83</sup>

The effect of Benzalkonium Chloride (1.0 and 5.0%) on human epidermal mitosis was evaluated in healthy adult male subjects (number not stated). Both concentrations were applied (occlusive patches, upper back) over a period of 4 days, each being renewed at 1-day intervals. Each subject served as his own control. Patches were removed 6 h prior to the end of the exposure period, at which time excess compound was removed and 0.5% colcemid cream applied (occlusive patches). Patches remained for 6 h, after which 3 mm punch biopsy specimens taken. Specimens were subjected to the Feulgen test and 12 sections of each were then scanned for mitoses to obtain a mitotic index (mitoses per thousand viable cells). One percent Benzalkonium Chloride had no effect on mitosis. However, 5% Benzalkonium Chloride induced a 10-fold increase in the mitotic index, peaking at about 72 h after the initial application. The increase in the mitotic index was accompanied by intense erythema and occasional blistering (Table 11).<sup>84</sup>

Two-hundred subjects (16–29 years old) were patch tested with 0.5% Benzalkonium Chloride in water. Each patch was applied to the outer aspect of the right upper arm and removed after 48 h. Reactions were scored 24 h after removal according to the scale: 0 (no reaction) to 6 (infiltrated erythema with vesicles, pustules and/or erosion). A mean irritation score of 3 (erythema, homogeneous) for the 200 subjects was reported (Table 11).<sup>85</sup>

The skin irritation potential of a cream containing 0.1% Benzalkonium Chloride was evaluated in 10 subjects (18–59 years old). A closed patch

containing 0.2 ml of the cream was applied to the back of each subject. Patches were removed 23 h after application and sites were washed immediately. Reactions were scored 1 h after patch removal. The cream was applied to the same site on each subject for 21 consecutive days. The grading scale for cumulative irritation ranged from 0 (no irritation) to 630 (primary irritation). The total irritation score (all panelists) for the 21 applications was 20, which was interpreted as essentially no evidence of cumulative irritation (Table 11).<sup>86</sup>

### Skin Irritation and Sensitization

A skin irritation and sensitization study of a cream containing 0.1% Benzalkonium Chloride was conducted with 101 men and women (18–65 years old). The cream (approx. 0.1 ml) was applied via a semioclusive patch to the back or arm of each subject during a 6-week period. During the first 3 weeks of testing, patches were applied on Mondays, Wednesdays, and Fridays and removed 24 h after application. Reactions were scored after patch removal according to the following scale: 0 (negative), 1+ (erythema), 2+ (erythema and edema or induration), 3+ (erythema, edema/induration and vesiculation), and 4+ (erythema, edema/induration, bulla, with or without ulceration). The last induction patches were applied on Monday of week 4 and removed 24 h later. Reactions were scored 48 h after patch removal. On Monday of week 6, a challenge patch was placed on each subject (new site) and removed 48 h later. Reactions were scored 48 and 72 h after application. No significant reactions were observed during induction or challenge phases. The cream was neither an irritant nor an allergic contact sensitizer (Table 11).<sup>87</sup>

### Skin Sensitization

A sensitization study was conducted with 100 patients who had been treated for conjunctivitis (3 months or longer) with different preparations containing Benzalkonium Chloride. All subjects were patch tested with 0.07% aqueous Benzalkonium Chloride at the conclusion of treatment. Six of the patients had positive reactions at 48 and 72 h. They were then tested with 0.05, 0.025, 0.01, and 0.005% Benzalkonium Chloride. All had positive reactions to 0.05, 0.025, and 0.01% Benzalkonium Chloride; two had positive reactions to 0.005% Benzalkonium Chloride (Table 11).<sup>88</sup>

An epidemiologic study was conducted with 2,806 patients (male and female) with eczema. The patients were patch tested with 0.1% Benzalkonium Chloride in petrolatum according to the standard International Contact Dermatitis Research Group procedure. Reactions were scored 48 and 96 h postapplication. For some patients, another scoring was done 8 days after patch removal. Sixty-six (2.13%) of the 2,806 patients were sensitive to Benzalkonium Chloride (Table 11).<sup>89</sup>

Patients with chronic external otitis (142; at least 3 months' duration) were patch tested with 0.1% Benzalkonium Chloride according to the chamber test procedure.<sup>94</sup> The test substance was applied to the back of each subject and removed after 24 h. Results were interpreted at 2, 4, and sometimes 7 days after application. Only edematous, infiltrative, or vesicular reactions, noted

after the 2nd day of application, were considered to be allergic reactions. Reactions of erythema only, or those that had disappeared by the 2nd day, were not considered to be positive responses. Benzalkonium Chloride induced allergic reactions in 9 (6.3%) of the 142 patients (Table 11).<sup>90</sup>

In another study, three of eight patients experienced allergic conjunctivitis after wearing contact lenses that had been soaked in a lens solution containing Benzalkonium Chloride. All three had positive patch test reactions to the solution and to 0.1% Benzalkonium Chloride at 48 h postapplication (Table 11).<sup>91</sup>

Five patients were patch tested with 0.1 and 0.01% Benzalkonium Chloride. The experimental procedure was not stated. All patients had a + or ++ allergic reaction to 0.1% Benzalkonium Chloride. Two patients had a + reaction to 0.01% Benzalkonium Chloride. To assess the incidence of Benzalkonium Chloride sensitivity in the general population, 130 normal subjects were patch tested with 0.1% Benzalkonium Chloride (procedure not stated). At a contact dermatitis clinic 110 patients were also patch tested with 0.1% Benzalkonium Chloride. Sensitivity to Benzalkonium Chloride was not detected in any of the normal subjects. However, a positive reaction was observed in 1 of the 110 patients. Prior to the test, this patient was diagnosed as having eczema secondary to proven contact sensitivity to cetrimide (Table 11).<sup>92</sup>

The skin sensitization potential of a moisturizing cream containing 0.13% Benzalkonium Chloride was evaluated using 150 subjects (18–65 years old). The cream was applied via occlusive patches to the upper back of each subject on Mondays, Wednesdays, and Fridays for 3 consecutive weeks. Each patch remained for 24 h and sites were scored prior to the next patch application. After a 2-week nontreatment period, two challenge patches were applied consecutively to sites adjacent to the original induction sites; patches remained for 48 h. Reactions were scored 48 and 96 h after patch application according to the scale: 0 (no reaction) to 4 (bullae or extensive erosions). No positive reactions to the moisturizing cream were observed.<sup>95</sup> In a similar study (same procedure), a moisturizing cream containing 0.13% Benzalkonium Chloride and a local antiseptic did not induce positive reactions when applied to 155 subjects (18–65 years old).<sup>96</sup>

## SUMMARY

Benzalkonium Chloride is a mixture of alkylbenzyltrimethylammonium chlorides. One method of production entails treatment of a solution of *N*-alkyl-*N*-methylbenzylamine in a suitable organic solvent with methyl chloride; Benzalkonium Chloride precipitates as it is formed. As of 1986, Benzalkonium Chloride was present in 83 cosmetic formulations at concentrations ranging from  $\leq 0.1\%$  to 5%. Its cosmetic uses include foaming and cleansing agent, conditioner, and bactericide. Noncosmetic uses of Benzalkonium Chloride include preservative in ophthalmic solutions, spermicide, and sanitizer for chemically clean surfaces.

Benzalkonium Chloride was not detected in either venous blood or breast milk from women using tampons containing Benzalkonium Chloride (60 mg). Following the instillation of [ $^{14}\text{C}$ ]Benzalkonium Chloride solution onto the corneal surface of rabbits, radioactivity was detected in the corneal epithelium, endothelium, and stroma, and in the bulbar and palpebral conjunctivae. At no time was radioactive material found in the aqueous humor or in any tissues.

No adverse effects were noted when rats and hamsters inhaled a conditioner containing 0.1% Benzalkonium Chloride over a period of 13 consecutive weeks (4 h/day).

Acute oral  $\text{LD}_{50}$ s for rats dosed with Benzalkonium Chloride ranged from 342 to 525 mg/kg.

In a subchronic toxicity study, Benzalkonium Chloride solutions were administered via stomach tube to 40 albino rats for 12 weeks (once/day) at dosages of 50.0 mg/kg (1:20 dilution) and 100.0 mg/kg (1:10 dilution). Two of 20 animals receiving the 100.0 mg/kg dosage died.

In a chronic toxicity study, Benzalkonium Chloride (10.0%) was administered via stomach tube to 18 beagle dogs at dosages of 12.5, 25.0, and 50.0 mg/kg for 52 weeks (once daily). One of six dogs receiving 50 mg/kg dosages and three of six dogs receiving 25 mg/kg dosages died.

The application of 0.1% Benzalkonium Chloride to the round window membrane (middle ear) in guinea pigs resulted in fibrosis of the tympanic cavity, cochlea, and vestibulum, and destruction of vestibular neuroepithelia. Of the 3 exposure periods, 10, 30, and 60 min, the most damage was noted after 60 min.

Of 96 mice receiving dermal applications of 6.5 and 50% Benzalkonium Chloride, 29 died within 72 h after application.

At concentrations of 0.033 and 0.10%, Benzalkonium Chloride caused significant growth retardation of cardiac fibroblasts (rat). Benzalkonium Chloride (1.5 M) was toxic to murine suspension cultures of the P815 tumor cell line. Benzalkonium Chloride concentrations ranging from 0.000022 to 0.000042 M induced hemolysis in defibrinated blood from rabbits.

Benzalkonium Chloride 1% and 2.0% aqueous induced severe iritis and severe conjunctival injection, respectively, when instilled into the conjunctival sac of rabbits twice daily for 7 days. Benzalkonium Chloride (0.3%) induced minimal ocular irritation when instilled once into the eyes of rabbits. Single instillations of 0.1% Benzalkonium Chloride into the conjunctival sac of albino rabbits did not cause ocular irritation. The instillation of 0.1% Benzalkonium Chloride into the conjunctival sacs of rabbits 5 times daily for 1 week resulted in corneal damage. The instillation of 0.01% Benzalkonium Chloride into the conjunctival sacs of rabbits (5 min–6-h period) resulted in corneal damage.

In *in vivo* intraocular toxicity studies, Benzalkonium Chloride concentrations ranging from 0.007 to 10.0% were tested. Four hours after the instillation of 0.5, 1.0, and 10% Benzalkonium Chloride, corneal damage was noted in rabbits and guinea pigs. The ocular administration of 0.5, 1.0, and 2.0% solutions twice daily for 7 days caused conjunctival damage in rabbits. Following the daily administration of 0.007 and 0.1% Benzalkonium Chloride for 2 weeks, retinal detachment was observed in pigmented but not albino rabbits.

In *in vitro* intraocular toxicity studies, the exposure of rabbit corneas to Benzalkonium Chloride concentrations ranging from 0.0001 to 0.01% resulted in corneal damage. Exposure periods ranged from 2 min (0.01%) to 110 min (0.0001%). The longest exposure was 180 min (0.0065% Benzalkonium Chloride).

Benzalkonium Chloride concentrations of 1.0–50% induced reactions ranging from erythema to necrosis when applied (duration not stated) to the skins of rabbits. In another study, 24-h applications of 1.0 to 10.0% Benzalkonium Chloride to the skins of rabbits resulted in severe induration. Benzalkonium Chloride concentrations of 1.0 and 5.0% induced epidermal necrosis when applied (24-h exposure) to the skins of albino guinea pigs. Applications of 2.0% Benzalkonium Chloride to the skins (abraded and intact) of rabbits resulted in severe erythema (2-day application period). Slight erythema was noted 7 days after application. Applications of 1.0% Benzalkonium Chloride to the skins of white rats during a 2-month period caused hyperemia and necrosis. Following applications of 0.5% Benzalkonium Chloride to the skins of rabbits (24 h exposure), severe erythema, moderate edema, and eschar formation were observed. Benzalkonium Chloride (0.5%) resulted in practically no skin irritation when applied to the skins of albino rabbits (24-h exposure). When 0.1% Benzalkonium Chloride was applied to the skins of rabbits (5-day contact period), slight erythema and necrosis were observed. These reactions were observed for 3 weeks posttreatment.

The instillation of 100 or 200 mg/kg of aqueous Benzalkonium Chloride into the vaginas of pregnant rats resulted in sternal defects in the offspring.

Benzalkonium Chloride was not mutagenic to *Salmonella typhimurium* strains TA 1535, TA 1536, TA 1537, and TA 1538 and *E. coli* strains B/r WP2 hcr<sup>+</sup> and WP2 hcr<sup>-</sup> in microbial test systems making up the rec-assay in combination with reverse mutation systems. Mutagenic activity also was not demonstrated in reversion assays involving strains TA 1535, TA 1536, TA 1537, and TA 1538 of *Salmonella typhimurium*, and, in the rec-assay, with *Bacillus subtilis* strains H17 Rec<sup>+</sup> and M45 Rec<sup>-</sup>. In the plate incorporation assay, Benzalkonium Chloride was not mutagenic to *Salmonella typhimurium* strains TA 98, TA 1538, TA 1537, and TA 100. In the *E. coli* DNA polymerase assay Benzalkonium Chloride induced repairable DNA damage in strains W3110 (pol A<sup>+</sup>) and p3478 (pol A<sup>-</sup>).

The dermal application of 8.5 and 17% Benzalkonium Chloride to rabbits and mice did not result in tumor formation or systemic toxic effects, but did produce ulceration and inflammation at the application sites.

Slight conjunctival hyperemia was observed in 1 of 51 human subjects who had received ocular instillations of 0.02% Benzalkonium Chloride.

Cutaneous reactions were observed in 2 of 399 dermatitis patients patch tested with Benzalkonium Chloride over a period of 64 months. In separate studies, primary irritant dermatitis was observed in 13 patients and 12 patients patch tested with 10.0% Benzalkonium Chloride (24-h exposure). In another study, erythema was observed in 33 of 70 leprosy patients patch tested with 2.5% Benzalkonium Chloride. Benzalkonium Chloride concentrations of 0.5, 1.0, and 2.0% induced several pustular and/or bullous reactions in 26 of 55 patients (48-h exposures).

The application of 17.0% Benzalkonium Chloride (24-hour period) to the skin of each of 21 subjects resulted in well-defined erythema (13 subjects). Confluent erythema and edema were noted in the skin of subjects tested with 5.0 and 2.5% Benzalkonium Chloride (12-h exposure). Results from a 21-day skin irritation study of a cream containing 0.1% Benzalkonium Chloride indicated essentially no cumulative irritation.

A cream containing 0.1% Benzalkonium Chloride did not induce skin irritation or sensitization reactions in 101 subjects patch tested during a 6-week period (24-h exposures).

Sensitization reactions were observed in 6 of 100 patients patch-tested with 0.07% Benzalkonium Chloride. The 6 patients also had positive reactions to 0.05, 0.025, and 0.01% Benzalkonium Chloride. Sixty-six of 2,806 patients were sensitive to 0.1% Benzalkonium Chloride. In another study, allergic reactions were observed in 9 of 142 patients patch tested with 0.1% Benzalkonium Chloride. Sensitization reactions were not observed in normal subjects patch-tested with 0.1% Benzalkonium Chloride.

## DISCUSSION

Skin irritation studies in humans involved patients and normal subjects. Patients were tested with Benzalkonium Chloride concentrations ranging from 0.1 to 10.0%, and normal subjects with concentrations of 0.1 to 17.0%. Skin irritation was noted in both populations after applications of Benzalkonium Chloride concentrations greater than 0.1%. Skin irritation and ocular irritation were usually noted in animals when Benzalkonium Chloride was tested at concentrations greater than 0.1%.

Skin sensitization was noted in patients tested with Benzalkonium Chloride concentrations ranging from 0.01 to 0.7%. However, there was no incidence of skin sensitization in a population of normal subjects tested with 0.1% Benzalkonium Chloride. Individuals with diseased skin may be at risk for sensitization to Benzalkonium Chloride.

The Expert Panel recognizes that some of the products tested contained concentrations of Benzalkonium Chloride greater than 0.1%. If these products contain proteins or other agents that bind Benzalkonium Chloride, Benzalkonium Chloride concentrations greater than 0.1% would have to be added to yield 0.1% free Benzalkonium Chloride. It is important to note that only free Benzalkonium Chloride is effective as an antimicrobial agent and, also, that the free agent induces dermal toxicity.

## CONCLUSION

On the basis of the data presented in this report, the CIR Expert Panel concludes that Benzalkonium Chloride, at concentrations up to 0.1% free, active ingredient, is safe as a cosmetic ingredient as presently used.

## ACKNOWLEDGMENT

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## REFERENCES

1. GOSSELIN, R.E., HODGE, H.C., SMITH, R.P., and GLEASON, M.N. (1976). *Clinical Toxicology of Commercial Products*. Baltimore, Williams & Wilkins Co., Section III, p. 59, Section II, p. 182.
2. ESTRIN, N.F., CROSLLEY, P.A., and HAYNES, C.R. (1982). *Cosmetic Ingredient Dictionary*. Washington, DC, The Cosmetic, Toiletry and Fragrance Association, Inc., p. 25.
3. THE NATIONAL FORMULARY. (1979). United States Pharmacopeial Convention, Inc., p. 1211.
4. COSMETIC, TOILETRY AND FRAGRANCE ASSOCIATION (CTFA). (no date). *Cosmetic Ingredient Chemical Description of Benzalkonium Chloride*. CTFA Code No. 3-20-5.\*
5. OSOL, A., CHASE, G.D., GENNARO, A.R., GIBSON, M.R., GRANBERG, C.B., HARVEY, S.C., KING, R.E., MARTIN, A.N., SWINYARD, E.A., and ZINK, G.L. (1980). *Remington's Pharmaceutical Science*, 16th edition. Philadelphia, Philadelphia College of Pharmacy and Science, p. 1100.
6. CTFA. (1988). Submission of unpublished data. UV spectral analysis of Benzalkonium Chloride. No CTFA Code No.\*
7. HUNTING, A.L.L. (1983). *Encyclopedia of Shampoo Ingredients*. Micelle Press, Inc., p. 158.
8. WINDHOLZ, M., BUDAVARI, S., BLUMETTI, R.F., and OTTERBEIN, F.A. (1983). *The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals*, 10th edition. Rahway, N.J., Merck and Co., Inc., p. 150.
9. KABARA, J.J. (1984). *Cosmetic and Drug Preservation Principles and Practice*, New York, Marcel Dekker, Volume 1, p. 731.
10. TOXICOLOGY DATA BANK. (1986). Properties of Benzalkonium Chloride. National Library of Medicine computer printout.
11. CHRAI, S., GUPTA, S., and BRYCHTA, K. (1977). Interaction of label adhesive with benzalkonium chloride—preserved ophthalmic solutions packaged in plastic bottles. *Bull. Parenter. Drug Assoc.* **31**(4), 195–200.
12. KAWASE, S., and UKAI, S. (1981). Forensic chemical studies on drugs. V. Gas chromatographic analysis of Benzalkonium Chloride. *Eisei Kagaku* **27**(5), 296–302.
13. CYBULSKI, Z.R. (1984). Determination of Benzalkonium Chloride by gas chromatography. *J. Pharm. Sci.* **73**(12), 1700–2.
14. SRIEWOFIAN, S., and CURTO, M. (1984). Determination of benzocaine and Benzalkonium Chloride by gas chromatography. *Acta Pharm. Indones.* **9**(4), 164–5.
15. MEYER, R.C. (1980). Determination of Benzalkonium Chloride by reversed phase high pressure liquid chromatography. *J. Pharm. Sci.* **69**, 1148–50.
16. MARSH, D.F., and TAKAHASHI, L.T. (1983). Determination of Benzalkonium Chloride in the presence of interfering alkaloids and polymeric substrates by reverse-phase high-performance liquid chromatography. *J. Pharm. Sci.* **72**(5), 521–5.
17. EURBY M.R. (1985). High performance liquid chromatography of Benzalkonium Chlorides—variations in commercial preparations. *J. Clin. Hosp. Pharm.* **10**, 73–77.
18. NAGAYOSHI, M., SUZUKI, K., and KASHIWA, T. (1975). Systemic qualitative and quantitative analysis of pesticides. Part 10. Classification of pesticides by thin-layer chromatography. *Noyaku Kensasho Hokoku (Bull. Agric. Chem. Inspect. Sth.)* **15**, 22–31.
19. DAOUD, N.N., CROOKS, P.A., SPEAK, R., and GILBERT, P. (1983). Determination of Benzalkonium Chloride by chemical ionization mass spectroscopy. *J. Pharm. Sci.* **72**(3), 290–2.
20. LOWRY, J.B. (1979). Direct spectrophotometric assay of quaternary ammonium compounds using bromthymolblue. *J. Pharm. Sci.* **68**(1), 110–1.
21. FOOD AND DRUG ADMINISTRATION (FDA). (March 13, 1986). *Cosmetic product formulation data*. FDA computer printout.

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\*Available for review: Director, Cosmetic Ingredient Review, 1110 Vermont Ave, N.W., Suite 810, Washington, D.C. 20005

22. FDA. (1981). Indirect food additives: adjuvants, production aids, and sanitizers. Fed. Reg. **46**(38), 60566–67.
23. BROTHERTON, J. (1977). Assessment of spermicides by a stripping technique against human spermatozoa. J. Reprod. Fertil. **51**(2), 383–91.
24. MAIBACH, H.I., and GELLIN, G.A. (1982). *Occupational and Industrial Dermatology*. Chicago, Year Book Medical Publishers, p. 223.
25. FDA. (1982). List of inactive ingredients for approved prescription drug products.
26. FDA. (1984). The Division of Over-the-Counter Drug Evaluation Ingredient Status Report. National Center for Drugs and Biologics. (HFN-510). Food and Drug Administration, Washington, D.C.
27. BLEAU, G. (1983). Recherche du benzalkonium dans le sang de femmes utilisant le tampon pharmatex.
28. BLEAU, G. (1983). Mesure du benzalkonium dans le sang et le lait de femmes utilisant le tampon pharmatex.
29. GREEN, K., and CHAPMAN, J.G. (1986). Benzalkonium Chloride kinetics in young and adult albino and pigmented rabbit eyes. J. Toxicol.—Cut. and Ocul. Toxicol. **5**(2), 133–42.
30. READ, G.W., and KIEFER, E.F. (1979). Benzalkonium Chloride: selective inhibitor of histamine release induced by compound 48/80 and other polyamines. J. Pharmacol. Exp. Ther. **211**(3), 711–15.
31. COLEMAN, J.W. (1982). Neuraminidase- and Benzalkonium Chloride-dependent inhibition of basic peptide-induced rat mast cell secretion. Immunol. Lett. **5**(4), 197–201.
32. FELDBAU, E., and SCHWABE, C. (1971). Selective inhibition of serine proteases by alkyl-dimethylbenzylammonium chloride. Biochemistry **10**(11), 2131–8.
33. CTFA. (1979). Submission of unpublished data. Thirteen week subacute inhalation toxicity in the albino rat and golden hamster. CTFA Code No. 3-20-4.\*
34. GUCKLHORN, I.R. (1969). Antimicrobials in cosmetics. Part I. Mfg. Chemist Aerosol News **40**, 23–30.
35. CUMMINS, L.M., and KIMURA, E.T. (1971). Safety evaluation of selenium sulfide antidandruff shampoos. Toxicol. Appl. Pharmacol. **20**(1), 89–96.
36. CTFA. (1985). Submission of unpublished data by CTFA. Acute oral toxicity of a moisturizing cream containing 0.13% benzalkonium chloride. No CTFA Code No.\*
37. CTFA. (1980). Submission of unpublished data. Acute oral toxicity testing of a cream containing 0.1% Benzalkonium Chloride. No CTFA Code Number.\*
38. COULSTON, F., DROBECK, H.P., MIELENS, Z.E., and GARVIN, P.J. (1961). Toxicology of benzalkonium chloride given orally in milk or water to rats and dogs. Toxicol. Appl. Pharmacol. **3**, 584–94.
39. AURNES, J. (1982). Ototoxic effect of quaternary ammonium compounds. Acta Otolaryngol (Stockh.) **93**(5-6), 421–33.
40. SERRANO, L.J. (1972). Dermatitis and death in mice accidentally exposed to quaternary ammonium disinfectant. J. Am. Vet. Med. Assoc. **161**(6), 652–5.
41. BENGMARK, S., INGEMANSSON, B., and KALLEN, B. (1959). Endocrine dependence of rat prostatic tissue in vitro. Acta Endocrinol. (Copenh.) **30**, 459–71.
42. BENGMARK, S., and RYDBERG, B. (1968). Cytotoxic action of cationic detergents Acta Chir. (Scand.) **134**(1), 1–5.
43. TAKAHASHI, N. (1982). Quantitative cytotoxicity of preservatives evaluated in cell culture with Chang's human conjunctival cells—effect of temperature on cytotoxicity. Jpn. J. Ophthalmol. **26**(2), 234–8.
44. SHADDUCK, J.A., EVERITT, J., and MECCOLI, R.A. (1985). *In vitro* and *in vivo* eye irritation of six surface active agents. Soap Cosmet. Chem. Spec. **61**(11), 36–8, 56.
45. ANSEL, H.C., and CABRE, G.E. (1970). Influence of dimethyl sulfoxide on the hemolytic activity of antimicrobial preservatives. I. J. Pharm. Sci. **59**(4), 478–81.
46. GASSETT, A.R., ISHII, Y., KAUFMAN, H.E., and MILLER, T. (1974). Cytotoxicity of ophthalmic preservatives. Am. J. Ophthalmol. **78**(1), 98–105.
47. DRAIZE, J.H. (1959). In: *Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics*. Austin, TX, The Association of Food and Drug Officials of the United States.
48. CTFA. (1977). Submission of unpublished data. Ocular irritation study of 50.0% Benzalkonium Chloride. CTFA Code No. 3-20-1.\*
49. CTFA. (1985). Submission of unpublished data by CTFA. Ocular irritation study of a moisturizing cream containing 0.13% benzalkonium chloride. No CTFA Code No.\*
50. DRAIZE, J.H., WOODARD, G., and CALVERY, H.O. (1944). Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J. Pharmacol. Exp. Ther. **82**, 377–90.
51. GRIFFITH, J.F., NIXON, G.A., BRUCE, R.D., REER, P.J., and BANNAN, E.A. (1980). Dose-response studies

- with chemical irritants in the albino rabbit eye as a basis for selecting optimum testing conditions for predicting hazard to the human eye. *Toxicol. Appl. Pharmacol.* **55**, 501–13.
52. MATSUURA, T., TSUYOSI, T., and MASAMOTO, Y. (1983). Study for the influence of the surfactants on rabbit and guinea pig eyes. *J. Soc. Chem. Jpn.* **17**(2), 153–63.
  53. PFISTER, R.R. (1973). The normal surface of corneal epithelium: a scanning electron microscopic study. *Invest. Ophthalmol.* **12**, 654.
  54. PFISTER, R.R., and BURSTEIN, N. (1976). The effects of ophthalmic drugs, vehicles and preservatives on corneal epithelium: a scanning electron microscope study. *Invest. Ophthalmol.* **15**(4), 246–59.
  55. LAVINE, J.B., BINDER, P.S., and WICKHAM, W.G. (1979). Antimicrobials and the corneal endothelium. *Ann. Ophthalmol.* **11**(10), 1517–28.
  56. CHOU, A., HORI, S., and TAKASE, M. (1985). Ocular toxicity of beta-blockers and Benzalkonium Chloride in pigmented rabbits: electrophysiological and morphological studies. *Jpn. J. Ophthalmol.* **29**(1), 13–23.
  57. BURSTEIN, N.L., and KLYCE, S.D. (1977). Electrophysiologic and morphologic effects of ophthalmic preparations on rabbit cornea epithelium. *Invest. Ophthalmol. Vis. Sci.* **16**(10), 899–911.
  58. GREEN, K., HULL, D.S., VAUGHN, E.D., MALIZIA, A.A., and BOWMAN, K. (1977). Rabbit endothelial response to ophthalmic preservatives. *Arch. Ophthalmol.* **95**(12), 2218–21.
  59. BERNHOLC, N.M. (1985). Benzalkonium Chloride. Health hazard evaluation report. Gov. Rep. Announ. **24**, 1–15.
  60. WAHLBERG, J.E., and MAIBACH, H.I. (1981). Sterile cutaneous pustules: a manifestation of primary irritancy. Identification of contact pustulogens. *J. Invest. Dermatol.* **76**, 381–83.
  61. CTFA. (1976). Submission of unpublished data. Primary skin irritation study of 50.0% Benzalkonium Chloride. CTFA Code No. 3-20-3.\*
  62. CTFA. (1977). Submission of unpublished data. Primary skin irritation study of 50.0% Benzalkonium Chloride. CTFA Code No. 3-20-2.\*
  63. GISSL'EN, H., and MAGNUSSON, B. (1966). Effects of detergents on guinea pig skin. Histological studies after single exposure to anionic, cationic and non-ionic surfactants. *Acta Derm. Venereol. (Stockh.)* **46**(4), 269–74.
  64. BEREZOVSKAYA, I.V., BELOSHAPKO, A.A., VLASOVA, M.E., RABINKOV, A.G., RYMARTSEV, V.I., SHIUKASHVILI, N.N., and YATSENKO, I.E. (1978). The overall toxic action of the antiseptics Catamin and Roccal. *Pharm. Chem. J.* **12**, 1593–98.
  65. CTFA. (1985). Submission of unpublished data by CTFA. Skin irritation study of a moisturizing cream containing 0.13% benzalkonium chloride. No CTFA Code No.\*
  66. WILSON, J.G. (1965). Methods for administering agents and detecting malformations in experimental animals. In: Wilson, J.G., and Warkany, J. (ed.). *Teratology: Principles and Techniques*. Chicago: The University of Chicago Press.
  67. BUTTAR, H.S. (1985). Embryotoxicity of Benzalkonium Chloride in vaginally treated rats. *J. Appl. Toxicol.* **5**(6), 398–401.
  68. KADA, T., TUTIKAWA, K., and SADAIE, Y. (1972). *In vitro* and host-mediated rec-assay procedures for screening chemical mutagens and phloxine, a mutagenic red dye detected. *Mutat. Res.* **16**, 165–74.
  69. SHIRASU, Y. (1975). Significance of mutagenicity testing on pesticides. *Environ. Qual. Safety* **4**, 226–31.
  70. SHIRASU, Y., MORIYA, M., KATO, K., FURUHASHI, A., and KADA, T. (1976). Mutagenicity screening of pesticides in the microbial system. *Mutat. Res.* **40**, 19–30.
  71. LOVELY, T.J., LEVIN, D.E., and KLEKOWSKI, E. (1982). Light-induced genetic toxicity of thimerosal and Benzalkonium Chloride in commercial contact lens solution. *Mutat. Res.* **101**(1), 11–18.
  72. AMES, B.N., McCANN, J., YAMASAKI, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian microsome mutagenicity test. *Mutat. Res.* **31**, 347–64.
  73. ROSENKRANZ, H.S., GUTTER, B., and SPECK, W.T. (1976). Microbial assay procedures: Experience with two systems. In: F.J. DeSerres et al. (eds.). *In Vitro Metabolic Activation in Mutagenesis Testing*. Amsterdam: Elsevier, pp. 337–61.
  74. STENBACK, F. (1977). Local and systemic effects of commonly used cutaneous agents: lifetime studies of 16 compounds in mice and rabbits. *Acta Pharmacol. Toxicol.* **41**(5), 417–31.
  75. BARKMAN, R., GERMANIS, M., KARPE, G., and MALMBORG, S. (1969). Preservatives in eye drops. *Acta Ophthalmol. (Kbh)* **47**(3), 461–75.
  76. ALANKO, H.I., and AIRAKSINEN, P.J. (1983). Effects of topical timolol on corneal endothelial cell morphology *in vivo*. *Am. J. Ophthalmol* **96**(5), 615–21.

77. NORTH AMERICAN CONTACT DERMATITIS GROUP. (1985). A five-year study of cosmetic reactions. *J. Am. Acad. Dermatol.* **13**, 1062–9.
78. SONDERGAARD, J., GREAVES, M.W., and JORGENSEN, H.P. (1974). Recovery of prostaglandins in human primary irritant dermatitis. *Arch. Dermatol.* **110**(4), 556–8.
79. KASSIS, V., MORTENSEN, T., and SONDERGAARD, J. (1981). Prostaglandin E1 in suction-separated human epidermal tissue in primary irritant dermatitis. *Acta Derm. Venereol. (Stockh.)* **61**(5), 429–31.
80. MENEGHINI, C.L., ANGELINI, G., LOSPALLUTI, M., and TRIMIGLIOZZI, G. (1974). Cutaneous responses to irritants and microbial antigens in lepromatous leprosy. *Trans. St. Johns Hosp. Dermatol. Soc.* **60**(1), 91–3.
81. WAHLBERG, J.E., WRANGSJO, K., and HIETASALO, A. (1985). Skin irritancy from nonanoic acid. *Contact Derm.* **13**, 266–69.
82. BRITZ, M.B., and MAIBACH, H.I. (1979). Human cutaneous vulvar reactivity to irritants. *Contact Derm.* **5**(6), 375–77.
83. BARR, R.M., BRAIN, S., CAMP, R.D., CILLIERS, J., GREAVES, M.W., MALLET, A.I., and MISCH, K. (1984). Levels of arachidonic acid and its metabolites in the skin in human allergic and irritant contact dermatitis. *Br. J. Dermatol.* **111**(1), 23–8.
84. FISHER, L.B., and MAIBACH, H.I. (1975). Effect of some irritants on human epidermal mitosis. *Contact Derm.* **1**(5), 273–6.
85. HOLST, R., and MOLLER, H. (1975). One hundred twin pairs patch tested with primary irritants. *Br. J. Dermatol.* **93**(2), 145–9.
86. HILL TOP RESEARCH INC. (1981). Submission of unpublished data by CTFA. Report of a human skin test of cumulative irritation of a cream containing 0.1% Benzalkonium Chloride. No CTFA Code Number.\*
87. CTFA. (1982). Submission of unpublished data. Repeat insult patch testing of a cream containing 0.1% Benzalkonium Chloride. No CTFA Code No.\*
88. AFZELIUS, H., and THULIN, H. (1979). Allergic reactions to Benzalkonium Chloride. *Contact Derm.* **5**(1), 60.
89. CAMARASA, J.M. (1979). First epidemiological study of contact dermatitis in Spain—1977. Spanish Contact Dermatitis Research Group. *Acta Derm. Venereol. [Suppl.] (Stockh.)* **59**(85), 33–7.
90. FRAKI, J.E., KALIMO, K., TUOHIMAA, P., and AANTAA, E. (1985). Contact allergy to various components of topical preparations for treatment of external otitis. *Acta Otolaryngol. (Stockh.)* **100**, 414–18.
91. FISHER, A. (1985). Allergic reactions to contact lens solutions. *Cutis* **36**(3), 209–11.
92. LOVELL, C.R., and STANFORTH, P. (1981). Contact allergy to Benzalkonium Chloride in plaster of Paris. *Contact Derm.* **7**(6), 343–4.
93. ALANKO, H.I. (1983). Microcomputer analysis for corneal endothelial cell morphology. *Acta Ophthalmol.* **61**, 229.
94. PIRILA, V. (1975). Chamber test versus patch test for epicutaneous testing. *Contact Derm.* **1**:48.
95. CTFA. (1985). Submission of unpublished data by CTFA. Repeated insult patch testing of a moisturizing cream containing 0.13% benzalkonium chloride. No CTFA Code No.\*
96. CTFA. (1986). Submission of unpublished data by CTFA. Repeated insult patch testing of a moisturizing cream containing 0.13% benzalkonium chloride and a local antiseptic. No CTFA Code No.\*

## RE-REVIEW DOCUMENT ON BENZALKONIUM CHLORIDE

Benzalkonium Chloride (mixture of alkylbenzyltrimethylammonium chlorides) is listed in the *International Cosmetic Ingredient Dictionary and Handbook* (**Gottschalck and McEwen, 2006**). A CIR Final Safety Assessment on these ingredients was published with the following conclusion: On the basis of the data presented in this report, the CIR Expert Panel concludes that Benzalkonium Chloride, at concentrations up to 0.1% free, active ingredient, is safe as a cosmetic ingredient as presently used. (**Elder, 1989**).

An updated search of the literature was performed to identify studies on Benzalkonium Chloride that have been published since the Panel's Final Safety Assessment was issued or were omitted from that report. These studies are summarized in this re-review document.

### CHEMISTRY

#### DEFINITION

Benzalkonium Chloride (CAS No. 8001-54-5) is a mixture of alkylbenzyltrimethylammonium chlorides (**Gottschalck and McEwen, 2006**).

#### CHEMICAL AND PHYSICAL PROPERTIES

According to **Smith et al. (2002)**, Benzalkonium Chloride can have different alkyl chain lengths ranging from C<sub>8</sub>H<sub>17</sub> to C<sub>18</sub>H<sub>37</sub>, with chain length greatly affecting its chemical properties. The Benzalkonium Chloride materials investigated were mixtures of C<sub>12</sub>H<sub>25</sub> and C<sub>14</sub>H<sub>29</sub> as well as C<sub>14</sub>H<sub>29</sub> on its own. The diaphragm diffusion technique was investigated for its applicability to the measurement of diffusion coefficients of molecules with surfactant properties and the ability to form micelles. The average value of the membrane cell integral diffusion coefficient for Benzalkonium Chloride was 7.78 x 10<sup>-6</sup> cm<sup>2</sup>s<sup>-1</sup> at 25°C, and there was no significant effect of alkyl chain length on the measured value of the membrane cell integral diffusion coefficient.

#### UV ABSORPTION

In a UV spectral analysis of Benzalkonium Chloride by **Withrow et al. (1989)**, the absorption peak was well below 300 nm. There was no measurable absorption above 310 nm.

**Table 1.** Historical and current cosmetic product uses and concentrations for Benzalkonium Chloride

Product Category	1986 uses (Elder, 1989)	2005 uses (FDA, 2005)	1986 concentrations (Elder, 1989) (%)	2006 concentrations (CTFA, 2006) (%)
<b>Baby products</b>				
Lotions, oils, powders, and creams	4*	2	≤0.1 to 1*	
Other				
<b>Eye makeup</b>				
Eye lotion	-	1	-	
Eye makeup remover	-	10	-	
Other	6	-	≤0.1 to 1	
<b>Fragrance Products</b>				
Colognes and toilet waters	-	1	-	
<b>Non-coloring hair products</b>				
Conditioners		12		
Rinses		2		
Tonics, dressings, etc.	45*	7	≤0.1 to 5*	
Shampoos		5		
Other		5		
<b>Hair coloring products</b>				
Bleaches	-	1	-	
<b>Nail care products</b>				
Creams and lotions	-	1		
Other	-	1		
<b>Personal hygiene products</b>				
Underarm deodorants	11*	1	≤0.1 to 1*	
Other		1		
<b>Skin care products</b>				
Skin cleansing creams, lotions, liquids, and pads	6	17	≤0.1 to 1	
Face and neck creams, lotions, powder and sprays		3		
Body and hand creams, lotions, powder and sprays		3		
Foot powders and sprays	-	1	-	
Moisturizers	4	1	≤0.1 to 1	
Night creams, lotions, powders and sprays	22	1	≤0.1 to 1	
Paste masks/mud packs	-	3	-	
Skin fresheners	-	5	-	
Other	7	2	≤0.1 to 1	
<b>Suntan products</b>				
Suntan gels, creams, and liquids	-	1	-	
<b>Total uses/ranges for Benzalkonium Chloride</b>	<b>83</b>	<b>89</b>	<b>≤0.1 to 5</b>	

\* This category was combined when the original safety assessment was performed and is now two or more separate categories.

Benzalkonium Chloride is an ingredient of quasi drugs (in products to be used directly on the body) that are marketed in Japan. Quasi-drugs, by definition, must have a mild effect on the body, but are neither intended for the diagnosis, prevention, or treatment of disease, nor to affect the structure or function of the body (MHLW, 2000).

#### NONCOSMETIC USE

According to Keser et al. (2005), alcohols, chlorhexidine gluconate (CHG) (Hibiscrub), and

## BIOLOGICAL PROPERTIES

### DISTRIBUTION

In a study by **Xue et al. (2004)**, the distribution and disposition of Benzalkonium Chloride after the following routes of administration were evaluated: oral, intravascular (jugular vein, JV), femoral artery (FA), femoral vein (FV) and jugular artery (JA). Seventy-four male Sprague-Dawley rats were used in the study.

Twelve rats (weights = 384 to 440 g) and 18 rats (weights = 310 to 470 g) were used to determine the elimination kinetics and distribution of Benzalkonium Chloride, respectively. Each rat in the kinetic study was implanted with two catheters for drug administration and blood sampling. The rats in the distribution study were implanted with a single catheter for administration. A dose of 15 mg/kg of 1% Benzalkonium Chloride solution was infused over 1 minute via JV or FA. In the kinetic study, the blood samples of 0.1, 0.3, 0.5, 0.5, 0.8, 1.0, and 1.5 ml were collected from the femoral vein at 2, 5, 10, 30, 60, 120, and 180 minutes, respectively.

The fatal effects of Benzalkonium Chloride appeared soon in JV- FV- or JA-rats, but took hours in PO or FA rats. No rat receiving Benzalkonium Chloride via FA survived longer than one day. The PO-rats that aspirated Benzalkonium Chloride into their lungs had some systemic symptoms and higher blood and tissue concentrations of Benzalkonium Chloride. The blood Benzalkonium Chloride levels and kinetics were similar among the different routes of intravascular administration, but the lung and kidney levels were higher in JV-rats. Pathological examinations confirmed severe congestion and edema in the lungs and kidneys. These results suggest the following: (1) the toxic effects of Benzalkonium Chloride varied depending on the route of administration. (2) The degree of toxicity correlated with peak blood and tissue concentrations in orally dosed rats. (3) Different toxicological progressions and manifestations were observed in FA- and JV-dosed rats, even though these groups had similar blood concentration profiles. (4) the lung and kidney are reservoirs for Benzalkonium Chloride and considered to be the target organs of Benzalkonium Chloride (**Xue et al., 2004**).

### SKIN PENETRATION ENHANCEMENT

**van der Bijl et al. (2002)** showed that the permeation of cyclosporin A through intact and de-epithelialized human vaginal mucosa can be enhanced by 0.01% Benzalkonium Chloride. Specimens

were obtained from excess tissue removed from eight postmenopausal patients (mean age: 57 ± 16 years) following vaginal hysterectomies.

#### EFFECT ON CYTOKINES

**Wilmer et al. (1994)** examined qualitative and quantitative changes in selected intracellular and secreted cytokines in human keratinocyte cultures (normal human epidermal keratinocytes from breast skin of adult females) in response to the ulcerative agent Benzalkonium Chloride (dissolved in 0.1% aqueous CrO<sub>3</sub>). The keratinocytes were exposed to Benzalkonium Chloride anywhere from 3 to 48 hours. At a non-cytotoxic concentration (0.1 µg/ml), Benzalkonium Chloride stimulated the production and intracellular accumulation of IL-1α. It appears that the autocrine stimulation of IL-1α by TNFα cannot explain the apparent intracellular increase of IL-1α that was stimulated by Benzalkonium Chloride, which did not induce TNFα production.

Benzalkonium Chloride (concentrations up to 30% in distilled water) was also applied to mouse (specific-pathogen-free BALB/c ANCr female mice; 10 to 16 weeks old) skin to assess whether the chemical-specific pattern of inflammation correlated with the *in vitro* production of keratinocyte-derived cytokines. Specifically, the test substance was applied to the dorsal surface of both ears. Ear thickness was measured using a micrometer, and one ear per mouse was examined microscopically. Although Benzalkonium Chloride elicited neutrophils to the site of chemical application, time-dependent and chemical-specific patterns of inflammation could not be detected (**Wilmer et al., 1994**).

**Corsini et al. (1998)** investigated the effects of five relevant skin allergens (dinitrochlorobenzene, oxazolone, nickel sulfate, penicillin G, and eugenol), two skin irritants (aqueous Benzalkonium Chloride and methylsalicylate) and two compounds with no sensitizing activity (glycerol, and ethanol) on interleukin-1α (IL-1) production in HEL30 cells (C3H mouse-derived keratinocyte cell line). Twenty-four hours following treatment, both IL-1 release in conditioned media and cell-associated IL-1 were measured by a specific sandwich ELISA. Under experimental conditions, only contact sensitizers were able to increase in a dose-dependent fashion cell-associated IL-1. Both skin irritants and allergens induced the release of IL-1, because of the irritative properties of both chemicals, while ethanol and glycerol failed to induce changes in IL-1 production. Taken together, these data indicate

that it may be realistic to consider potential skin allergens those chemicals that are able to increase cell-associated IL-1, to consider skin irritants those chemicals that induce only IL-1 release, and to exclude as potential allergens or irritants those chemicals that fail to induce changes in IL-1 production.

#### INHIBITION OF HISTAMINE RELEASE

In a study by **Niitsuma et al. (1996)**, Benzalkonium Chloride inhibited histamine release from rat peritoneal macrophages induced by plasma bradykinin (BK), oligomer-specific lectin *Datura stramonium* agglutinin, (DSA) synthetic compound 48/80 (48/80), and a polyethylenimine with a molecular weight of 600 (PEI<sub>6</sub>), resulting in 50% inhibition (IC<sub>50</sub>) values of  $0.63 \pm 0.13 \mu\text{g/ml}$  (n = 6),  $3.04 \pm 0.25 \mu\text{g/ml}$  (n = 8),  $1.56 \pm 0.12 \mu\text{g/ml}$  (n = 8), and  $0.48 \pm 0.01 \mu\text{g/ml}$ , respectively. At concentrations of 3 and 6  $\mu\text{g/ml}$ , Benzalkonium Chloride almost completely inhibited the histamine release induced by BK and 48/80. Histamine release induced by DSA was also almost completely inhibited by 6  $\mu\text{g/ml}$  Benzalkonium Chloride.

#### SCLERAL PERMEABILITY ENHANCEMENT

**Okabe et al. (2005)** investigated the effect and safety of Benzalkonium Chloride on transscleral drug delivery in the rabbit after continuous intrascleral administration using *in vitro* and *in vivo* tests. Twenty-five eyes of 30 albino rabbits were used in the *in vitro* scleral permeability experiment. In the experiment investigating the *in vivo* ocular permeability of large molecules, 24 eyes of 24 albino rabbits were used.

Betamethasone 21-phosphate (BP) aqueous solutions, with or without Benzalkonium Chloride (0.01% or 0.05%), were continuously administered to albino rat sclera with an osmotic pump for 1 week. To investigate the effect of Benzalkonium Chloride on the scleral permeability of Benzalkonium Chloride *in vitro*, the penetration of BP aqueous solution with or without Benzalkonium Chloride across the rabbit sclera was evaluated using a two-chamber Ussing apparatus. To determine the effect of Benzalkonium Chloride on transscleral delivery of large molecules, 20- and 70-k-Da fluorescein isothiocyanate aqueous solutions, with or without Benzalkonium Chloride, were continuously administered to the sclera by an osmotic pump.

Benzalkonium Chloride increased concentrations of BP in the vitreous and retina-choroid

compared with the control (physiological saline). BP was not detected in the aqueous humor. In the *in vitro* study, Benzalkonium Chloride did not increase the scleral permeability of BP. In the retina-choroid, Benzalkonium Chloride significantly increased concentrations of FD-20, but did not increase concentrations of FD-20 or -70 in the vitreous. No substantial toxic reactions were observed in the retina in electrophysiological or histologic examinations after the addition of Benzalkonium Chloride.

The results of this study demonstrate that Benzalkonium Chloride may improve the ocular penetration of a drug in a transscleral drug delivery system without producing toxic reactions (**Okabe et al., 2005**).

#### EFFECT ON ENZYME ACTIVITY

**Wenzel et al. (1990)** conducted a systematic study comprising 28 synthetic ionic and cationic surfactants in order to examine their effect on the activity of elastase and cathepsin G from human leukocytes against 4-nitroanilide substrates. The whole spectrum, ranging from a complete loss to a pronounced rise in enzymatic activity, was observed at a 0.1% (w/v) surfactant concentration. Most significantly, Benzalkonium Chloride led to a five-fold increase in elastase activity.

**Jaganathan and Boopathy (2000)** studied the effect of Benzalkonium Chloride on the esterase and aryl acylamidase activities of butyrylcholinesterase (BChE). Acetylcholinesterase (AChE) and BChE from vertebrates display aryl acylamidase activity (AAA) that is capable of hydrolyzing the synthetic substrate *o*-nitroacetanilide to *o*-nitroaniline. This AAA activity is strongly inhibited by classical cholinesterase (ChE) inhibitors. Benzalkonium ( $\leq 4$  to  $16 \mu\text{M}$ ) inhibited the esterase activity of human serum and horse serum BChEs and AChEs from electric eel and human erythrocytes in a concentration-dependent manner. The remarkable property of Benzalkonium Chloride was its ability to profoundly activate the AAA activity of human serum and horse serum BChEs, but not the AAA activity of AChEs. Thus, Benzalkonium Chloride seems to preferentially activate the AAA activity of BChEs alone. The authors noted that this is the first report of a compound that inhibits the esterase activity, while simultaneously activating the AAA activity, of BChEs.

#### EFFECT ON DNA, RNA, AND PROTEIN SYNTHESIS

In a study by **Kajino (1987)**, The exposure of cultured Chinese hamster lung fibroblast cells to Benzalkonium Chloride at doses of 3 to  $30 \mu\text{g/ml}$  for 2 hours elicited inhibition of DNA-, RNA-, and

protein-syntheses, in a dose-related manner. The cytotoxicity of Benzalkonium Chloride was also studied, and the results of the experiment are included in the section on Cytotoxicity later in the report text.

## TOXICOLOGY

A toxicity profile on Benzalkonium Chloride by the British Industrial Biological Research Association (BIBRA) is available, and has been ordered from British Columbia (**BIBRA, 1989**).

### KINETIC CHARACTERISTICS AND TOXIC EFFECTS

**Xue et al. (2004)** investigated the kinetic characteristics and toxic effects of Benzalkonium Chloride following injection via the jugular vein (JV), femoral artery (FA) and oral administration (PO) using male Sprague-Dawley rats (body weights = 310 to 470 g). Both the FA and JV infusion experiments involved 15 rats. The oral dosing experiment involved 34 rats.

In the JV experiment, the elimination kinetics and distribution of Benzalkonium Chloride were studied. The elimination kinetics experiment involved six rats and the distribution experiment involved nine rats. Both groups were infused with a dose of 15 mg/kg, after which blood samples were collected. In the FA experiment (15 rats), the study design was identical to that of the JV experiment, except the sites for a dose (left FA) and blood sampling (right femoral vein). The Benzalkonium Chloride concentrations in blood and tissues (lung, liver, and kidney) were determined by high-performance liquid chromatography with solid phase extraction.

The fatal effects appeared soon after dose administration in JV rats, while the effects were delayed in FA- or PO-rats. After JV administration, most of the rats stopped breathing immediately after the dose, but recovered in 30 to 40 seconds. However, three rats died within 11 minutes, without recovery. After FA administration, all rats appeared to be normal in the initial period, but did not urinate after the dose and gradually became cyanotic. No FA rat died within 4 hours, but all three rats assigned for the distribution study over 24 hours died at 7, 10, and 13 hours (no sample at 24 hours). The blood Benzalkonium Chloride concentrations and the elimination half-lives were similar between JV- and FA-rats, while the distribution of Benzalkonium Chloride in tissues was slightly different.

In the oral dosing experiment (34 rats), fasted rats received a dose of 250 mg/kg

Benzalkonium Chloride by stomach tube. Blood samples were collected for up to 24 hours and the animals were killed. Approximately half of the rats appeared to be normal during the experimental period, while the other half coughed or vomited some of the dose, followed by sneezing or difficulty in breathing. Four rats died at 1, 6, 7, and 24 hours by respiratory irritation. The rats with some symptoms had significantly higher concentrations in the blood and tissues than the rats that appeared normal. The concentrations of Benzalkonium Chloride in the rats that died before their due times were higher than the others, except for the one rat that died at 24 hours. The Benzalkonium Chloride concentrations in blood and tissues were significantly higher in the aspirated PO-rats. The toxic degree of Benzalkonium Chloride was correlated with the Benzalkonium Chloride concentration in orally dosed rats. The lung and kidney had higher Benzalkonium Chloride concentrations, compared to the blood or liver, and they could be target organs of Benzalkonium Chloride (**Xue et al., 2004**).

#### ACUTE ORAL TOXICITY

In the oral administration study by **Xue et al. (2004)**, 30 fasted rats (weights = 330 to 368 g) were used. A dose of 250 mg/kg was given by stomach tube. Fifteen of the 30 rats dosed orally had symptoms such as sneezing, diarrhea, or difficulty in breathing. Erosion and petechial hemorrhages were observed in the gastrointestinal tract of all rats that were killed after 4 hours. Four rats died at 1, 6, 7, and 24 hours of respiratory irritation. These results were reported in a distribution and disposition study on Benzalkonium Chloride that is included earlier in the report text (See section on Biological Properties).

#### SHORT-TERM TOXICITY

**Kligman and Kligman (1998)** evaluated the short-term toxicity of Benzalkonium Chloride and other chemicals using groups of three hairless albino female mice (Skh:Hairless-1; weights not stated). The mice were treated daily, on five weekdays for 8 weeks, with 1.5% Benzalkonium Chloride. At the end of the treatment period, full thickness excisions were made across the mid-back, horizontal to the cephalad-caudal axis. The specimens were fixed in formalin and processed for light microscopy. An intense inflammatory infiltrate in the dermis resulted in a migration of neutrophils through the epidermis to form a huge exudate within a parakeratotic horny layer. The keratinocytes showed marked cytotoxic changes. There was an increased number of fine elastic fibers in the

dermis.

#### OCULAR IRRITATION/TOXICITY

**Maurer et al. (1998)** studied the ocular irritation potential of Benzalkonium Chloride using 5 adult male Sprague-Dawley rats. The right eye of each animal was treated by placing 10 µl directly on the cornea. Untreated left eyes served as controls. At 3 hours and days 1, 2, 3, 4, 7, 14, 21, 28, and 35 post-treatment, the eyes were macroscopically examined for irritation and reactions were scored according to the Draize scale (0 to 110). The eyes and eyelids were collected for microscopic examination. The total mean Draize score for Benzalkonium Chloride (highest mean score ≈40 at 3 hours and day 1; < 10 on days 14-35) was higher at the very early time points and decreased over time. At microscopic examination, changes in the conjunctiva (erosion/attenuation, denudation, and necrosis), cornea (epithelial cell loss), and iris/ciliary body (inflammation) were evident. Collectively, the macroscopic and microscopic changes suggest that Benzalkonium Chloride causes mild ocular irritation.

In another test (**Stern et al., 1998**), a mean Draize score of 6 (at 24 hours) was reported for 50% Benzalkonium Chloride.

In a study by **Krysiak et al. (1998)**, following the administration of 0.01% and 0.05% aqueous Benzalkonium Chloride into the eyes of rabbits, a weak irritating and rapidly reversible effect was revealed. This text is taken from the English abstract in a Polish publication.

In a study by **Furrer et al. (2000)**, 0.5% aqueous Benzalkonium Chloride was instilled into the eyes of six NMRI albino mice (1 µl instilled) and three male New Zealand white rabbits (10 µl instilled). The corneas were evaluated *in vivo* for ocular tolerance using a confocal laser scanning ophthalmoscope. Mean values for % of the corneal surface that was damaged were:  $50.55 \pm 1.66$  (mice) and  $57.0 \pm 2.0$  (rabbits). Values for 0.9% saline were:  $7.28 \pm 0.24$  (mice) and  $1.15 \pm 0.23$  (rabbits).

The influence of the concentration of Benzalkonium Chloride on ocular irritation potential in mice and rabbits was also studied. Over the concentration range of 0.01% to 0.5%, the test substance was applied to the corneas of mice (1 µl) and rabbits (10 µl) four times per day during three days. The irritation index of Benzalkonium Chloride increased with the test concentration. At the

lowest concentration (0.01%), the concentration at which Benzalkonium Chloride is commonly used as a preservative for aqueous eye drops, Benzalkonium Chloride was not more irritating to the mouse eye than the saline solution. In the case of the rabbit, 0.01% Benzalkonium Chloride was only slightly more irritating than the saline reference (5.78% of corneal surface fluorescent versus 1.15%) (**Furrer et al., 2000**).

**de Saint Jean et al. (2000)** studied the toxicity of preserved and unpreserved beta-blocker eye drops in an *in vitro* model for human conjunctival cells. Chang's conjunctival cell line (ATCC CCL 20.2) was treated for 15 minutes with 0.1%, 0.25% or 0.4% timolol with or without Benzalkonium Chloride and examined immediately or 24 hours later. Cell viability, chromatin condensation, mitochondrial mass and activity, and free radicals production were studied by microplate cold light cytometry. Relative cell number was evaluated using a crystal violet colorimetric test. Additionally, cell size and the expression of an apoptotic marker Apo2.7 were studied by flow cytometry.

Timolol with Benzalkonium Chloride induced a rapid decrease in cell viability, ranging from 40% immediately after treatment to 85% 24 hours later. A small, significantly less important decrease in cell viability was also observed with all tested concentrations of timolol without Benzalkonium Chloride. At 24 hours post-treatment with 0.25% timolol with Benzalkonium Chloride, the relative cell number was reduced by 55%, whereas it did not vary after 0.25% timolol without Benzalkonium Chloride treatment. Only timolol with Benzalkonium Chloride induced chromatin condensation, a decrease in mitochondrial membrane potential, and cell size reduction. Also, reactive oxygen species (ROS) production was significantly more important after cell exposure to timolol with Benzalkonium Chloride.

In this model of conjunctival cells *in vitro*, timolol with Benzalkonium Chloride induced irreversible cytotoxic damage with some characteristics of apoptosis. The authors noted that the active compound of timolol without Benzalkonium Chloride could be responsible for ROS production and for cell viability variations. Oxidative stress could also play a role in timolol with Benzalkonium Chloride-induced toxicity. The authors also noted that *in vitro* toxic effects of antiglaucoma drugs could, in part, explain some ocular surface disorders in long-term treated patients (**de Saint Jean et al., 2000**).

**Noecker et al. (2004)** studied the ocular toxicity of the following glaucoma medications containing Benzalkonium Chloride as the preservative: Bimatoprost 0.03% (contains Benzalkonium Chloride; pH 6.7 to 7.8), Dorzolamide 2% (0.008% Benzalkonium Chloride; pH 5.6), Timolol maleate 0.5% (0.01% Benzalkonium Chloride; pH 6.5 to 7.5), and Latanoprost 0.005% (0.02% Benzalkonium Chloride; pH 6.7). Fifteen New Zealand white male rabbits with normal findings on slit-lamp biomicroscopy were used. The upper and lower eyelids of each rabbit were separated, and a single drop was instilled and allowed to moisten the entire cornea. The lids were manually closed, and the rabbit was then allowed to blink normally. Once-daily drops were administered between 7 and 8 a.m. Twice-daily regimens were administered at 7 to 8 a.m. and at 7 to 8 p.m. for 30 days. At the end of treatment, the eyes were enucleated and photomicrographs of the cornea were taken. Corneal damage was graded according to the following scale: 0 (no damage) to 5 (severe damage). Conjunctival specimens were stained and mounted on slides.

Bimatoprost, which contains the lowest Benzalkonium Chloride concentrations of the evaluated medications (0.005%), was associated with significantly less damage than latanoprost, timolol, or dorzolamide ( $P = 0.002$ ). Interestingly, dorzolamide, with a Benzalkonium Chloride concentration of 0.0075%, induced more corneal epithelial damage than either latanoprost or timolol, both of which contain higher levels of Benzalkonium Chloride. These findings suggest that dorzolamide-induced corneal damage may be secondary to low pH (5.6) and not solely to the preservative. Bimatoprost produced significantly less lymphocytic infiltration than latanoprost ( $P = 0.033$ ). Taken together, the findings indicate that 1-month treatment with glaucoma medications containing higher levels of Benzalkonium Chloride resulted in more corneal damage and conjunctival lymphocytic infiltration than lower levels of Benzalkonium Chloride (**Noecker et al., 2004**).

**Denoyer et al. (2006)** conducted an *in vivo* assessment of the corneal epithelial toxicity of timolol with Benzalkonium Chloride using very-high-frequency ultrasound imaging. A solution of timolol with 0.01% Benzalkonium Chloride was applied twice daily in the test eyes of ten rabbits, and a Benzalkonium Chloride-free solution of timolol in the control eyes, for 56 days. A 60-MHz ultrasound device was used to evaluate the epithelial damage in Benzalkonium Chloride-exposed eyes, compared to control eyes.

The clinical findings were conjunctival redness, corneal staining and instability of the tear film. *In vivo* VHF ultrasound revealed a thinning of the epithelium of test eyes (from  $40.9 \pm 1.6 \mu\text{m}$  at day 0 to  $31.8 \pm 3.4 \mu\text{m}$  at day 56;  $p = 0.0006$  for day 0 versus day 56), while the epithelium of control eyes remained unchanged. Ultrasound epithelial thickness was correlated with corneal staining (at day 34 and day 56;  $p = 0.0025$  and  $0.0377$ , respectively) and histological epithelial pachymetry ( $p = 0.0176$  for control and  $0.0505$  for tested epithelium). Qualitative VHF ultrasound imaging of early epithelial damage was also reported (**Denoyer et al., 2006**).

#### EFFECT ON MUCOUS MEMBRANES

In a study by **Collin and Carroll (1986)**, ten normal and 20 keratectomized corneas received hourly drops of vehicle with or without Benzalkonium Chloride (0.02% Benzalkonium Chloride or 0.01% Benzalkonium Chloride plus 0.1% disodium ethylenediamine tetraacetate) over the daylight hours of 2 days (18 applications). When applied to the intact cornea, Benzalkonium Chloride with or without  $\text{Na}_2\text{EDTA}$  caused only slight clarification of the endothelial cytoplasm, while a few mitochondrial cristae were displaced. In the keratectomized corneas receiving Benzalkonium Chloride, the majority of the mitochondria of the corneal endothelial cells were pale and swollen, or even disrupted. Many contained aggregations of membranous material either within the mitochondrion or at its outer membrane. The peripheral endothelium was much less affected than the central area of endothelium behind the keratectomy. These results suggest that Benzalkonium Chloride should not be administered to corneas in which the anterior epithelial barrier is incomplete.

**Patton et al. (1999)** studied the effects of multiple applications of Benzalkonium Chloride and nonoxynol-9 on the vaginal epithelium in the pigtailed macaque (*Macaca nemestrina*). Fourteen sexually mature female pig-tailed macaques were used in the experiments. All were assigned to 1 of 3 treatment groups. A commercially available spermicide containing 4% nonoxynol-9 and one containing 1.2% Benzalkonium Chloride were tested, and each product individually as well as a combination of the products were administered intravaginally. Five macaques received 1.5 ml dose volumes of the Benzalkonium Chloride product (daily for 4 days); 4 received 1.5 ml dose volumes of the nonoxynol-9 product; and 5 received dose volumes of Benzalkonium Chloride product (0.75 ml)

mixed with the nonoxynol-9 product (0.75 ml).

Cervical erythema and vaginal erythema were observed in all three treatment groups. Vaginal epithelial disruption was noted in both the Benzalkonium Chloride and nonoxynol-9 + Benzalkonium Chloride groups. Cervical biopsy specimens from each group revealed acute inflammatory infiltrates with occasional plasma cells and lymphoid follicles. Detection of most microorganisms, including viridans streptococci, decreased in the Benzalkonium Chloride and the nonoxynol-9 + Benzalkonium Chloride groups. It was concluded that Benzalkonium Chloride not only may damage epithelial tissues, but also appears to reduce the population of potentially protective *Lactobacillus* species in the vagina (**Patton et al., 1999**).

**Cho et al. (2000)** evaluated the effect of long-term use of Benzalkonium Chloride on rat nasal respiratory mucosa using 40 Sprague-Dawley rats (4 to 5 weeks old; weights = 120 to 150 g). The following two Benzalkonium Chloride solutions was administered to 2 groups of 9 rats, respectively, and 4 rats comprised the saline-treated control group: Two test solutions, a 0.01 w/v% Benzalkonium Chloride solution that is commonly used as a preservative for nasal drops and a 0.1 w/v% Benzalkonium Chloride solution that could induce dermatitis in humans were made from Benzalkonium Chloride. Each solution (7  $\mu$ l) was administered into the nasal cavities through both nostrils. Specimens of the head (including nasal cavity) were prepared for microscopic examination. Nasal wheezing was observed in groups that received 0.1 w/v% Benzalkonium Chloride for 2 and 4 weeks. No specific symptoms were observed in the control group.

The control group showed normal respiratory mucosa consisting of pseudostratified columnar epithelium in the nasal septum. In Benzalkonium Chloride treatment groups, inflammatory cell infiltration and the proliferation of intraepithelial glands increased with one week of administration. These histological changes appeared in severe degrees of proliferation of intraepithelial glands, inflammatory cell infiltration, and vascular hyperplasia (**Cho et al., 2000**).

**Lebe et al. (2004)** studied the effects of Benzalkonium Chloride in nasal formulations on the mucosal integrity. Benzalkonium (0.01%) was administered to the nostrils of male rats (two groups of 5; weights = 350 to 450 g). One of the groups received 0.01% w/v Benzalkonium Chloride for one

week and the other group received 0.01% Benzalkonium Chloride for four weeks. Clinical symptoms were recorded during treatment and light and electron microscopic examinations were carried out on samples taken from one third central and lower regions of the noses at the end of the treatment periods. Symptomatic changes such as sneezing and nasal rubbing were observed in almost all of the groups, starting from the 6<sup>th</sup> day of administration. Light and electron microscopy showed histological changes and nasal lesions. The symptomatic and histological changes were more pronounced with prolonged duration of administration. Therefore, it has been concluded that *in vivo* administration of Benzalkonium Chloride may be irritating to the respiratory epithelium of rats.

#### SKIN IRRITATION/SENSITIZATION

**Woolhiser et al. (1998)** combined the parameters of a murine local lymph node assay and a mouse ear swelling irritancy assay in an effort to establish a single, rapid screening procedure for the sensitization and irritancy potential of chemicals. In order to validate this assay, a range of chemical irritants and sensitizers was evaluated for their ability to elicit responses in B6C3F1 female mice. The chemicals were administered for four consecutive days to the dorsal and ventral surfaces of each ear. Benzalkonium Chloride was administered at concentrations up to 5.0% in acetone. Ear thickness served to predict irritancy, whereas [<sup>3</sup>H]thymidine uptake by cervical draining lymph nodes suggested sensitization.

All chemicals known to be potent chemical sensitizers (oxazolone, 2,4-dinitrofluorobenzene, and toluene diisocyanate) produced a marked lymph node cell proliferation in this assay. Animals exposed to irritating agents (sodium lauryl sulfate, croton oil, tetradecane, nonanoic acid, and Benzalkonium Chloride) experienced a significant increase in ear swelling. In addition, these irritating agents elicited low-level lymphocyte proliferation. The authors noted that the combined LLNA/ear swelling assay appears to be a reliable predictor of sensitization and irritancy potential, since it identified the activity of all 8 chemicals tested (**Woolhiser et al., 1998**).

**Herouet et al. (1999)** studied the capacity of sensitizers, irritants, and neutral chemicals to modulate the surface marker expression and morphology of pure mature Langerhans cells *in vitro*. Contact with four sensitizers (2,4-dinitrobenzenesulfate, 4-ethoxymethylene-2-phenyl-2-oxazolin-5-one,

p-Phenylenediamine, mercaptobenzo-thiazole) resulted in a rapid, specific, marked fall in 33D1 expression, a murine specific dendritic cell marker. No effect was observed with two neutral chemicals (sodium chloride, methyl nicotinate) or two irritants (dimethyl sulfoxide, Benzalkonium Chloride). Benzalkonium Chloride and dimethyl sulfoxide were tested at a concentration of 0.2 mg/ml. Collectively, the sensitizers were tested at concentrations up to 5 mg/ml. Sodium lauryl sulfate (0.05 mg/ml), a very irritant detergent, altered morphology and down-regulated all membrane markers (Herouet et al., 1999).

These preliminary data suggest that *in vitro* modulation of 33D1 expression by strong sensitizers may be an approach to the development of an *in vitro* model for the identification of chemicals that have the potential to cause skin sensitization and to distinguish them as far as possible from irritants.

#### SKIN SENSITIZATION

Goh (1989) evaluated the sensitization potential of Benzalkonium Chloride using a modified Beuhler's technique involving groups of 10 guinea pigs. The induction phase consisted of three weekly 6-hour applications of 10% Benzalkonium Chloride in petrolatum (with Hill Top chambers, under occlusion) to the left shoulder (shaved; same site for all applications). Both 10% Benzalkonium Chloride and 10% Benzalkonium Chloride in petrolatum were tested. The animals remained immobilized for 6 hours and the chambers were removed. This procedure was repeated once per week for the next two weeks, and the induction phase was followed by a two-week non-treatment period.

During the challenge phase, performed at week 5, the test substance (0.5% in petrolatum) was applied to a new site (shaved) on the right flank. The site remained under occlusion for 6 hours. Reactions were scored at 24 hours and 48 hours according to the following scale: 0 (no reaction) to 3 (strong erythema, gross edema). Grades of 1 or greater indicated sensitization. Benzalkonium Chloride induced sensitization in 2 of 10 guinea pigs. None of the 10 guinea pigs in the petrolatum control group had sensitization reactions. The sensitization rate in the dinitrochlorobenzene control group was 9 of 10 guinea pigs (Goh, 1989).

Krysiak et al. (1998) studied the sensitization potential of Benzalkonium Chloride in guinea

pigs. A sensitizing effect of 0.5%, 0.1%, and 0.05% Benzalkonium Chloride aqueous solutions was indicated. The effect differed depending on the concentration that was used. The evaluation covered the proportion of sensitized animals, the nature and intensity of dermal reaction, as well as peripheral blood eosinophil and basophil tests. The results showed that 0.1% aqueous Benzalkonium Chloride solution is an optimum concentration for monitoring the sensitizing effect in humans that are in contact with this substance.

**Pichowski et al. (2001)** studied allergen-induced changes in interleukin 1 $\beta$  (IL-1 $\beta$ ) mRNA expression by human blood-derived dendritic cells. Twelve healthy male and female volunteers (mean age: 48 years, range = 39 to 60) participated in the study. Following five days of culture, the dendritic cells were treated for 30 minutes with 13.89  $\mu$ M Benzalkonium Chloride (in 0.01% DMSO in medium). Cultures were also treated with known sensitizers such as dinitrofluorobenzene. Data from this study confirm that, under conditions where contact allergens induce increased IL-1 $\beta$  mRNA expression by dendritic cells derived from responsive donors, non-sensitizing skin irritants (in this case, Benzalkonium Chloride) are without effect.

In a study by **Hirota and Moro (2005)**, in order to seek a novel biomarker for predicting skin sensitization, changes in the gene expression profile of THP-1 cells (leukemic cell line, of monocytic origin) on exposure to 2,4-dinitrochlorobenzene (DNCB), p-Phenylenediamine (pPD) and nickel sulfate (Ni) were assayed using oligo-DNA microarrays. While the change in gene expression varied depending on the sensitizers, up-regulation of MIP-1 $\beta$  mRNA expression was detected in both DNCB-treated and Ni-treated THP-1 cells. This finding was validated by RT-PCR and confirmed at the protein level by ELISA. Secretion of microphage inflammatory protein 1 $\beta$  from THP-1 was detected after 24 hours of treatment with sensitizers such as DNCB, Ni, 2-mercaptobenzothiazole and cobalt sulfate, while pPD and nonsensitizers such as sodium dodecyl sulfate and Benzalkonium Chloride (at 1  $\mu$ g/ml) had no effect.

#### CYTOTOXICITY

In a study by **Neville et al. (1986)**, cultured human and rat corneal epithelial cells were used as a model to test cytolytic action of four common preservatives according to the method of Brunner et al. 1968). Benzalkonium Chloride (0.0075 to 0.15%) was found to lyse > 40% of the cells (human and rat) when incubated for 15 minutes at concentrations that are in clinical use in topical ophthalmic

medication. This was not true for 0.0015% Benzalkonium Chloride.

In a study by **Kolde and Knop (1987)**, 15% Benzalkonium Chloride in acetone was painted on to the ears of female BaLB/c mice (8 to 10 weeks old; number of animals not stated). Specimens were examined using electron microscopy. The application of Benzalkonium Chloride induced injury to Langerhans cells that was detectable as early as 1-hour post-application. The Langerhans cells were characterized by mitochondrial swelling and irregular cytoplasmic vacuolization, followed by membrane disruption and disorganization of cellular components.

**Kajino (1987)** studied the effect of Benzalkonium Chloride on cultured Chinese hamster lung fibroblast V79 cells. The growth of V79 cells was completely inhibited by treatment with Benzalkonium Chloride (30 µg/ml) for 24 to 48 hours. When V79 cells were treated with Benzalkonium Chloride (3 µg/ml) for 2 to 24 hours, the cell survivals were over 75% of untreated cells. The survival of cells exposed at 10 µg/ml for 2 hours, 6 hours, or 12 hours was approximately 65, 30, or 0% of untreated cells, respectively. Treatment of cells with Benzalkonium Chloride at a dose of 30 µg/ml for 2 hours resulted in 100% inhibition of cell survival. The effect of Benzalkonium Chloride on DNA-, RNA-, and protein-syntheses is included in the section on Effects on DNA, RNA, and Protein Synthesis earlier in the report text.

**Eun et al. (1994)** conducted a comparative study of the cytotoxicity of skin irritants on cultured human oral and skin keratinocytes. Keratinocytes were exposed to sodium lauryl sulfate (SLS) and Benzalkonium Chloride at concentrations of  $10^{-4}$  to  $10^{-7}$  M for 24 hours. Cytotoxicity was evaluated by changes in mitochondrial metabolic activity (MTT) and plasma membrane integrity (lactate dehydrogenase leakage). The decrease in the number of viable cells was statistically significant at a concentration of  $1 \times 10^{-5}$  M ( $P < 0.05$ ), and there was almost total loss of viability at  $1 \times 10^{-4}$  M ( $P < 0.05$ ). There was also a significant decrease in viable cells at  $1 \times 10^{-6}$  M ( $P < 0.05$ ) and at a concentration of  $1 \times 10^{-5}$  M. Benzalkonium Chloride was nearly 100% cytotoxic to both cell types. At concentrations of  $1 \times 10^{-4}$  M SLS and  $1 \times 10^{-5}$  M Benzalkonium Chloride, both irritants increased LDH leakage significantly in both cell types.

**Debbasch et al. (1999)** studied the cytotoxicity of quaternary ammonium compounds. Cytotoxicity tests were done on a continuous human conjunctival cell line using microplate cold light cytofluorimetry. Membrane integrity (neutral red test), DNA condensation, and reactive oxygen

species (ROS) production were evaluated using living cells treated with different concentrations of Benzalkonium Chloride, benzododecinium bromide, and cetrimide (0.00001 to 0.01%) after 15 minutes of treatment or 15 minutes and 24 hours of cell recovery.

All of the compounds tested showed similar *in vitro* effects. Using the neutral red test, a decrease in membrane integrity was observed, even at concentrations of 0.005% and 0.01% ( $p < 0.001$ ) and after a short time (15 minutes). A stimulation of ROS production ( $H_2O_2$  and  $O_2$ ) was observed at concentrations of 0.00001% and above ( $p < 0.001$ ), associated with chromatin condensation due to an apoptotic phenomenon. It was concluded that a necrotic phenomenon is suggested at high concentrations of quaternary ammonium preservatives, whereas an apoptotic mechanism exists for lower concentrations. The authors also stated that this toxicity observed *in vitro* can explain some of the ocular surface damage that is caused by long-term use of preserved eye-drops (Debbash et al., 1999).

In a study by Moreno (2000), the cytotoxicity of Benzalkonium Chloride and other surfactants was evaluated *in vitro* using murine Swiss albino 3T6 fibroblasts. Cell viability was evaluated by measuring the cellular retention of neutral red. Controls and cells incubated with surfactant were loaded for 3 hours with medium containing 0.05% neutral red. After cell lysis, the absorbance of neutral red trapped in cells was read at 535 nm. In the lactate dehydrogenase (LDH) assay, cytotoxicity was measured using the index of lactate dehydrogenase leakage from damaged cells, and was expressed as a percentage of total cellular activity. Arachidonic Acid (AA) release and prostaglandin  $E_2$  ( $PGE_2$ ) synthesis were also evaluated using 3T6 fibroblast cultures.

Benzalkonium Chloride elicited a concentration-dependent inhibition of neutral red uptake (data not shown), and the concentrations that produced a 10 and 50% decrease ( $NR_{90}$  and  $NR_{50}$  values, respectively) relative to control cultures were  $1.1 \pm 0.2 \mu\text{g/ml}$  and  $5.5 \pm 0.2 \mu\text{g/ml}$ , respectively. In the lactate dehydrogenase leakage assay, the cytotoxic effect of Benzalkonium Chloride was dose-dependent, and the lowest concentration that was able to cause a significant increase in LDH was  $1 \mu\text{g/ml}$ . The dose-response curves clearly demonstrated that 3T6 fibroblasts responded to Benzalkonium Chloride treatment with a concentration dependent increase in  $[^3\text{H}]AA$  release,  $[^3\text{H}]AA$  metabolism, and  $PGE_2$  formation. Benzalkonium Chloride induced significant  $[^3\text{H}]AA$  mobilization and metabolism and  $PGE_2$  at the dose of  $1 \mu\text{g/ml}$ . Interestingly,  $[^3\text{H}]AA$  and  $PGE_2$  release occurred at

concentrations of Benzalkonium Chloride that did not cause significant cytotoxicity (**Moreno, 2000**).

**De Saint Jean et al. (2002)** studied the *in vitro* toxicity of Benzalkonium Chloride using a human conjunctival cell line. Benzalkonium Chloride was dissolved in serum-free medium at the following concentrations: 0.1% (1 mg/ml), 0.05% (500 µg/ml), 0.01% (100 µg/ml), 0.005% (50 µg/ml), 0.001% (10 µg/ml), and 0.0001% (1 µg/ml). Cells were treated for 10 minutes. Cells were examined prior to Benzalkonium Chloride treatment and at 24, 48, and 72 hours after re-exposure to normal cell culture conditions.

Cell viability decreased significantly ( $p < 0.001$ ), in a dose-dependent manner, after 10 minutes of treatment with Benzalkonium Chloride at concentrations of 0.0001%, 0.0005%, 0.001%, 0.005%, and 0.01%. Only 0.01% Benzalkonium Chloride induced a significant chromatin condensation immediately after treatment. Free radical production was observed after treatment with Benzalkonium Chloride at 0.001% and 0.0001%. After prolonged treatment with Benzalkonium Chloride (15 to 30 minutes), reactive oxygen species production leveled off, or even diminished, which was probably due to the significant cytotoxicity of drugs utilized during long-term exposure (greater than 10 minutes).

Cells were examined 24 hours after treatment with Benzalkonium Chloride. As demonstrated by anticytokeratin and phalloidin labelling, about 75% of cells treated with 0.01% Benzalkonium Chloride presented shrunken cytoskeleton, compared to nontreated cells. With DAPI staining, these cells presented chromatin condensation and fragmentation and reduced nuclear size when compared to the control. Cells treated with 0.001% Benzalkonium Chloride showed mildly diminished cell and nuclear sizes. Chromatin condensation was less frequent, and many cells died without the usual indications of apoptosis. There was neither cell size reduction nor chromatin condensation in 0.0001% Benzalkonium Chloride-treated cells (**De Saint Jean et al., 2002**).

**Boston et al. (2002)** examined the morphological changes, cell viability, and lactate dehydrogenase (LDH) activity of human neutrophils exposed to either nasal saline spray (NSS - contains 0.01% Benzalkonium Chloride) or buffered saline without preservatives. Heparinized venous samples were obtained from healthy adult volunteers. The blood was separated, resulting in a cell preparation that was >95% neutrophils. Neutrophils were exposed to one of two commercially available NSS containing Benzalkonium Chloride, one of two control solutions containing phosphate

buffered saline (PBS) without Benzalkonium Chloride or a "homemade" saline solution (HSS) without Benzalkonium Chloride. The final concentration of Benzalkonium Chloride in the experimental conditions was 0.0001% to 0.002%.

In one of the *in vitro* experiments, morphological changes were studied by exposing neutrophils to NSS or PBS at concentrations of 1% to 20% for 3 minutes. Because the nasal spray contains 0.01% Benzalkonium Chloride, the neutrophils were actually exposed to Benzalkonium Chloride at concentrations ranging from 0.0001% to 0.002%. Cells were analyzed for morphological changes by light microscopy.

In a second *in vitro* experiment, a quantitative determination of neutrophil cell lysis was performed using an optimized bioassay to detect LDH that was released from lysed cells. Neutrophils were exposed to the same concentrations of NSS or PBS. LDH released by disrupted neutrophils into solution was measured by spectrophotometry.

Neutrophil morphology was dramatically altered by exposure to NSS. Cells exposed to 20% PBS solution for three minutes exhibited normal cellular architecture with sharp, distinct cell membrane borders, characteristic staining patterns, and typical multi-lobulated nuclei. Neutrophils exposed to 20% NSS for three minutes demonstrated severe disruption of cell membranes and intracellular structures. Similar effects were seen at lower concentrations of NSS, although more intact cells survived. Neutrophils exposed to 20% PBS remained viable and did not stain with trypan blue, while cells exposed to 20% NSS stained dark blue, indicating disruption of their membranes. Similar effects were seen with lower concentrations of NSS, but there was less extensive staining at lower concentrations.

Average LDH activity resulting from neutrophil lysis was graphed as a function of increasing concentration of NSS and PBS, with a constant incubation time of 3 minutes. Neutrophils exposed to NSS at increasing concentrations demonstrated a significant increase in LDH activity at concentrations above 5% ( $p < 0.05$ ). The cells exposed to PBS showed small amounts of LDH activity and little increase in activity with increasing concentrations of PBS.

This study demonstrates that NSS containing Benzalkonium Chloride, even at concentrations far lower than those in commercially available preparations, alters neutrophil morphology, decreases cell viability, and increases neutrophil LDH activity in a concentration- and time-dependent manner. It

was concluded that multi-use preparations of NSS are toxic to human neutrophils, and that substances used to preserve NSS, particularly Benzalkonium Chloride, are responsible for the toxicity (**Boston et al., 2002**).

In a study by **Goto et al. (2003)**, cells from a human lens epithelial cell line (SrA01/04) were cultured in Dulbecco minimum essential medium supplemented with 5% fetal bovine serum and Benzalkonium Chloride (200 mg/ml). After 7 days of culture, cell morphological changes were assessed using phase-contrast microscopy, and cell-free supernatants were collected for prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), interleukin 1 $\alpha$  (IL-1 $\alpha$ ), and interleukin 6 (IL-6) iodine 125 radioimmunoassay, enzyme-linked immunosorbent assay, and chemiluninescent enzyme immunoassay, respectively. All cells that were cultured with Benzalkonium Chloride detached from the culture dish and died within 3 days. Secretions of PGE<sub>2</sub>, IL-1 $\alpha$ , and IL-6 were 190 to 305 times higher at a x180 dilution of Benzalkonium Chloride compared to controls.

#### OTOTOXICITY

**Barlow et al. (1994)** compared the cochlear and middle ear toxicity of the following topical otomicrobial agents: cortisporin otic solution (COS), 0.3% gentamicin ophthalmic solution (GOS), Benzalkonium Chloride (0.026% and 0.05%), and 1% Ofloxacin, a new quinoline antibiotic. Saline (0.9%) was used as the control. The agent was instilled daily for 7 days into the bulla of juvenile guinea pigs (male and female juvenile Hartley strain guinea pigs; starting weight = 250 to 400 g). The animals were killed on the 14<sup>th</sup> day. The organ of Corti was examined using light microscopy and scanning electron microscopy. The tympanic membrane (TM) and adjacent middle ear mucosa were examined with light microscopy.

The average cochlear hair cell damage was 66% for COS, 6.5% for GOS, and 1% for Ofloxacin, Benzalkonium Chloride, and saline. COS and Benzalkonium Chloride produced moderate mucosal thickening and inflammation. However, this was not statistically different from the mild mucosal thickening produced by saline, GOS, Ofloxacin, and 0.026% Benzalkonium Chloride. There was statistically significant mild to moderate thickening of the TM for all agents, compared to the saline control (**Barlow et al., 1994**).

#### TOXICITY TO BONE/SOFT TISSUES

In a study by **Tarbox et al. (1998)**, sixteen rats were separated randomly into two groups:

Benzalkonium Chloride and normal saline. All of the rats had undergone surgical and irritation procedures. A 4-cm incision had been made over the lower lumbar region and the incision was then irrigated with either 0.1% Benzalkonium Chloride or saline. The wound was then closed. Two rats from each group were killed on postoperative days 1, 3, 5, and 7. The surgical wounds were excised and prepared for microscopic examination. Microscopic examination of tissues from the surgical wounds of animals irrigated with 0.1% Benzalkonium Chloride or with normal saline revealed no significant difference in wound inflammation or injury between the Benzalkonium Chloride group and the normal saline group. The bone and cartilage that were present in the sections appeared normal in both groups. Changes were observed in the soft tissues between the various times; however, these changes were similar in both groups and were considered evidence of the normal wound healing process.

#### NEUROTOXICITY

**Goto and Grosfeld (1989)** studied the effect of Benzalkonium Chloride on the lower esophagus. Following midline laparotomy, topical Benzalkonium Chloride (0.5%) was applied to the muscularis of the lower 1.0 cm of the esophagus (30-minute application) in 38 Sprague-Dawley rats (weights = 200 g). Thirty-eight additional rats served as controls (unoperated, n = 19; sham laparotomy, n = 19). At three months, the esophagus was evaluated for histologic study and acetylcholinesterase staining. Aganglionosis with positive cholinesterase staining fibers was observed at microscopic examination of the esophagus from rats treated with Benzalkonium Chloride. Topical Benzalkonium Chloride resulted in distal esophageal aganglionosis, characterized by distal narrowing, proximal dilatation, decreased food intake, and limited weight gain, when compared to controls.

In a study by **Zucoloto et al. (1991)**, the jejunum of rats (87 male Wistar rats used in study; weights  $\approx$  100g) was treated by serosal application of a 0.2% solution of Benzalkonium Chloride for 30 minutes. Control animals were treated with saline. The animals were allocated to 8 groups of 10 rats each and killed 15, 30, 45, and 60 days after treatment. Segments were removed from the jejunum for neuronal counting, measurement of the smooth muscle area and morphokinetic study of the epithelium. There was a significant reduction in neuron number in the myenteric plexus at 30 days post-treatment. Thickening of smooth muscle was noted at 15 to 60 days post-treatment. No change in epithelial cell proliferation in the jejunum was noted at day 30 or days 15 to 60 post-treatment.

**Holle (1998)** denervated a region of the small intestine by treating the prepared segment (serosal surface) with a 0.062% Benzalkonium Chloride solution. Male Wistar rats (mean body weight = 350 g) were used. The intestine was then washed with saline and peritoneal and abdominal cavities were closed by sutures. The abdominal cavity was opened again at 20 to 27 days, and, in some animals, seven days after Benzalkonium Chloride treatment.

Following Benzalkonium Chloride, in three cases of the duodenum, jejunum, and ileum, respectively, the myenteric neurons and nerve strands were completely eliminated in the Benzalkonium Chloride segment of the 21-day-treated animals and, in one case, a seven-day-treated animal. In all other cases, including the seven-day-treated animals, neurons and nerves were markedly reduced, damaged, or degenerating in the myenteric plexus, longitudinal muscle layer, and, to a lesser extent, in the circular muscle layer. The most frequent sign of degeneration was vacuolization of the cytoplasm, and, less frequently, of the nucleus. In addition, loss of mitochondrial cristae, damaged or greatly altered organelles, lysis of cell membrane or nuclear membrane, edematous swelling, collagen invasion, and the presence of cellular debris were observed. Fibroblasts as well as macrophages and other phagocytosing cells (e.g. white blood cells) could be observed between the damaged neurons and smooth muscle (**Holle, 1998**).

**Kaya et al. (2005)** studied the effects of Benzalkonium Chloride on the rat bladder using 15 adult female Wistar rats (weights = 250 to 280 g). The rats were divided into two groups (10 test, 5 control), and the bladders were treated with a local application of 0.3% Benzalkonium Chloride for 30 minutes in the denervation group and saline in the control group. Before and at the 8<sup>th</sup> week after treatment, cystometry was performed in all rats, and results were recorded. The bladders were removed and whole-mount sections were prepared and evaluated by immunocytochemistry. Rats treated (denervated) with Benzalkonium Chloride showed a significant increase in bladder capacity ( $p < 0.05$ ), but no significant change was observed in other cystometric parameters ( $p > 0.05$ ) when compared with controls. Immunocytochemistry results for rats treated with Benzalkonium Chloride indicated none or very few intramural nerve plexuses within the dome of the bladder. The results of this study show that denervation caused by Benzalkonium Chloride is useful for augmentation of the rat bladder. Bladder capacity was increased by the local application of Benzalkonium Chloride without impairing bladder functions.

## MUTAGENICITY/GENOTOXICITY

**Fukuda (1987)** assessed the carcinogenic hazard of 6 substances used in dental practices using Syrian hamster embryo (SHE) cells in culture. Morphological transformation (MT), unscheduled DNA synthesis (UDS) and sister chromatid exchanges (SCEs) induced in the cells following treatment were used as markers for the risk assessment. MT, which shows a good correlation with carcinogenicity in animals, was induced by treatment with carbol camphor (CC, 0.001 ~0.003 v/v%), eugenol (0.0001~0.001 v/v%) or thymol (3 ~30 µg/ml) for 48 hours. Treatment with EDTA (0.03 µg/ml), Benzalkonium Chloride (0.3 ~3 µg/ml) or benzethonium chloride (0.3 ~ 3 µg/ml) failed to induce MT. All of the three MT-positive substances resulted in UDS, which shows DNA synthesis for repair of DNA damage, indicating that MT by the three substances was initiated by DNA damage.

Exposure of SHE cells to six substances tested, including both MT-positive and -negative substances, for 18 ~ 20 hours elicited a significant level of SCEs, but the inducibility did not exceed twice that of the control level (**Fukuda, 1987**).

**Sakagami et al. (1988)** evaluated the genotoxicity of Benzalkonium Chloride using the *umu* test system (Nakamura et al., 1987). Doses up to 5 µg/ml were tested with and without metabolic activation. This method is based on the ability of toxic chemicals to induce *umu* gene expression in *Salmonella typhimurium* TA1535/pSK 1002 in which a plasmid psK1002 carrying a fused gene *umuC'*-*'lacZ* has been introduced. The results for Benzalkonium Chloride were negative with and without metabolic activation. Glutaraldehyde induced potent genotoxic activity, independent of the metabolic activation system, and the activity was increased within the concentration range of 10 to 50 µg/ml.

**Sakagami et al. (1988)** studied the DNA-damaging capacity and mutagenicity of 10% Benzalkonium Chloride using the liquid rec-assay and Ames test (Ames, et al., 1975) , respectively. *Bacillus subtilis* suspensions were used in the rec-assay and *Salmonella typhimurium* strains TA 98 and TA 100 were used in the Ames test. Minimum inhibitory concentrations were determined according to the method of Kosaki et al. (1974), by adding the test substance to the brain-heart infusion agar plates and growing the bacteria on them. For the rec-assay, DNA-damaging capacity was estimated by the growth of tester bacteria, measuring either the ratio of growth rate (Matsui et al., 1980) or the time lag of the growth (Sakagami et al., 1981). The liquid rec-assay revealed the DNA-damaging capacity of Benzalkonium Chloride, which was not positively detected by the minimum

inhibitory concentration method and the Ames test.

**Withrow et al. (1989)** evaluated the cytotoxicity and mutagenicity of ophthalmic solution preservatives and UVA radiation in L5178Y cells. The following four preservatives commonly used in ophthalmic solutions were tested for their toxic and mutagenic potential in mouse lymphoma cells with and without exposure of the cells to UVA radiation: Benzalkonium Chloride, chlorhexidine, thimerosal, and EDTA. Cell survival and mutagenesis were measured using the L5178Y mouse lymphoma (TK<sup>+/+</sup>) system. Cells were exposed to varying amounts of preservatives for 1 hour, and aliquots were then irradiated with UVA radiation (during exposure to the preservative). Cells were then assayed for survival, and for mutagenesis at the thymidine kinase (TK) locus.

The concentration required to kill 50% of the cells as a result of a 1-hour exposure was approximately 20 µg/ml for chlorhexidine, >3 µg/ml for thimerosal, and 15 µg/ml for Benzalkonium Chloride. Chlorhexidine alone had little effect on the mutation rate, but when combined with UVA exposure, there was approximately a three-fold increase over background in the number of mutants. Thimerosal by itself appears to be weakly mutagenic. When cells were exposed to thimerosal plus UVA treatment, there was approximately a 6-fold increase in the number of mutants over the control value. Treatment of cells with Benzalkonium Chloride alone or in combination with UVA did not induce any mutations. Similarly, EDTA with or without UVA exposure did not change the mutation rate

At concentrations commonly found in ophthalmic solutions, Benzalkonium Chloride, chlorhexidine, and thimerosal were toxic to cells, and thimerosal was slightly mutagenic. When cells were exposed to preservative and UVA irradiation, chlorhexidine was mutagenic and the mutagenic activity of thimerosal was enhanced (**Withrow et al., 1998**).

**Hikiba et al. (2005)** assessed the genotoxicity of 14 chemical agents that are used in dental practice using the chromosome aberrations assay involving Syrian hamster embryo (SHE) cells. Statistically significant increases in the frequencies of chromosome aberrations were induced in SHE cells treated with the following chemicals (without metabolic activation): carbol camphor, *m*-cresol, eugenol, guaiacol, zinc oxide, hydrogen peroxide, formaldehyde, and iodine. The results were negative for the following chemicals: thymol, glutaraldehyde, iodoform, Benzalkonium Chloride (test concentrations up to 30 µM), benzethonium chloride, and chlorhexidine. Only thymol induced chromosome aberrations in the presence of exogenous metabolic activation.

## REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

In a study by **Momma et al. (1987)**, relatively high doses of Benzalkonium Chloride (3, 10, and 30 mg/kg) were given by gavage daily at an early stage of pregnancy (0 to 6 days). Animals were killed on the 13<sup>th</sup> day. Significant changes were not found in body weight, food consumption and general appearance in dams. Also, the number of implantations, number of live fetuses, sex ratio, and body weight in fetuses were not significantly changed. However, there was a tendency toward a slight decrease in pregnancy rate of dams in the groups treated with 10 and 30 mg/kg and a slight increase in the number of dead or resorbed fetuses in the group treated with 30 mg/kg.

In the second experiment, groups of animals were treated with Benzalkonium Chloride at lower doses (1, 50, and 100 µg/kg) during pregnancy (0 to 6 days), while other groups were treated with 1 and 50 µg/kg throughout the entire pregnant period (0 to 18 days). Significant changes were not observed in both treated groups when compared to controls for dams and fetuses.

These results indicate that Benzalkonium Chloride can cause implantation disturbance and/or abortion at a relatively high dose (30 mg/kg), but that it has no reproductive functions at lower dose levels (less than 100 µg/kg) (**Momma et al., 1987**).

## CLINICAL ASSESSMENT OF SAFETY

### OCULAR IRRITATION/TOXICITY

**Liu et al. (2001)** observed a cluster of 12 eyes out of 19 routine cataract surgeries (by phaco-emulsification), performed on a single day, that developed permanent corneal decompensation postoperatively. No eye was identified as having preexisting corneal endothelial disease. An investigation revealed that the cause was inadvertant intracameral use of balanced salt solution preserved with 0.013% Benzalkonium Chloride. In the initial 48 hours after surgery, although only 2 patients reported blurring of vision, corneal edema was present and persisted beyond the 2-week follow-up in 12 of 19 patients. The postoperative appearance of the cornea in the other 7 patients was unremarkable. It was concluded that Benzalkonium Chloride in concentrations that are commonly used extraocularly is highly toxic to the corneal endothelium.

**Pisella et al. (2003)** conducted a retrospective study using glaucoma patients treated with 0.5% timolol (with [15 patients] or without 0.01% Benzalkonium Chloride [17 patients]). The two

groups were comparable for age and duration of treatment, lasting at least one year. Specimens were analyzed by flow cytometry for inflammatory profile (using antibodies directed against human leukocyte antigen [HLA] DR and intercellular adhesion molecule-1 [ICAM-1] and mucin detection [anti-M1/MUC5AC antibody]). Impression cytology analyses showed a significant increase in the expression of the two inflammatory markers, HLA DR and ICAM-1 in the timolol + Benzalkonium Chloride group and also a significant decrease in goblet cell density in the same group, as compared to the timolol - Benzalkonium Chloride group. It was concluded that the use of long-term preserved beta-blocker in glaucoma patients is associated with a direct subclinical toxicity in the conjunctiva.

#### MUCOUS MEMBRANE IRRITATION

**Hallén and Graf (1995)** studied the effect of an oxymetazoline nasal spray (decongestive spray) with and without Benzalkonium Chloride on the nasal mucosa. Twenty healthy volunteers participated in the study. They used the nasal sprays (one dose in each nostril) three times a day for 10 days. Ten of the subjects used a nasal spray composed of oxymetazoline (0.5 mg/ml) and Benzalkonium Chloride (0.1 mg/ml), and the remaining 10 used oxymetazoline nasal spray (0.5 mg/ml) alone. The present trial was performed 3 months after the subjects had used the nasal sprays three times per day during 4 weeks.

The group treated with oxymetazoline nasal spray containing Benzalkonium Chloride developed nasal mucosa swelling during the period of 10 days. The authors stated that the development of nasal mucosa swelling may have been due to vasodilation or to interstitial edema. The group treated with oxymetazoline nasal spray also developed no significant nasal mucosa swelling. Based on the results of this study, the authors stated that Benzalkonium Chloride in decongestant sprays seems to have a long-term effect on the nasal mucosa, at least for three months. Furthermore, they recommended that long-term treatment with nasal decongestive sprays, especially when they contain Benzalkonium Chloride, should be avoided (**Hallén and Graf, 1987**).

In a study by **Graf et al. (1999)**, 18 patients received oxymetazoline hydrochloride (0.5 mg/ml) nasal spray containing the preservative Benzalkonium Chloride (0.5 mg/ml), and the other 17 were treated with oxymetazoline nasal spray without Benzalkonium Chloride. Before and after the treatment, recordings of the nasal mucosa and minimal cross sectional area were made with rhinostereometry and acoustic rhinometry, followed by histamine hydrochloride challenge tests.

No rebound swelling was found after the 10-day treatment in the two groups with either of the methods or as estimated by symptom scores. In the group receiving oxymetazoline containing Benzalkonium Chloride, but not in the other group, the histamine sensitivity was significantly reduced after treatment ( $p < 0.001$ ). Specifically, in this group, the mean mucosal swelling after histamine hydrochloride challenge before treatment was 1.4 mm with a dose of 1 mg/ml, 1.8 mm with 2 mg/ml, and 2.2 mm with 4 mg/ml. After 10 days of treatment, the corresponding values for mucosal swelling were 0.5, 0.8, and 1.1 mm. The reduction in mucosal swelling after histamine challenge was significant at all 3 histamine provocation levels (analysis of variance,  $p < 0.001$ ). The mean mucosal swelling after 10 days was 0.21 mm, compared with the reference value before starting the medication.

The authors noted that it is safe to use topical nasal oxymetazoline with or without Benzalkonium Chloride for 10 days in patients with vasomotor rhinitis; however, this study indicates that Benzalkonium Chloride in nasal decongestant sprays affects the nasal mucosa also after short-term use (**Graf et al., 1999**).

#### SKIN IRRITATION

In a study by **Willis et al. (1986)**, 17 non-atopic, healthy adult patients (ages not stated) were patch-tested (occlusive Finn chambers) with 0.5% Benzalkonium Chloride for a maximum of 48 hours. The Finn chambers were applied to the left forearm. Irritant contact dermatitis was induced in the patients.

**Agner and Serup (1988)** studied the skin irritation potential of Benzalkonium Chloride using 16 healthy volunteers. Closed patch tests containing 3% Benzalkonium Chloride (in distilled water) were applied to both arms (antero-lateral surface of upper arm) using Finn chambers on Scanpor tape. Patches were removed at 24 hours post-application. Reactions were scored at 24 and 96 hours post-application. Reactions were scored according to the following scale: 0 (no reaction) to 3 (strong, positive reaction - advanced erythema, infiltration, possibly vesicles, bullae, pustules and/or pronounced crustation). Median visual scores of 1.5 and 0 were reported at 24 and 96 hours post-application. Scores of 1 and 2 are defined as weak positive reaction and moderate positive reaction, respectively.

**Willis et al. (1988a)** evaluated the skin irritation potential of Benzalkonium Chloride and other

mononuclear cells. Occasional foci of necrotic damage were evident in the upper stratum spinosum. Ultrastructurally, the affected keratinocytes were characterized by shrunken pyknotic nuclei, disrupted organelles, and membranes and considerable intracytoplasmic vacuolation. Lipid accumulation within keratinocytes was rarely observed (**Willis et al., 1989**).

**Willis et al. (1990)** studied the behavior of epidermal CD1+ cells in experimentally induced irritant contact dermatitis. Ten healthy non-atopic male volunteers were patch tested (on the volar aspect of the forearm using Finn chambers) with 0.5% (w/v) aqueous Benzalkonium Chloride. After 48 hours of contact, the patches were removed and reactions were scored 1 hour later according to the following scale 0 (negative) to 3 (erythema, edema, and vesiculation). Half of the volunteers received vehicle control patch tests of yellow soft paraffin and propan-1-ol, with the remaining half receiving patch tests of distilled water and occlusion alone. Punch biopsies were obtained from the test and control sites and examined microscopically. The majority of the irritation reactions were mild to severe. All controls were negative during visual assessment. Benzalkonium Chloride (0.5% aqueous) induced irritant contact dermatitis.

For Benzalkonium Chloride-treated sites, the numbers of CD1+ cells within the epidermis were not significantly different from those of their appropriate control group. Regarding the dendrite cell length of CD1+ cells, the dendrites present in the reaction with Benzalkonium Chloride and, also, in the vehicle and occlusion controls were not significantly different from those of normal skin. Qualitative assessment of the distribution of CD1+ cells within the epidermis suggested that there was little or no difference between Benzalkonium Chloride-treated and normal skin. That is, the majority of CD1+ cells were located in the mid or basal epidermis. Ultrastructural examination of Benzalkonium Chloride patch test sites indicated that the majority of epidermal cells with Langerhans cell-like characteristics were found to possess Birbeck granules. Some of these cells resembled those in normal skin, but many exhibited morphologic features that were indicative of metabolic activation, or, less commonly, of cellular damage (**Willis et al., 1990**).

In a study by **Krysiak et al. (1998)**, the threshold irritating effect of Benzalkonium Chloride on rabbit skin was determined. As a threshold concentration of Benzalkonium Chloride evoking slight inflammatory reaction on the skin in a single, closed exposure and in multiple, open exposure, the following values were adopted for its aqueous solutions: >0.5 to 1% and 0.05%, respectively. This

text is taken from the English abstract in a Polish publication.

**Wallengren (2000)** applied 1% aqueous Benzalkonium Chloride to the dorsal skin of 21 subjects using Al-tests®. The study group consisted of 25 female patients with nickel contact allergy (mean age =  $47 \pm 10$  years) and 8 patients (3 men, 5 women; mean age =  $39 \pm 14$  years) with birch-pollen-induced allergic rhinitis. Details regarding the composition of the group of 21 patients were not included. The test substance was applied for 48 hours and the reaction was examined 24 hours after patch removal. The response area was estimated planimetrically. Benzalkonium Chloride induced a sharply demarcated homogenous erythema, sometimes with edema. Further details were not provided.

**Santucci et al. (2003)** evaluated cutaneous responses to irritants. From January to March of 2001, 520 subjects (40 healthy volunteers; 480 affected by active skin diseases) were tested. All subjects were simultaneously patch tested with 15  $\mu$ L of 1% aqueous Benzalkonium Chloride. The following groups were tested: Group 1 (40 healthy nonatopic volunteers), Group 2 (40 atopic subjects with active dermatitis), Group 3 (57 patients with active psoriasis), Group 4 (124 eczematous subjects with positive patch test responses), Group 5 (140 eczematous subjects with no patch test responses), Group 6 (79 patients with active urticaria), and Group 7 (40 subjects with generalized pruritus). Patch tests were applied to both sides of the upper back for two days using Al-test on Fixomul. Readings were performed one hour after removal of the strips. Results were scored according to the following scale: - (no visible reaction) to ++++ (erythema with vesicles or bullae).

Isolated positive reactions to 1% aqueous Benzalkonium Chloride occurred in: 42% of the healthy subjects, 40% of eczema patients with positive patch tests, 38% of patients with both psoriasis and eczema with negative patch tests, 33% of patients with urticaria, 30% of patients with atopic dermatitis, and 27% of patients with generalized pruritus (**Santucci et al., 2003**).

**Marriott et al. (2005)** conducted two 23-hour patch tests on Benzalkonium Chloride (0.1% and 0.2%) to determine irritant susceptibility. Fifty-eight healthy adults (19 males, 39 females; ages = 22 to 58, mean age of 39 years) participated in the study. Benzalkonium Chloride (50  $\mu$ l) was applied to patches consisting of Finn chambers on Fixomull tape. Each panelist had two patches (containing 0.1% and 0.2% Benzalkonium Chloride, respectively) applied, one on each upper outer arm. The patch with the lower concentration also held the negative control, ultrapure water. The patches were

applied for 23 hours, after which they were removed. The second patches were then applied to the same sites for a further period of 23 hours. Erythema, dryness, and other visual parameters were assessed by trained skin assessors using the grading scheme by Basketter et al. (1997).

Panelists with an erythema grade of <6 were as follows: 18 subjects (0.1% Benzalkonium Chloride) and 14 subjects (0.2% Benzalkonium Chloride). The following panelists had an erythema grade of >6: 39 subjects (0.1% Benzalkonium Chloride) and 43 (0.2% Benzalkonium Chloride). There were also 7 panelists who also developed an erythema grade of >6 to ultrapure water (**Marriott et al., 2005**).

#### REDUCTION OF SKIN IRRITATION POTENTIAL

**McFadden et al. (2000)** conducted a study to determine whether or not Benzalkonium Chloride reduces the skin irritation potential of sodium dodecyl sulfate. Fifty-four non-atopic adult volunteers (16 males, mean age = 34.7; 38 females, mean age = 34.6). Sodium dodecyl sulfate (20%) was applied for 2 hours under occlusion (Finn chamber on Scanpor). Benzalkonium Chloride (1%) was then applied to the same site. Various controls, including sodium dodecyl sulfate application followed by water for 2 hours, were included. The irritant reaction was assessed at 24 hours and 48 hours. Forty of the 54 subjects had some reaction when Sodium dodecyl sulfate was applied for 2 hours, followed by either Benzalkonium Chloride or the water control under occlusion. In comparison to the water control, where Benzalkonium Chloride was applied after sodium dodecyl sulfate, 20 of the 40 responders had a weaker reaction, but only 4 had a stronger response. This study shows that Benzalkonium Chloride applied to skin exposed to sodium dodecyl sulfate attenuates the resulting irritant reaction.

#### SKIN SENSITIZATION

**De Groot et al. (1986)** studied the prevalence of contact allergies to commonly used preservatives, including Benzalkonium Chloride. During the period from January 1 to April 30, 1985, 627 consecutive patients were patch-tested with 0.1% aqueous Benzalkonium Chloride according to ICDRG recommendations. Irritant or dubious (?+) reactions were observed frequently (27 patients). The frequency of positive reactions to 0.1% aqueous Benzalkonium Chloride was 8/627 or 1.3%.

**Kokelj and Cantarutti (1986)** evaluated the incidence of sensitization reactions to Benzalkonium Chloride and numerous other ingredients in a vehicle test series and the more common

allergens that are contained in creams and ointments. Twenty-five patients with leg ulcers (10 males, 15 females; average age: 69 years; range: 45 to 87 years). Patch test evaluations were performed according to the procedure of Wilkinson et al., 1970, and test results indicated one positive reaction to Benzalkonium Chloride. The greatest number of positive reactions was induced by balsam of Peru (7 reactions) and neomycin (7 reactions).

In a study by **Klein et al. (1991)**, five positive reactions to 0.1% Benzalkonium Chloride in petrolatum were observed in a population of 32 patients that were tested with an eye or preservatives series. All reactions were ++ or +++ and considered to be allergic, by virtue of erythema, infiltration and sometimes vesicles, spreading over the margins of the test area, and a crescendo from days 2 to 3. In one patient (elderly), the reaction was clearly relevant, because he did not tolerate Benzalkonium Chloride-containing-eyedrops, showed no reaction to all other compounds that he used, and his conjunctivitis and eyelid eczema cleared after removal of the eyedrops. The other 4 patients were young nurses who suffered from recurrent hand eczema and had contact with Benzalkonium Chloride-containing surface disinfectants.

Three nurses were willing to undergo additional tests. Two had an additional patch test and showed positive reactions to higher dilutions and all 3 performed a use test by applying an aqueous solution of Benzalkonium Chloride on a small circumscribed area of the volar aspect of the forearm 5 x/day for 4 days. The use test was positive only in one of the patients, whose eczema improved after removal of the Benzalkonium Chloride-containing disinfectant from her working environment. In contrast, the other two neither reacted to a 0.05% (which is the working concentrations in surface-disinfectant solutions) nor to a 0.1% solution. Despite their positive patch test reactions, the two nurses with negative use tests were therefore considered not to be allergic to Benzalkonium Chloride.

Benzalkonium Chloride may thus cause sensitization in hospital personnel in rare instances, but positive patch test reactions must be interpreted with great caution, even if they occur in higher dilutions than recommended in the literature and show the typical characteristics of allergic reactions. Patients should be encouraged to perform use tests, which can conveniently be performed on the forearms (**Klein et al. 1991**).

**Fuchs et al. (1993)** conducted a multicenter study to determine whether Benzalkonium Chloride is a relevant contact allergen or irritant. At eight dermatological clinics, a total of 2146

patients was tested with 0.1% Benzalkonium Chloride between May of 1990 and December of 1991. In 225 cases, an allergic reaction was proven, with 258 irritant reactions in addition. Only 12 cases were clinically relevant. Therefore, Benzalkonium Chloride is considered a weak allergen.

In a study by **Perrenoud et al. (1994)**, from February 1989 to January 1990, the Swiss Contact Dermatitis Research Group conducted a 1-year study to examine the frequency of sensitization to a series of 13 common preservatives. A group of 2295 consecutive outpatients with suspected allergic contact dermatitis (age range: 7 to 90 years; mean age: 42; 911 males, 1384 females) was tested. The percentages of positive reactions to the preservatives studied were as follows, in descending order: formaldehyde (5.7%), 0.1% aqueous Benzalkonium Chloride (5.5%), Kathon CG (5.5%), thimerosal (4.2%), chlorhexidine digluconate (2.0%), dmdm hydantoin (1.7%), paraben mix (1.7%), chloroacetamide (1.5%), bronopol (1.2%), imidazolidinyl urea (1.0%), quaternium 15 (1.0%), triclosan (0.8%), and 2,4-dichlorobenzyl alcohol (0.4%).

In a study by **Stables et al. (1996)**, 92 children (45 girls, 47 boys; ages: 3 to 14, mean age of 9.3 years) were referred for patch testing at the Regional Contact Dermatitis Investigation unit in Glasgow, UK during the period from 1979 to 1993. The European standard series of allergens was applied to all children, together with any additional series as indicated from the history. Patch testing was performed on the unaffected skin of the upper part of the back using Finn chambers on Scanpor tape. Readings were graded according to the ICDRG recommendations (2- and 4-day readings). Relevance of each positive reaction was determined by reviewing the patient's history.

At the time of referral for patch testing, 45 of the 92 children were diagnosed as having atopic dermatitis. Twenty-six had localized dermatitis affecting the face or perineum or hands and/or feet only (with no associated personal or family features of atopy). Fifteen had juvenile plantar dermatosis (JPD), and there were 2 each with orofacial granulomatosis, vaccination reaction, and atypical psoriasis. Patch test results indicated that nickel was the most common allergen (10 of 92 patients or 10.9%). A single positive reaction to Benzalkonium Chloride was reported (**Stables et al., 1996**).

**Aoki (1997)** studied the clinical manifestations and causative agents in cases with allergic contact dermatitis due to eye drops in patients who consulted the Department of Dermatology, Nippon Medical School during the period of 9 years between January of 1987 and December of 1995. Among the 66,165 cases who visited the department, 3,903 were suspected of having contact dermatitis or

drug eruption and underwent patch testing. Of these, 141 (3.6%) were patch tested with eye drops and 49 cases (34.8%) reacted positively and were diagnosed as having allergic contact dermatitis.

The 49 cases diagnosed as having allergic contact dermatitis from eye drops included 17 males and 32 females. 81.6% of the patients were over 40 years of age. The most frequent ages were between 60 and 69 years. The period from onset to the first visit to the dermatology department was less than 2 weeks in 51.4% of the cases. However, three patients consulted the department after more than six months. The determined allergens were noted in the order of frequency as follows: fradiomycin sulfate (14 cases), ketotifen fumarate (6 cases), dibekacin sulfate (3 cases), befunolol hydrochloride (3 cases), phenylephrine hydrochloride (3 cases), and Benzalkonium Chloride, preservative in eye drops, (3 cases) (Aoki, 1997).

Schnuch et al. (1998) collected patch test data and data from the patients' history from 24 departments participating in the Information Network of Departments of Dermatology from January 1, 1990 to December 31, 1994. Patch test data from 28,349 patients tested with preservatives of the standard series (SS), from 11,485 patients tested additionally with a preservative series (PS), and from 1787 patients tested with an industrial baccata tray (IB) were evaluated. 11,308 patients were patch-tested with Benzalkonium Chloride. Patch tests were performed according to recommendations of the International Contact Dermatitis Research Group and the German Contact Dermatitis Group. Finn chambers on Scanpor were used in 19 departments and other systems were used in five departments. The patch test concentrations were as follows: Benzalkonium Chloride (0.1% in petrolatum), alkylaminobenzoate (0.3% in petrolatum), MDBGN/PE (0.5% in petrolatum), chloroacetamide (0.2% in petrolatum), diazolidinyl urea (2% in petrolatum), octylgallate (0.3% in petrolatum), and Bronopol™ (0.5% in petrolatum).

Nine of the 24 centers applied patch tests for 24 hours, and, the remainder (15 of 24) for 48 hours. Readings were done until at least 72 hours after application of the test chambers. For this study, only readings at 72 hours were considered.

The most important allergens of the PS were, in women, alkylaminobenzoate (contained in milking fat) (2.5%), Methylidibromoglutaronitrile/phenoxyethanol (MDBGN/PE, 2.2%), Benzalkonium Chloride (1.8% - 207 patients with positive reactions), chloroacetamide (1.4%), diazolidinyl urea (1.3%), octylgallate (1.2%), and 2-bromo-2-nitropropane-1,3-diol (Bronopol™, 1.1%). In men, rates

differed only with regard to alkylaminobenzoate (0.9%) (**Schnuch et al., 1998**).

**Nettis et al. (2002)** studied occupational irritant and allergic contact dermatitis among healthcare workers. The authors reviewed their database for data from 1994 to 1998 and selected 360 consecutive patients working in healthcare environments and experiencing contact dermatitis of the hands, wrists, and forearms. The authors found that allergic contact dermatitis and irritant contact dermatitis were considered work-related in 16.5% (72/436) and 44.4% (194/436) of diagnoses, respectively. In this study, the major relevant etiological agents that induced occupational allergic contact dermatitis were: nickel sulfate (41 patch positivities), glutaraldehyde (5), Benzalkonium Chloride (7), thiuram mix (15), carba mix (9), and tetramethylthiuram monosulfide (6).

**Herbst et al. (2004)** evaluated patch test results of the Information Network of the Departments of Dermatology. During a 5-year period (1995-1999), of a total of 49,256 patch-tested patients, 1053 (2.1%) were eventually diagnosed as allergic periorbital dermatitis (APD) and 588 (1.2%) as non-allergic periorbital dermatitis (NAPD). Patient characteristics between APD, NAPD and other cases (OCs) differed with respect to sex (19.7% male in both periorbital groups versus 36.3% in OCs), atopic dermatitis (10.4% in APD versus 60.2% in NAPD versus 16.9% in OCs) and age, APD being substantially more often (68.2%), ages 40 and above, than NAPD (52.6%). For the APD group, of the 893 patients patch tested with 0.1% Benzalkonium Chloride in petrolatum, 1.9% positive reactions. For the NAPD group, of the 491 patients patch-tested with 0.1% Benzalkonium Chloride in petrolatum, 0.3% had positive reactions. For the OC patients, of the 27,122 patients patch-tested with 0.1% Benzalkonium Chloride in petrolatum, 0.9% had positive reactions.

**Saap et al. (2004)** patch-tested 54 leg ulcer patients (19 men, 35 women; mean age = 65.24 ± 13.96 years), with the NACDG standard series and a comprehensive supplemental series of 52 allergens. Patch tests (Finn chambers) were applied to hairless skin of the back of each patient. Patch test results were read 48 hours and 96 or 120 hours after application and reactions were scored according to the following scale: 1+ (weak positive) to 3+ (extremely strong positive). Sixty-three percent (n = 34) of the patients had one or multiple positive patch test results, and 37% (n = 20) had no positive patch test result. The most common allergens were balsam of Peru (30% of the patients, [16/54]), bacitracin (24% [13/54]), fragrance mix (20% [11/54]), propylene glycol (14% [7/52]), Benzalkonium Chloride (13% [7/54]), carba mix (11% [6/54]), nickel sulfate (11% [6/54]), and control

gel hydrocolloid (11% [6/54]).

According to a study by **Dastychova et al. (2004)**, a group of 514 patients (178 men, 336 women; average age = 42.8 years) suffering from chronic eczema was tested by means of epicutaneous tests for contact hypersensitivity to selected auxiliary substances of dermatological external and cosmetic preparations. The testing took place from April of 2001 to December of 2002. In 194 patients, the principal diagnosis was atopic eczema. Of the preservatives, the most frequently sensitizing agents were as follows: thiomersal (in 13.6% of the patients); phenylmercuric acetate (in 7.8%); formaldehyde (in 5.6%); Bronopol (in 5.1%); chlorhexidine (in 3.3%); dibromodicyanobutane/phenoxyethanol (in 2.9%); chloroacetamide (in 2.1%); Kathon CG and parabens (in 1.9%); imidazolidinyl urea and diazolidinyl urea (in 1.4%); glutaraldehyde (in 1.2%); DMDM Hydantoin (in 1.0%); dichlorophen (in 0.8%); sorbic acid, phenoxyethanol, and triclosan (all in 0.6%); Benzalkonium Chloride, quaternium-15, and chlorocresol (all in 0.4%); and chloroquinaldol (in 0.2% of the group of patients).

**Pratt et al. (2004)** reported the results of patch testing from January 1, 2001 to December 31, 2002, by the North American Contact Dermatitis Group. For this study period, Benzalkonium Chloride was one of the new additions to the NACDG screening tray. Up to 4,913 patients (2 to 97 years old) were patch-tested with a screening series of 65 allergens. Patch testing was done with a standardized technique using Finn Chambers on Scanpor tape. The patches remained in place for 48 hours, and test sites were evaluated initially at 48 to 72 hours, and again between 72 and 168 hours after placement. A positive reaction was interpreted as +, ++, or +++, manifested by erythematous papules, vesicles and/or a spreading reaction, sometimes with crusting and ulceration. The clinical relevance of the positive patch test reactions was determined by the patient's history and clinical skin examination findings. Present relevance was divided into definite, probable, and possible relevance.

Relevance was considered definite if the reaction to a use test with the putative item containing the suspected allergen was positive or if a positive patch-test reaction to the object or product was obtained. Relevance was considered probable if the substance identified by patch testing could be verified as present in the known skin contactants of the patient, and if the clinical distribution fit. Relevance was considered possible if the patient was exposed to circumstances in which skin contact with materials known to contain the putative allergen likely occurred.

Of the 4,892 patients patch tested with 0.1% aqueous Benzalkonium Chloride, 4.3% had positive reactions, 2.8% had irritant reactions, and 1.5% had reactions that were classified as unknown. Relevance was summarized as follows: definite relevance (0.9%), probable relevance (5.2%), possible relevance (20.8%), and past relevance (8.5%). 2.5% nickel sulfate yielded the highest frequency of positive reactions (16.7%) in 4,901 patients; 0.4% had irritant reactions (**Pratt et al., 2004**).

A study by **Boyvatz et al. (2005)** was designed to evaluate the frequency of contact sensitivity to 14 common preservatives among patients with contact dermatitis in Turkey. From 2000 to 2004, 308 patients with a diagnosis of contact dermatitis were patch tested in the Department of Dermatology of Ankara University School of Medicine. All patients were patch tested with the European standard series. Of the 308 patients suspected of having contact dermatitis, 23 patients were found to have positive reactions to one or more preservatives. The preservatives that were the most frequent cause of positive reactions were as follows: thimerosal (1.6%), Benzalkonium Chloride (5 positive reactions or 1.6%), formaldehyde (1.3%), and MDBGN/PE (0.9%).

#### CASE REPORTS

In a case report by **Fisher (1987)**, a 36-year-old operating nurse acquired a pruritic eczematous eruption around an abrasion after he had applied a medicated adhesive strip containing Benzalkonium Chloride to the inner aspect of the thigh. A strongly positive patch test result was obtained with a 1:1000 aqueous solution of Benzalkonium Chloride.

**Miszkiel et al. (1988)** investigated the possibility that Benzalkonium Chloride-induced bronchoconstriction results from the endogenous release of histamine by examining the effect of the selective histamine antagonists terfenadine and astemizole on the airways' response to inhaled Benzalkonium Chloride and histamine in 12 asthmatic subjects (6 men, 6 women; mean age =  $29 \pm 4$  years). All were atopic, demonstrating a greater than 3 mm wheal following skin prick test to at least two common allergens and had a mean baseline forced expiratory volume ( $FEV_1$ ) of  $94 \pm 7\%$  predicted values. Airway calibre was measured before and during the inhalation challenge as the  $FEV_1$ . On each challenge day, Benzalkonium Chloride was dissolved in 0.9% sodium chloride to produce a range of doubling concentrations of 0.4 to 50 mg/ml. Histamine monophosphate was dissolved in 0.9% sodium chloride to produce a range of doubling concentrations of 0.03 to 64 mg/ml.

Benzalkonium Chloride and histamine caused concentration-related decreases in FEV<sub>1</sub> in all of the subjects, with Benzalkonium Chloride being 7.4 times less potent as a bronchoconstrictor agonist than histamine. Terfenadine displaced to the right the Benzalkonium Chloride and histamine concentration response curves by 3.7- and 111-fold, respectively. Terfenadine attenuated the initial (5 minutes) bronchoconstrictor response to Benzalkonium Chloride by 40%. However, over the whole 45-minute period, the response was reduced by only 13%, compared with 86% inhibition of the response to histamine.

Eight of the 12 subjects undertook a time course study with inhaled Benzalkonium Chloride after pretreatment with the chemically unrelated histamine antagonist astemizole. Astemizole inhibited Benzalkonium Chloride-induced bronchoconstriction to an almost identical degree as that achieved with terfenadine.

It was concluded that the initial bronchoconstrictor effect of Benzalkonium Chloride is due, in part, to histamine release (**Miszkiel et al., 1988**).

**Trevisan et al. (1988)** reported a case of a 6-year-old girl affected by an itching erythematous, vesiculous, desquamative dermatitis of the ears and cheeks. Dermatitis appeared after applications of earrings and a diagnosis of contact dermatitis to metal was made. Patch testing with metals (including gold chloride) gave negative results. On the contrary, positive results were achieved with Lysoform Medical (1:20 and 1:1000), a liquid disinfectant used to clean earring pins, and with Benzalkonium Chloride present in the Lysoform.

In a case report by **Okamoto et al. (1991)**, a 37-year-old female presented with a scaly, erythematous eruption on the second and third fingers of the left hand over a three-month period. She had been working in the ophthalmology department of a hospital for two years, and her routine work there was to instill mydiratic agents into patients with eye diseases, as part of their fundoscopic examination. The eyedrops that were used contained Benzalkonium Chloride and other ingredients. Patch test results for Benzalkonium Chloride were negative.

According to **Boucher et al. (1992)**, a 64-year-old male was admitted to the Ottawa General Hospital because of an exacerbation of chronic obstructive airway disease. His presenting symptoms were increasing shortness of breath and productive cough. The patient was started on intermittent albuterol, being exposed to 600 µg of Benzalkonium Chloride per treatment. The drug combination

was administered every 1 to 2 hours initially, but was changed to every four hours because the patient was complaining of increased difficulty in breathing after treatments. The patient improved and early discharge was anticipated, however, he began to complain of increased shortness of breath after each nebulized treatment.

Considering the time course of the bronchoconstriction, the amount of preservative present in the nebulized solution, and the lack of reaction to the drugs administered via metered-dose inhalation, the authors noted that this case is suggestive of a preservative-induced severe paradoxical bronchoconstriction. The authors noted that two classes of preservative agents have been mostly involved in bronchoconstriction reactions, sulfite derivatives (sodium metabisulfite and sodium bisulfite) and Benzalkonium Chloride (**Boucher et al., 1992**).

**Ponder and Wray (1993)** reported a case of a 58-year-old gynecologic nurse with a long history of allergic rhinitis, chronic sinusitis, and relative airway disease. The patient was scheduled for sinus surgery and, during a preoperative jet nebulization treatment with Proventil (contains Benzalkonium Chloride), flushing and itching of the face and neck were observed. The patient was initially tested (epicutaneous method) with 0.001% and 0.01% Benzalkonium Chloride (diluted with saline), and the results were negative. Subsequently, when tested intradermally with the 0.001% Benzalkonium Chloride solution, a positive response was reported. Within a few minutes of the intradermal test, the patient experienced a systemic reaction consisting of runny nose, throat fullness, and facial flushing (with pitting edema of the face and a severe cough). The results for three healthy control subjects were negative in both epicutaneous and intradermal tests.

In a case report by **Corazzo and Virgili (1993)**, a 48-year old female farm worker (assigned to pig breeding) had a 1-year history of widespread recurrent vesicular eczema of the hands, face, neck, thighs, axillae, groins and abdominal folds. She was exposed to detergents and disinfectants on the job, and was subsequently patch tested with the European standard series and the antimicrobials and preservatives series. The following positive reactions were reported: 5% nickel sulfate in petrolatum (++) on day 2; (+++) on day 3), 0.1% aqueous Benzalkonium Chloride (- on day 2; ++ on day 3), and 0.01% aqueous Benzalkonium Chloride (?+ on day 2; + on day 3). Patch tests with Benzalkonium Chloride at a concentration of 0.01% aqueous and two products at a concentration of 10% aqueous were negative in five controls. The authors stated that the positive reaction to

Benzalkonium Chloride at the lower test concentration and the close relationship between flares of eczema and use of the products are sufficient evidence for the diagnosis of occupational allergic contact dermatitis from Benzalkonium Chloride.

In a case report by **Cusano and Luciano (1993)**, a 36-year-old nurse was seen for an intensely itchy eczema, which had begun on the hands and forearms about 4 months previously and had worsened in the last month, spreading to the upper arms and face. Patch test results were positive on day 2 (+++) and day 3 (+++). Because of extreme bullous reactions to Benzalkonium Chloride two healthy volunteers and 8 patients with contact dermatitis were patch tested. Positive reactions were not observed.

According to **Corazza and Virgili (1993)**, a 48-year-old female had a one-year history of widespread recurrent vesicular eczema of the hands, face, neck, thighs, axillae, groins, and abdominal folds. She was a farm worker assigned to pig breeding, and used detergents and disinfectants. Patch test results for 0.1% aqueous Benzalkonium Chloride were negative on day 2 and ++ on day 3. Subsequent patch testing with 0.01% Benzalkonium Chloride yielded the following results: ?+ on day 2 and + on day 3.

In a case report by **Cox (1994)**, a 78-year-old female presented with marked periorbital erythema and edema, and erythema of the nail folds of both hands. The clinical differential diagnosis of the facial rash included contact allergy, drug eruption, and dermatomyositis. Patch testing to the European standard series and an eyedrop/contact lens series was carried out, when her general condition permitted, demonstrating a ++ reaction to Benzalkonium Chloride. The patient had used Teoptic eyedrops (cartereol 2%, with Benzalkonium Chloride preservative) for glaucoma. The facial and nailfold eruptions settled rapidly with moderate-potency topical corticosteroid, and the eyedrops were changed to metipranolol 0.3%. No relapse occurred during the following two months.

According to **Stanford and Georgouras (1996)**, a 14-year-old boy presented with severe dermatitis of the right forearm, where it had been in contact with a plaster cast over the previous month. Upon removal of the cast, the underlying skin was erythematous and edematous with vesicles and bullae. There were no systemic symptoms, no past or family history of atopy and no previous exposure to plaster casts. Antiseptic solution had not been used prior to application of the cast. On examination, a severe dermatitis was evident around the right forearm. A milder eczematous area on

the abdomen corresponded to the area where the cast had rested while in the sling. The patient developed a strong allergic reaction to the Gypsona® plaster, as well as to both 0.1% aqueous and 0.01% aqueous Benzalkonium Chloride.

In a case report by **Gonzalo Garijo et al. (1996)**, a 37-year-old male with a history of allergic rhinitis from sensitization to dust mites experienced urticaria, dyspnea, wheezing, rhinorrhea, and dysphonia 20 minutes after intraarticular administration of mepivacaine hydrochloride and paramethasone acetate (includes the excipients Benzalkonium Chloride, polysorbate 80, and povidone) in his right knee. After treatment, symptoms resolved uneventfully over the next 24 hours. Two months after discharge from the hospital, the patient was seen in the allergy department.

Because of suspected anaphylactic shock, the patient was admitted for controlled provocation tests. Prior *in vitro* studies with these substances (histamine release test, basophil degranulation test, and lymphoblast transformation test - test details not provided) were negative. Results of provocation testing polysorbate 80 and Benzalkonium Chloride were negative, but were positive with povidone. The reaction to povidone appeared 5 minutes after intramuscular administration of the pure preparation (1 mL), and included aqueous rhinorrhea, conjunctival reddening, dysphagia, dyspnea, rash on the back, and discrete edema of the uvula and anterior pillars (**Gonzalo Garijo et al., 1996**).

In a case report by **Ortiz-Frutos (1996)**, a 41-year-old clerk, with no prior diseases, presented with itchy erythematous lesions, with vesicles and exudation, on the neck. Two days prior to onset, she had begun to apply a scarf soaked in commercial ethanol (96%) to treat pharyngitis. She had previously used commercial ethanol as a disinfectant, with no problems. After the first outbreak had been cured, she began to spray a perfume on her neck, which caused fresh eczema to break out. The perfume company confirmed the presence of Benzalkonium Chloride as a denaturant of the ethanol that was used in the manufacture of her perfume. The following positive patch reactions to Benzalkonium Chloride were reported: 0.1% Benzalkonium Chloride (++) on days 2 and 4) and 0.01% aqueous Benzalkonium Chloride (+ on day 2 and ++ on day 4)

According to **Krogsrud and Larsen (1997)**, a laboratory technician, without previous skin diseases and non-atopic, developed urticaria on her face and neck 4 to 6 hours after washing a laminar air flow (LAF) bench with Benzalkonium Chloride solution. Itching began on the same evening. The skin became red and vesicles appeared on the chin and on the front of the neck. The

the abdomen corresponded to the area where the cast had rested while in the sling. The patient developed a strong allergic reaction to the Gypsona® plaster, as well as to both 0.1% aqueous and 0.01% aqueous Benzalkonium Chloride.

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symptoms lasted for 2 days. No symptoms were recognized on the hands or the arms, and she showed no symptoms of the airways and mucosa. Patch test (\*Finn chambers on Scanpor tape) results for 0.1% aqueous Benzalkonium Chloride were negative. Patches were applied for 2 days and readings were done at days 2 and 3.

According to case reports by **Purohit et al. (2000)**, three female nurses manifested asthma symptoms upon handling disinfectant solutions containing Benzalkonium Chloride. Two of the nurses handled solutions containing 10% and 40% Benzalkonium Chloride, respectively. The concentration of Benzalkonium Chloride in the solution handled by the third nurse was not stated. A work-related decrease in peak expiratory flow rates was observed in all three nurses. The diagnosis was confirmed by challenge tests where the patients were exposed, in a closed chamber, to the suspect disinfectant contained in a tray. All of the nurses developed early or delayed symptoms upon exposure. Similar challenge tests to placebo or other disinfectants devoid of quaternary ammonium compound were negative.

**According to Park et al. (2000)**, a 35-year-old female presented with an 8-month history of intensely pruritic eczema, beginning on the feet and spreading to the lower legs. The lesions were erythematous to dark-brownish, edematous, lichenified, and exudative. There were no systemic symptoms and no personal or family history of atopy. She had been treated intermittently with Triaxin® , an antifungal solution. On patch testing with the ingredients of Triaxin®, Benzalkonium Chloride (0.1% aqueous, 0.01% aqueous) elicited ++ reactions on days 2 and 4. Patch tests with Benzalkonium Chloride (0.1% aqueous and 0.01% aqueous) in 5 control subjects were negative. A focal flare of skin lesions was noted during patch testing. Distilled water produced negative results.

In a case report by **Kanerva et al. (2000)**, a 32-year-old non-atopic cook was referred for investigation of a papular eruption on the backs of her hands and lower arms and a facial dermatitis, after cleaning the oven at work with a disinfecting detergent for sanitary and foodstuff areas. She had worked in the same occupation for 10 years and had twice earlier had symptoms from a similar product for cleaning ovens. Neomycin sulfate, nickel sulfate, and Benzalkonium Chloride (Epikon, 0.1% aqueous) provoked +++ reactions.

In a second case, a 42-year-old patient, cleaner with hand dermatitis, had a ++ patch test reaction to Benzalkonium Chloride (0.1% aqueous) and a +++ reaction to formaldehyde (1%

aqueous). Both reactions were considered relevant and caused by detergents that she had used at work (**Kanerva et al., 2000**).

In a case report by **Wong and Watson (2001)**, an 81-year-old female developed multiple large, tense, hemorrhagic bullae on the palm, an acute vesicular eczematous eruption on the forearm, after the application of a Plaster of Paris splint. Subsequent patch testing revealed positive reactions to both the Plaster of Paris bandage and to Benzalkonium Chloride. The patient was patch tested with Benzalkonium Chloride (0.1% in petrolatum), and readings were performed at 48 and 96 hours, and a 1+ reaction was reported.

In a case report by **Chowdhury and Statham (2002)**, a 65-year-old man presented with an acute erythematous rash after using Timodine® cream. There was no personal or family history of atopy. Patch test (Finn chambers) results indicated a ++ reaction to Timodine® cream and a + reaction to 0.1% aqueous Benzalkonium Chloride.

**Walker et al. (2004)** reported a case of a 47-year-old varnish maker with a bilateral hyperkeratotic palmar dermatitis for 8 months. In addition to a standard series, the patient was patch-tested with his own medicaments, gloves, and components of face, eye, and coolant series. The patient had ++ reactions to 0.05% 1,2-benzisothiazolin-3-one in petrolatum and 0.1% Benzalkonium Chloride in petrolatum at 2 and 4 days.

**Kim and Ahn (2004)** reported a case of anaphylactic shock in a 23-year-old asthmatic female, following an intradermal skin test with a salbutamol solution containing Benzalkonium Chloride. Since the patient complained of cough and dyspnea after inhalation therapy with a nebulizer solution, an intradermal skin test was conducted using the same solution (serial dilution), which contained Benzalkonium Chloride. A positive reaction was obtained with a 1:10 dilution of the solution (wheal: 4 x 5 mm in diameter, flare: 18 x 20 mm). Approximately 10 minutes later, the patient reported dizziness, palpitations, and dyspnea. On examination, tachycardia, tachypnea, and hypotension were found. She was resuscitated with a subcutaneous injection of epinephrine and an infusion of saline. One month later, a bronchial provocation test with Benzalkonium Chloride was conducted and the patient showed a positive response. In the bronchial provocation test, the patient inhaled 3 ml of 0.9% NaCl solution containing 600 µg of Benzalkonium Chloride.

In a case report by **Oiso et al. (2005)**, a 62-year-old female was treated for itchy

erythematous macules on the face, especially on the forehead and the neck, after using a shampoo containing Benzalkonium Chloride. Patch tests involving the cosmetics, shampoo, and rinse that were used by the patient were performed. The patient was also patch tested with the ingredients of the shampoo. A positive reaction to the shampoo (at 100 times dilution, + at day 2 and day 3) or 0.1% aqueous Benzalkonium Chloride (+ at day 2 and day 3) was observed. However, no reaction to the shampoo (at 1000 times dilution) or to 0.01% aqueous Benzalkonium Chloride was observed on days 2, 3, and 4.

## REFERENCES

- Agner, T. And J. Seruo. 1988. Contact thermography for assessment of skin damage due to experimental irritants. *Acta Dermato-Venereol* 68:192-195.
- Allen, M.H., S.H. Wakelin, D. Holloway, S. Lisby, O. Baadsgaard, J.N. Barker, and J.P. McFadden. 2000. Association of TNFA gene polymorphism at position -308 with susceptibility to irritant contact dermatitis. *Immunogenetics* 51:201-205.
- Aoki, J. 1997. Allergic contact dermatitis due to eye drops. Their clinical features and the patch test results. *Nippon Ika Daigaku Zasshi* 64:232-237.
- Barlow, D.W., L.G. Duckert, C.S. Kreig, and G.A. Gates. 1995. Ototoxicity of topical otomicrobial agents. *Acta Otolaryngol* 115:231-235.
- British Industrial Biological Research Association (BIBRA). 1989. Benzalkonium chloride. Toxicity profile. BIBRA 309. BIBRA Toxicology International, British Industrial Biological Research Association, Carshalton, United Kingdom.
- Boston, M.E. 2002. Effects of nasal saline spray on human neutrophils. NTIS Report No. ADA406691.
- Boucher, M., M.T. Roy, and J. Henderson. 1992. Possible association of benzalkonium chloride in nebulizer solutions with respiratory arrest. *Ann Pharmacother* 26:772-774.
- Boyvatz, A., A. Akyol, and E. Gurgey. 2005. Contact sensitivity to preservatives in Turkey. *Contact Dermatitis* 52:329-332.
- Cho, J.H., Y.S. Kwun, H.S. Jang, J.M. Kang, Y.S. won, and H.R. Yoon. 2000. Long-term use of preservatives on rat nasal respiratory mucosa: effects of benzalkonium chloride and potassium sorbate. *Laryngoscope* 110:312-317.
- Chowdhury, M.M. and B.N. Statham. 2002. Allergic contact dermatitis from dibutyl phthalate and benzalkonium chloride in Timodine cream. *Contact Dermatitis* 46:57.
- Collin, H.B. and N. Carroll. 1986. Ultrastructural changes to the corneal endothelium due to benzalkonium chloride. *Acta Ophthalmol* 64:226-231.
- Corazza, JM. And A. Virgilio. 1993. Airborne allergic contact dermatitis from benzalkonium chloride. *Contact Dermatitis* 28:195-196.
- Corsini, E., A. Primavera, M. Marinovic, and C.L. Galli. 1998. Selective induction of cell-associated interleukin-1 alpha in murine keratinocytes by chemical allergens. *Toxicology* 129:193-200.
- Cox, N.H. 1994. Allergy to benzalkonium chloride simulating dermatomyositis. *Contact Dermatitis* 31:50.
- Cusano, F. and S. Luciano. 1993. Contact allergy to benzalkonium chloride and glutaraldehyde in a dental nurse. *Contact Dermatitis* 28:127.
- Dastychova, E., M. Necas, K. Pencikova, and P. Ceerny. 2004. Contact sensitization to pharmaceutical aids in dermatologic cosmetic and external use preparations. *Ceska Slov Farm* 53:151-156.
- Debbasch, C. M. De Saint Jean, P.J. Pisella, P. Rat, J.M. Warnet, and C. Baudouin. 1999. Quaternary ammonium cytotoxicity in a human conjunctival cell line. *J Fr Ophthalmol* 22:950-958.
- DeGeorge, G.L., T.L. Ripper, S. Young, and D.R. Cerven. 2004. Alternative photosensitization assay in the mouse. *Toxicologist* 78:270.

- De Groot, A.C., J.W. Weyland, J.D. Bos, and B.A. Jagtman. 1986. Contact allergy to preservatives I. *Contact Dermatitis* 14:120-122.
- Denoyer, A., F. Ossant, B. Arbeille, F. Fetissof, F. Patat, and P.J. Pisella. 2006. In vivo assessment of corneal epithelial toxicity of timolol with benzalkonium chloride using very-high-frequency ultrasound imaging. *J Fr Ophthalmol* 29:11-18.
- De Saint Jean, M., C. Debbasch, F. Brignole, J.M. Warnet, and C. Baudouin. 2002. Relationship between in vitro toxicity of benzalkonium chloride. *Adv Exp Med Biol* 506:697-702.
- De Saint Jean, M., C. Debbasch, F. Brignole, P. Rat, J.M. Warnet, and C. Baudouin. 2000. Toxicity of preserved and unpreserved beta-blocker eyedrops in an in vitro model of human conjunctival cells. *J Fr Ophthalmol* 23:111-121.
- Elder, R.L. 1989. Final report on the safety assessment of benzalkonium chloride. *JACT* 8:589-625.
- Eun, H.C., J.H. Chung, S.Y. Jung, K.H. Cho, and K.H. Kim. 1994. A comparative study of the cytotoxicity of skin irritants on cultured human oral and skin keratinocytes. *Br J Dermatol* 130:24-28.
- European Economic Community. 2005. Consolidated version of the EEC Cosmetics Directive 76/768/EEC, containing the 7<sup>th</sup> amendment and some subsequent technical adaptations up to 9 September 2005. Annex III. Part 1. List of substances which cosmetic products must not contain except subject to the restrictions and conditions laid down. Annexes VI. Part 1. List of preservatives allowed. Brussels:EEC.
- Fisher, A.A. 1987. Allergic contact dermatitis and conjunctivitis from benzalkonium chloride. *Cutis* 39:381-383.
- Food and Drug Administration (FDA). 2005. Frequency of use of cosmetic ingredients. *FDA database*. Washington:FDA.
- Food and Drug Administration (FDA). 2006. OTC Drug Review Ingredient Report. Internet site accessed April, 2006. <http://www.fda.gov/cder/offices/otc/industry.htm>.
- Fuchs, T., A. Meinert, W. Aberer et al. 1993. Is benzalkonium chloride a relevant contact allergen or irritant? Results of a multicentre study conducted by German contact Allergy Group (DKG). *Hautarzt* 44:699-702.
- Fukuda, S. 1987. Assessment of the carcinogenic hazard of 6 substances used in dental practices. (1) Morphological transformation, DNA damage and sister chromatid exchanges in cultured Syrian hamster embryo cells induced by carbol camphor, eugenol, thymol, EDTA, benzalkonium chloride and benzethonium chloride. *Shigaku* 74:1365-1384.
- Furrer, P., B. Plazonnet, J.M. Mayer, and R. Gurny. 2000. Application of in vivo confocal microscopy to the objective evaluation of ocular irritation induced by surfactants. *Int J Pharm* 207:89-98.
- Goh, C.L. 1989. Contact sensitivity to topical antimicrobials. (II). Sensitizing potentials of some topical antimicrobials. *Contact Dermatitis* 21:166-171.
- Gonzalo Garijo, M.A., J.A. Duran Quintana, P. Bobadilla Gonzalez, and P. Maiquez Asuero. 1996. Anaphylactic shock following povidone. *Ann Pharmacother* 30:37-40.
- Goto, S. And J.L. Grosfeld. 1989. The effect of a neurotoxin benzalkonium chloride on the lower esophagus. *J Surg Res* 47:117-119.
- Goto, Y., N. Ibaraki, and K. Miyake. 2003. Human lens epithelial cell damage and stimulation of their secretion of chemical mediators by benzalkonium chloride rather than latanoprost and timolol. *Arch Ophthalmol* 121:835-839.

- Gottschalck, T.E. and G.N. McEwen, Jr., eds. 2006. International Cosmetic Ingredient Dictionary and Handbook, 11<sup>th</sup> ed. Washington, D.C.:CTFA, 227-228.
- Graf, P., J. Enerdal, and H. Hallen. 1999. Ten days' use of oxymetazoline nasal spray with or without benzalkonium chloride in patients with vasomotor rhinitis. *Arch Otolaryngol Head Neck Surg* 125:1128-1132.
- Hallen, H. and P. Graf. 1995. Benzalkonium chloride in nasal decongestive sprays has a long-lasting adverse effect on the nasal mucosa of healthy volunteers. *Clin Exp Allergy* 25:401-405.
- Herbst, R.A., W. Uter, C. Pirker, J. Geier, and P.J. Frosch. 2004. Allergic and non-allergic periorbital dermatitis: patch test results of the Information Network of the Departments of Dermatology during a 5-year period. *Contact Dermatitis* 51:13-19.
- Herouet, C., M. Cottin, P. Galanaud, J. Leclaire, and F. Rousset. 1999. Contact sensitizers decrease 33D1 expression on mature Langerhans cells. *Eur J Dermatol* 9:185-190.
- Hikiba, H., E. Watanabe, J.C. Barrett, and T. Tsutsui. 2005. Ability of fourteen chemical agents used in dental practice to induce chromosome aberrations in Syrian hamster embryo cells. *J Pharmacol Sci* 97:146-152.
- Hirota, M. and O. Moro. 2005. MIP-1beta, a novel biomarker for in vitro sensitization test using human monocytic cell line. *Toxicol In Vitro* [Not in print]
- Holle, G.E. 1998. Changes in muscularis externa of rat small intestine after myenteric ablation with benzalkonium chloride: electron microscopic and morphometric study. *Dig Dis Sci* 43:2666-2675.
- Jaganathan, L. and R. Boopathy. 2000. Distinct effect of benzalkonium chloride on the esterase and aryl acylamidase activities of butyrylcholinesterase. *Bioorg Chem* 28:242-251.
- Kajino, T. 1987. Effect of benzalkonium chloride on cultured V79 cells. *Shigaku* 75:63-74.
- Kanerva, L., R. Jolanki, and T. Estlander. 2000. Occupational allergic contact dermatitis from benzalkonium chloride. *Contact Dermatitis* 42:357-358.
- Kaya, M., F. Baba, M. Deniz, S. Baykara, and S. Yucesan. 2005. Effects of benzalkonium chloride application on the rat bladder. A functional and histopathological study. *Urol Int* 74:74-78.
- Keser, A., M. Bozkurt, O.F. Taner, B. Yorgancigio, M. Dogan, and O. Sensoz. 2005. Evaluation of antiseptic use in plastic and hand surgery. *Ann Plast Surg* 55:490-494.
- Kim, S.H. and Y. Ahn. 2004. Anaphylaxis caused by benzalkonium in a nebulizer solution. 2004. Anaphylaxis caused by benzalkonium in a nebulizer solution. *J Korean Med Sci* 19:289-290.
- Klein, G.F., N. Sepp, and P. Fritsch. 1991. Allergic reaction to benzalkonium chloride? Do the use test. *Contact Dermatitis* 25:269-270.
- Kligman, A.M. and L.H. Kligman. 1998. A hairless mouse model for assessing the chronic toxicity of topically applied chemicals. *Food and Chemical Toxicology* 36:867-878.
- Kokelj, F. and A. Cantarutti. 1986. Contact dermatitis in leg ulcers. *Contact Dermatitis* 15:47-49.
- Kolde, G. and J. Knop. 1987. Different cellular reaction patterns of epidermal Langerhans cells after application of contact sensitizing, toxic, and tolerogenic compounds. A comparative ultrastructural and morphometric time-course analysis. *Journal of Investigative Dermatology* 89:19-23.

- Krogsrud, N.E. and A.I. Larsen. 1997. Airborne irritant contact dermatitis from benzalkonium chloride. *Contact Dermatitis* 36:112.
- Krysiak, B., K. Rydzynski, and M. Kiec-Swierczynska. 1998. The evaluation of the irritating and sensitizing effects of benzalkonium chloride. *Med Pr* 49:371-379. Erratum in *Med Pr* 49:456.
- Krogsrud, N.E. and A.I. Larsen. 1997. Airborne irritant contact dermatitis from benzalkonium chloride. *Contact Dermatitis* 36:112.
- Lebe, E., M. Baka, A. Yavasoglu, H. Aktug, U. Ates, and Y. Uyanikgil. 2004. Effects of preservatives in nasal formulations on the mucosal integrity: an electron microscopic study. *Pharmacology* 72:113-120.
- Liu, H., I. Routley, and K.D. Teichmann. 2001. Toxic endothelial cell destruction from intraocular benzalkonium chloride. *J Cataract Refract Surg* 27:1746-1750.
- Marriott, M. J. Holmes, L. Peters, K. Cooper, M. Rowson, and D.A. Basketter. 2005. The complex problem of sensitive skin. *Contact Dermatitis* 53:93-99.
- Maurer, J.K., R.D. Parker, and G.J. Carr. 1998. Ocular irritation: Pathological changes occurring in the rat with surfactants of unknown irritancy. *Toxicologic Pathology* 26:226-233.
- McFadden, J.P., D.B. Holloway, E.G. Whittle, and D.A. Basketter. 2000. Benzalkonium chloride neutralizes the irritant effect of sodium dodecyl sulfate. *Contact Dermatitis* 34:264-266.
- Ministry of Health, Labour and Welfare (MHLW). (March 23, 2005). MHW Ordinance No. 331. Appendices 2-4. Restricted lists. Ministry of Health Labour and Welfare, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Tokyo, Japan.
- MHLW. (September 29, 2000). MHW Ordinance No. 332. Ingredients of quasi-drugs. Products to be used directly on the body. Ministry of Health Labour and Welfare, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Tokyo, Japan.
- Miszkiel, K.A., r. Beasley, P. Rafferty, and S.T. Holgate. 1988. The contribution of histamine release to bronchoconstriction provoked by inhaled benzalkonium chloride in asthma. *Br J Clin Pharmacol* 25:157-163.
- Momma, J., K. Takada, Y. Aida et al. 1987. Effects of benzalkonium chloride on pregnant mice. *Eisei Shikenjo Hokoku* 105:20-25.
- Moreno, J.J. 2000. Arachidonic acid release and prostaglandin E2 synthesis as irritant index of surfactants in 3T6 fibroblast cultures. *Toxicology* 143:275-282.
- Nettis, E., M.C. Colanardi, A.L. Soccio, A. Ferrannini, and A. Tursi. 2002. Occupational irritant and allergic contact dermatitis among healthcare workers. *Contact Dermatitis* 46:101-107.
- Neville, R., P. Dennis, D. Sens, and R. Crouch. 1986. Preservative cytotoxicity to cultured corneal epithelial cells. *Curr Eye Res* 5:367-372.
- Nitsuma, A., M.K. Uchida, and t. Suzuki-Nishimura. 1996. Benzalkonium chloride inhibited the histamine release from rat peritoneal mast cells induced by bradykin and GlcNAc oligomer-specific lectin Datura stramonium agglutinin, but heparin did not. *Gen Pharmacol* 27:123-128.
- Noecker, r.J., L.A. Herrygers, and R. Anwaruddin. 2004. Corneal and conjunctival changes caused by commonly used glaucoma medications. *Cornea* 23:490-496.
- Oiso, N., K. Fukai, and M. Ishii. 2005. Irritant contact dermatitis from benzalkonium chloride. In a shampoo. *Contact Dermatitis* 52:54.

- Okabe, K., H. Kimura, J. Okabe et al. 2005. Effect of benzalkonium chloride on transscleral drug delivery. *Invest Ophthalmol Vis Sci* 46:703-708.
- Okamoto, H. and S. Kawai. 1991. Allergic contact sensitivity to mydriatic agents on a nurse's fingers. *Cutis* 47:357-358.
- Ortiz-Frutos, F.J., D. Argila, r. Rivera, O. Zamorro, and S. Miguelez. 1996. Allergic contact dermatitis from benzalkonium chloride used as a denaturant of ethanol. *Contact Dermatitis* 35:302.
- Park, H.J., H.A. Kang, J.Y. Lee, and H.O. Kim. 2000. Allergic contact dermatitis from benzalkonium chloride in an antifungal solution. *Contact Dermatitis* 42:306-307.
- Patton, D.L., G.G. Kidder, Y.C. Sweeney, L.K. Rabe, and S.L. Hillier. 1999. Effects of multiple applications of benzalkonium chloride and nonoxynol-9 on the vaginal epithelium in the ;igtailed macaque (Macaca nemestrina). *Am J Obstet Gynecol* 180:1080-1087.
- Perrenoud, D., A. Bircher, T. Hunziker. 1994. Frequency of sensitization to 13 common preservatives in Switzerland. Swiss Contact Dermatitis Research Group. *Contact Dermatitis* 30:276-279.
- Pichowski, J.S., M. Cumberbatch, R.J. Dearman, D.A. Basketter, and I. Kimber. 2001. Allergen-induced changes in interleukin 1 beta (IL-1 beta) mRNA expression by human blood-derived dendritic cells: interindividual differences and relevance for sensitization testing. *J Appl Toxicol* 21:115-121.
- Pisella, P.J., E. Lala, V. Parier, F. Brignole, and C. Baudouin. Effect of preservatives on he conjunctiva: a comparative study o beta-blocker eye drops with and without preservatives in glaucoma patients. *J Fr Ophthalmol* 26:675-679.
- Ponder, R.D. and B.B. Wray. 1993. A case report: sensitivity to benzalkonium chloride. *J Asthma* 30:229-231.
- Pratt, M.D., D.V. Belsito, V.A. DeLeo. 2004. North American Contact Dermatitis Group patch-test results, 2001-2002 study period. *Dermatitis* 15:176-183.
- Purohit, A., M.C. Kopferschmitt-Kubler, C. Moreau, E. Popin, M. Blaumeiser, and G. Pauli. 2000. Quaternary ammonium compounds and occupational asthma. *Int Arch Occup Environ Health* 73:423-427.
- Rizova, H., P. Carayon, A Barbier, F. Lacheretz, L. Dubertret, and L. Michel. 1999. Contact allergens, but not irritants, alter receptor-mediated endocytosis by human epidermal Langerhans cells. *British Journal of Dermatology* 140:200-209.
- Saap, L., S. Fahim, E. Arsenault, M. Pratt, T. Pierscianowski, V. Falanga, and A. Pedvis-Leticik. 2004. Contact sensitivity in patients with leg ulcerations: a North American study. *Arch Dermatol* 140:1241-1246.
- Sakagami, Y., H. Yamazaki, N. Ogasawara, H. Yokoyama, Y. Ose, and T. Sato. 1988. The evaluation of genotoxic activities of disinfectants and their metabolites by UMU test. *Mutat Res* 209:155-160.
- Sakagami, Y., Y. Yamasaki, H. Yokoyama, Y Ose, and T. Sato. 1988. DNA repair test of disinfectants by liquid rec-assay. *Mutat Res* 193:21-30.
- Santucci, B., C. Cannistraci, I. Lesnoni et al. 2003. Cutaneous response to irritants. *Contact Dermatitis* 48:69-73.
- Schnuch, A., J. Geier, W. Uter, and P.J. Frosch. 1998. Patch testing with preservatives, antimicrobials and industrial biocides. Results from a multicentre study. *Br J Dermatol* 138:467-476.

- Smith, M.J., T.H. Flowers, M.J. Cowling, and H.J. Duncan. 2002. Method for the measurement of the diffusion coefficient of benzalkonium chloride. *Water Research* 36:1423-1428.
- Stables, G.I., A. Forsyth, and R.S. Lever. 1996. Patch testing in children. *Contact Dermatitis* 34:341-344.
- Stanford, D., and K. Georgouras. 1996. Allergic contact dermatitis from benzalkonium chloride in plaster of Paris. *Contact Dermatitis* 35:371-372.
- Stern, M., M. Klausner, R. Alvarado, K. Renskers, and M. Dickens. 1998. Evaluation of the EpiOcular tissue model as an alternative to the Draize eye irritation test. *Toxicology In Vitro* 12:455-461.
- Storer, E., K.J. Koh, and L. Warren. 2004. Severe contact dermatitis as a result of an antiseptic bath oil. *Australas J Dermatol* 45:73-75.
- Tarbox, B.B., B.P. Conroy, E.S. Malicky et al. 1998. Benzalkonium chloride. A potential disinfecting irrigation solution for orthopaedic wounds. *Clin Orthop Relat Res* 346:255-261.
- Trevisan, G., F. Kokelj, and E. Briscik. 1988. Contact dermatitis caused by benzalkonium chloride mimicking metal dermatitis. *G Ital Dermatol Venereol* 123:513-515.
- Van der Bijl, P., A.D. Van Eyk, A.A. Gareis, and I.O. Thompson. 2003. Enhancement of transmucosal permeation of cyclosporine by benzalkonium chloride. *Oral Dis* 8:168-172.
- Walker, S.L., J.A. Yell, and M.H. Beck. 2004. Occupational allergic contact dermatitis caused by 1,2-benzisothiazolin-3-one in a varnish maker, followed by sensitization to benzalkonium chloride in Oilatum Plus bath additive. *Contact Dermatitis* 50:104-105.
- Wallengren, J. 2000. Dual effects of CGRP-antagonist on allergic contact dermatitis in human skin. *Contact Dermatitis* 43:137-143.
- Wenzel, H.R., A. Feldman, S. Engelbrecht, and H. Tschesche. 1990. Activation of the human leukocyte proteinases elastase and cathepsin G by various surfactants. *Biol Chem Hoppe Seyler* 371:721-724.
- Willis, C.M., E. Young, D.R. Brandon, and J.D. Wilkinson. 1986. Immunopathological and ultrastructural findings in human allergic and irritant contact dermatitis. *British Journal of Dermatology* 115:305-316.
- Willis, C.M., C.J.M. Stephens, and J.D. Wilkinson. 1988a. Experimentally-INDUCED irritant contact dermatitis. Determination of optimum irritant concentrations. *Contact Dermatitis* 18:20-24.
- Willis, C.M., C.J.M. Stephens, and J.D. Wilkinson. 1988b. Assessment of erythema in irritant contact dermatitis. Comparison between visual scoring and laser doppler flowmetry. *Contact Dermatitis* 18:138-142.
- Willis, C.M., C.J.M. Stephens, and J.D. Wilkinson. 1989. Epidermal damage induced by irritants in man: A light and electron microscopic study. *Journal of Investigative Dermatology* 93:695-699.
- Willis, C.M., C.J.M. Stephens, and J.D. Wilkinson. 1990. Differential effects of structurally unrelated chemical irritants on the density and morphology of epidermal Cd1+ cells. *Journal of Investigative Dermatology* 95:711-716.
- Wilmer, J.L., F.G. Burlison, F. Kayama, J. Kanno, and M.I. Luster. 1994. Cytokine induction in human epidermal keratinocytes exposed to contact irritants and its relation to chemical-induced inflammation in mouse skin. *J Invest Dermatol* 102:915-922.

- Withrow, T.J., V.M. Hitchins, A.G. Strickland, and N.T. Brown. 1989. Cytotoxicity and mutagenicity of ophthalmic solution preservatives and UVA radiation in L5178Y cells. *Photochem Photobiol* 50:385-389.
- Wong, D.A. and A.B. Watson. 2001. Allergic contact dermatitis due to benzalkonium chloride in plaster of Paris. *Australas J Dermatol* 45:73-75.
- Woolhiser, M.R., B.B. Hayes, and B.J. Meade. 1998. A combined murine local lymph node and irritancy assay to predict sensitization and irritancy potential of chemicals. *Toxicology Methods* 8:245-256.
- Xue, Y., Y. Hieda, K. Kimura, K. Takayama, J. Fujihara, and Y. Tsujino. 2004. Kinetic characteristics and toxic effects of benzalkonium chloride following intravascular and oral administration in rats. *J Chromatogr B Analyt Technol Biomed Life Sci.* 811:53-58.
- Xue, Y., Y. Hieda, Y. Saito et al. 2004. Distribution and disposition of benzalkonium chloride following various routes of administration in rats. *Toxicol Lett* 148:113-123.
- Zucoloto, S., J.C. Silva, J.S.M. Oliveira, and G. Muccillo. 1991. The chronological relationship between the thickening of smooth muscle, epithelial cell proliferation and myenteric neural denervation in the rat jejunum. *Cell Proliferation* 24:15-20.

## DISCUSSION

~~Arachidyl Propionate was used in 31 cosmetic products in 1981, based on voluntary reports provided to FDA by industry, with concentrations of use ranging from 3% to 10% (Elder 1990). In 2005, Arachidyl Propionate was reportedly used in 47 cosmetic products (FDA 2006). Data from an industry survey in 2006 indicated that Arachidyl Propionate was used at concentrations ranging from 0.001% to 7% (CTFA 2006).~~

## REFERENCES

~~Cosmetic, Toiletry, and Fragrance Association (CTFA). 2006. Concentration of use survey results. Unpublished data submitted by CTFA.<sup>2</sup>~~  
~~Elder, R. L., ed. 1990. Final report on the safety assessment of arachidyl propionate. *J. Am. Coll. Toxicol.* 9:143–52.~~  
~~Food and Drug Administration (FDA). 2006. Frequency of use of cosmetic ingredients. *FDA database*. Washington, DC: FDA.~~

## Benzalkonium Chloride

## CONCLUSION

In a safety assessment of Benzalkonium Chloride (Elder 1989), the Cosmetic Ingredient Review (CIR) Expert Panel stated that Benzalkonium Chloride, at concentrations up to 0.1% free, active ingredient, is safe as a cosmetic ingredient as presently used. The Expert Panel reviewed newly available studies since that assessment, along with updated information regarding types and concentration of use, noting that these studies were similar to those already included in the original safety assessment and, therefore, raised no new safety issues. The Panel confirmed the safety of Benzalkonium Chloride at concentrations up to 0.1% free, active ingredient and did not reopen the safety assessment.

## DISCUSSION

Benzalkonium Chloride was used in 83 products in 1986, based on voluntary reports provided to FDA by industry, at concentrations of  $\leq 0.1\%$  to 5% (Elder 1989). Data provided to FDA in 2006 indicated that Benzalkonium Chloride was used in 89 products (FDA 2006). Current use concentration data from a cosmetics industry survey indicated that Benzalkonium Chloride is being used in cosmetics at concentrations ranging from 0.01% to 0.5% (CTFA 2006). The available usage and use concentration data are given in Table 4 as a function of product category.

It appears that the maximum reported use concentration of 0.5% (i.e., 0.5% Benzalkonium Chloride in a liquid towelette [personal hygiene product]) exceeds the Panel's 0.1% concentration limit for Benzalkonium Chloride, which is based on skin irritation and sensitization potential. However, it was determined that this is not a concern because Benzalkonium Chloride is bound in the liquid towelette product, and, therefore, the concentration that comes in contact with the skin would be expected to be  $< 0.1\%$ .

The Panel recognizes that there are data gaps regarding use and concentration of this ingredient. However, the overall information available on the types of products in which this ingredient is used and at what concentration indicate a pattern of use, which was considered by the Expert Panel in assessing safety.

The Panel noted that Benzalkonium Chloride can increase the dermal penetration of other chemicals (e.g., betamethasone phosphate). The CIR Expert Panel advised formulators to consider this if the other ingredients in a formulation include those found safe by CIR on the basis that they did not penetrate the skin.

## REFERENCES

Agner, T., and J. Seruo. 1988. Contact thermography for assessment of skin damage due to experimental irritants. *Acta Dermato-Venerol.* 68:192–195.

Allen, M. H., S. H. Wakelin, D. Holloway, S. Lisby, O. Baadsgaard, J. N. Barker, and J. P. McFadden. 2000. Association of TNFA gene polymorphism at position –308 with susceptibility to irritant contact dermatitis. *Immunogenetics* 51:201–205.

Aoki, J. 1997. Allergic contact dermatitis due to eye drops. Their clinical features and the patch test results. *Nippon Ika Daigaku Zasshi.* 64:232–237.

Barlow, D. W., L. G. Duckert, C. S. Kreig, and G. A. Gates. 1995. Ototoxicity of topical otomicrobial agents. *Acta Otolaryngol.* 115:231–235.

British Industrial Biological Research Association (BIBRA). 1989. Benzalkonium chloride. Toxicity profile. BIBRA 309. BIBRA Toxicology International, British Industrial Biological Research Association, Carshalton, United Kingdom.

Boston, M. E. 2002. Effects of nasal saline spray on human neutrophils. NTIS Report No. ADA406691.

Boucher, M., M. T. Roy, and J. Henderson. 1992. Possible association of benzalkonium chloride in nebulizer solutions with respiratory arrest. *Ann. Pharmacother.* 26:772–774.

Boyvat, A., A. Akyol, and E. Gurgey. 2005. Contact sensitivity to preservatives in Turkey. *Contact Dermatitis* 52:329–332.

Cho, J. H., Y. S. Kwun, H. S. Jang, J. M. Kang, Y. S. won, and H. R. Yoon. 2000. Long-term use of preservatives on rat nasal respiratory mucosa: Effects of benzalkonium chloride and potassium sorbate. *Laryngoscope* 110:312–317.

Chowdhury, M. M., and B. N. Statham. 2002. Allergic contact dermatitis from dibutyl phthalate and benzalkonium chloride in Timodine cream. *Contact Dermatitis* 46:57.

Collin, H. B., and N. Carroll. 1986. Ultrastructural changes to the corneal endothelium due to benzalkonium chloride. *Acta Ophthalmol.* 64:226–231.

Corazza, J. M., and A. Virgilio. 1993. Airborne allergic contact dermatitis from benzalkonium chloride. *Contact Dermatitis* 28:195–196.

Corsini, E., A. Primavera, M. Marinovich, and C. L. Galli. 1998. Selective induction of cell-associated interleukin-1 alpha in murine keratinocytes by chemical allergens. *Toxicology* 129:193–200.

Cosmetic, Toiletry, and Fragrance Association (CTFA). 2006. Use concentration data on sorbic acid and potassium sorbate from industry survey. Unpublished data submitted by CTFA. 2 pages.<sup>2</sup>

Cox, N. H. 1994. Allergy to benzalkonium chloride simulating dermatomyositis. *Contact Dermatitis* 31:50.

Cusano, F., and S. Luciano. 1993. Contact allergy to benzalkonium chloride and glutaraldehyde in a dental nurse. *Contact Dermatitis* 28:127.

Dastychova, E., M. Necas, K. Pencikova, and P. Ceerny. 2004. Contact sensitization to pharmaceutical aids in dermatologic cosmetic and external use preparations. *Ceska. Slov. Farm.* 53:151–156.

Debbasch, C. M. De Saint Jean, P. J. Pisella, P. Rat, J. M. Warnet, and C. Baudouin. 1999. Quaternary ammonium cytotoxicity in a human conjunctival cell line. *J. Fr. Ophthalmol.* 22:950–958.

**TABLE 4**  
Historical and current cosmetic product uses and concentrations for Benzalkonium Chloride

Product category	1986 uses (Elder 1989)	2006 uses (FDA 2006)	1986 concentrations (Elder 1989) (%)	2006 concentrations (CTFA 2006) (%)
Baby products	4 <sup>a</sup>		≤ 0.1 – 1 <sup>a</sup>	
Shampoos		—		0.03
Lotions, oils, powders, and creams		2		0.03–0.1
Other		2		0.03
Bath products				
Soaps and detergents	—	—	—	0.1
Eye makeup	6 <sup>a</sup>		≤ 0.1 – 1 <sup>a</sup>	
Eyebrow pencils		—		0.02
Eyeliners		—		0.02
Eye shadow		—		0.02
Eye lotion		1		0.02
Eye makeup remover		10		0.01–0.05
Mascaras		—		0.02–0.1
Other		—		0.02
Fragrance Products				
Colognes and toilet waters	—	1	—	0.1
Perfumes	—	—	—	0.1
Powders	—	—	—	0.08–0.1
Sachets	—	—	—	0.1
Other	—	—	—	0.1
Noncoloring hair products	45 <sup>a</sup>		≤ 0.1 – 5 <sup>a</sup>	
Conditioners		12		0.05
Straighteners		—		0.1
Permanent waves		—		0.1
Rinses		2		0.1
Shampoos		—		0.1
Tonics, dressings, etc.		7		0.02–0.05
Hair-coloring products				
Dyes and colors	—	—	—	0.02
Tints	—	—	—	0.02
Rinses	—	—	—	0.02
Color sprays	—	—	—	0.02
Lighteners with color	—	—	—	0.02
Bleaches	—	1	—	0.02
Makeup				
Blushers	—	—	—	0.1
Face powders	—	—	—	0.1
Foundations	—	—	—	0.1
Makeup bases	—	—	—	0.1
Makeup fixatives	—	—	—	0.1
Other	—	—	—	0.1
Nail care products				
Cuticle softeners	—	—	—	0.1
Creams and lotions	—	1	—	—
Other	—	1	-	0.01–0.1

(Continued on next page)

**TABLE 4**  
Historical and current cosmetic product uses and concentrations for Benzalkonium Chloride (*Continued*)

Product category	1986		1986	2006
	uses (Elder 1989)	2006 uses (FDA 2006)	concentrations (Elder 1989) (%)	concentrations (CTFA 2006) (%)
Oral hygiene products				
Mouthwashes and breath fresheners	—	—	—	0.03
Personal hygiene products	11 <sup>a</sup>		≤ 0.1 – 1 <sup>a</sup>	
Underarm deodorants		1		0.1
Douches		—		0.1
Feminine deodorants		—		0.1
Other		1		0.1–0.5 <sup>b</sup>
Shaving products				
Aftershave lotions	—	—	—	0.1
Shaving cream	—	—	—	0.1
Shaving soap	—	—	—	0.1
Skin care products				
Skin cleansing creams, lotions, liquids, and pads	6	17	≤ 0.1 – 1	0.05–0.1
Depilatories	—	—	—	0.1
Face and neck creams, lotions, powder, and sprays	—	3	—	0.06–0.1
Body and hand creams, lotions, powder, and sprays	—	3	—	0.09–0.1
Foot powders and sprays	—	1	—	0.08–0.1
Moisturizers	4	1	≤ 0.1 – 1	0.1
Night creams, lotions, powders, and sprays	—	1	≤ 0.1 – 1	0.1
Paste masks/mud packs	—	3	—	0.1
Skin fresheners	—	5	—	0.1
Other	7	2	≤ 0.1 – 1	0.1
Suntan products				
Suntan gels, creams, and liquids	—	1	—	—
Total uses/ranges for Benzalkonium Chloride	83	89	≤ 0.1 – 5	0.01–0.5

<sup>a</sup>These categories were combined when the original safety assessment was performed and are now two or more separate categories.

<sup>b</sup>0.5% in a towelette product.

- DeGeorge, G. L., T. L. Ripper, S. Young, and D. R. Cerven. 2004. Alternative photosensitization assay in the mouse. *Toxicologist* 78:270.
- De Groot, A. C., J. W. Weyland, J. D. Bos, and B. A. Jagtman. 1986. Contact allergy to preservatives I. *Contact Dermatitis* 14:120–122.
- Denoyer, A., F. Ossant, B. Arbeille, F. Fetissof, F. Patat, and P. J. Pisella. 2006. In vivo assessment of corneal epithelial toxicity of timolol with benzalkonium chloride using very-high-frequency ultrasound imaging. *J. Fr. Ophthalmol.* 29:11–18.
- De Saint Jean, M., C. Debbasch, F. Brignole, J. M. Warnet, and C. Baudouin. 2002. Relationship between in vitro toxicity of benzalkonium chloride. *Adv. Exp. Med. Biol.* 506:697–702.
- De Saint Jean, M., C. Debbasch, F. Brignole, P. Rat, J. M. Warnet, and C. Baudouin. 2000. Toxicity of preserved and unpreserved beta-blocker eyedrops in an in vitro model of human conjunctival cells. *J. Fr. Ophthalmol.* 23:111–121.
- Elder, R. L. 1989. Final report on the safety assessment of benzalkonium chloride. *J. Am. Coll. Toxicol.* 8:589–625.
- Eun, H. C., J. H. Chung, S. Y. Jung, K. H. Cho, and K. H. Kim. 1994. A comparative study of the cytotoxicity of skin irritants on cultured human oral and skin keratinocytes. *Br. J. Dermatol.* 130:24–28.
- European Economic Community. 2005. Consolidated version of the EEC Cosmetics Directive 76/768/EEC, containing the 7th amendment and some subsequent technical adaptations up to 9 September 2005. Annex III. Part 1. List of substances which cosmetic products must not contain except subject to the restrictions and conditions laid down. Annexes VI. Part 1. List of preservatives allowed. Brussels: EEC.
- Fisher, A. A. 1987. Allergic contact dermatitis and conjunctivitis from benzalkonium chloride. *Cutis* 39:381–383.
- Food and Drug Administration (FDA). 2006. Frequency of use of cosmetic ingredients in 2005. *FDA database*. Washington, DC: FDA.
- Food and Drug Administration (FDA). 2006. OTC Drug Review Ingredient Report. Internet site accessed April, 2006. <http://www.fda.gov/cder/offices/otc/industry.htm>.
- Fuchs, T., A. Meinert, W. Aberer et al. 1993. Is benzalkonium chloride a relevant contact allergen or irritant? Results of a multicentre study conducted by German contact Allergy Group (DKG). *Hautarzt.* 44:699–702.
- Fukuda, S. 1987. Assessment of the carcinogenic hazard of 6 substances used in dental practices. (1) Morphological transformation, DNA damage and sister chromatid exchanges in cultured Syrian hamster embryo cells induced

- by carbol camphor, eugenol, thymol, EDTA, benzalkonium chloride and benzethonium chloride. *Shigaku* 74:1365–1384.
- Furrer, P., B. Plazonnet, J. M. Mayer, and R. Gurny. 2000. Application of in vivo confocal microscopy to the objective evaluation of ocular irritation induced by surfactants. *Int. J. Pharm.* 207:89–98.
- Goh, C. L. 1989. Contact sensitivity to topical antimicrobials. (II). Sensitizing potentials of some topical antimicrobials. *Contact Dermatitis* 21:166–171.
- Gonzalo Garijo, M. A., J. A. Duran Quintana, P. Bobadilla Gonzalez, and P. Maiquez Asuero. 1996. Anaphylactic shock following povidone. *Ann. Pharmacother.* 30:37–40.
- Goto, S., and J. L. Grosfeld. 1989. The effect of a neurotoxin benzalkonium chloride on the lower esophagus. *J. Surg. Res.* 47:117–119.
- Goto, Y., N. Ibaraki, and K. Miyake. 2003. Human lens epithelial cell damage and stimulation of their secretion of chemical mediators by benzalkonium chloride rather than latanoprost and timolol. *Arch. Ophthalmol.* 121:835–839.
- Gottschalck, T. E., and G. N. McEwen, Jr., eds. 2006. *International cosmetic ingredient dictionary and handbook*, 11th ed., 227–228. Washington, DC: CTFA.
- Graf, P., J. Enerdal, and H. Hallen. 1999. Ten days' use of oxymetazoline nasal spray with or without benzalkonium chloride in patients with vasomotor rhinitis. *Arch. Otolaryngol. Head Neck Surg.* 125:1128–1132.
- Hallen, H., and P. Graf. 1995. Benzalkonium chloride in nasal decongestive sprays has a long-lasting adverse effect on the nasal muclsa of healthy volunteers. *Clin. Exp. Allergy.* 25:401–405.
- Herbst, R. A., W. Uter, C. Pirker, J. Geier, and P. J. Frosch. 2004. Allergic and non-allergic periorbital dermatitis: patch test results of the Information Network of the Departments of Dermatology during a 5-year period. *Contact Dermatitis* 51:13–19.
- Herouet, C., M. Cottin, P. Galanaud, J. Leclaire, and F. Rousset. 1999. Contact sensitizers decrease 33D1 expression on mature Langerhans cells. *Eur. J. Dermatol.* 9:185–190.
- Hikiba, H., E. Watanabe, J. C. Barrett, and T. Tsutsui. 2005. Ability of fourteen chemical agents used in dental practice to induce chromosome aberrations in Syrian hamster embryo cells. *J. Pharmacol. Sci.* 97:146–152.
- Holle, G. E. 1998. Changes in muscularis externa of rat small intestine after myenteric ablation with benzalkonium chloride: Electron microscopic and morphometric study. *Dig. Dis. Sci.* 43:2666–2675.
- Jaganathan, L., and R. Boopathy. 2000. Distinct effect of benzalkonium chloride on the esterase and aryl acylamidase activities of butyrylcholinesterase. *Bioorg. Chem.* 28:242–251.
- Kajino, T. 1987. Effect of benzalkonium chloride on cultured V79 cells. *Shigaku* 75:63–74.
- Kanerva, L., R. Jolanki, and T. Estlander. 2000. Occupational allergic contact dermatitis from benzalkonium chloride. *Contact Dermatitis* 42:357–358.
- Kaya, M., F. Baba, M. Deniz, S. Baykara, and S. Yucesan. 2005. Effects of benzalkonium chloride application on the rat bladder. A functional and histopathological study. *Urol. Int.* 74:74–78.
- Keser, A., M. Bozkurt, O. F. Taner, B. Yorgancigio, M. Dogan, and O. Sensoz. 2005. Evaluation of antiseptic use in plastic and hand surgery. *Ann. Plast. Surg.* 55:490–494.
- Kim, S. H., and Y. Ahn. 2004. Anaphylaxis caused by benzalkonium in a nebulizer solution. 2004. Anaphylaxis caused by benzalkonium in a nebulizer solution. *J. Korean Med. Sci.* 19:289–290.
- Klein, G. F., N. Sepp, and P. Fritsch. 1991. Allergic reaction to benzalkonium chloride? Do the use test. *Contact Dermatitis* 25:269–270.
- Kligman, A. M., and L. H. Kligman. 1998. A hairless mouse model for assessing the chronic toxicity of topically applied chemicals. *Food Chem. Toxicol.* 36:867–878.
- Kokelj, F., and A. Cantarutti. 1986. Contact dermatitis in leg ulcers. *Contact Dermatitis* 15:47–49.
- Kolde, G., and J. Knop. 1987. Different cellular reaction patterns of epidermal Langerhans cells after application of contact sensitizing, toxic, and tolerogenic compounds. A comparative ultrastructural and morphometric time-course analysis. *J. Invest. Dermatol.* 89:19–23.
- Krogsrud, N. E., and A. I. Larsen. 1997. Airborne irritant contact dermatitis from benzalkonium chloride. *Contact Dermatitis* 36:112.
- Krysiak, B., K. Rydzynski, and M. Kiec-Swierczynska. 1998. The evaluation of the irritating and sensitizing effects of benzalkonium chloride. *Med. Pr.* 49:371–379. Erratum in *Med. Pr.* 49:456.
- Krogsrud, N. E., and A. I. Larsen. 1997. Airborne irritant contact dermatitis from benzalkonium chloride. *Contact Dermatitis* 36:112.
- Lebe, E., M. Baka, A. Yavasoglu, H. Aktug, U. Ates, and Y. Uyanikgil. 2004. Effects of preservatives in nasal formulations on the mucosal integrity: an electron microscopic study. *Pharmacology* 72:113–120.
- Liu, H., I. Routley, and K. D. Teichmann. 2001. Toxic endothelial cell destruction from intraocular benzalkonium chloride. *J. Cataract Refract. Surg.* 27:1746–1750.
- Marriott, M. J. Holmes, L. Peters, K. Cooper, M. Rowson, and D. A. Basketter. 2005. The complex problem of sensitive skin. *Contact Dermatitis* 53:93–99.
- Maurer, J. K., R. D. Parker, and G. J. Carr. 1998. Ocular irritation: Pathological changes occurring in the rat with surfactants of unknown irritancy. *Toxicol. Pathol.* 26:226–233.
- McFadden, J. P., D. B. Holloway, E. G. Whittle, and D. A. Basketter. 2000. Benzalkonium chloride neutralizes the irritant effect of sodium dodecyl sulfate. *Contact Dermatitis* 34:264–266.
- Ministry of Health, Labour and Welfare (MHLW). (March 23, 2005). MHW Ordinance No. 331. Appendices 2–4. Restricted lists. Ministry of Health Labour and Welfare, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Tokyo, Japan.
- MHLW. (September 29, 2000). MHW Ordinance No. 332. Ingredients of quasi-drugs. Products to be used directly on the body. Ministry of Health Labour and Welfare, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Tokyo, Japan.
- Miszkiel, K. A., R. Beasley, P. Rafferty, and S. T. Holgate. 1988. The contribution of histamine release to bronchoconstriction provoked by inhaled benzalkonium chloride in asthma. *Br. J. Clin. Pharmacol.* 25:157–163.
- Momma, J., K. Takada, Y. Aida, et al. 1987. Effects of benzalkonium chloride on pregnant mice. *Eisei Shikenjo Hokoku.* 105:20–25.
- Moreno, J. J. 2000. Arachidonic acid release and prostaglandin E2 synthesis as irritant index of surfactants in 3T6 fibroblast cultures. *Toxicology* 143:275–282.
- Nettis, E., M. C. Colanardi, A. L. Soccio, A. Ferrannini, and A. Tursi. 2002. Occupational irritant and allergic contact dermatitis among healthcare workers. *Contact Dermatitis* 46:101–107.
- Neville, R., P. Dennis, D. Sens, and R. Crouch. 1986. Preservative cytotoxicity to cultured corneal epithelial cells. *Curr. Eye Res.* 5:367–372.
- Nitsuma, A., M. K. Uchida, and T. Suzuki-Nishimura. 1996. Benzalkonium chloride inhibited the histamine release from rat peritoneal mast cells induced by bradykin and GlcNAc oligomer-specific lectin *Datura stramonium* agglutinin, but heparin did not. *Gen. Pharmacol.* 27:123–128.
- Noecker, r.J., L. A. Herrygers, and R. Anhwarruddin. 2004. Corneal and conjunctival changes caused by commonly used glaucoma medications. *Cornea* 23:490–496.
- Oiso, N., K. Fukai, and M. Ishii. 2005. Irritant contact dermatitis from benzalkonium chloride in a shampoo. *Contact Dermatitis.* 52:54.
- Okabe, K., H. Kimura, J. Okabe, et al. 2005. Effect of benzalkonium chloride on transscleral drug delivery. *Invest. Ophthalmol. Vis. Sci.* 46:703–708.
- Okamoto, H., and S. Kawai. 1991. Allergic contact sensitivity to mydriatic agents on a nurse's fingers. *Cutis.* 47:357–358.
- Ortiz-Frutos, F. J., D. Argila, R. Rivera, O. Zamarro, and S. Miguelez. 1996. Allergic contact dermatitis from benzalkonium chloride used as a denaturant of ethanol. *Contact Dermatitis* 35:302.
- Park, H. J., H. A. Kang, J. Y. Lee, and H. O. Kim. 2000. Allergic contact dermatitis from benzalkonium chloride in an antifungal solution. *Contact Dermatitis* 42:306–307.
- Patton, D. L., G. G. Kidder, Y. C. Sweeney, L. K. Rabe, and S. L. Hillier. 1999. Effects of multiple applications of benzalkonium chloride and nonoxynol-9

- on the vaginal epithelium in the pigtailed macaque (*Macaca nemestrina*). *Am. J. Obstet. Gynecol.* 180:1080–1087.
- Perrenoud, D., A. Bircher, and T. Hunziker. 1994. Frequency of sensitization to 13 common preservatives in Switzerland. Swiss Contact Dermatitis Research Group. *Contact Dermatitis* 30:276–279.
- Pichowski, J. S., M. Cumberbatch, R. J. Dearman, D. A. Basketter, and I. Kimber. 2001. Allergen-induced changes in interleukin 1 beta (IL-1 beta) mRNA expression by human blood-derived dendritic cells: interindividual differences and relevance for sensitization testing. *J. Appl. Toxicol.* 21:115–121.
- Pisella, P. J., E. Lala, V. Parier, F. Brignole, and C. Baudouin. Effect of preservatives on the conjunctiva: a comparative study of beta-blocker eye drops with and without preservatives in glaucoma patients. *J. Fr. Ophthalmol.* 26:675–679.
- Ponder, R. D., and B. B. Wray. 1993. A case report: sensitivity to benzalkonium chloride. *J. Asthma* 30:229–231.
- Pratt, M. D., D. V. Belsito, and V. A. DeLeo. 2004. North American Contact Dermatitis Group patch-test results, 2001–2002 study period. *Dermatitis* 15:176–183.
- Purohit, A., M. C. Kopferschmitt-Kubler, C. Moreau, E. Popin, M. Blaumeiser, and G. Pauli. 2000. Quaternary ammonium compounds and occupational asthma. *Int. Arch. Occup. Environ. Health* 73:423–427.
- Rizova, H., P. Carayon, A. Barbier, F. Lacheretz, L. Dubertret, and L. Michel. 1999. Contact allergens, but not irritants, alter receptor-mediated endocytosis by human epidermal Langerhans cells. *Br. J. Dermatol.* 140:200–209.
- Saap, L., S. Fahim, E. Arsenault, M. Pratt, T. Pierscianowski, V. Falanga, and A. Pedvis-Leticik. 2004. Contact sensitivity in patients with leg ulcerations: A North American study. *Arch. Dermatol.* 140:1241–1246.
- Sakagami, Y., H. Yamazaki, N. Ogasawara, H. Yokoyama, Y. Ose, and T. Sato. 1988. The evaluation of genotoxic activities of disinfectants and their metabolites by UMU test. *Mutat. Res.* 209:155–160.
- Sakagami, Y., Y. Yamasaki, H. Yokoyama, Y. Ose, and T. Sato. 1988. DNA repair test of disinfectants by liquid rec-assay. *Mutat. Res.* 193:21–30.
- Santucci, B., C. Cannistraci, I. Lesnani, et al. 2003. Cutaneous response to irritants. *Contact Dermatitis* 48:69–73.
- Schnuch, A., J. Geier, W. Uter, and P. J. Frosch. 1998. Patch testing with preservatives, antimicrobials and industrial biocides. Results from a multicentre study. *Br. J. Dermatol.* 138:467–476.
- Smith, M. J., T. H. Flowers, M. J. Cowling, and H. J. Duncan. 2002. Method for the measurement of the diffusion coefficient of benzalkonium chloride. *Water Res.* 36:1423–1428.
- Stables, G. I., A. Forsyth, and R. S. Lever. 1996. Patch testing in children. *Contact Dermatitis* 34:341–344.
- Stanford, D., and K. Georgouras. 1996. Allergic contact dermatitis from benzalkonium chloride in plaster of paris. *Contact Dermatitis* 35:371–372.
- Stern, M., M. Klausner, F. Alvarado, K. Renskers, and M. Dickens. 1998. Evaluation of the EpiOcular tissue model as an alternative to the Draize eye irritation test. *Toxicol. In Vitro* 12:455–461.
- Storer, E., K. J. Koh, and L. Warren. 2004. Severe contact dermatitis as a result of an antiseptic bath oil. *Australas. J. Dermatol.* 45:73–75.
- Tarbox, B. B., B. P. Conroy, E. S. Malicky et al. 1998. Benzalkonium chloride. A potential disinfecting irrigation solution for orthopaedic wounds. *Clin. Orthoped. Relat. Res.* 346:255–261.
- Trevisan, G., F. Kokelj, and E. Briscik. 1988. Contact dermatitis caused by benzalkonium chloride mimicking metal dermatitis. *G. Ital. Dermatol. Venereol.* 123:513–515.
- Van der Bijl, P., A. D. Van Eyk, A. A. Gareis, and I. O. Thompson. 2003. Enhancement of transmucosal permeation of cyclosporine by benzalkonium chloride. *Oral Dis.* 8:168–172.
- Walker, S. L., J. A. Yell, and M. H. Beck. 2004. Occupational allergic contact dermatitis caused by 1,2-benzisothiazolin-3-one in a varnish maker, followed by sensitization to benzalkonium chloride in Oilatum Plus bath additive. *Contact Dermatitis* 50:104–105.
- Wallengren, J. 2000. Dual effects of CGRP-antagonist on allergic contact dermatitis in human skin. *Contact Dermatitis* 43:137–143.
- Wenzel, H. R., A. Feldman, S. Engelbrecht, and H. Tschesche. 1990. Activation of the human leukocyte proteinases elastase and cathepsin G by various surfactants. *Biol. Chem. Hoppe Seyler* 371:721–724.
- Willis, C. M., E. Young, D. R. Brandon, and J. D. Wilkinson. 1986. Immunopathological and ultrastructural findings in human allergic and irritant contact dermatitis. *Br. J. Dermatol.* 115:305–316.
- Willis, C. M., C. J. M. Stephens, and J. D. Wilkinson. 1988a. Experimentally-INDUCED irritant contact dermatitis. Determination of optimum irritant concentrations. *Contact Dermatitis* 18:20–24.
- Willis, C. M., C. J. M. Stephens, and J. D. Wilkinson. 1988b. Assessment of erythema in irritant contact dermatitis. Comparison between visual scoring and laser doppler flowmetry. *Contact Dermatitis* 18:138–142.
- Willis, C. M., C. J. M. Stephens, and J. D. Wilkinson. 1989. Epidermal damage induced by irritants in man: A light and electron microscopic study. *J. Invest. Dermatol.* 93:695–699.
- Willis, C. M., C. J. M. Stephens, and J. D. Wilkinson. 1990. Differential effects of structurally unrelated chemical irritants on the density and morphology of epidermal Cd1+ cells. *J. Invest. Dermatol.* 95:711–716.
- Wilmer, J. L., F. G. Bureson, F. Kayama, J. Kanno, and M. I. Luster. 1994. Cytokine induction in human epidermal keratinocytes exposed to contact irritants and its relation to chemical-induced inflammation in mouse skin. *J. Invest. Dermatol.* 102:915–922.
- Withrow, T. J., V. M. Hitchins, A. G. Strickland, and N. T. Brown. 1989. Cytotoxicity and mutagenicity of ophthalmic solution preservatives and UVA radiation in L5178Y cells. *Photochem. Photobiol.* 50:385–389.
- Wong, D. A., and A. B. Watson. 2001. Allergic contact dermatitis due to benzalkonium chloride in plaster of Paris. *Australas. J. Dermatol.* 45:73–75.
- Woolhiser, M. R., B. B. Hayes, and B. J. Meade. 1998. A combined murine local lymph node and irritancy assay to predict sensitization and irritancy potential of chemicals. *Toxicol. Methods* 8:245–256.
- Xue, Y., Y. Hieda, K. Kimura, K. Takayama, J. Fujihara, and Y. Tsujino. 2004. Kinetic characteristics and toxic effects of benzalkonium chloride following intravascular and oral administration in rats. *J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci.* 811:53–58.
- Xue, Y., Y. Hieda, Y. Saito, et al. 2004. Distribution and disposition of benzalkonium chloride following various routes of administration in rats. *Toxicol. Lett.* 148:113–123.

### Cetearyl Alcohol, Cetyl Alcohol, Isostearyl Alcohol, Myristyl Alcohol, and Behenyl Alcohol

#### CONCLUSION

~~In a safety assessment of Cetearyl Alcohol, Cetyl Alcohol, Isostearyl Alcohol, Myristyl Alcohol, and Behenyl Alcohol (Elder, 1988), the Cosmetic Ingredient review (CIR) Expert Panel stated these cosmetic ingredients were safe in the present practices of use. The Expert Panel reviewed newly available studies since that assessment, along with updated information regarding types and concentrations of use. The Panel confirmed the safety of Cetearyl Alcohol, Cetyl Alcohol, Isostearyl Alcohol, Myristyl Alcohol, and Behenyl Alcohol in the practices of use and concentrations as given in Table 5, and did not reopen the safety assessment.~~

#### DISCUSSION

~~Cetearyl Alcohol was used in 56 cosmetic products in 1982, based on voluntary reports provided to FDA by industry, with use concentrations ranging from >1% to 25% (Elder 1988). In 2006, Cetearyl Alcohol was reportedly used in 1435 cosmetic products (FDA 2006). Data from an industry survey in 2005 indicated~~