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# Final Report on the Safety Assessment of Benzalkonium Chloride

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

## CHEMISTRY

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

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

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

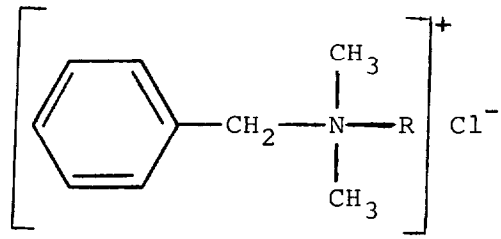

FIG. 1. Benzalkonium Chloride.

TABLE 1. Properties of Benzalkonium Chloride

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

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

## METHODS OF PRODUCTION

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

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

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

## REACTIVITY

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

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

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

## ANALYTICAL METHODS

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

## IMPURITIES

Information concerning impurities in Benzalkonium Chloride is not available.

## USE

### Purpose in Cosmetics

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

### Scope and Extent of Use in Cosmetics

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

**TABLE 3.** Product Formulation Data[21]

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

## Surfaces to which Applied

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

## Frequency and Duration of Application

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

## Noncosmetic Use

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

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

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

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

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

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

## BIOLOGICAL PROPERTIES

### Absorption and Distribution

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

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

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

### Effect on Histamine Release

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

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

### Effect on Enzymatic Activity

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

## ANIMAL TOXICOLOGY

### Subchronic Inhalation Toxicity

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

## Acute Oral Toxicity

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

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

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

## Subchronic Oral Toxicity

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

## Chronic Oral Toxicity

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

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

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

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

## Ototoxicity

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

## Dermal Toxicity

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

## Cytotoxicity

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

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

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

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

The hemolytic activity of Benzalkonium Chloride ($4.2 \times 10^{-5}$ 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).[45]

## Ocular Irritation

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

**TABLE 6.** Cytotoxicity of Benzalkonium Chloride

| *Cells tested* | *Test substance* | *Methodology* | *Results* | *References* |
|---|---|---|---|---|
| Epithelial cells (prostate gland) and fibroblasts (heart) from rats | 0.10, 0.033, 0.02 and 0.01% Benzalkonium Chloride | Cell cultures incubated with Benzalkonium Chloride for 10 days | 0.10 and 0.033% Benzalkonium Chloride caused significant growth retardation | 42 |
| Human conjunctival cells | 0.007% Benzalkonium Chloride | Cell cultures exposed to 0.5 ml of test solution for 16 min | Exposure period resulting in 50% cellular damage was 98.9 $\pm$ 12.1 s at 37°C | 43 |
| Murine suspension cultures of P815 tumor cell line | 1.5 M Benzalkonium Chloride | 0.5, 2, and 4-h exposures | Dosages resulting in 50% cytotoxicity were: 7.2 ppm (at 0.5 h), 3.5 ppm (at 2 h), and 0.6 ppm (at 4 h) | 44 |
| Defibrinated blood from rabbits | $4.2 \times 10^{-5}$ 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).[46]

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

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

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

### Intraocular Toxicity

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

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

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

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

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

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

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

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

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

The effect of Benzalkonium Chloride on corneal morphology was evaluated in New Zealand White rabbits (number not stated). Corneas were removed and bathed in aerated solutions (34°C, pH 7.4) of the following composition: 103.4 mM NaCl, 15.3 mM $Na_2SO_4$, 10 mM $NaHCO_3$, 2.2 mM $K_2HPO_4$, 0.5 mM $KH_2PO_4$, 5.24 mM $H_3PO_4$, 0.61 mM $MgSO_4$, 0.7 mM calcium gluconate, 26 mM glucose, and 20 mM tris-(hydroxymethyl) aminomethane (Tris). Benzalkonium Chloride was added to solutions at concentrations of 0.001, 0.0004, and 0.0001%; solutions remained in contact with the epithelial surface for 30–110 min. Corneas were then fixed and examined via SEM. One hour after exposure to 0.001% Benzalkonium Chloride, corneal surface cells were loosened or removed, exposing second- and third-layer cells. Also, the plicate appearance of surface cells was lost and some deeper cells had abnormally long microvilli. An increase in the number of cells with peripheral loss of microvilli and microplicae was noted 1 h after exposure to 0.0004% Benzalkonium Chloride. No discernible modification of surface morphology was noted after 2 h of exposure to 0.0001% Benzalkonium Chloride (Table 8).[57]

The effect of Benzalkonium Chloride on the corneal endothelium was investigated using albino rabbits (number not stated). The eyes were enucleated (complete with conjunctival sac and eyelids) and corneas were prepared and mounted in a specular microscope. After a 1 h stabilization period, the corneal endothelium was perfused for 3 h with Benzalkonium Chloride at concentrations ranging from $6.5 \times 10^{-6}$ 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 $\mu$m/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).[58]

## Skin Irritation

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

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

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

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

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

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

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

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

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

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

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

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

## Teratogenicity

Single doses (0, 25, 50, 100, and 200 mg/kg) of aqueous Benzalkonium Chloride solutions were instilled (1 ml/kg) into the vaginas of adult, nulliparous female Wistar rats (169–203 g; groups of 6–8 rats). Dams were killed via $CO_2$ inhalation on day 21 of gestation. The fetuses were removed by cesarean section and all were examined for viability and external malformations. Two-thirds of the live fetuses from each litter were examined stereomicroscopically for skeletal abnormalities after staining with Alizarin red S. The remainder were fixed in Bouin's fluid and examined for visceral anomalies, using the

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

## Mutagenicity

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

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

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

**TABLE 10.** Mutagenicity of Benzalkonium Chloride

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

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

### Tumorigenicity

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

## CLINICAL ASSESSMENT OF SAFETY

### Ocular Irritation and Intraocular Toxicity

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

**TABLE 11.** Clinical Assessment of Safety

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

**TABLE 11.** *Continued*

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

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

### Skin Irritation

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

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

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

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

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

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

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

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

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

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

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

### Skin Irritation and Sensitization

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

### Skin Sensitization

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

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

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

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

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

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

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

## SUMMARY

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

## DISCUSSION

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

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

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

## CONCLUSION

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

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