JOURNAL OF THE AMERICAN COLLEGE OF TOXICOLOGY
Volume 4, Number 5, 1985
Mary Ann Liebert, Inc., Publishers

3

# Final Report on the Safety Assessment of Benzethonium Chloride and Methylbenzethonium Chloride

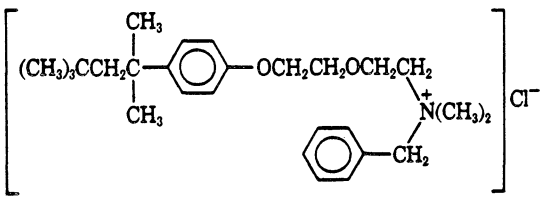
**Benzethonium Chloride and Methylbenzethonium Chloride are quaternary ammonium salts used in cosmetics primarily as preservatives and secondarily as cationic surfactants, usually at concentrations below 1 percent. They can be irritating to the skin at concentrations of greater than 5 percent. Chronic and subchronic feeding studies indicated little or no toxic effects for both ingredients. Benzethonium Chloride was nonmutagenic in microbial systems and shown to be noncarcinogenic in rodent studies.**

**In clinical studies, Benzethonium Chloride produced mild skin irritation at 5 percent but not at lower concentration. Neither ingredient is considered to be a sensitizer.**

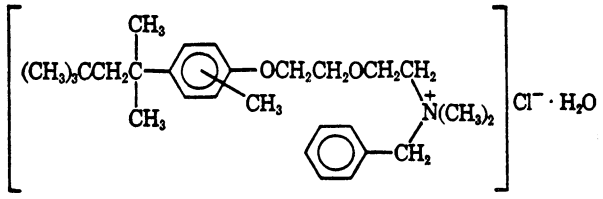
**It is concluded that both compounds are safe at concentrations of 0.5 percent in cosmetics applied to the skin. A maximum concentration of 0.02 percent is safe for cosmetics used in the eye area.**

## CHEMICAL AND PHYSICAL PROPERTIES

Benzethonium Chloride, also known as diisobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride and phemerol, and Methylbenzethonium Chloride, also known as diisobutyl cresoxy ethoxy ethyl dimethyl benzyl ammonium chloride, are synthetic quaternary ammonium salts.[1,2] These two compounds conform to the following structural formulas:[3]




Benzethonium Chloride



Methylbenzethonium Chloride

Benzethonium Chloride and Methylbenzethonium Chloride occur as colorless to white, odorless to mildly odorous, and bitter tasting monohydrate crystals. Benzethonium Chloride, when crystallized from benzene, chloroform, or ether, forms thin hexagonal plates. Both compounds are soluble in water, the lower alcohols, glycols, ethyleneglycol monomethyl ether, tetrachloroethane, and benzene and miscible in ethylene dichloride and carbon tetrachloride. Benzethonium Chloride is additionally soluble in acetone and chloroform, and methylbenzethonium Chloride in cellosolve. Solutions of these compounds are stable within the pH range of 4.8 to 7.01.[1,4-8] Ultraviolet (UV) spectra of Benzethonium Chloride and Methylbenzethonium Chloride indicate that these compounds have a peak absorbance at approximately 274 and 275 nm, respectively.[9,10] The physicochemical properties of these compounds are presented in Table 1.

Quaternary ammonium compounds can be synthesized via multiple routes, beginning with fatty acids, fatty alcohols, or a hydrocarbon source. However, the exact methods of manufacture for Benzethonium Chloride and Methylbenzethonium Chloride are proprietary information. After developing the appropriate tertiary amine, Benzethonium Chloride can be prepared by heating the amine with the quaternizing agent benzyl chloride at 60 to 80°C in the presence of water. Manufacturers use a 5 to 25 percent excess of the stoichiometric quantity of tertiary amine to ensure complete consumption of the toxic benzyl chloride. Hydrochloric acid is then added to convert the unreacted tertiary amine to its hydrochloride salt. Formulations incorporating Benzethonium Chloride and Methylbenzethonium Chloride must therefore have an acid pH to prevent conversion back to the free amine. Methylbenzethonium Chloride is a methyl derivative of Benzethonium Chloride with the methyl group on the benzene ring containing the phenoxy group.[7,8,14-16]

These commercial preparations contain a large number of functional groups and impurities including unreacted starting materials and side reaction products, such as amides and primary and secondary amines. Impurities from the quaternizing agent benzyl chloride may include traces of benzyl chloride itself, benzal chloride, benzyl alcohol, and benzaldehyde. Other impurities include benzylamine derivatives, sodium chloride, and hydrochloric acid. These impurities contribute greatly to the properties of the quaternary ammonium product. Gerstein[15] even states: "The behavior of a quaternary material is not influenced, but rather determined, by the impurities and additives." No data are available on nitrosamine impurities.

Qualitative and quantitative determinations of Benzethonium Chloride and

**TABLE 1.** Physicochemical Properties of Benzethonium Chloride and Methylbenzethonium Chloride

| | *Value* | | |
|---|---|---|---|
| *Property* | *Benzethonium Chloride* | *Methylbenzethonium Chloride* | *Reference* |
| Empirical formula | | | |
| Anhydrous | $C_{27}H_{42}ClNO_2$ | $C_{28}H_{44}ClNO_2$ | 3 |
| Molecular weight | | | |
| Anhydrous | 448.09 | 462.11 | 3, 11 |
| Monohydrate | 466.11 | 480.13 | |
| Melting point (°C) | 158–166 (sinters at 120) | 159–163 | 1, 3, 4, 6 |
| Density (lb/ft$^3$) | 27.5 | 27.5 | 7, 8 |
| Assay as ingredient (anhydrous basis) | 97.0–103.0% | 97.0–103.0% | 3 |
| Loss on drying (105° for 4 hours) | ≤5% | ≤5% | 3, 12 |
| Residue on ignition | ≤0.1% | ≤0.1% | 3 |
| Solubility* | | | |
| Water | s, vs | s, vs | 4, 6–8, 13 |
| Alcohol | s | s, vs | 4, 6–8, 13 |
| Acetone | s | – | 4, 6 |
| Benzene | s | vs (hot) | 1, 4, 6 |
| Carbon tetrachloride | m | m, i | 4, 7, 8 |
| Cellosolve | – | s, vs | 4, 6, 8 |
| Chloroform | s | i, vs | 4, 6, 13 |
| Ether | ss, s | i, s, vs | 4, 7, 8, 13 |
| Ethylene dichloride | m | m | 7, 8 |
| Glycols | s | s | 7, 8 |
| Tetrachloroethane | s | s | 7, 8 |
| pH | | | |
| 10% solution | 7.01 | – | 5 |
| 1 % solution | 4.8 to slightly alkaline | Neutral to slightly alkaline | 6, 13 |
| Surface tension (10% solution) | 36 dynes/cm | – | 5 |
| Wetting power (0.1% solution) | 142 seconds | – | 5 |
| Foam height (1.0% solution) | 268 nm | – | 5 |
| UV spectra-peak absorbance (nm) | 274 | 275 | 9, 10 |

*i, insoluble; m, miscible; s, soluble; ss, slightly soluble; vs, very soluble.

Methylbenzethonium Chloride have been made by many methods, including colorimetry, chromatography, multiphase titrations, extraction and spectrophotometry, electrophoresis, ultraviolet spectroscopy, potentiometric assays, and various other physical and chemical assays. Many of these depend on the formation of a relatively stable ion-pair complex. Tanaka et al.[17] report that the extractability of quaternary ammonium compounds as an ion-pair complex with bromophenol blue is determined by the lipophilic character of the ion and the steric effect around the cationic head. Table 2 lists the reported analytical methods for Benzethonium Chloride and Methylbenzethonium Chloride determination.

The reactivity of Benzethonium Chloride and Methylbenzethonium Chloride is determined for the most part by their cationic properties. They are inactivated by and are incompatible with soaps, anionics, organic material, nitrates, iodides, hexachlorophene, potassium chromate, potassium dichromate, sodium hepta-

**TABLE 2.** Analytical Methods for the Determination of Benzethonium Chloride and Methylbenzethonium Chloride

| *Method* | *Reagents and Specifics* | *Interference* | *Reference* |
|---|---|---|---|
| Agar gel electrophoresis | | | 24 |
| Colorimetric | Bromophenol blue, sodium hydroxide | | 13, 17, 25 |
| | Sodium alizarine sulfonate | | 26 |
| | Eosin | | 27 |
| Colorimetric/ion exchange/gas-liquid chromatography (GLC) | Bromophenol blue, sodium hydroxide | | 25 |
| Electrophoresis | Iron or aluminum anodes | | 28 |
| Extraction/spectrophotometric | 2,6-dibromophenol indophenol, 1,2-dichloroethane, maximum absorption of extraction at 640 nm | None at pH 5.6; other quaternary ammonium salts and amines at pH 8.2 | 29 |
| | 2,6-dichloroindophenol, nitrobenzene, maximum absorption of extraction at 650 nm | None from common inorganic salts; slight from amines and alkaloids | 30 |
| Gas chromatography (GC); mass spectrometry | | | 31 |
| Ion-pair atomic absorption | Sodium dioctylsulfosuccinate, cupric orthophenanthroline, methyl isobutyl ketone | | 32 |
| Multiphase titrations | BC: Water, chloroform, sodium tetraphenylborate, bromophenol blue, sodium hydroxide, water, hydrochloric acid, (HCl), sodium tetraphenylboron, methyl orange | | 12, 13, 33 |
| | MBC: Water, chloroform, potassium iodide, potassium iodate, HCl | | 13 |
| Potentiometric assay | Mercury-coated platinum, potassium ion selective, or silver electrodes, sodium tetraphenylborate | None from alcohol, acetone, sodium phosphates, disodium edetate, nonionic surfactants | 34–37 |
| Reverse phase ion-pair chromatography | Perchloric acid, methane-sulfonic acid | | 38 |
| Spectrophotometric assay | Bromthymol blue at pH 7.5 | None from epinephrine bitartrate, phenylephrine-HCl, pilocarpine-HCl, polyvinyl alcohol | 39 |
| Thin-layer chromatography (TLC) | | | 40 |
| TLC/fluorescence/refractive index | | | 41 |
| Ultraviolet spectroscopy | BC: Maximum absorption at 256–275 nm | | 12, 27 |
| Various other physical and chemical assays | | | 12, 13 |

phosphate, cotton fabrics, cellulose sponges, certain plastics, and other porous materials. Benzethonium Chloride (in greater than 2 percent concentrations) is precipitated from mineral acids and salt solutions as an oil that recrystallizes on drying. Both compounds are considered biologically active due to their precipitation, denaturation, redispersion, and complex formation reactions with proteins. Benzethonium Chloride and Methylbenzethonium Chloride are readily adsorbed onto a variety of surfaces, including proteinaceous surfaces, gauze, cork, plastics, and cellulose. As antimicrobial agents they adsorb onto the negatively charged cell wall of microorganisms, interrupt normal cell metabolism, and lead to death or growth inhibition.[1,4,6-8,15,18-23]

Benzethonium Chloride and Methylbenzethonium Chloride are relatively stable compounds; both aqueous and alcoholic solutions are stable in light, air, and temperatures up to 100°C. They are stabilized in detergents, cosmetics, and pharmaceuticals containing oxygen-forming substances by the presence of organotin compounds. Gamma-irradiation was found to decrease the antibacterial activity of Benzethonium Chloride, although this effect became less severe as the concentration of Benzethonium Chloride increased. Irradiation also increased the surface tension of 0.01 to 0.1 percent solutions; however, little effect was noted on 1 percent solutions.[1,22,42,43]

In a study on sarcosinate-cationic creme rinse shampoos, Benzethonium Chloride was compatible with many sarcosinate surfactants while retaining its antimicrobial activity.[44] Methylbenzethonium Chloride had an increase in bacteriostatic action when combined with sodium lauroyl sarcosinate.[45] These quaternary compounds also molecularly bind other formulation ingredients to the surface of the hair, thus intensifying the effects of fatty alcohols and esters, perfume oils, and other waxy and oily compounds.[15]

Benzethonium Chloride loses its antimicrobial activity when formulated with such cosmetic ingredients as lecithin (0.3 percent), polysorbate 80 (1.0 percent), and sodium sulfite (0.1 percent).[46] Methylbenzethonium Chloride in a 0.1 percent aqueous solution was inactivated by adsorption onto a variety of powders, including calamine, heavy and light kaolin, and magnesium trisilicate after 18 hours of storage at 22°C. Autoclaving Methylbenzethonium Chloride with the powders for 15 minutes at 121°C decreased the amount of Methylbenzethonium Chloride adsorbed.[47] Germicidal activity of both quaternary ammonium compounds was decreased by metallic ions in cosmetic formulations. This decrease was proportional to the valence of the ion, which interfered by competing for the negative sites on the microbial cell wall.[27]

## USE

### Cosmetic Use

Benzethonium Chloride and Methylbenzethonium Chloride are used in cosmetics as preservatives, antimicrobials, and cationic surfactants. They are usually found at concentrations below 1 percent in the following product categories: baby, bath, eye makeup, personal cleanliness, fragrance, noncoloring hair, shaving, skin, and suntan preparations.[15,44,48]

The FDA product formulation data for Benzethonium Chloride and Methylbenzethonium Chloride are compiled through voluntary filing of such data in accordance with Title 21 part 720.4 of the Code of Federal Regulations[49] (Table 3). Ingredients are listed in prescribed concentration ranges under specific product type categories.[48] Since certain cosmetic ingredients are supplied by the manufacturer at less than 100 percent 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

**TABLE 3.** Product Formulation Data[48]

| *Product Category** | *Total No. of Formulations in Category* | *Total No. Containing Ingredient* | *No. of Product Formulations Within Each Concentration Range (%)** | | |
|---|---|---|---|---|---|
| | | | *>1–5* | *>0.1–1* | *≤0.1* |
| *Benzethonium Chloride* | | | | | |
| Baby products | 15 | 1 | – | 1 | – |
| Bath preparations | 132 | 1 | – | – | 1 |
| Eyeliner | 396 | 2 | – | 2 | – |
| Colognes and toilet waters | 1120 | 6 | – | 6 | – |
| Perfumes | 657 | 3 | – | 3 | – |
| Fragrance powders (dusting and talcum, excluding aftershave talc) | 483 | 1 | – | 1 | – |
| Hair conditioners | 478 | 2 | – | 2 | – |
| Hair sprays (aerosol fixatives) | 265 | 1 | – | – | 1 |
| Hair rinses (noncoloring) | 158 | 3 | – | – | 3 |
| Hair shampoos (noncoloring) | 909 | 1 | – | 1 | – |
| Tonics, dressings, and other hair grooming aids | 290 | 1 | – | – | 1 |
| Wave sets | 180 | 1 | – | – | 1 |
| Other hair preparations (noncoloring) | 177 | 2 | – | – | 2 |
| Deodorants (underarm) | 239 | 11 | – | 8 | 3 |
| Douches | 26 | 7 | 4 | 3 | – |
| Feminine hygiene deodorants | 21 | 3 | – | – | 3 |
| Other personal cleanliness products | 227 | 7 | – | 3 | 4 |
| Aftershave lotions | 282 | 2 | – | – | 2 |
| Men's talcum | 13 | 2 | – | 1 | 1 |
| Preshave lotions (all types) | 29 | 1 | – | 1 | – |
| Skin cleansing preparations (cold creams, lotions, liquids, and pads) | 680 | 5 | – | 1 | 4 |
| Face, body, and hand skin care preparations (excluding shaving preparations) | 832 | 7 | – | 2 | 5 |
| Moisturizing skin care preparations | 747 | 2 | – | – | 2 |
| Paste masks (mud packs) | 171 | 2 | – | – | 2 |
| Skin fresheners | 260 | 13 | – | 1 | 12 |
| Other skin care preparations | 349 | 3 | – | – | 3 |
| Suntan gels, creams, and liquids | 1642 | 2 | – | – | 2 |
| Indoor tanning preparations | 15 | 1 | – | – | 1 |
| 1981 TOTALS | | 93 | 4 | 36 | 53 |

**TABLE 3.** (Continued)

| *Product Category** | *Total No. of Formulations in Category* | *Total No. Containing Ingredient* | *No. of Product Formulations Within Each Concentration Range (%)** | | |
|---|---|---|---|---|---|
| | | | *>1–5* | *>0.1–1* | *≤0.1* |
| *Methylbenzethonium Chloride* | | | | | |
| Baby lotions, oils, powders, and creams | 56 | 2 | – | 1 | 1 |
| Colognes and toilet waters | 1120 | 1 | – | – | 1 |
| Hair conditioners | 478 | 1 | – | – | 1 |
| Hair sprays (aerosol fixatives) | 265 | 6 | – | – | 6 |
| Deodorants (underarm) | 239 | 5 | – | 4 | 1 |
| Douches | 26 | 1 | – | 1 | – |
| Feminine hygiene deodorants | 21 | 2 | – | – | 2 |
| Other personal cleanliness products | 227 | 1 | – | – | 1 |
| Aftershave lotions | 282 | 4 | – | 3 | 1 |
| Other shaving preparation products | 29 | 1 | – | – | 1 |
| Skin cleansing preparations (cold creams, lotions, liquids, and pads) | 680 | 1 | – | – | 1 |
| Face, body, and hand skin care preparations (excluding shaving preparations) | 832 | 1 | – | – | 1 |
| Moisturizing skin care preparations | 747 | 1 | – | – | 1 |
| Skin fresheners | 260 | 3 | – | – | 3 |
| Suntan gels, creams, and liquids | 164 | 2 | – | – | 2 |
| Other suntan preparations | 28 | 1 | – | – | 1 |
| 1981 TOTALS | | 33 | 0 | 9 | 24 |

*Preset product categories and concentration ranges in accordance with federal filing regulation (21 CFR 720.4).

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.

In 1981, approximately 96 and 97 percent of the formulations containing Benzethonium Chloride and Methylbenzethonium Chloride, respectively, incorporated these ingredients at concentrations of 1 percent or less. Furthermore, 57 and 71 percent of the total 93 Benzethonium Chloride and 34 Methylbenzethonium Chloride formulations, respectively, contained concentrations of 0.1 percent or less.[48]

The European Economic Community (EEC) has approved a maximum concentration of 0.1 percent for Benzethonium Chloride as a provisionally permitted preservative in cosmetics.[50] No further limitations or label requirements of any kind are listed.

The formulation data presented in Table 3 indicate that cosmetic products containing Benzethonium Chloride and Methylbenzethonium Chloride may

contact all external body surfaces and hair, as well as the eyes and mucosal membranes. These products may be used daily or occasionally over a period of up to several years. The frequency and length of application could result in continuous exposure.

## Noncosmetic Use

Benzethonium Chloride and Methylbenzethonium Chloride are widely used in disinfectants, germicides, herbicides, bactericides, topical antiinfectives, as cationic detergents, and preservatives.[4,6,14-16,20,27,51-60] Their varied applications as disinfectants encompass use in restaurants (on eating equipment), dairy farms (on milking equipment), janitorial purposes, food plants, hospitals, barber and beauty shops, textile factories, food storage rooms, and swimming pools. Additionally, the veterinary and agricultural uses of Benzethonium Chloride include sanitizing chicken drinking water, in egg handling, as general disinfectants, and as topical antibacterials. As cationic surfactants, these compounds are used in ore flotation, fabric softening, colloid flocculation processes, asphalt emulsification, corrosion inhibition, paper processing wood pulp slurries, and as pigment wetting and grinding aids in the production of thixotropic paints and printing inks.

Benzethonium Chloride is or has been used in various pharmaceuticals primarily as a preservative. Benzethonium Chloride has been incorporated into vaccines, preparations for treating cardiovascular disorders, anesthetics, and injectable solutions and is also used in the production of heparin derivatives. Benzethonium Chloride concentrations vary from product to product but seldom exceed 1 percent.[27,61-67] In addition, 0.2 percent aqueous or alcoholic solutions of Benzethonium Chloride have been used in the treatment of hydrofluoric acid burns.[68,69]

Benzethonium Chloride and Methylbenzethonium Chloride are currently under evaluation by the FDA Over-the-Counter (OTC) Drug Review Program. These compounds were assigned to 8 of the 17 advisory panels pertaining to their use as antimicrobials, in contraceptives and other vaginal products, dentifrices and dental care agents, miscellaneous external, ophthalmic, and oral cavity drug products, topical analgesics, antirheumatics, otic, burn, and sunburn treatment and prevention products.[70] The Ophthalmic Panel found Benzethonium Chloride satisfactory as a preservative at maximum concentrations of 0.01 percent for preparations used directly in the eye and at a maximum of 0.02 percent for preparations not for direct use in the eye.[71] The Antimicrobial I Panel also concluded that Benzethonium Chloride and Methylbenzethonium Chloride are safe and effective for use as detergents in skin wound cleansers at a maximum concentration of 0.13 percent; no claims of antimicrobial activity are associated with this use.[72] Several panels noted that although quaternary ammonium compounds were embraced as disinfectants upon their appearance in 1935, subsequent reviews have produced significant doubt as to their safety and antimicrobial effectiveness.[21,71-73] Controversy surrounds their microbial spectrum (particularly gram-negative bacteria) and inactivation by a substantial number of compounds. Consequently, the major classification of Benzethonium Chloride and Methylbenzethonium Chloride as used in OTC products falls in Category III: insufficient data available for final evaluation of safety and effectiveness. Benzethonium Chloride and Methylbenzethonium Chloride have been classified as

inactive ingredients in contraceptives: Benzethonium Chloride in dentifrices, ophthalmic solutions, and hair growth and hair loss prevention products, and Methylbenzethonium Chloride in acne treatment products.[21,70,71,74,75] Table 4 presents a synopsis of the status of Benzethonium Chloride and Methylbenzethonium Chloride in the OTC drug review.

Benzethonium Chloride and Methylbenzethonium Chloride are included in the listing of quaternary ammonium chlorides (hexadecyl, octadecyl derivative) as indirect food additives, limited to use as preservatives only in adhesives used in packaging, transporting, or holding food.[76] The literature also contains references to the use of Benzethonium Chloride in India as a plant growth regulator applied to sugar cane foliage. Five to six weeks treatment with 1000 and 2500 ppm Benzethonium Chloride improved the sugar cane purity coefficient and increased the percentage of fiber.[77,78]

Benzethonium Chloride and Methylbenzethonium Chloride are also used in several analytical methods: an aqueous Zimmermann reaction test for the determination of 17-ketosteroids (Benzethonium Chloride), a microturbidimetric method for the determination of protein in cerebrospinal fluid and urine (Benzethonium Chloride), and in combination with toluene and octoxynol-9 in a scintillator for colloidal counting of plasma and urine (Methylbenzethonium Chloride).[83–85]

## GENERAL BIOLOGY

### Antimicrobial

The antimicrobial properties of Benzethonium Chloride and Methylbenzethonium Chloride have been extensively studied. Their lack of odor, color, instability, and toxicity (at effective levels) has resulted in their widespread use as disinfectants and preservatives since 1935.[22,73] However, varying experimental and in-use results have engendered controversy over the scope of their microbial spectrum and their inactivation by a large number of materials. This has led the majority of FDA OTC Drug Review Panels to conclude that insufficient data are available to determine the safety and efficacy of Benzethonium Chloride and Methylbenzethonium Chloride as used in OTC drug products (Table 4).

Benzethonium Chloride and Methylbenzethonium Chloride are inactivated by soaps, anionics, phospholipids, proteins, nitrates, iodides, polysorbate 80, sodium sulfite, magnesium, calcium, and iron salts.[1,6,20,22,46] Hard water, acidity, and the presence of organic matter also generally reduce antimicrobial effectiveness. The surface-active nature of these compounds, causing them to be readily adsorbed on glass or plastic surfaces, also accounts for some reduction in effectiveness.[20–22,73]

The germicidal action of Benzethonium Chloride and Methylbenzethonium Chloride has generally been credited to their ability to disrupt cell membrane permeability and the subsequent loss of intracellular materials. Many other factors are believed to add to the sum total mechanism, varying in relative influence with changing conditions. These include lysis, protein denaturation, oxidation and enzyme inhibition, effects on activating ions, and interference with growth and reproduction.[23,73,86–89]

**TABLE 4.** Status of Benzethonium Chloride and Methylbenzethonium Chloride in the OTC Drug Review[70,79]

| *Ingredient* | *Advisory Review Panel* | *Active (A) or Inactive (I)** | *Use/Comment* | *Recommended Category†* | *Reference Document‡* | *Date* |
|---|---|---|---|---|---|---|
| Benzethonium Chloride and Methylbenzethonium Chloride | Antimicrobial I | A | Antimicrobial soap/physical and/or chemical incompatibility in formulation | II SE | TFM (73) | 1/6/78 |
| Benzethonium Chloride and Methylbenzethonium Chloride | Antimicrobial I | A | Surgical hand scrub | III SE | TFM (73) | 1/6/78 |
| Benzethonium Chloride and Methylbenzethonium Chloride | Actimicrobial I | A | Skin wound cleanser (maximum concentration of 1/750) | I | TFM (preamble Proposal (72) | 9/13/74 |
| Benzethonium Chloride and Methylbenzethonium Chloride | Antimicrobial I | A | Skin antiseptic | III E | TFM (73) | 1/6/78 |
| Benzethonium Chloride and Methylbenzethonium Chloride | Antimicrobial I | A | Health care personnel handwash | III SE | TFM (73) | 1/6/78 |
| Benzethonium Chloride and Methylbenzethonium Chloride | Antimicrobial I | A | Skin and protectant | III E | TFM (73) | 1/6/78 |
| Benzethonium Chloride and Methylbenzethonium Chloride | Antimicrobial I | A | Patient preoperative skin preparation | III SE | TFM (73) | 1/6/78 |
| Benzethonium Chloride and Methylbenzethonium Chloride | Contraceptives and other vaginal drug products | I | Cationic surfactant in douches (BC) and contraceptives (MBC); preservative in vaginal preparations | III SE | Proposals (21) | 12/12/80 |
| Benzethonium Chloride | Antimicrobial II | A | Antifungal | III SE | OTC Panel Mtg. (28th) | 8/26–27/77 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Benzethonium Chloride | Dentifrices and dental care agents | I | Ingredient contained in marketed products submitted for review, considered inactive | – | Proposal (74) | 3/28/80 |
| Benzethonium Chloride | Miscellaneous external drug products | A | Styptic | II | OTC Panel Mtg. (40th) | 8/3–4/80 |
| Benzethonium Chloride | Miscellaneous external drug products | A | Antimicrobial (any concentration) as an aid in prevention of diaper rash, cradle cap, excoriating skin conditions, and to stimulate healing | III E | OTC Panel Mtg. (41st) | 10/5–6/80 |
| Benzethonium Chloride | Miscellaneous external drug products | I | Hair grower and hair loss prevention products | – | Proposal (75) | 11/7/80 |
| Benzethonium Chloride | Ophthalmic drug products | I, A | Preservative agent in maximum concentrations of 0.01% in the eye considered inactive only when used as a formulation agent and when no labeling claims are made | – | Proposals (75) | 5/6/80 |
| Benzethonium Chloride | Oral cavity drug products | A | Antimicrobial used for oral health care | III SE | OTC Panel Mtgs. (27th, 28th) | 8/14/79, 12/12–14/79 |
| Benzethonium Chloride | Topical analgesics, antirheumatics, otic, burn, sunburn treatment and prevention products | | Deferred to Antimicrobial Panel | – | Proposal (80) | 12/4/79 |
| Benzethonium Chloride (In Karaya/Tragacanth as a vehicle) | Miscellaneous external drug products | A | Antiinfective. Discussion delayed until receipt of further information | – | OTC Panel Mtg. (21st) | 9/30–10/1/77 |
| Benzethonium Chloride and Captan | Miscellaneous external drug products | A | Panel undecided on the effect of combined active ingredients as an antidandruff treatment | – | OTC Panel Mtg. (38th) | 4/20–21/80 |

**TABLE 4.** (Continued)

| *Ingredient* | *Advisory Review Panel* | *Active (A) or Inactive (I)** | *Use/Comment* | *Recommended Category†* | *Reference Document‡* | *Date* |
|---|---|---|---|---|---|---|
| Methylbenzethonium Chloride | Antimicrobial II | I | Inactive ingredient in treatment of acne | – | OTC Panel Mtg. (43rd) | 7/20/79 |
| Methylbenzethonium Chloride | Miscellaneous external drug products | A | Treatment of cradle cap (seborrheic dermatitis of the scalp in infants) | III S | OTC Panel Mtg. (40th) | 8/3–4/80 |
| Methylbenzethonium Chloride | Miscellaneous external drug products | A | Corn and callus remover | II SE | Proposal (FDA, 1982) | 1/5/82 |
| Quaternary ammonium compounds (includes Benzethonium Chloride and Methylbenzethonium Chloride) | Antimicrobial I | A | Use concentration not greater than 0.13% | I | TFM (FDA, 1978) | 1/6/78 |
| Quaternary ammonium compounds (includes Benzethonium Chloride and Methylbenzethonium Chloride) | Contraceptives and other vaginal products | | BC considered safe and effective in a vaginal douche in recommended dosage dilutions and with directions for intermittent usage, no claims of antiseptic or disinfectant activity | – | OTC Panel Mtg. (19th) | 6/23–24/75 |
| Quaternary ammonium compounds (includes Benzethonium Chloride and Methylbenzethonium Chloride) | | | MBC considered effective but of unproven safety as cleaning and deodorizing components of vaginal suppositories, unreliable as bacteriocides | – | OTC Panel Mtg. (19th)<br>OTC Panel Mtg. (30th) | 12/16–17/76 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Quaternary ammonium compounds (includes Benzethonium Chloride and Methylbenzethonium Chloride) | Ophthalmic drug products | A | Incompatible with serum. Serum and/or yeast cells may inactivate preservative system | – | OTC Panel Mtg. (24th) | 2/3–4/78 |
| Quaternary ammonium compounds (includes Benzethonium Chloride and Methylbenzethonium Chloride) | Oral cavity drug products | A | Antimicrobial for use on oral and pharyngeal mucous membranes | III SE | OTC Panel Mtg. (27th) | 8/14/79 |

*Active ingredient: "any component that is intended to furnish pharmacological activity or other direct effect in the diagnosis, cure, mitigation, treatment, or prevention of disease, or to affect the structure or any function of the body of man or other animals. The term includes those components that may undergo chemical change in the manufacture of the drug product and be present in the drug product in a modified form intended to furnish the specified activity or effect."[81] Inactive ingredient, "any component other than an 'active ingredient.' "[82]

†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.
Categories II and III: reason for categorization may be symbolized by S (safety) and/or E (effectiveness).
– Indicates no categorization at this time.

‡TFM, Tentative final monograph.

Benzethonium Chloride and Methylbenzethonium Chloride are effective against many gram-positive and some gram-negative bacteria, the former generally being more susceptible to their germicidal action, whereas many strains of the latter, particularly *Pseudomonas aeruginosa*, are resistant.[1,22,23,73,90-92] Christensen[93] obtained satisfactory bactericidal activity (at 37°C) with concentrations of Benzethonium Chloride ranging from 0.005 to 0.01 percent; concentrations of 0.0025 percent Benzethonium Chloride, intended for use in vaccines, had insufficient antibacterial effects. Rawlins et al.[94] and Joslyn et al.,[1] on the other hand, have reported satisfactory activity (at 20°C) with Benzethonium Chloride concentrations of 0.00125 to 0.0083 percent.

Benzethonium Chloride and Methylbenzethonium Chloride have less fungicidal than bactericidal activity.[73] Concentrations of Benzethonium Chloride ranging from 0.1 to 0.2 percent were effective against some fungi and ineffective against others.[1,94]

Benzethonium Chloride has synergistic antibacterial and antifungal activity with acylated peptides,[95] chlorhexidine gluconate,[96] candicidin, a polyene macrotide antifungal antibiotic,[97] and thimerosol.[93]

Benzethonium Chloride has been tested widely for its potential to reduce bacterial plaque accumulation. Results of numerous studies indicate that solutions or mouthrinses containing Benzethonium Chloride (0.075 to 0.1 percent) give a moderate to significant reduction in plaque accumulation.[98-102] One clinical study found no significant reduction in existing plaque accumulations.[103] Benzethonium Chloride was slightly less effective in inhibiting plaque formation than chlorhexidine and chlorhexidine gluconate, whereas zinc chloride reduced the antiplaque potency of this compound.[99,104,105] Several investigators have reported yellow-brown tooth and tongue discolorations associated with the use of Benzethonium Chloride-containing dentifrices.[99,106,107] Gaffar and Volpe[108,109] reported that the dental staining can be prevented by the incorporation of a polyamine polyphosphonate without inactivating Benzethonium Chloride.

A process has been developed for incorporating Corobex CP-4 containing 0.075 percent Benzethonium Chloride into polymerized methyl methacrylate used in contact lenses. The treated lenses had reduced numbers of organisms, and thus treatment would lessen bacterial and other contamination of ocular tissues.[110]

Benzethonium Chloride has also been used for many years as a bacterial inactivator in the manufacture of vaccines. Marked losses in vaccine potency, first reported in 1960, necessitated numerous studies on the antimicrobial effectiveness of Benzethonium Chloride in the poliomyelitis, pertussis, diphtheria-pertussis-tetanus (DPT), and the combined DPT-poliomyelitis (DPTP) vaccines.[19,27,61,111,112] Pivnick et al.[61] found Benzethonium Chloride, at 25 ppm, to be an ineffective inhibitor of gram-negative bacteria in both the poliomyelitis and DPTP vaccines. However, Benzethonium Chloride did inhibit growth of gram-positive bacteria, yeasts, and mold while also increasing the antifungal activity of other preservatives in the vaccines.

## Biochemical Effects

Benzethonium Chloride inhibited proteolytic enzymes, including brain aminopeptidases and arylamidases, trypsin (Methylbenzethonium Chloride in-

hibits also), and bovine hypothalamus acid proteinase.[86,113-117] Stedman et al., in their studies on *Serratia marcescens*, emphasized the contributing role this enzyme activity loss plays in the cytotoxic effects of Benzethonium Chloride. They found that approximately 50 percent enzyme inhibition was achieved with a Benzethonium Chloride concentration that gave less than a 2 percent loss in cell viability. Makinen[118] found that Benzethonium Chloride markedly inhibited the enzymatic hydrolysis of all but 1 tested amino acid, 2-naphthylamides purified from human saliva. Kinetic data indicated that the inhibition was competitive and effective under high substrate conditions.

Sugiura and Ogiso[119] studied the effect of Benzethonium Chloride on the enzymatic hydrolysis of olive oil by *Mucor* lipase. Benzethonium Chloride at a maximum concentration of 0.0065 percent enhanced the rate of hydrolysis. The investigators found that the increased rate depended on the oil:surfactant ratio and that a small amount of Benzethonium Chloride increased lipase adsorption at the oil-water interface. However, Benzethonium Chloride concentrations greater than 0.0065 percent resulted in lipase inhibition.

Several studies have been conducted on the effects of Benzethonium Chloride on acetylcholinesterase. Addition of Benzethonium Chloride to electric eel spinal cord or tissue preparations decreased the proportion of solubilized enzyme as globular species and slowed or inhibited the conversion of "native" species into globular forms.[113,120] Benzethonium Chloride also had an inhibitory effect on acetylcholinesterase in homogenates from rabbit or ox caudate nuclei. The investigators suggested that inhibition was achieved through the complexing of the benzethonium cation with the anionic enzyme site.[121]

Several other biochemical effects of Benzethonium Chloride have been studied. Benzethonium Chloride (0.02 M) in a phosphate-buffered solution did not inhibit the photodecomposition of flavin adenine dinucleotide (FAD) after irradiation for 2 hours.[122] Benzethonium Chloride also induced UV spectral changes in drugs, such as thiamylal and thiopental, and enhanced ampicillin partition behavior.[123,124]

## Cellular Effects

Benzethonium Chloride and Methylbenzethonium Chloride are known to cause cytolytic injury by disrupting the permeability properties of cellular membranes with a subsequent loss of intracellular materials. Absolute cell density and the weight ratio of preservative to cells dictates the amount of lysis obtained. Significant reduction in either the cell density or in the weight ratio also reduces the amount of lysis. Other cytotoxic effects include protein denaturation, oxidation and enzyme inhibition, effects on activating ions, and interference with growth and reproduction.[23,73,86-89,97,125,126]

In one of the many studies on Benzethonium Chloride, the compound was toxic at a concentration of 10 μg/ml to 3 types of cultured human cells, whereas, 1 μg/ml inhibited cell growth.[127] In another test for adjuvant activity using diphtheria toxoid in guinea pigs, Benzethonium Chloride was an active adjuvant, hemolytic, and disruptive to cellular cytoplasm.[126]

In an ultrastructural study, suspensions of ram spermatozoa and avian erythrocytes were coincubated (45 minutes at 22°C) in the presence of Benzethonium

Chloride and Methylbenzethonium Chloride at concentrations ranging from 0 to 177 μg/ml. Erythrocyte swelling was induced as a result of increased membrane permeability. This, in turn, created a situation of close proximity with localized regions of membrane fusion and, in some cases (all concentrations of Benzethonium Chloride, only the highest concentration of Methylbenzethonium Chloride), erythrocyte-erythrocyte fusion. Spermatozoa with intact acrosomes were also observed embedded in erythrocyte cytoplasm; adjacent membranous vesicles were believed to represent the fused cellular membranes. Benzethonium Chloride and Methylbenzethonium Chloride have been used to accelerate the acrosome reaction in guinea pig spermatozoa and to produce acrosomal vesiculation in bovine sperm.[125]

Benzethonium Chloride and Methylbenzethonium Chloride are also potent spermicides. A foam containing 0.2 percent Benzethonium Chloride was an effective spermicide.[128] Brotherton[129] tested Benzethonium Chloride and Methylbenzethonium Chloride for spermicidal activity by titration against human spermatozoa and found both effective. She also found that slight "variations" in chemical structure resulted in large potency differences: Methylbenzethonium Chloride, with an extra ring methyl group, was 3 times more potent than Benzethonium Chloride (1.82 pmol/cell Methylbenzethonium Chloride compared to 6.03 pmol/cell Benzethonium Chloride necessary for 100 percent stripping of spermatozoa).

Fur depigmentation has been noted in a number of studies on Benzethonium Chloride. In a study in which Benzethonium Chloride was injected subcutaneously into 50 black mice after an injection of dibenzo(a,i)pyrene (DBP), all of the mice receiving 2 injections of 0.7 mg Benzethonium Chloride at Days 1 and 8 had decolorization of the fur at the site of injection. Of those 50 receiving 0.35 mg Benzethonium Chloride on Days 1, 8, and 15, 97.4 percent had depigmentation. In another study, spotty depigmentation occurred 10 days after treatment in 2 of 3 mice painted with 140 mg/kg Benzethonium Chloride in tricaprylin. Other mice in this study received doses ranging from 8.75 to 280 mg/kg and did not have depigmentation. Hair exposed in vitro for 48 hours was not bleached by concentrations of Benzethonium Chloride up to 280 mg/kg. One hundred mice injected subcutaneously in the groin with 0.7 mg Benzethonium Chloride (in tricaprylin) also had fur depigmentation. In another study, all of the 8 mice receiving repeated subcutaneous injections of 70 mg/kg Benzethonium Chloride had depigmentation near the injection site at 34 days; 5 of the 8 mice receiving 35 mg/kg had a similar spotty depigmentation. When the depigmented fur was plucked, the new hair growth was also depigmented.[130]

## Tissue Effects

Several studies have been conducted on the tissue effects of Benzethonium Chloride. The ciliary activity of isolated mouse tracheal mucosa was weakened after a 15-minute contact with a Benzethonium Chloride concentration of 0.01 percent in Locke-Ringer's solution.[131] A final Benzethonium Chloride dilution of 1:40,000 in Parker's medium 199 was toxic to tissue cultures of primary monkey kidney cells. The toxic action was also found to be time dependent.[132]

The apparent exsorption rate constant K (excretion into the intestinal lumen through the intestinal wall) of sulfaguanidine administered intravenously to rats

was used to measure the influence of Benzethonium Chloride upon the intestinal mucosa. The perfusion of Benzethonium Chloride in an isotonic (pH 7.4) phosphate buffer through the intestine at a rate of 4 ml/minute for 10 minutes greatly increased K and resulted in histological changes in the intestinal mucosa. The diffusion of sulfaguanidine from the blood vessel to the intestinal lumen was increased. The K values for Benzethonium Chloride were independent of the concentration (5 mM and 10 mM), and the investigators believed this was due either to good tissue permeability or a rate determining effect of blood or lymph flow.[133]

The effects of Benzethonium Chloride on tone and motility of isolated segments of rabbit and rat ileum were studied by the Magnus technique. Benzethonium Chloride was added to the muscle preparation to give effective concentrations ranging from 0.00005 to 0.5 percent; fresh segments were used for each test concentration. Benzethonium Chloride inhibited the motility of the smooth muscle of the rabbit ileum. Concentrations of 0.002 to 0.5 percent totally inhibited muscle contractions and markedly decreased muscle tone. Some motility remained at 0.001 percent concentration, but muscle tone was equally depressed. Decreasing concentrations produced decreasing effects. At 0.0002 percent concentrations, tone was still depressed, but motility was only slightly affected; 0.0001 percent had only a slight effect on tone and no effect on motility. A concentration of 0.00005 percent produced no effects. The effects of Benzethonium Chloride on rat ileum were similar in their progression, but the results were indicative of a slightly greater sensitivity of the rat ileum. The investigators suggested that Benzethonium Chloride had a specific toxic action and reported that the inhibitory effect at concentrations as high as 0.005 percent could be reversed by several changes of Locke-Ringer's solution.[60]

### Absorption, Metabolism, and Excretion

Labeled $^{14}$C-Benzethonium Chloride at doses of 1.13 and 3.56 mg/kg per day was administered orally to pregnant rats on Days 6 through 15 of gestation. The fetal absorption of $^{14}$C-Benzethonium Chloride was variable, and most of the radioactivity remained within the dam.[134]

## ANIMAL TOXICOLOGY

### Acute Toxicity

#### Oral

The reported acute oral $LD_{50}$ values in rats for Benzethonium Chloride include 368, 420 ± 25, 450, and 665 mg/kg.[60,135-138] These values place Benzethonium Chloride in the moderately and slightly toxic categories according to Hodge and Sterner.[139] In the tests conducted by Finnegan and Dienna,[60] a few deaths occurred within 24 hours, but approximately half of the animals died after 1 week; the longest survival period was 21 days. All deaths were preceded by severe depression.

Rosen et al.[138] conducted a study to determine the influence of dimethyl-

sulfoxide (DMSO) on the permeability and absorption of Benzethonium Chloride and other drugs. The acute oral $LD_{50}$s for Benzethonium Chloride in both distilled water and in 50 percent DMSO were determined in rats and mice. Male albino rats of the Charles River CD strain and Charles River CD-1 albino male and female mice were used. All animals were fasted 18 hours prior to oral dosing; a constant volume of 16 ml/kg, previously determined to be well tolerated, was used for both mice and rats. A total of 220 mice and rats were used: approximately 10 animals per dose group with 5 or 6 dose groups in each experiment. The acute oral $LD_{50}$s for Benzethonium Chloride administered in water and DMSO, respectively, were 665 and 368 mg/kg in rats and 485 and 338 mg/kg in mice. The lower values (more toxic) obtained with use of DMSO were statistically significant ($p < 0.05$). The investigators concluded that DMSO used as a solvent increased the oral toxicity of Benzethonium Chloride in both rats and mice.

### Subcutaneous

Mason et al.[140] subcutaneously injected 5 groups of 4 Fischer 344 rats with varying doses of Benzethonium Chloride; the acute $LD_{50}$ was 119.0 mg/kg.

Gall[126] tested Benzethonium Chloride for adjuvant activity with a purified diphtheria toxoid. A 0.2 ml dose of 1 Lf in borate-succinate buffer was mixed with 0.1 mg Benzethonium Chloride, gently agitated for 1 hour on a turntable, and then subcutaneously injected into the abdominal wall of 5 albino guinea pigs. Twenty-eight days later, the animals received a second injection of the diphtheria toxoid alone. Diphtheria antitoxin titers were measured by the guinea pig intracutaneous method. Benzethonium Chloride was a moderately active adjuvant. No mention was made of any specific toxic effects due to the administration of Benzethonium Chloride alone.

### Intraperitoneal

Finnegan and Dienna[60] reported an intraperitoneal $LD_{50}$ of 33.1 ± 2.5 mg/kg in male albino rats. Death usually occurred in 24 hours and was preceded by severe depression.

The lowest intraperitoneal lethal doses in mice were 7.813[141] and 8 mg/kg.[135]

### Intravenous

The acute intravenous toxicity of Benzethonium Chloride in mice was studied by Arro and Salenstedt.[132] Twofold dilutions of a 10 percent Benzethonium Chloride solution were prepared, and 0.5 ml of each was injected into 5 mice. Five control animals were administered the diluent, Parker's medium 199. Observation continued for 14 days. The mean intravenous $LD_{50}$ in 2 experiments was 35 mg/kg.

The intravenous $LD_{50}$ of Benzethonium Chloride in male albino rats was 19.1 ± 0.8 mg/kg. Most of the animals died within 10 minutes, although a few deaths occurred after several hours. All deaths were preceded by severe depression. Hematuria was noted in these animals immediately after dosing, but erythrocyte numbers determined at 48 hours were only slightly lower than normal.[60]

### Intranasal

Benzethonium Chloride was tested in vitro and in vivo for activity against influenza A virus.[142] Four mice under light ether anesthesia were inoculated intranasally with 0.05 ml of a mixture of equal volumes of Benzethonium Chloride and virus in saline. Deaths and the degree of lobar consolidation of the lungs were recorded for the next 10 days. Benzethonium Chloride at a concentration of 0.0125 percent completely inhibited influenza A virus (no deaths or lobar consolidation), and 0.00625 percent gave partial inhibition (no deaths but presence of some lobar consolidation). These concentrations of Benzethonium Chloride were above the toxic range for mice. Concentrations of Benzethonium Chloride ranging from 0.25 to 4 percent were initially tested also but were toxic, causing death with lobar consolidation similar to an influenzal pneumonia.

In the in vivo methods, lightly etherized mice were exposed to Benzethonium Chloride preceding and following the test virus dose of 10 MLD. Preliminary doses were administered into the intranasal passage by means of a pasteur pipet; however, subsequent doses were administered via a spray chamber. Five mice were exposed to 2 percent Benzethonium Chloride for 9 minutes before and ater the 10 minute virus dose. Five additional mice were exposed only to the test virus dose (in the same chamber) as controls. This was repeated with 5 mice exposed to 0.4 percent Benzethonium Chloride for 60 minutes before and after the virus dose. In both cases, Benzethonium Chloride had no protective activity: 2 and 3 mice of the 2 percent Benzethonium Chloride group died on Days 6 and 7, respectively, whereas 2 and 3 of the 0.4 percent group died on Days 5 and 7, respectively. Based on these results and those of a similar study on Sephiran and pneumococcus, Benzethonium Chloride appears to be rapidly inactivated upon contact with the lung.[142]

## Irritation

### Topical

A percutaneous toxicity study was conducted by Finnegan and Dienna[137] to determine the local and systemic effects of Benzethonium Chloride and Methylbenzethonium Chloride. The hair of 12 healthy albino rabbits was clipped from the back and sides over an area extending from the neck to the hind legs. Two milliliters of a 0.1 percent solution of Benzethonium Chloride were applied to the clipped area of the skin of 6 rabbits once daily, 5 days per week for 4 weeks. Similarly, a 0.1 percent solution of Methylbenzethonium Chloride was applied to the other 6 rabbits. The animals were closely watched for signs of irritation, but none were observed during this period.

Groups of 3 male C57BL/6 mice (black mice) were given single doses of 8.75, 17.5, 35, 70, 140, and 280 mg/kg Benzethonium Chloride (in tricaprylin) by application with a camel's hair brush. A control group was painted with tricaprylin alone. Severe local blistering occurred at the 2 highest doses, more moderate local reactions occurred at the 70 and 35 mg/kg doses, and no visible reactions occurred at the 2 lowest doses. The mice had no immediate bleaching. Ten days later, spotty depigmentation occurred at the site of painting in 2 of the mice of the 140 mg/kg group. Hair exposed in vitro for 48 hours was not bleached by the same concentrations of Benzethonium Chloride as used in this experiment.[130]

### Ocular

Benzethonium Chloride and Methylbenzethonium Chloride were evaluated for ocular irritancy by an "irritant threshold" test.[137] The test solutions were introduced into the conjunctival sac of the rabbit eye, and observations of edema, erythema, and increased secretions were recorded for 1 hour. Five rabbits were used at each concentration (not specified) within a selected significant range. The threshold concentration, defined as the highest concentration not producing irritation in 3 or more of the 5 test rabbits was determined for each compound. Benzethonium Chloride and Methylbenzethonium Chloride were quite irritating, with a threshold concentration of 0.03 percent.

A Draize eye irritation test was conducted on Benzethonium Chloride. Three groups of 3 albino rabbits each received a 0.1 ml instillation of Benzethonium Chloride in distilled water into the conjunctival sac of 1 eye; the other eye served as the control. In the first group of 3 rabbits, the treated eye was not rinsed. The treated eyes of the second and third groups were rinsed with 20 ml of lukewarm water 2 and 4 seconds after instillation, respectively. Reactions were recorded at 24, 48, and 72 hours and 4 and 7 days following treatment or until such time as all signs of irritation had disappeared. Benzethonium Chloride had a maximum tolerated concentration, defined as that concentration at which no corneal or iridic lesions are present at the seventh day reading, of 0.5 percent.[143]

In a subchronic eye irritation study, a 0.1 percent solution of Benzethonium Chloride was instilled into the conjunctival sac of rabbit eyes 2 to 3 times per day for 1 to 3 months. Only the superficial layers of the cornea and conjunctiva were affected: the corneal epithelium was thick and rough, with slight vascularization of the corneal stroma. The deep layers of the cornea and the intraocular tissues were not damaged, as determined by slit lamp or microscopic examination.[144]

A cologne stick containing 0.5 percent Benzethonium Chloride was evaluated for ocular irritation in 3 rabbits. A 0.1 g sample of the cologne was instilled into 1 eye of each rabbit; the other eye served as the control. Eyes were scored according to Draize at 1 hour and daily thereafter for up to 7 days. The highest daily scores for each rabbit were 4, 4, and 6 (max, 110); all eyes were normal by Days 4, 4, and 5, respectively. The cologne stick was minimally irritating according to the Draize standard of classification.[145]

### Intracutaneous

An intracutaneous irritation study was conducted on four quaternaries, including Benzethonium Chloride.[132] Intracutaneous injections of 0.1 ml of serial 3-fold dilutions of each compound were administered to rabbits. Corresponding dilutions of each compound, as well as the diluent (Parker's medium 199) alone, were injected on the same side in the same rabbit, and 4 rabbits were used for each of the 6 dilutions. Rabbits were observed daily for erythema and infiltration, and observations were recorded on Days 1, 2, 4, 6, and 13. Numerical scores of 0, 1, 2, and 3 were given for no visible reaction, slight, moderate, and severe reactions, respectively. All inoculations with the diluent alone produced no skin reactions. A concentration of 1.0 percent Benzethonium Chloride produced a reaction of grade 1 on Days 1 and 2, 3 on Days 6 and 13; 0.33 percent Benzethonium Chloride produced a reaction of grade 0.5 on Day 1, 1 on Day 2, and 3 on Days 6 and 13; 0.11 percent Benzethonium Chloride produced a reaction of grade 0 on Day 1, 0.1 on Days 2, 6, and 13; 0.037 percent Benzethonium Chlo-

ride produced a reaction of grade 0 on Days 1 and 6 and 0.5 on Days 2 and 13.

The investigators ranked the four compounds tested for relative toxicity. Benzethonium Chloride received the score of 3 on a relative scale of 3:3:1:2. They also commented that this test was not very sensitive, as similar scores were obtained with all the 4 compounds tested, and they suggested that the rabbits could be interfering with the results by scratching of the injection sites.[132]

### Vaginal

A contraceptive foam containing 0.2 percent Benzethonium Chloride produced no signs of vaginal mucosal irritation after 10 and 15 injections in rabbits and dogs, respectively, over a 3-week period.[128]

In a test for the British Family Planning Association, a contraceptive foam containing 0.2 percent Benzethonium Chloride was applied to the vaginas of 2 monkeys 5 times per week for 4 to 6 months. The monkeys had no signs of vaginal irritation.[128]

## Subchronic Toxicity

### Oral

Oral doses of Benzethonium Chloride ranging from 1.13 to 35.58 mg/kg were administered to both rats and rabbits. Little or no toxic effects were noted at any dose except for the highest. Average body weight was reduced in rats receiving 35.58 mg/kg Benzethonium Chloride over a subchronic exposure period (experimental period unspecified).[136]

### Percutaneous

A percutaneous toxicity study was conducted on 0.1 percent solutions of Benzethonium Chloride and Methylbenzethonium Chloride. The hair of 12 albino rabbits was clipped from the back and sides over an area extending from the neck to the hind legs. Two millilters of the Benzethonium Chloride solution were applied to the clipped area of the skin of 6 rabbits once daily 5 days per week for 4 weeks. Similarly, 2 ml of the Methylbenzethonium Chloride solution were applied to the other 6 rabbits. The animals were observed for signs of systemic toxicity, including weight loss. At termination, all the animals were killed, and representative tissues were examined microscopically. No systemic effects were noted for either compound.[137]

### Subcutaneous

Sixty Fischer 344 rats were separated into 5 groups of 6, 12, 24, 12, and 6 with equal numbers of males and females, and administered twice-weekly subcutaneous injections of Benzethonium Chloride in saline for 4 weeks.[140] The doses of Benzethonium Chloride were separated by quarter- or half-log intervals. The maximum tolerated dose (by repeated injections) was 3.0 mg/kg.

Groups of 8 C57BL/6 mice (black mice) were given subcutaneous injections of Benzethonium Chloride (in tricaprylin) ranging from 17.5 to 180 mg/kg. The maximum tolerated dose for repeated injection was 35 mg/kg. At injection sites some large ulcers were found, and these healed within approximately 4 weeks. In all of the mice receiving 70 mg/kg Benzethonium Chloride, depigmentation of the fur near the site of injection was noted after 34 days. Five of the eight mice receiving 35 mg/kg had a similar spotty depigmentation. When the depigmented fur was plucked, the new hair growth was also depigmented.[130]

## Chronic Toxicity

### Oral

A 1-year chronic oral feeding study was conducted using 9 adult mongrel dogs.[60] Benzethonium Chloride was mixed with Purina dog chow meal to give concentrations of 5, 100, and 500 ppm. Each diet was fed to 3 dogs for 1 year. All animals appeared well and gained weight during the test period. Prior to the start of the experiment and during the sixth and twelfth months, hemoglobin and complete blood counts were determined; all values were within normal limits. At termination, the dogs were necropsied, and the following organs were preserved for histopathological examination: heart, liver, lungs, thyroid, stomach, small intestine, cecum, large intestine, spleen, pancreas, kidneys, adrenals, and gonads. No gross or microscopic abnormalities were noted (Table 5).

A 2-year chronic oral feeding study was conducted using albino rats.[60] Benzethonium Chloride was mixed with finely ground Purina dog chow meal to give concentrations of 50, 200, 1000, 2500, and 5000 ppm. Sixty male and sixty female rats were divided into 12 groups of 10 (separated as to sex) and were individually housed. One group of each sex was fed one of the diets, and one served as the control group. Mortality was increased only at the 5000 ppm concentration between Weeks 10 and 30 in both males and females. Similarly, body weights were not significantly reduced ($p < 0.05$) except at the 5000 ppm concentration during the first week. The erythrocyte and differential white blood cell counts and the hemoglobin values taken during the 11th and 23rd months were within normal limits. All rats dying on test (not autolyzed) and all survivors at termination were necropsied, and the following organs were preserved for histopathological examination: heart, liver, lungs, thyroid, stomach, small intestine, cecum, large intestine, spleen, pancreas, kidneys, adrenals, and gonads. Microscopic examination was made on tissues from the survivors and those that died just prior to termination. One male (of 6 examined) at the 2500 ppm level, and two (of 3 examined) at the 500 ppm level had testicular atrophy. At the first necropsies, greatly distended ceca were observed in the higher dietary groups (1000, 2500, and 5000 ppm). This condition became progressively worse with increasing Benzethonium Chloride concentration and apparently occurred less than a week after treatment initiation. Thinning of the cecal wall, but no other abnormalities, were found at microscopic examination (Table 5).

Chronic (experimental period unspecified) oral doses of Benzethonium Chloride ranging from 1.13 to 35.58 mg/kg were administered to rats and rabbits. Few, if any, toxic effects were observed at doses other than the highest. A reduction in average body weight was noted in rats receiving a dose of 35.58 mg/kg Benzethonium Chloride[136] (Table 5).

### Subcutaneous

A 1-year subcutaneous toxicity and carcinogenicity study was conducted on Benzethonium Chloride.[140] Groups of 20, 40, 60, and 80 Fischer 344 rats received approximately 0.25 ml doses of 0.1, 0.3, 1.0, and 3.0 mg/kg Benzethonium Chloride in saline, respectively, twice weekly for 52 weeks. Animals were held for observation another 6 months after treatment. Three controls were used: a vehicle control of twice weekly 0.25 ml saline injections (60 males and 60 fe-

**TABLE 5.** Chronic Toxicity

| *Ingredient* | *Concentration and/or Dose* | *Length of Study* | *Species* | *Number of Animals* | *Results* | *References* |
|---|---|---|---|---|---|---|
| *Oral* | | | | | | |
| Benzethonium Chloride | 5, 100, and 500 ppm in the diet | 1 year | Dog | 3 in each conc. group | No gross or microscopic abnormalities | 60 |
| Benzethonium Chloride | 50, 200, 1000, 2,500, and 5000 ppm in the diet | 2 years | Rat | 20 in each conc. group | Increased mortality and decreased body weight at 5000 ppm; thinning of cecal wall at 1000, 2500, and 5000 ppm; no other abnormalities | 60 |
| Benzethonium Chloride | 1.125–35.576 mg/kg | Chronic, unspecified | Rat and rabbit | Unspecified | Few toxic effects at doses other than the highest; decreased average body weight in rats at highest dose | 136 |
| *Subcutaneous* | | | | | | |
| Benzethonium Chloride in saline | 0.1, 0.3, 1.0, and 3.0 mg/kg | 1 year—twice weekly injections; observed for 18 months | Rat | 80, 60, 40, and 20 in the high to low dose groups, respectively | 14% reduction in weight gain at highest dose at 12 months, decreasing to 12% at 18 months; no other adverse effects | 140 |
| Benzethonium Chloride in saline | 0.0034 and 0.034 mg | Single injection, observed for 15 months | Mice | 100 in each dose group | Slight decrease in highest dose female body weights; slight increase in mortality of high dose group; no significant compound-related effects | 146 |

males), a negative control (60 males and 60 females), and a positive control using predetermined fixed doses of nickel sulfide (80 males and 80 females). Toxicity was determined by survival time, weight changes, and drug-related lesions. The animals were necropsied either at 12 or 18 months, as planned, and tissues from all spontaneous deaths, moribund rats, those rats with gross lesions or abnormal organ weights, and randomly selected rats were examined microscopically. Mortality for the first 12 months was only 1.5 percent, compared to 75 percent for the positive control. Mortality of Benzethonium Chloride-treated rats at 18 months was 7.5 percent, compared to 5.8 to 8.3 percent for the negative and vehicle controls and 90 percent for the positive controls. Benzethonium Chloride, at the highest dose, causes a 14 percent reduction in weight gain over 12 months compared to untreated and vehicle controls. The rats recovered body weight slightly by 18 months, with a reduction of 12 percent. Lower doses resulted in less reductions in body weight gains (Table 5).

Newborn Swiss mice were injected subcutaneously with 2 doses of Benzethonium Chloride, 1 set at 10 percent of the $LD_{50}$ (0.0034 mg) and 1 set at the approximate $LD_{50}$ (0.034 mg). A single injection was administered to each mouse in groups of 50 males and 50 females per dose. A saline vehicle control group and positive control groups of dibenz(a,h)anthracene (DBA) in both corn oil and saline were used. Injections were made at the base of the tail, and the needle was inserted along the hypodermal layer to the area of the neck and shoulders. The entire mouse population was afflicted with a respiratory condition at 15 weeks, causing some mortality. The animals were also treated with a DDT dust preparation at 15 and 19 weeks for sarcoptic mange mites. Animals were observed for 15 months and then sacrificed. Body weights were in the normal range. Females receiving the higher dose of Benzethonium Chloride had slightly decreased body weights. No mortality trends were noted. However, the higher dose Benzethonium Chloride group had more deaths than did the high dose DBA group. Survival did not appear to be correlated with compound administration. No compound-related nonneoplastic lesions were noted at termination. An insignificant number of tumors were found (except for injection site tumors in positive controls) distributed among test and control groups. These were typical spontaneous tumors in mice, and no correlation could be made between Benzethonium Chloride treatment and neoplasm formation[146] (Table 5).

## Teratogenesis

A series of studies designed to determine the teratogenic effect of Benzethonium Chloride in rats were conducted by Gilman and DeSalva.[147] Pregnant rats were administered doses of Benzethonium Chloride up to 35.58 mg/kg per day by gastric intubation on Days 6 through 15 of gestation. Animals were killed on Day 20, and the fetuses were examined. The high dose (35.58 mg/kg per day) of Benzethonium Chloride produced lower mean body weights and delayed ossification. This finding was confirmed in a second study. No clinical manifestations of skeletal deformity were observed in rat fertility, perinatal, and postnatal studies. The investigators concluded that delayed ossification was not an expression of skeletal teratogenic changes but was most likely related to maternal toxicity and secondarily to reduced fetal maturation.

The Chemical Evaluation Committee (CEC) of the National Toxicology Program (NTP) has recommended testing Benzethonium Chloride for reproductive effects.[148]

## Mutagenesis

The Ames test with *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100 was used to study the mutagenic potential of Benzethonium Chloride; assays were conducted both with and without rat liver S-9 fraction. Benzethonium Chloride, at a maximum dose of 100 nmol/plate (due to bacterial toxicity), was nonmutagenic both with and without metabolic activation.[149]

Benzethonium Chloride was nonmutagenic in the NTP mutagenicity testing program using *S. typhimurium*.[150]

## Carcinogenesis

One hundred C57BL/6 male mice were injected subcutaneously in the groin with 0.7 mg Benzethonium Chloride (in tricaprylin). Five weeks later, the injection sites were excised, minced in 6 ml of Ringer's solution, and pooled. The resulting mix was injected subcutaneously into 25 C57BL/6 mice of the same age. All animals were killed after 18 weeks and examined grossly and microscopically for tumors. Positive dibenzo(a,i)pyrene and negative (tricaprylin) controls were also subjected to the same treatment. No significant mortality was observed in the Benzethonium Chloride-treated group. The injection sites had granulation tissue and multiple granulomas with numerous giant cells. Scar tissue and numerous cysts, lined by single layers of endotheliumlike cells or granulation tissue with numerous giant cells, frequently contained cholesterol clefts. Some cysts were filled with granular or reticular amorphous material. None of the fibroblasts in this granulation and scar tissue had features suggestive of malignant transformation. Similar foreign body reactions were observed in the negative controls. All of the mice receiving Benzethonium Chloride had local depigmentation of the fur. However, depigmentation was not seen in any of the secondary hosts. Benzethonium Chloride was not found to be carcinogenic under these test conditions.[130]

In another study, reportedly "the most sensitive system" for carcinogen detection, 0.35 mg Benzethonium Chloride were injected as a single dose into the tail vein of each of 50 CF-1 and 50 A/Jax female mice. Twenty additional CF-1 female mice were given 7 injections at monthly intervals. Positive dibenzo(a,i)pyrene and negative (Ringer's solution) controls were used for the single dose groups. All mice were killed at 7 months. The lungs were inflated with formaldehyde and examined under a dissecting microscope for tumors visible on the lung surface. Benzethonium Chloride did not induce a significant number of pulmonary tumors when compared to controls.[130]

In a cocarcinogen study, 50 C57BL/6 male mice were each injected subcutaneously with 12.5 $\mu$g dibenzo(a,i)pyrene (DBP) in tricaprylin. Twenty-four hours and again 8 days later, 0.70 mg of Benzethonium Chloride was injected into the same site. An additional series of Benzethonium Chloride at one-half the maxi-

mum tolerated dose, 0.35 mg/mouse, was started approximately 6 weeks later. This group received 0.35 mg Benzethonium Chloride 1, 8, and 15 days after the injection of DBP. Positive (croton oil) and negative controls were used. All animals in the 0.70 mg group were killed at 29 weeks; those in the 0.35 mg group at 23 weeks. Tissues from all were examined microscopically for tumors; tumors found were fibrosarcomas produced by DBP. Benzethonium Chloride was not cocarcinogenic. However, the study was considered inconclusive due to the fact that croton oil, the positive control, failed to have any cocarcinogenic action. The investigator commented on the "significant" inhibiting effect of Benzethonium Chloride upon tumor formation following the injection of DBP. Benzethonium Chloride groups had 34.1 and 0 percent cumulative tumor incidence for the high and low dose groups, respectively, compared to 48.8 and 52.0 percent for the positive control (croton oil and DBP) and the DBP control. All mice receiving 0.7 mg Benzethonium Chloride and 97.4 percent of the 0.35 mg Benzethonium Chloride group had depigmented fur.[130]

Newborn Swiss mice were injected subcutaneously with 2 doses of Benzethonium Chloride, a low dose (0.0034 mg) set at 0.1 of the $LD_{50}$ and a high dose set at the approximate $LD_{50}$ (0.034 mg). A single injection was administered to each mouse of groups of 50 males and 50 females per test dose. Positive (DBA) and vehicle (saline) control groups were used. Animals were observed for 15 months, killed, and tissues were examined microscopically. No compound-related nonneoplastic lesions were noted at termination. An insignificant number of tumors was found distributed among the test and control groups (with the exception of injection site tumors in positive controls). These were typical spontaneous tumors for mice, and no correlation could be made between Benzethonium Chloride treatment and neoplasm formation. Under these study conditions, Benzethonium Chloride was not a chemical carcinogen.[146]

Weanling Swiss and Balb/c male and female mice were to be given multiple injections of Benzethonium Chloride (concentration not given) either subcutaneously or intraperitoneally every 2 weeks for 20 injections. Difficulties ensued so that only 15 to 16 injections were given. Animals were observed for 18 months. Similar groups of mice were given a single injection of Benzethonium Chloride as controls. A significant number of tumors did not occur in the treated mice as compared to controls. Benzethonium Chloride was not carcinogenic in these mice.[151]

In a carcinogenicity study, groups of 20, 40, 60, and 80 (equal males and females) Fischer weanling rats were injected subcutaneously with 0.1, 0.3, 1.0, and 3.0 mg/kg Benzethonium Chloride in saline, respectively, twice weekly for 52 weeks.[140] Animals were held for observation another 6 months after treatment. Positive (nickel sulfide), negative, and vehicle (saline) controls were used. All rats were necropsied at the time of death or at the 18-month sacrifice and tissues were examined microscopically for tumor formation. The treated rats had 26 sarcomas at injection sites (13 percent) compared to 0 and 1 tumors in the vehicle and negative controls (0 to 2 percent) and 90 percent incidence in the positive controls. A high incidence of granulomatous reactions occurred at the sites of the subcutaneous injection of Benzethonium Chloride, and these were dose related. The tumors were principally fibrosarcomas; none metastasized, but some did grow to a large size. This type of induced neoplasm has been described as arising from mesenchymal cells in the area of repeated irritation. Based on the incidence

of dose-related tumors at the injection sites, Benzethonium Chloride should be classified as a weak carcinogen under the classification system of Grasso and Golberg.[152] Clayson,[153] however, regards the induction of localized sarcomas in mice upon repeated subcutaneous injection of test solutions as "notoriously unreliable as an indicator of carcinogenicity." Furthermore, he considers "the results of individual experiments as extremely variable."

A 2-year chronic oral feeding study was conducted using albino rats.[60] Benzethonium Chloride was mixed in the diet to give concentrations of 0, 50, 200, 1000, 2500, and 5000 ppm. One group of 10 males and one of 10 females were fed each dietary concentration. Rats that died and those killed at termination were necropsied and tissues were examined microscopically. Three mammary gland fibroadenomas were found; none occurred in the 2 highest dose groups. The investigators commented that this tumor occurrence of 6.5 percent was low for rats of that age. One subcutaneous reticulum cell sarcoma was found in a male of the 200 ppm group after 53 weeks, but the occurrence of this tumor was unrelated to treatment because no such tumors were found in higher dose groups over a longer period of time.

The Chemical Evaluation Committee of the NTP has recommended Benzethonium Chloride for carcinogenicity testing.[150]

## CLINICAL ASSESSMENT OF SAFETY

### Irritation and Sensitization

An occlusive patch containing 0.1 ml of a 5 percent aqueous Benzethonium Chloride solution was applied to the upper back of each of 100 volunteer white males. Patches were removed after 48 hours, and the sites were evaluated 0, 1, 24, 48, and 72 hours later. Fifty-one of the 100 subjects had irritant reactions, defined as "redness without vesiculation or infiltration, declining in intensity 24 hours after removal, nonitching, and not spreading beyond the patch." In doubtful cases, the patches were repeated on the forearms. The investigators noted that Benzethonium Chloride is a known irritant, not a sensitizer[154] (Table 6).

In clinical studies designed to determine the antiseptic properties of Benzethonium Chloride, observations were made on skin sensitivity or other toxic reactions. The conditions of the test conformed to hospital preoperative skin preparation procedures. The abdominal skin of each patient was first scrubbed with a tincture of green soap and water, cleansed with alcohol, and then painted with either an aqueous solution of Benzethonium Chloride (0.2 percent) or a tincture of Benzethonium Chloride (0.2 percent) on one side only. Three hundred obstetric deliveries and 100 surgical cases were evaluated. The skin of each patient was carefully observed for several days after treatment; no evidence of irritation, desquamation, or other reactions was noted. A tincture of Benzethonium Chloride was further studied to determine the effect of heat and sweating on its antiseptic properties. Both thighs of 10 patients were painted with the Benzethonium Chloride tincture and a heat cradle was placed over 1 thigh of each for 2½ hours. No irritation or rise in bacterial count was noted[155] (Table 6).

An aerosol antiperspirant and a deodorant, each containing 0.12 percent Benzethonium Chloride, were evaluated in a repeated insult patch test for irrita-

**TABLE 6.** Clinical Irritation and Sensitization

| *Ingredient* | *Type of Test* | *Number of Humans* | *Results/Comments* | *References* |
|---|---|---|---|---|
| Benzethonium Chloride in 5% aqueous solution | 48-hour occlusive patch | 100 | 51 exhibited irritant reactions, "redness without vesiculation or infiltration, declining in intensity 24 hours after removal, nonitching, and not spreading beyond the patch"; irritant | 154 |
| Benzethonium Chloride in 0.2% aqueous solution or tincture of green soap and water | Single topical application on scrubbed abdominal skin (hospital preoperative skin preparation procedures) | 400 | No irritation, desquamation, or other reactions noted | 155 |
| Benzethonium Chloride in 0.2% tincture of green soap and water | Single topical application on thighs with heat administered for 2½ hours | 10 | No irritation | 155 |
| Benzethonium Chloride, 0.12% in an aerosol antiperspirant | RIPT* | 50 | No reactions; no effects indicative of a primary irritant, fatiguing agent, or senitizer | 156 |
| Benzethonium Chloride, 0.12% in a deodorant | RIPT | 50 | No reactions; no effects indicative of a primary irritant, fatiguing agent, or sensitizer | 157 |
| Benzethonium Chloride, 0.1% in an aerosol foam | Clinical test for symptomatic control in patients with dermatological disorders; applications every 2 hours for 6 days (average) | 98 | One case of slight stinging with first application in a patient with pruritis; no other side effects; nonirritant | 158 |
| Methylbenzethonium Chloride, 0.5% in a skin cleanser | Irritation–single patch | 18 | No reactions; no difference in irritancy between product and control; nonirritant | 159 |
| Methylbenzethonium Chloride, 0.5% in a skin cleanser | RIPT with rechallenge test | 100 | A total of 10 barely perceptible or mild (1+ on scale of 0–4) reactions observed in 7 subjects during induction and in 6 subjects at challenge; 3 subjects were rechallenged, resulting in 1 mild reaction considered due to the abrasive nature of the cleanser; investigators concluded the cleanser did not induce sensitization; mild irritant; nonsensitizer | 160 |

*RIPT, repeated insult patch test.

tion and sensitization.[156,157] Each product (full strength) was applied using a 24-hour occlusive patch on 50 human subjects. Each site was examined at the time of patch removal and graded on a scale of 0 to 4, representing no response to a severe response. Patches were applied Monday through Thursday for 2 weeks, followed by a 2-week rest period and a challenge patch application to the same area in the fifth week of the study. Challenge patches were removed after 24 hours, and the sites treated with the aeorsol antiperspirant were examined at removal and after 24, 48, and 96 hours. Sites treated with the deodorant were examined 10 minutes, 24, 48, and 72 hours after removal of the patches. All 50 subjects in each test had no reactions during the induction or challenge phases. The investigators concluded that, under these test conditions, neither product was a primary irritant, fatiguing agent, or sensitizer in any of the subjects. They also predicted with 95 percent certainty that 92.89 percent of a general population would not be sensitized by these products (Table 6).

A hydrocortisone-containing aerosol foam with 0.1 percent Benzethonium Chloride was tested clinically for symptomatic control in 98 patients with dermatological disorders. These consisted of contact dermatitis (70), pruritis ani (14), neurodermatitis (6), and miscellaneous disorders (8). In most cases, the aerosol was applied every 2 hours for an average time of 6 days. With the exception of 1 case of slight stinging with the first appication in a patient with pruritis, no other side effects were reported[158] (Table 6).

A skin cleanser containing 0.5 percent Methylbenzethonium Chloride was tested for skin irritation in 18 human subjects.[159] A patch containing a 0.1 ml dose of the cleanser was applied to the volar surface of the forearm or inner aspect of the arm. A comparable product was used as a control. Reactions were graded 2 and 24 hours after patch removal on a scale of 0 to 4, representing no response to a severe response. All 18 panelists had no reactions. Type of patch, application, and duration of application were unspecified. The control product was also nonirritating (Table 6).

A skin irritation and allergic contact sensitization test was conducted to evaluate a cleanser containing 0.5 percent Methylbenzethonium Chloride.[160] Twenty-four hour occlusive patches were applied to the backs of 100 panelists (96 females, 4 males). Each patch contained a 0.1 ml dose of the full-strength cleanser, and applications were made every Monday, Wednesday, and Friday at the same site for 3 consecutive weeks. Responses were scored on a scale of 0 to 4 just prior to applications 2 through 9 and the next test date after application 9. Following a 2-week nontreatment period, a 24-hour challenge patch was applied to a previously unpatched site, and reactions were scored 24 and 48 hours after removal. Any subjects with reactions at challenge indicative of possible induced sensitization were retested 1 week later. This follow-up testing consisted of occlusive patches with the cleanser full-strength and in a 1:3 dilution, and an example of exaggerated use in which the cleanser was applied full-strength to the flex area of the arm 3 times daily for 5 days. A total of 10 reactions were observed in 7 subjects during the induction phase: 5 barely perceptible and 5 mild. Six subjects had a total of 10 reactions at challenge, 4 of whom had no reactions during induction. The reactions observed were mild (1+) or barely perceptible, and 3 of these subjects were selected for rechallenge. All 3 subjects gave no response 24, 48, and 72 hours after the full strength and diluted patch applications. One subject had a mild reaction on Day 3 of the 5-day exaggerated use test. However,

this subject had been vigorously rubbing the "abrasive cleanser" onto her arm, causing noticeable abrasions considered irritant in nature. The majority of reactions during induction and challenge were apparently due to this same grain pressure effect. This reactivity was not confirmed in the 3 rechallenged subjects. Under these test conditions, the cleanser had no potential for inducing sensitization (Table 6).

Fisher[161] reported that Benzethonium Chloride is rarely a sensitizer to either skin or oral membranes. Topical oral application may produce allergic sensitization.

## Case Reports

Three cases of Benzethonium Chloride sensitization have been reported. A laborer using Benzethonium Chloride and benzalkonium chloride applied topically as disinfectants for many years developed a contact dermatitis. On patch testing, he was sensitive to both compounds in dilutions of 1:100,000.[162] A 23-year-old woman acquired a severe edematous, pruritic, vulvar and perivulvar eruption after using a hygiene spray containing Benzethonium Chloride for 1 month. This was superimposed upon a seborrheic dermatitis in the inguinal area. A 1:1000 aqueous solution of both Benzethonium Chloride and benzalkonium chloride produced a strongly positive reaction in this patient. A 29-year-old man developed penile and scrotal dermatitis after each act of sexual intercourse with a woman who used a spray containing Benzethonium Chloride. This man also gave strongly positive reactions to patch tests with 1:1000 aqueous solutions of Benzethonium Chloride and benzalkonium chloride.[161] Fisher suggests that individuals may have cross sensitization reactions to Benzethonium Chloride and benzalkonium chloride.

## Irritation – Ocular, Vaginal, Oral, and Otic

Benzethonium Chloride is used as a preservative in ophthalmic preparations. Over a period of 5 years, various wetting agents, including Benzethonium Chloride, were administered to "hundreds" of patients at an eye clinic. Instillations varied from a single administration to 2 to 4 times daily for periods up to 3 years. The cornea were examined by slitlamp, and patients were questioned regarding irritation. Solutions of 0.1 percent administered as a single 0.50 ml drop into the conjunctival sac of volunteers produced conjunctival reactions consisting of hyperemia, edema, and thickening and reduced transparency of the superficial layers associated with dilation of capillaries. Other effects noted were profuse lacrimation, enlargement of deeper-lying vessels in some cases, and frequently, desquamation of the conjunctival epithelium. Corneal lesions developed 10 minutes after instillation. Solutions of approximately 0.03 to 0.04 percent instilled 3 to 4 times daily for 2 to 8 weeks produced similar but much less severe corneal reactions. In most cases, recovery time was 12 hours or less. The investigator reported that individuals had varied responses to these solutions but that the minimal concentrations producing irritation were greater than those found therapeutically effective. Surface activity, pH variation, and osmotic pressure were cited as possible causes of irritation. Frequent or prolonged administration greatly increased the severity of the irritation.[144]

Benzethonium Chloride has been added to several ophthalmic solutions used to reduce intraocular pressure. A carbostyril derivative containing 0.1 mg

Benzethonium Chloride was placed in the eye of a person with secondary glaucoma, and no irritation was reported.[163] Bupranolol solutions (35 μl of 1 percent or 0.5 percent) containing 0.01 g Benzethonium Chloride were instilled in 1 eye of each of 12 normal humans and 30 patients with either ocular hypertension or primary open-angle glaucoma. The other eye of the 12 served as controls, whereas the other eye of the 30 received a placebo instillation. No side effects were reported. Similarly, 10 patients received 4 times daily instillations of the 1 percent Bupranolol solution for 6 months. Two of the patients also received a 1 percent pilocarpine solution. No side effects were reported, with the exception of a stinging sensation at the time of instillation and lasting for several seconds. At the end of 6 months, no change in isopters or abnormalities in tear secretions were found.[164]

In a review of Benzethonium Chloride as a preservative in ophthalmic preparations, the Ophthalmic Panel of the FDA OTC Drug Review Program found Benzethonium Chloride satisfactory as a preservative at maximum concentrations of 0.01 percent for preparations used directly in the eye and 0.02 percent for preparations not for direct use in the eye.[71]

A number of studies have been conducted on a vaginal aerosol foam contraceptive containing 0.2 percent Benzethonium Chloride and 8.0 percent nonyl phenoxypolyethoxyethanol in an oil in water dispersion. One hundred thirty women of childbearing age used the foam over an average period of 20.4 months (1 to 57 months), and no clinical evidence of irritation, sensitization, or other abnormalities resulted.[165] Sobrero[166] encountered no reports of burning, itching, irritation, or unfavorable reactions by husbands to the foam in postcoital tests with 22 women. He also found that daily vaginal injections in 12 volunteer women over approximately 3 weeks produced no evidence of irritation by inspection or papanicolaou smear. In another study, 142 couples used the foam for a month or more; 1 couple discontinued the test because of urethal irritation in the wife.[128] Six percent (15 of 247) of the volunteers in a trial of the foam complained of postcoital irritation.[167] A study was conducted involving 2932 women who used the foam over an average period of 270 days (1076 used the foam for 1 year or more). Of the 1994 women who discontinued the study, 107 did so because of vaginal irritation and 17 discontinued because of irritation of the genitalia of the male partner. There were no reports of severe local reaction[168] (Table 7).

A 3-year trial of a spermicidal cap containing 4.5 mg Benzethonium Chloride and 39.0 mg nonoxynol 9 was conducted with 326 couples, of which 230 couples completed the test. Average use was 14 months. Mild redness, burning, and local irritation occurred in 18 couples (5.5 percent) and caused one couple to discontinue the test. Variation in vaginal pH and inflammatory changes were not of clinical significance. No evidence of neoplastic changes was found in cells of the portio or of the vagina in cytologic preparations obtained from these areas.[169]

In a study of odor changes following the intravaginal administration of a suppository containing 0.4 mg Methylbenzethonium Chloride to 10 women, no vaginal irritation was reported.[170]

Numerous clinical studies have been conducted to determine the effect of antimicrobial formulations containing Benzethonium Chloride on the onset of caries, calculus, and gingival inflammation. Benzethonium Chloride generally re-

**TABLE 7.** Clinical Vaginal Irritation

| *Ingredient* | *Type of Test* | *Number of Humans* | *Results/Comments* | *References* |
|---|---|---|---|---|
| Benzethonium Chloride, 0.2% in an aerosol foam contraceptive | Normal use for an average period of 20.4 months | 130 women | No evidence of irritation, sensitization, or other abnormalities | 164 |
| Benzethonium Chloride, 0.2% in an aerosol foam contraceptive | Postcoital test | 22 couples | No reports of burning, itching, irritation, or unfavorable reactions by husbands | 165 |
| Benzethonium Chloride, 0.2% in an aerosol foam contraceptive | Daily vaginal injection for 3 weeks | 12 women | No evidence of irritation | 128 |
| Benzethonium Chloride, 0.2% in an aerosol foam contraceptive | Normal use for 1 or more months | 142 couples | 1 couple discontinued test due to urethal irritation in wife | 166 |
| Benzethonium Chloride, 0.2% in an aerosol foam contraceptive | Postcoital trial | 247 women | 15 complaints of irritation | 167 |
| Benzethonium Chloride, 0.2% in an aerosol foam contraceptive | Normal use for an average period of 270 days | 2932 women | 107 cases of irritation; 7 cases of irritation to male genitalia; no instances of severe local reactions | 168 |
| Benzethonium Chloride, 4.5 mg in a spermicidal cap | Normal use for an average period of 14 months | 230 couples | Mild redness, burning, and local irritation in 18 couples, causing 1 to discontinue the test; no evidence of neoplastic changes in cells of the portio or vagina | 169 |
| Methylbenzethonium Chloride, 0.4 mg in a vaginal suppository | Single application | 10 women | No vaginal irritation | 170 |

duced plaque accumulation but had no significant effect on gingivitis. No signs of oral mucosal irritation were reported.[98,99,101,103]

In several studies otic solutions containing approximately 0.02 percent Benzethonium Chloride were used both prophylactically and in the treatment of otitis externa. Drops were instilled in the ears of a total of 1282 humans as frequently as 4 times daily and for periods up to 16 days. No irritation or other toxic effects were reported.[171–175]

## SUMMARY

Benzethonium Chloride and Methylbenzethonium Chloride are synthetic quaternary ammonium salts occurring as colorless to white, slightly odorous, and bitter tasting monohydrate crystals. Both compounds are soluble in water, the lower alcohols, glycols, ether, and benzene. The exact methods of manufacture of these compounds are proprietary. Commercial preparations contain a large number of functional groups and impurities that contribute to the physicochemical properties of the product. Analytical procedures for the detection and quantification of Benzethonium Chloride and Methylbenzethonium Chloride are numerous, and most depend on the formation of a relatively stable ion-pair complex.

Benzethonium Chloride and Methylbenzethonium Chloride are relatively stable compounds. Their reactivity is determined for the most part by their cationic properties. They are inactivated by and adsorbed onto a variety of substances.

Benzethonium Chloride and Methylbenzethonium Chloride are used in cosmetics primarily as preservatives and secondarily as cationic surfactants, usually at concentrations below 1 percent. In 1981, Benzethonium Chloride and Methylbenzethonium Chloride were used in 93 and 33 formulations, respectively. Cosmetic products containing these compounds may contact all external body surfaces and hair, as well as ocular and vaginal mucosae. Frequency and length of application could result in continuous exposure. Benzethonium Chloride and Methylbenzethonium Chloride also are used widely in industry and in pharmaceuticals and are indirect food additives (limited to use as preservatives in adhesives).

Benzethonium Chloride and Methylbenzethonium Chloride have been extensively used and studied as antimicrobials. Controversy surrounds the scope of their microbial spectrum and their inactivation by a large number of materials. They are considered more bactericidal than fungicidal, and their antimicrobial activity has been credited generally to their ability to disrupt permeability of cell membranes. Benzethonium Chloride and Methylbenzethonium Chloride cause additional cytolytic injury by protein denaturation, by oxidation and enzyme inhibition, by effects on activating ions, and by interference with growth and reproduction. Tissue effects of Benzethonium Chloride include weakened activity of cilia of mucosal cells of the isolated trachea of the mouse and inhibition of smooth muscle contraction in rabbit ileum.

Fur depigmentation was noted in black mice either receiving Benzethonium Chloride injections or painted with the compound.

In acute toxicity studies, Benzethonium Chloride was moderately to slightly toxic when administered orally or intraperitoneally and mildly toxic to mice treated intranasally with concentrations of 0.25 to 4 percent. The subcutaneous $LD_{50}$ for Benzethonium Chloride was 119.0 mg/kg in rats and the intravenous $LD_{50}$ was 35 and 19.1 mg/kg in mice and rats, respectively.

Benzethonium Chloride and Methylbenzethonium Chloride were both irritating to the rabbit eye at concentrations greater than 0.03 percent. Additionally, Benzethonium Chloride produced ocular irritation in rabbits at a concentration of 0.1 percent and gave a maximum tolerated concentration (no corneal or iridic lesions at 7 days) of 0.5 percent. A cologne stick containing 0.5 percent Benzethonium Chloride was minimally irritating to rabbit eyes.

Both Benzethonium Chloride and Methylbenzethonium Chloride were nonirritating when applied topically (0.1 percent) to the skin of rabbits, although mice had local reactions to the moderate and high doses (35 to 280 mg/kg) of Benzethonium Chloride in a skin-painting study. Benzethonium Chloride was slightly irritating when administered intracutaneously to rabbits and nonirritating in tests of vaginal contraceptives with rabbits, dogs, and monkeys.

In subchronic studies, little or no toxic effects were found when Benzethonium Chloride was administered orally or percutaneously. Methylbenzethonium Chloride was also nontoxic when administered percutaneously. The maximum tolerated dose of Benzethonium Chloride administered subcutaneously was 3.0 and 35 mg/kg in rats and mice, respectively.

Chronic oral studies were also generally negative for toxic effects due to Benzethonium Chloride except for a slight increase in mortality and reduced body weight in rats at the high dietary concentration (5000 ppm) in one study. Greatly enlarged ceca were also noted in this latter study. In 1 of 2 chronic subcutaneous studies on Benzethonium Chloride, reduced weight gain was found at the high dose only, and a 13 percent incidence of sarcomas at injection sites was dose related.

Placental transport of Benzethonium Chloride was variable and inconsistent. Some delayed ossification was noted in fetuses. However, this change was attributed to maternal toxicity and only secondarily to reduced fetal maturation.

Benzethonium Chloride was nonmutagenic in a microbial sensor system and in the Ames test both with and without metabolic activation. No evidence of carcinogenicity of Benzethonium Chloride was found in 6 studies with mice and rats. However, in a seventh study, Benzethonium Chloride was classified as a weak carcinogen in rats due to a high incidence (13 percent) of compound-related tumors at injection sites. Benzethonium Chloride was not cocarcinogenic in combination with 3,4,9,10-dibenzpyrene (DBP) in mice, and in fact had a significant inhibiting effect upon tumor formation following injection of DBP.

In clinical studies, Benzethonium Chloride produced mild skin irritation at 5 percent and at lower concentrations produced no irritation. An aerosol antiperspirant and a deodorant, each containing 0.12 percent Benzethonium Chloride, produced no irritation or sensitization reactions when tested in human volunteers. Two skin cleansers, each containing 0.5 percent Methylbenzethonium Chloride, were tested for irritation, and irritation and sensitization, respectively. No irritation resulted from use of the first cleanser; irritant reactions caused by the second were apparently due to the "abrasive" nature of the cleanser, and no

sensitization was noted with either product. Three cases of Benzethonium Chloride sensitization have been documented.

Benzethonium Chloride is used as a preservative in ophthalmic preparations with maximum concentrations of 0.1 percent for use directly in the eye and 0.02 percent for use not directly in the eye. In various clinical uses of ophthalmic wetting agents containing Benzethonium Chloride, individuals varied in their irritant response to these solutions, although a general dose-response was seen. Increasing irritation was observed at concentrations greater than those found antibacterially effective.

In numerous studies of a vaginal aerosol foam contraceptive containing 0.2 percent Benzethonium Chloride, no irritation was found in 3 and no sensitization in 1. In others 0.7, 4, and 6 percent irritation were reported. Methylbenzethonium Chloride produced no vaginal irritation when used in a suppository. Benzethonium Chloride (4.5 mg) in a spermicidal cap produced mild irritation in 5.5 percent of the test population after an average use period of 14 months. No neoplastic changes were found in cells of the portio or vagina of these women.

No signs of oral mucosal irritation were noted in studies with antimicrobial formulations containing Benzethonium Chloride. Also, no irritation or toxic effects were produced by otic solutions containing Benzethonium Chloride.

## DISCUSSION

Benzethonium Chloride and Methylbenzethonium Chloride are weak antimicrobial agents. They can be irritating to the skin at concentrations of 5 percent or greater. However, at the concentrations used in cosmetics, neither compound produces significant irritation. These compounds have been reported to be ocular and vaginal irritants. In one study, fur depigmentation was noted in black mice upon subcutaneous injection or topical application of Benzethonium Chloride.

## CONCLUSION

On the basis of the available animal and clinical data, the Expert Panel concludes that Benzethonium Chloride and Methylbenzethonium Chloride are safe at concentrations of 0.5 percent in cosmetics applied to the skin. A maximum concentration of 0.02 is safe for cosmetics used in the eye area.

## ACKNOWLEDGMENT

Elizabeth Meerman Santos, Scientific Analyst and writer, prepared the literature review and technical analysis used by the Expert Panel in developing this report.

## REFERENCES


1. JOSLYN, D.A., YAW, K., and RAWLINS, A.L. (1943). Germicidal efficacy of phemerol. J. Am. Pharm. Assoc. Sci. Ed. **32,** 49–51.
2. ESTRIN, N.F., CROSLEY, P.A., and HAYNES, C.R. (eds.). (1982). *CTFA Cosmetic Ingredient Dictionary,* 3rd ed. Washington, DC: Cosmetic, Toiletry and Fragrance Association.
3. UNITED STATES PHARMACOPEIA (USP). (1979). 20th ed. Rockville, MD: United States Pharmacopeial Convention, Inc.
4. HAWLEY, G.G. (ed.). (1971). *Condensed Chemical Dictionary,* 8th ed. New York: Van Nostrand Reinhold.
5. HAZLETON, L.W. (May 1952). Relation of surface active properties to irritation of the rabbit eye. Proc. Sci. Sec., Toilet Goods Assoc., No. 17.
6. WINDHOLZ, M. (ed.). (1976). *The Merck Index,* 9th ed. Rahway, NJ: Merck and Co.
7. COSMETIC, TOILETRY and FRAGRANCE ASSN. (CTFA). (Oct. 8, 1981). Submission of unpublished data by CTFA. Cosmetic ingredient chemical description, Benzethonium Chloride (2-15-12).*
8. CTFA. (Oct. 8, 1981). Submission of unpublished data by CTFA. Cosmetic ingredient chemical description, Methylbenzethonium Chloride (2-15-13).*
9. CTFA. (March 23, 1983). Submission of unpublished data by CTFA. UV absorbance data on Benzethonium Chloride and Methylbenzethonium Chloride.*
10. CTFA. (March 28, 1983). Submission of unpublished data by CTFA. UV Absorbance data on Benzethonium Chloride.*
11. GRANT, J. (ed.). (1972). *Hackh's Chemical Dictionary,* 4th ed. New York: McGraw-Hill.
12. JAPAN COSMETIC INDUSTRY ASSOCIATION (JCIA). (1979). *Japanese Standards of Cosmetic Ingredients,* 1st ed. Tokyo, Japan: Yakuji Nippo, Ltd.
13. AMERICAN PHARMACEUTICAL ASSOCIATION (APA). (1974). National Formulary Board. *National Formulary XIV.* Washington, DC: American Pharmaceutical Association.
14. JUNGERMANN, E. (1979). Fat-based surface-active agents, in: D. Swern (ed.). *Bailey's Industrial Oil and Fat Products,* 4th ed. New York: Wiley, Vol. 1, pp. 642–3.
15. GERSTEIN, T. (Nov. 1979). An introduction to quaternary ammonium compounds. Cosmet. Toilet. **94,** 33–41.
16. GOSSELIN, R.E., HODGE, H.C., SMITH, R.P., and GLEASON, M.N. (1976). *Clinical Toxicology of Commercial Products,* 4th ed. Baltimore, MD: Williams & Wilkins.
17. TANAKA, K., HIOKI, M., and SHINDO, H. (1974). Determination of pancuronium bromide and its metabolites in human urine by dye-extraction method–relation between the extractability and structure of quaternary ammonium ions. Chem. Pharm. Bull. (Jpn.) **22**(11), 2599–606.
18. ROSEN, M.J. (1978). *Surfactants and Interfacial Phenomena.* New York: Wiley.
19. CAMERON, J. (1974). Preservative systems compatible with DPT-Polio (Salk) and TABTD-Polio (Salle) vaccines. Dev. Biol. Stand. **24,** 155–65.
20. ROSSOFF, I.S. (1974). *Handbook of Veterinary Drugs.* New York: Springer.
21. FOOD AND DRUG ADMINISTRATION. (FDA). (Dec. 12, 1980). Establishment of a monograph and proposed rulemaking on vaginal contraceptive drug products for over-the-counter human use. Fed. Reg. **45**(241), 82017, 82042.
22. SCHIMMEL, J., and SLOTSKY, M.N. (1974). Preservation of Cosmetics, in: M.S. Balsam and E. Sagarin (eds.). *Cosmetics Science and Technology* **3,** 391–470. New York: Wiley.
23. AMERICAN MEDICAL ASSOCIATION (AMA). (1977). *AMA Drug Evaluations,* 3rd ed. Chicago, IL, p. 890.
24. MOORE, K.E., and STRETTON, R.J. (1978). Detection of preservatives in pharmaceutical and cosmetic products using agar gel electrophoresis. J. Chromatogr. **156**(1), 211–7.
25. SENZEL, A.J. (ed.). (1977). *Newburger's Manual of Cosmetic Analysis,* 2nd ed. Washington, DC: Assoc. of Official Analytical Chemists.
26. BALEUX, B., and CAUMETTE, P. (1977). Biodegradation of some cationic surfactants. Water Res. **11**(9), 833–42.
27. OLSON, B.H., ELDERING, G., and GRAHAM, B. (1964). Stabilization of pertussis vaccine in the presence of Benzethonium Chloride. J. Bacteriol. **87,** 543–6.


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

28. BERSET, C., JASKIEWICZ, H., TRAN MANH SUNG, G., and SANDRET, F. (1979). Electrolytic purification methods for waste water. Elimination of synthetic surfactants. Trib. Cebedean (French) **32**(424), 79.
29. TSURUBOU, S., OHNO, N., and SAKAI, T. (1980). Extraction-spectrophotometric determination of benzethonium in the presence of quaternary ammonium salts with 2,6-dibromophenolindophenol. Nippon Kagaku Kaishi (Jpn.) **6,** 828–32.
30. SAKAI, T., TSUBOUCHI, M., and ZAECHI, Y. (1976). Spectrophotometric determination of cationic surfactants with 2,6-di-chloroindophenol by solvent extraction. Bunseki Kagaku (Jpn.) **25**(10), 675–9.
31. BIANCHI, W., BONIFORTI, L., and DI DOMENICO, A. (1974). Analysis of some quaternary ammonium compounds by gas chromatography-mass spectrometry. Mass. Spectrom. Biochem. Med. Symp., pp. 183–96.
32. ALARY, J., ROCHAT, J., VILLET, A., and COEUR, A. (1976). Microdetermination of organic bases by atomic absorption. I. Assay of quaternary ammonium [compounds]. Ann. Pharm. Fr. (French) **34**(9–10), 345–53.
33. HIROSE, S., YOSHIDA, S., and ATOMORI, M. (1977). Determination of a disinfectant by complementary tristimulus colorimetry and diphasic titration. Yakuzaigaku (Jpn.) **37**(1), 38–45.
34. PINZAUTI, S., LA PORTA, E., PAPESCHI, G., and BIFFOLI, R. (1980). A mercury-coated platinum electrode in the direct potentiometric determination of quaternary ammonium salts. J. Pharm. Belg. **35**(4), 281–4.
35. PINZAUTI, S., and LA PORTA, E. (1977). Use of the silver electrode in the potentiometric determination of quaternary ammonium compounds. Analyst (London) **102**(1221), 938–42.
36. PINZAUTI, S., and LA PORTA, E. (1979). Potentiometric analysis with a silver electrode in stability control of quaternary ammonium salt disinfectant solutions during storage in plastic and glass containers. J. Pharm. Pharmacol. **31**(2), 123–4.
37. PINZAUTI, S., and LA PORTA, E. (1979). The potassium ion selective electrode as a tetraphenylborate sensor for quaternary ammonium salts analysis. J. Pharm. Pharmacol. **31**(8), 573–4.
38. JOHNSON, E. (1978). Reverse phase-ion pair chromatography of high molecular weight quaternary amines. Ind. Res. Dev. **20**(6), $C_2$, $C_6$.
39. LOWRY, J.B. (1979). Direct spectrophotometric assay of quaternary ammonium compounds using Bromthymol Blue. J. Pharm. Sci. **68**(1), 110–1.
40. AHREND, K.F., and TIESS, D. (1973). Thin-layer chromatographic parameters of 180 toxicologically significant compounds with low volatility in four simple systems with instructions for practical application. Wiss. Z. Univ. Rostock, Math.-Naturwiss. Reiche (German) **22**(9), 951–63.
41. TACHIZAWA, M. (1976). Use of mixed fluorescent materials for thin-layer chromatographic drug analysis. Iyakuhin Kenkyu (Jpn.) **7**(4), 570–7.
42. HOSOBUCHI, K., and SATO, K. (1978). Effect of gamma-irradiation on disinfectants. 4. Benzalkonium Chloride, Benzethonium Chloride, and Cetylpyridium Chloride cationic surfactants. Bokin Bobai **6**(4), 154–60.
43. KAO SOAP CO. (Jan. 1980). Stable Quaternary Ammonium Chloride Compositions. Jpn. Kokai. Tokkyo Koho Patent No. 80-07219.
44. HART, J.R., and LEVY, E.F. (1977). Sarcosinate-cationic creme rinse shampoo. Soap Cosmet. Chem. Spec. **53**(8), 31–4, 60B-60C.
45. BOUCHAL, A.W. (Jan. 19, 1960). US Patent 2,921,855. Colgate-Palmolive Co.
46. REYBROUCK, G. (1979). Efficacy of inactivators against 14 disinfectant substances. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1: Orig. Reihe B. **168**(5–6), 480–92.
47. MYBURGH, J.A., and McCARTHY, T.J. (1980). Inactivation of preservatives in the presence of particulate solids. Pharm. Weekbl. Sci. Ed. **2**(5), 137–42.
48. FDA. (Dec. 1981). Product formulation data. Computer printout.
49. CODE OF FEDERAL REGULATIONS (CFR). (1979). Title 21, Part 720.4. Washington, DC.
50. EUROPEAN ECONOMIC COMMUNITY (EEC). (June 15, 1982). List of preservatives which cosmetic products may contain. Annex 4, Annex VI, of a Council Directive of May 17, 1982, amending Directive 76/768/EEC. Off. J. Eur. Communities: No. L167/1–31.
51. DREWE, N.W., PARKER, R.J., and TADROS, T.F. (Aug. 2, 1973). Herbicidal quaternary bipyridylium salts. Ger. Offen. Patent No. 2304204 (Imperial Chemical Industries).
52. FORD, R.R., and TADROS, T.F. (Aug. 21, 1975). Herbicidal compositions containing bipyridylium salts. Ger. Offen. Patent No. 2506834 (Imperial Chemical Industries).
53. YAMASHITA, S., TSUYUKI, H., FURULAWA, T., KUWATA, T., and HISAYAMA, H. (Jan. 12, 1979). Germicide composition. Jpn. Kokai Tokkyo Koho Patent No. 79 04287 (Tokyo Organic Chemical Industries).

54. UENO, S., and UENO, Y. (Feb. 3, 1978). Luminous deodorant, disinfectant, and insecticidal compositions for toilets. Jpn. Kokai Patent No. 7812432.
55. KOSE, Y., KAWAMURA, H., OZAWA, M., and OHTSUKA, K. (Jan. 24, 1973). Slow-release pesticide. Jpn. Patent No. 73 02331 (Sankyo Co.).
56. KOSE, Y., KAWAMURA, H., OZAWA, M., and OHTSUKA, K. (Jan. 26, 1973). Sustained release type pesticide. Jpn. Patent No. 73 02775 (Sankyo Co.).
57. GOLDHAFT, T.M., KAIRZ, C., and MAIER, G.D. (May 10, 1977). Disinfectant composition comprising a quaternary ammonium compound, a phenol, and formaldehyde. U.S. Patent No. 4022911 (Damon Corp.).
58. EGUCHI, Y., and IIJIMA, E. (Jan. 30, 1980). Benzethonium disinfectants. Jpn. Kokai Tokkyo Koho Patent No. 80 13228 (Kao Soap Co.).
59. SHIRAHATA, A., NOZAKI, Y., HORIUCHI, T., ISHIKAWA, K., MUTO, M., MIZUTANI, K., OIKAWA, T., MIZUHARA, H., and MAEDA, N. (1975). Investigations of bacterial infection rate and bacterial flora of premature infants during no gowning period. Acta Paediat. Jpn. **17**(1), 31.
60. FINNEGAN, J.K., and DIENNA, J.B. (1954). Toxicity of quaternaries. Soap Sanit. Chem. **30,** 147, 149, 152–3, 157, 173, 175.
61. PIVNICK, H., TRACY, J.M., and GLASS, D.G. (1963). Studies of preservatives of poliomyelitis (Salk) vaccine. I. Benzethonium Chloride. J. Pharm. Sci. **52,** 883–8.
62. TSUKADA, K., and KATO, W. (Aug. 18, 1979). Stable injections of high concentrations of DOPA and related compounds. Jpn. Kokai Tokkyo Koho Patent No. 79105221 (Sankyo Co.).
63. TOMIBE, K., OHTSU, A., KONDO, N., and SUENAGA, E. (Dec. 2, 1976). Production of heparin derivatives. Jpn. Kokai Patent No. 76139884 (Teijin).
64. HASTINGS, S.G., POSCHEL, B.P.H., and BUTLER, D.E. (Dec. 6, 1977). Treating cardiovascular disorders. US Patent No. 4061756 (Parke, Davis and Co.).
65. CHRISTIE, G.J., and BUCKWALTER, F.H. (July 22, 1975). Ketamine hydrochloride-containing anesthetic. US Patent No. 3896221 (Bristol-Myers Co.).
66. BURNAP, R.W. (April 12, 1977). Anesthetic and sedative composition. US Patent No. 4017619.
67. BACHMAYER, H., and REEVE, P. (Feb. 1, 1979). Subunit Vaccines. Ger. Offen. Patent No. 2829089 (Sandoz-Patent-G.m.b.h.).
68. SHEWMAKE, S.W., and ANDERSON, B.G. (1979). Hydrofluoric acid burns. A report of a case and review of the literature. Arch. Dermatol. **115**(5), 593–6.
69. REINHARDT, C.F., HUME, W.G., LINCH, A.L., and WEATHERHOLD, J.M. (1966). Hydrofluric acid burn treatment. Am. Ind. Hyg. Assoc. J. **27,** 166–71.
70. CHECCHI, A.A. (1982). *OTC Drug Ingredient Index and Manual*. Washington, DC, pp. 92.0-.7; 92A-1.0, 2.0; 404.0-.3; 570.0-.3.
71. FDA. (May 6, 1980). Establishment of a monograph and proposed rulemaking on ophthalmic drug products for over-the-counter human use. Fed. Reg. **45**(89), 30005–6, 30017.
72. FDA. (Sept. 13, 1974). Establishment of monograph and use of certain halogenated salicylanilides as active or inactive ingredients. OTC Topical Antimicrobial Products and Drug and Cosmetic Products. Fed. Reg. **39**(179), 33116, 33131–2.
73. FDA. (Jan. 6, 1978). Tentative final monograph on over-the-counter drugs generally recognized as safe, effective, and not misbranded. OTC Topical Antimicrobial Products. Fed. Reg. **43**(4), 1218, 1227, 1229–30, 1236–37.
74. FDA. (March 28, 1980). Establishment of a monograph on anticaries drug products for over-the-counter human use; proposed rulemaking. Fed. Reg. **45**(62), 20670.
75. FDA. (Nov. 7, 1980). Proposed rulemaking on hair grower and hair loss prevention. Drug products for over-the-counter human use. Fed. Reg. **45**(218), 73957.
76. CFR. (1979). Title 21, Part 175.105(G)(5). Washington, DC.
77. KUMAR, A., and NARASIMHAN, R. (1977). Ripening in sugar cane with Polaris, Cetrimide, and Hyamine 1622. Indian Sugar **26**(12), 817–20.
78. KUMAR, A., and NARASIMHAN, R. (1977). Ripening in sugar cane with Polaris, Cetrimide, and Hyamine 1622. Indian Sugar **27**(8), 437–41.
79. FDA. (Nov. 13, 1980). Status of ingredients in the OTC drug review. Division of OTC Drug Evaluation (HFD-510), Bureau of Drugs.
80. FDA. (Dec. 4, 1979). Establishment of a monograph and notice of proposed rulemaking on external analgesic drug products for over-the-counter human use. Fed. Reg. **44**(234), 69772.
81. CFR. (1979). Title 21, Part 210.3(b)(7). Washington, DC.
82. CFR. (1979). Title 21, Part 210.3(b)(8). Washington, DC.

83. FELDKAMP, C.S., EPSTEIN, E., THIBERT, R.J., and ZAK, B. (1975). Spectrophotometric study of drug interferences in an aqueous 17 ketosteroid reaction. Microchem. J. **20**(4), 523–33.
84. IWATA, J., and NISHIKAZE, O. (July 1979). New microturbidimetric method for determination of protein in cerebrospinal fluid and urine. Clin. Chem. **25**(7), 1317–9.
85. WHYMAN, A.E. (Feb. 1970). Measurement of tritium in neat plasma and urine with a Toluene/Triton X-100/Hyamine 10-X Scintillant. Int. J. Appl. Radiat. Isot. **21**(2), 81–6.
86. STEDMAN, R.L., KRAVITZ, E., and KING, J.D. (1957). Studies on cell surface-germicide and enzyme-germicide reactions and their contribution to the lethal effect. J. Bact. **73,** 655.
87. HARVEY, C., and STUCKEY, R.E. (1962). Spermicidal activity of surface active agents. Reprod. Fert. **3,** 124–31.
88. LAWRENCE, A. (1950). *Surface-Active Quaternary Ammonium Germicides*. New York: Academic Press, pp. 113–26.
89. ROSENTHAL, M.W. (1972). Mouthwashes, in: M.S. Balsam and E. Sagarin (eds.). *Cosmetics: Science and Technology*, 2nd ed. New York: Wiley, Vol. I, Chap. 15, pp. 533–63.
90. NADA, T., ITO, H., YAMAMOTO, H., IMAI, J., and TAMURA, T. (1980). Bacteriostatic and bactericidal effects of ordinary disinfectants against glucose-nonfermenting gram-negative rods. Eisei Kensa. **29**(7), 929–37.
91. GOTO, S., KANEKO, Y., KAWASAKI, K., ANJO, S., KAGAMI, M., SATO, T., OKURA, M., MIYOSHI, Y., and MIYASHITA, S. (1977). Bactericidal activity of conventional disinfectants against clinically isolated strains of gram-negative rods. Rinsho Byori. **25**(8), 684–90.
92. NAITO, R., ITOH, T., HASEGAWA, E., ARIMURA, H., FUJITA, Y., HASEGAWA, K., INABA, T., KAGITANI, Y., KOMEDA, S., MATSUMOTO, T., OKAMOTO, H., OKANO, K., AGUKO, Y., and OGUSHI, T. (1974). Bronopol as a substitute for thimerosal. Dev. Biol. Stand. **24,** 39–48.
93. CHRISTENSEN, P.E. (1963). Evaluation of the antibacterial effect of preservatives, with special preference to phemerol and thiomersal. Acta. Pathol. Microbiol. Scand. **57,** 104–10.
94. RAWLINS, A.L., SWEET, L.A., and JOSLYN, D.A. (1943). Relationship of chemical structure to germicidal activity of a series of quaternary ammonium salts. J. Am. Pharm. Assoc. Sci. Ed. **32,** 11–6.
95. NIKKO CHEMICALS K.K. (April 1, 1981). Antimicrobial formulations with acylated peptides. Jpn. Kokai Tokkyo Koho Patent No. 81 32420.
96. KAWAMURA, M., TAKEDA, K., SHIMADA, T., and YAMAMOTO, Y. (1980). Synergistic effect of Hyamine-T solution and Hibitane Gluconate in controlling *Pseudomonas*, *E. coli* and *S. aureus*. Byoin Yakugaku. **6**(2), 127–33.
97. ITO, K., KITAMURA, J., and NOMURA, T. (1975). The effect of polyene antibiotics on the permeability of the cell membrane of *Candida albicans* and its polyene-resistant mutant euphoria cacophoria. **2,** 20–31.
98. VOLPE, A.R., KUPEZAK, L.J., BRANT, J.H., KING, W.J., KESTENBAUM, R.C., and SCHLISSEL, H.J. (1969). Antimicrobial control of bacterial plaque and calculus and the effects of these agents on oral flora. J. Dent. Res. **48,** 832–41.
99. COMPTON, F.H., and BEAGRIE, G.S. (1975). Inhibitory effect of Benzethonium and Zinc Chloride mouthrinses on human dental plaque and gingivitis. J. Clin. Periodontol. **2,** 33–43.
100. TANZER, J.M., SLEE, A.M., KAMAY, B., and SCHEER, E.R. (1979). In vitro evaluation of seven cationic detergents as antiplaque agents. Antimicrob. Agents Chemother. **15**(3), 408–14.
101. GOMER, R.M., HOLROYD, S.V., FEDI, P.F., and FERRIGNO, P.D. (March–April 1972). The effect of oral rinses. J. Am. Soc. Prevent. Dent. **2,** 7, 12, 55, 58.
102. GAFFAR, A., MARCUSSEN, H.W., SOLIS-GAFFAR, M.C., and RUSTOGI, K.N. (1980). Long term antiplaque gingivitis and calculus effects of Benzethonium Chloride in beagle dogs. J. Periodont. Res. **15**(1), 107–10.
103. CARTER, H.G., and BARNES, G.P. (May–June, 1975). Effects of three mouth-washes on existing dental plaque accumulations. J. Prev. Dent. **2**(3), 6–8, 10–11.
104. BAKER, P.J., COBURN, R.A., GENCO, R.J., and EVANS, R.T. (1978). The in vitro inhibition of microbial growth and plaque formation by surfactant drugs. J. Periodont. Res. **13**(5), 474–85.
105. COMPTON, F.H., BEAGRIE, G.S., CHERNECKY, R., and GLASSER, K. (1979). Effect of two antibacterial mouth sprays and dentifrices on dental plaque and gingivitis in beagle dogs. J. Dent. Res. **58**(5), 1471–7.
106. ERIKSEN, H.M., JEMTLAND, B., FINCKENHAGEN, H.J., and GJERMO, P. (1979). Evaluation of extrinsic tooth discoloration. Acta Odontol. Scand. **37,** 371–5.
107. GAFFAR, A., and GRECSEK, J.J. (Sept. 23, 1980). Antibacterial oral composition. US Patent No. 4224309 (Colgate-Palmolive Co.).
108. GAFFAR, A., and VOLPE, A.R. (Nov. 23, 1978). Antibacterial composition for mouth care. Ger. Offen. Patent No. 2722187 (Colgate-Palmolive Co.).

109. GAFFAR, A., and VOLPE, A.R. (Jan. 30, 1979). Antibacterial oral composition. US Patent No. 4137303 (Colgate-Palmolive Co.).
110. MOTE, E.M., SCHOESSLER, O.D., and HILL, R.M. (March 1969). Lens incorporated germicides. J. Am. Optom. Assoc. **40,** 291–3.
111. GARDNER, R.A., and PITTMAN, M. (1965). Relative stability of pertussis vaccine preserved with merthiolate, Benzethonium Chloride, or the parabens. Appl. Microbiol. **13**(4), 564–9.
112. WETTERLOW, L.H., and EDSALL, G. (1970). The effect of Benzethonium Chloride on the antigens of pertussis vaccine. Int. Arch. Allergy Appl. Immunol. **37**(2), 143–53.
113. RIEGER, F., TSUJI, S., and MASSONLIS, J. (1972). Formes Natives et Globulaires de l'acetylcholinesterase Dans la Moelle epiniere et le Cerveau de Gymnote, Electrophorus electricus. Eur. J. Biochim. **30,** 73.
114. MARKS, N., D'MONTE, B., and LAJTHA, A. (1973). Inhibition of cerebral proteolytic enzymes. Texas Rep. Biol. Med. **31,** 345–65.
115. CALANDRA, J.C., HARDT, L.L., and STANISH, E.S. (1951). The effect of various compounds on trypsin activity. Gastroenterology **19,** 5645.
116. BOEHME, D.H., UMEZAWA, H.L., HASHIM, G., and MARKS, H. (1978). Treatment of experimental allergic encephalomyelitis with an inhibitor of cathepsin (peptatin). Neurochem. Res. **3**(2), 185–94.
117. AKOPYAN, T.N., ARUTUNYAN, A.A., LAJTHA, A., and GALOYAN, A.A. (1978). Acid proteinase of hypothalamus purification. Some properties, and action on somatostatin and substance P. Neurochem. Res. **3**(1), 89–99.
118. MAKINEN, K.K. (1968). Inhibition of arylaminopeptidases and proline aminopeptidases of human whole saliva by Benzethonium Chloride Benzyldimethyl (2-(2(P-1,1,3,3-Tetramethylbutyl Phenoxy)Ethoxy)-Ethyl)Ammonium Chloride. Febs Lett. **2**(2), 101–4.
119. SUGIURA, M., and OGISO, T. (1969). Enzymes. XLVIII. Bile-sensitive lipase. Effect of surfactants on *Mucor* Lipase. Yakugaku Zasshi **89**(9), 1289–96.
120. CHEN, Y.T., ROSENBERRY, T.L., and CHANG, H.W. (1974). Subunit heterogeneity of acetylcholinesterase. Arch. Biochem. **161**(2), 479–87.
121. JACKSON, R.L., and APRISON, M.H. (1966). Mammalian brain acetylcholinesterase. Effects of surface-active agents. J. Neurochem. **13**(12), 1367–71.
122. NARA, T., TSUJI, K., and KOMATSU, T. (1974). Flavine. 9. Effect of preservatives and hydroxybenzoic acid derivaties on the photodecomposition of flavine adenine dinucleotide. Yakuzaigaku **34**(2), 65–71.
123. SAKURAI, T., MATSUMARU, H., and TSUCHIYA, S. (1977). Microenvironmental changes of thiobarbiturate molecules in protein binding. Yakuzaigaku **37**(4), 169–75.
124. HURWITZ, A.R., and CARNEY, C.F. (1978). Enhancement of ampicillin partition behavior. J. Pharm. Sci. **67**(1), 138–40.
125. HOLT, W.V., and DOTT, H.M. (1980). Chemically induced fusion between ram spermatozoa and avian erythrocytes: An ultrastructural study. J. Ultrastruct. Res. **71**(3), 311–20.
126. GALL, D. (1966). The adjuvant activity of aliphatic nitrogenous bases. Immunology **11**(4), 369–86.
127. KUWAHARA, K., HITOSHI, T., HAYASHI, H., and SATAKE, K. (1976). Effects of detergents on cultured human cells. Kyushu Yakugakkai Kaiho. **30,** 31–7.
128. PANIAQUA, M.E., RIO PIEDRIAS, P.R., VALLIANT, A.B., and GAMBLE, C.J. (1961). Field trial of a contraceptive foam in Puerto Rico. JAMA **177,** 125.
129. BROTHERTON, J. (1977). Assessment of spermicides by a stripping technique against human spermatozoa. J. Reprod. Fert. **51,** 383–97.
130. HOMBURGER, F. (1968). Carcinogenicity of several compounds. National Technical Information Service, PB. No. 183 027, 26 pp.
131. SCHLEPPY, C.A., and ETTER, J.C. (1976). Applications of the ciliary activity test of the mouse trachea to evaluate components used to formulate nasal preparations. Pharm. Acta Helv. **51**(10), 297–303.
132. ARRO, L., and SALENSTEDT, C.R. (1973). Evaluation of toxicity of some quaternary ammonium compounds. J. Biol. Stand. **1**(1), 87–99.
133. NADAI, T., KUME, M., TATEMATSU, A., and SEZAKI, H. (1975). Drug-induced histological changes and its consequences on the permeability of small intestinal mucosa. II. Chem. Pharm. Bull. **23**(3), 543–51.
134. LA ROSA, R.T., FINE, N., and DE SALVA, S.S. (1978). Rat maternal and fetal absorption of $^{14}$C-Benzethonium Chloride ($^{14}$C-BTC). Pharmacologist **20,** 254.
135. SAX, N.I. (1979). *Dangerous Properties of Industrial Materials*, 5th ed. New York: Van Nostrand Reinhold.
136. FDA. (Nov. 1979). Information copy of OTC topical antifungal report. Division of OTC Drug Evaluation (HFD-510), Bureau of Drugs.
137. FINNEGAN, J.K., and DIENNA, J.B. (1953). Toxicological observations on certain surface-active agents. Toilet Goods Assoc. Proc. Sci. Sec. **20,** 16–9.

138. ROSEN, H., BLUMENTHAL, A., PANASEVICH, R., and McCALLUM, J. (1965). Dimethylsulfoxide (DMSO) as a solvent in acute toxicity determinations. Proc. Soc. Exp. Biol. Med. **120,** 511–4.
139. HODGE, H.C., and STERNER, H. (1949). Tabulation of toxicity classes. Am. Indus. Hyg. Assoc. Quart. **10,** 93–6.
140. MASON, M.M., CATE, C.C., and BAKER, J. (1971). Toxicology and carcinogenesis of various chemicals used in the preparation of vaccines. Clin. Toxicol. **4**(2), 185–204.
141. CHEMICAL BIOLOGICAL COORDINATION CENTER. (Feb. 1950). Summary biological tests. Washington, DC: National Research Council.
142. KLEIN, M., and STEVENS, D.A. (1945). The in vitro and in vivo activity of synthetic detergents against influenza A virus. J. Immunol. **50,** 265–73.
143. DRAIZE, J.H., and KELLEY, E.A. (May 1952). Toxicity to eye mucosa of certain cosmetic preparations containing surface-active agents. Toilet Goods Assoc. Proc. Sci. Sect. **17,** 1–4.
144. SWAN, K.C. (1944). Reactivity of the ocular tissues to wetting agents. Am. J. Ophthalmol. **27,** 1118–22.
145. CTFA. (Aug. 16, 1983). Submission of unpublished data by CTFA. Rabbit eye irritation test.*
146. DERSE, P.H. (1970). Injection of Newborn Mice with Seven Chemical Adjuvants to Help Determine their Safety in Use in Biologicals. U.S. Clearinghouse Fed. Sci. Tech. Inform., PB Rep 195153:135 p., 1969. From U.S. Govt. Res. Develop. Rep. **70,** 56.
147. GILMAN, M.R., and DeSALVA, S.J. (1979). Teratology studies of Benzethonium Chloride, Cetyl Pyridinium Chloride and Chlorhexidine in rats. Toxicol. Appl. Pharmacol. **48,** A35.
148. NATIONAL TOXICOLOGY PROGRAM (NTP). (Aug. 1981). Technical Bulletin. Dept. of Health and Human Services, Public Health Service, 5.
149. DeFLORA, S. (1981). Study of 106 organic and inorganic compounds in the *Salmonella*/microsome test. Carcinogenesis (London) **2**(4), 283–98.
150. NTP. (April 1983). Technical Bulletin. Dept. of Health and Human Services, Public Health Service, 9.
151. KIRSCHSTEIN, R.L. (1974). Toxicology and carcinogenicity of preservatives used in the preparation of biological products. Dev. Biol. Stand. **24,** 203–12.
152. GRASSO, P., and GOLBERG. (1966). Subcutaneous sarcoma as an index of carcinogenic potency. Food Cosmet. Toxicol. **4,** 297–320.
153. CLAYSON, D.B. (1962). *Chemical Carcinogenesis*. Boston, MA: Little, Brown, p. 341.
154. KLIGMAN, A.M., and LEYDEN, J.J. (1979). Reactions to standard patch test materials. Acta Derm. Venereol. (Sweden) **59**[Suppl. 85], 101–3.
155. BROWN, W.E., GUNDERSON, M.F., SCHWARTZ, P., and WILDER, V.M. (1944). A clinical and bacteriological study of phemerol as a skin antiseptic. Surg. Gynecol. Obstet. **78,** 173–80.
156. FOOD AND DRUG RESEARCH LABORATORIES (FDRL). (Sept. 10, 1973). Evaluation of potential hazards by dermal contact with an aerosol antiperspirant, sample RG-385-1 (2-15-11).*
157. FDRL. (April 19, 1974). Evaluation of potential hazards by dermal contact with a deodorant concentrate, sample HRG-385-1 (2-15-10).*
158. WISE, R. (May 1968). Clinical trial of a unique hydrocortisone-containing topical aerosol in a variety of dermatologic disorders. Ill. Med. J. **133,** 611–3.
159. CTFA. (Dec. 5, 1980). Submission of unpublished data by CTFA. Clinical skin irritation test (2-15-4).*
160. CTFA. (April 3, 1981). Submission of unpublished data by CTFA. Allergic contact sensitization test (2-15-5).*
161. FISHER, A.A. (1973). Allergic reaction to feminine hygiene sprays. Arch. Dermatol. **108**(6), 801–2.
162. SHMUNES, E., and LEVY, E.J. (Jan. 1972). Quaternary ammonium compound contact dermatitis from a deodorant. Arch. Dermatol. **105,** 91–3.
163. TANAKA, N., NISHINAKAMURA, Y., and NAKAGAWA, K. (Aug. 28, 1980). Agent for treating glaucoma. Ger. Offen. Patent No. 3001011 (Otsuka Pharmaceutical Co.).
164. TAKASE, M., KOMURO, S., NANBA, H., and ARAIE, M. (1978). Effects of topical bupranolol hydrochloride on the intraocular pressure. Jpn. J. Ophthalmol. **22**(1), 142–54.
165. BUSHNELL, L.F. (1965). A practical and effective method of contraception. Pacif. Med. Surg. **73,** 353.
166. SOBRERO, A.J. (1960). Evaluation of a new contraceptive. Fertil. Steril. **11,** 518–24.
167. MEARS, E. (1962). Chemical contraceptive trial. II. J. Reprod. Fertil. **4,** 337.
168. BERNSTEIN, G.S. (Jan. 1971). Clinical effectiveness of an aerosol contraceptive foam. Contraception **3,** 37–43.
169. GIAROLA, A., PERNIOLA, L., GAZZANI, G., and MAGNI, E. (Nov. 1979). Long-term multicentre trial with TA-RO CAP, a new spermicidal product. Contraception **20**(5), 489–95.
170. DRAVNIEKS, A., KEITH, L., KROTOSZYNSKI, B.K., and SHAH, J. (Jan. 1974). Vaginal odors: GLC assay method for evaluating odor changes. J. Pharm. Sci. **63,** 36–40.

171. HEILIG, D., HEILIG, M.C., and GLASSMAN, J.M. (Dec. 1979). Prophylactic use of a topical nonaqueous acetic acid medication for the prevention of otitis externa (swimmer's ear): Two year study with follow-up. Curr. Ther. Res. Clin. Exp. **26,** 862–73.
172. ORDONEZ, G.E., KIME, C.E., UPDEGRAFF, W.R., GLASSMAN, J.M., and SOUKA, J.P. (May 1978). Effective treatment of acute, diffuse otitis externa. 1. Controlled comparison of hydrocortisone-acetic acid, nonaqueous and hydrocortisone-neomycin-polymyxin B otic solutions. Curr. Ther. Res. Clin. Exp. **23**(5) [Suppl.], 3–14.
173. KIME, C.E., ORDONEZ, G.E., UPDEGRAFF, W.R., GLASSMAN, J.M., and SOYKA, J.P. (May 1978). Effective treatment of acute, diffuse otitis externa. 2. Controlled comparison of hydrocortisone-acetic acid, nonaqueous and hydrocortisone-neomycin-colistin otic solutions. Curr. Ther. Res. Clin. Exp. **23**(5) [Suppl.], 15–28.
174. GARRITY, J.D., HALLIDAY, T.C., and GLASSMAN, J.M. (1974). Prevention of swimmer's ear by simple prophylactic regimen. Curr. Ther. Res. **16**(5), 437–41.
175. DADAGIAN, A.J., HICKS, J.J., ORDONES, G.E., and GLASSMAN, J.M. (1974). Treatment of otitis externa: A controlled bacteriological clinical evaluation. Curr. Ther. Res. **16**(5), 431–6.