proper test method, the compounds may appear as good disinfectants (Mallman et al., 1946), or that the phenol coefficient test cannot be used to test quaternary ammonium compounds (Sykes, 1965), or that disinfection end points obtained via inoculated carrier tests require much larger amounts of compound over those observed from phenol coefficient testing (Mallman and Hanes, 1945; Stuart et al., 1953), or that tests with quaternary ammonium compounds measure bacteriostatic activity instead of bactericidal activity (Klarmann and Wright, 1946) are grossly misleading unless some additional interpretive and qualifying information is provided. Unfortunately, a large amount of the experimental work of the middle 1930s, immediately following Domagk's publication (1935), did indeed indicate a widespread spectrum of antimicrobial activity, including sporicidal and tuberculocidal activity at extremely low concentrations of quaternary ammonium compounds. There is little doubt that this was a result of inadequate neutralization of the test material carried over to the subculture medium, so that a static condition produced in the subculture was mistaken for destructive activity.

However, it must be remembered that the entire area of neutralization to prevent carryover stasis was inadequately dealt with at the time. A firm distinction between the biostatic and biocidal activities of an antimicrobial was not always determined. The necessity to obviate stasis from the subculture medium was not always demanded as a requisite of antimicrobial testing, and the neutralization with specific chemical moieties used for individual classes of antimicrobials was not always insisted upon. Any neutralization that occurred utilized dilution of the carryover antimicrobial from the aliquot sample to the volume of subculture medium to obtain a less than static concentration of the antimicrobial in the subculture medium.

More recently, the successful identification of discrete chemicals as specific quaternary ammonium compound neutralizers, which may be incorporated into subculture media or into diluents, has made it mandatory that antimicrobial testing be performed only in the presence of such neutralizers (Quisno et al., 1946). Consequently, the more recent experimental results with quaternary ammonium compounds cannot be discredited as static instead of destructive. The statement that the quaternary ammonium compounds may be made to look good by the proper test selection (Mallman et al., 1946) may be applied to any antimicrobial chemical.

Certainly, a manipulation of the various test parameters such as type and amount of soil, selection of test organisms, and many others will bias the results, so that any chemical product will appear as a good or poor antimicrobial agent. Of the many inadequacies attributed to the phenol coefficient test method (Klarmann and Wright, 1946; McCulloch, 1947), the most pertinent objection was its use as an index of actual disinfection levels for quaternary ammonium compounds (Sykes, 1965). However, this same objection may be raised for the entire spectrum of modern disinfectant products, because extrapolation from the phenol coefficient to recommended use-dilution for a product by way of some arbitrary factor is invalid and untenable in any and every instance (Klarmann, 1959; Ortenzio et al., 1961). If the in-use characterization of a disinfectant is divorced from the phenol coefficient test, the method becomes entirely useful to describe the antimicrobial activity of a substance as the concentration that will destroy a bacterial suspension in 5-, 10-, or 15-minute contact periods. The method, which fixes many independent variables such as operation equipment, media, temperature, and volumes of test solution and inoculum, may be applied with some minor reservations to any species of microorganism. That carrier type test methods provide lower dilution end points than the phenol coefficient method has already been stated for quaternary ammonium products (Mallman and Hanes, 1945; Stuart et al., 1953) and, more importantly, for other kinds of chemical products (Klarmann, 1959; Ortenzio et al., 1961). The dynamics of testing carriers prepared with inocula as dried films of microorganisms provide a more severe test than the liquid inoculum system of the phenol coefficient method. This feature of carrier-type tests has led to the development and use of these tests by the United States regulatory agencies to measure bactericidal, sporicidal, tuberculocidal, and sanitizing activity (Official Methods, AOAC, 1984).

Of special note are the efforts to determine the validity and accuracy of product-label statements and claims made by manufacturers of disinfectant products for sale in the United States. This regulation of antimicrobials intended for treatment of inanimate surfaces was formerly exercised by the United States Department of Agriculture and currently by the United States Environmental Protection Agency. It includes deliberate scrutiny of a product for antimicrobial efficacy and toxicologic and chemical properties in compliance with the product-label claims and other attendant information—all prior to the initial sale of the product. Further, the agency is required to monitor future production obtained on a periodic inspection schedule. To facilitate the antimicrobial examination, a number of testing protocols are prescribed to examine specific antimicrobial activity (Greene and Petrocci, 1980).

For those catalogued as AOAC methods, the reader is referred to the AOAC publications (Official Methods of Analysis of the AOAC, 1984) for the purpose, scope, precise methods, and application areas served by these tests. In addition to the AOAC methods, the Environmental Protection Agency recommends methods to determine the sanitizing activity on inanimate, nonfood contact surfaces, the initial and residual bactericidal and bacteriostatic activity of treated laundry fabric, the sanitizing activity on carpeting, the virucidal activity on environmental surfaces, and the initial and residual fungicidal and fungistatic activity on inanimate surfaces. This requirement to test products by specified protocols pro-

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vides a useful means to collect data that may be compared, and so to more accurately characterize the antimicrobial activity of a substance. The recent installations of prescribed test methods by other agencies and in other countries is a most salutary development to eliminate the confusing conclusions developed heretofore from many different kinds of experimental studies.

Quaternary ammonium compounds have been tested by a multitude of different procedures, which may be generally classified as (1) In vitro static or minimum inhibitory level tests, usually as broth dilution tests; (2) In vitro or killing dilution tests, with phenol coefficient as an example; (3) Simulated use-dilution tests, including inoculated carriers of representative surfaces such as stainless steel, unglazed porcelain, and fabric; and (4) In-use tests, which use the product in the actual environment (Greene and Petrocci, 1980). The results of such testing with quaternary ammonium compounds may be summarized as follows: the active quaternary ammonium compounds sold as items of commerce are algistatic, bacteriostatic, tuberculostatic, sporostatic, and fungistatic at low concentration levels of 0.5 to 5 ppm (Hueck et al., 1966; Freelander, 1940; Schneider, 1935); they are algicidal, bactericidal, fungicidal, and virucidal against lipophilic viruses at medium concentration levels of 10 to 50 ppm (Lawrence, 1950; Petrocci et al., 1974; Klein and Deforest, 1963); they are not tuberculocidal or sporicidal or virucidal against hydrophilic viruses at high concentration levels (Klein and Deforest, 1963; Smith et al., 1950; Davies, 1949). They are bactericidal to both gram-positive and gram-negative bacteria, with some evidence for greater activity against the gram-positive bacteria (Quisno and Foter, 1946). In this regard, it should be remembered that the gram-negative bacteria as a group, and especially the pseudomonas species, are more resistant to all the antimicrobial compounds currently available.

The quaternary ammonium compounds are bactericidal agents in acid and alkaline environments, with some evidence for greater activity in the alkaline range (Gershenfeld and Perlstein, 1941). The following descriptions of antimicrobial results are presented as representative data for quaternary ammonium compounds. They are compiled and prepared from the published scientific literature, from Onyx (Stepan) unpublished work, from brochures and data sheets supplied by the quaternary ammonium compound manufacturers, and from patents issued for quaternary ammonium compounds. It is important to recognize the absence of bias in information supplied by the manufacturers' literature that is carefully scrutinized by the regulatory agencies in advance of the sale of the chemical. The bacteriostatic, fungistatic, and algistatic activities of fatty nitrogen compounds, including quaternary ammonium compounds, were described

Table 13–14. *Inhibiting Concentrations (in PPM) of Fatty Nitrogen Compounds For Some Bacteria, Fungi, and Algae*

	Bacteria			
	G–N		*G–P*	
Compound	*Escherichia coli*	*Pseudomonas fluorescens*	*Bacillus subtilis*	*Staphylococcus aureus*
Benzethonium chloride	1,000	300	3	3
Benzalkonium chloride	200	300	3	4
Dodecyltrimethyl ammonium chloride	500	500	5	5
Dodocylbenzyldimethyl ammonium chloride	750	750	2	2
Cocobenzyldimethyl ammonium chloride	225	225	2	2
Didecyldimethyl ammonium chloride	225	750	0.7	7

	Fungi			
Compound	*Aspergillus niger*	*C. globosum*	*M. verrucaria*	*T. viridae*
Benzethonium chloride	300	30	300	200
Benzalkonium chloride	60	10	40	80
Dodecyltrimethyl ammonium chloride	500	50	500	500
Dodocylbenzyldimethyl ammonium chloride	75	7	150	75
Cocobenzyldimethyl ammonium chloride	20	7	20	20
Didecyldimethyl ammonium chloride	75	7	20	20

	Algae			
Compound	*Ch. vulgaris*	*Stigeoclonium species*	*A. cylindrica*	*Os. tenuis*
Benzethonium chloride	3	1	1	1
Benzalkonium chloride	1	0.7	1	0.6
Dodecyltrimethyl ammonium chloride	50	5	5	0.5
Dodocylbenzyldimethyl ammonium chloride	0.2	0.2	0.2	0.2
Cocobenzyldimethyl ammonium chloride	2	0.5	2	0.7
Didecyldimethyl ammonium chloride	2	0.7	0.2	0.7

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by Hueck et al. (1966); data selected therefrom for the quaternary ammonium compounds are presented in Table 13–14. The killing dilutions for some chemically different, commercially available quaternary ammonium compounds after a 10-minute contact period at 20°C by the phenol coefficient method against the recommended test organisms *Staphylococcus aureus* and *Salmonella typhosa* are compiled from the manufacturers' brochures (e.g., Brochure: Hyamine, Brochure: Roccal, BTC 2125M BTC, Brochure: Bardac) as Table 13–15.

These are at high dilutions against both organisms, and they are at significantly different dilutions depending on the specific chemical structure of the quaternary compound. The effect of the size of the quaternary molecule on the antimicrobial activity of a homologous series of alkyldimethylbenzyl ammonium chlorides is well documented by Cutler et al. (1966). They demonstrated a maximum activity as the highest 10-minute killing dilution against *Staphylococcus aureus* and *Salmonella typhosa* and *Pseudomonas aeruginosa* for the C_{14} member compound tested. Their results are shown in Table 13–3. Additional evidence for the structure and antimicrobial activity relationship is offered by the work of Petrocci et al. (1974), who evaluated the interference of hard water with the sanitizing activity against *Escherichia coli* of a group of dialkyldimethyl ammonium chloride compounds of varying long-chain lengths. This comparison of the hard-water tolerances, which are defined as the maximum concentrations of synthetic hard water (as $CaCO_3$), in which 200 ppm of active quaternary compound will continue to effectively sanitize (at 99.999% reduction) a suspension of *Escherichia coli* after a 30-second contact period, are displayed in Table 13–16.

Quisno's work with cetyl pyridinium chloride (Quisno and Foter, 1946) illustrates several characteristics of quaternary ammonium compounds, such as the greater activity for gram-positive bacteria over gram-negative bacteria (Table 13–17), and the positive effect of temperature and the negative effect of organic matter on antimicrobial activity (Table 13–18).

Lawrence (1950) described the effect of pH on the antibacterial activity of an alkyldimethylbenzyl ammonium chloride at 5-, 10-, and 15-minute contact periods against *Staphylococcus aureus*. A portion of these data is available as Table 13–19. Over many years, Petrocci has examined the fungicidal activity of many quaternary structures against *T. interdigitale* (Petrocci, unpublished). Some of these data are available as Table 13–20. This small section of antimicrobial activity is only a minute portion of the total information that has been published and is available for examination. The space limitations of this chapter do not permit a complete listing of this information; instead, I have attempted to select and present representative data that are compatible with the bulk of current opinions in the field.

TOXICOLOGY

On March 4, 1987, the EPA issued a Data Call-In Notice requiring all registrants of antimicrobial active ingredients to submit subchronic and chronic toxicologic data to support the continued registration of their products. The EPA will permit registrants to select among several options for ways to comply with the data requirements of this notice. The EPA has concluded that it should be possible to evaluate risk by acquiring exposure data and by acquiring toxicity data under a tiered approach. The three-tier studies may be summarized as follows:

A. *The first-tier studies:*
   1. 90-days dermal
   2. 90-days inhalation
   3. Teratogenicity 1st
   4. Mutagenicity

B. *The second-tier studies:*
   1. Subchronic feeding

Table 13–15. *Killing Dilutions of Commercially Available Quaternary Ammonium Germicides for 10-Minute Contact Periods at 20°C from Phenol Coefficients in Manufacturers' Brochures*

Chemical	*Killing Dilutions vs.*	
	Staphylococcus aureus	*Salmonella typhosa*
Di-isobutylphenoxyethoxyethyl dimethyl benzyl ammonium chloride	1/25,000	1/25,000
40% Methyldodecylbenzyl trimethyl ammonium chloride, 10% methyl-dodecylxylylene bis (trimethyl ammonium chloride)	1/24,000	1/22,500
N-alkyl (50%C_{14}, 40%C_{12}, 10%C_{16}) dimethyl benzyl ammonium chlorides	1/45,000	1/45,000
Benzyldimethyltetradecyl ammonium chloride	1/42,800	1/67,500
N-alkyl (60%C_{13}, 30%C_{16}, 5%C_{12}, 5%C_{18}) dimethylbenzyl ammonium chlorides	1/44,000	1/39,600
25% *N*-alkyl (68%C_{12}, 32%C_{14}) dimethylethylbenzyl ammonium chlorides	1/55,500	1/54,300
N-alkyl (98%C_{12}, 20%C_{14}) dimethyl 1-naphthylmethyl ammonium chlorides	1/60,000	1/58,500
N-alkyl (50%C_{12}, 30%C_{14}, 17%C_{16}, 3%C_{18}) isoquinolinium bromides	1/38,000	1/40,000
Octyldodecyldimethyl ammonium chlorides	1/61,800	1/57,500
Alkyl (50%C_{14}, 40%C_{12}, 10%C_{16}) dimethylbenzyl ammonium saccharinates	1/43,500	1/40,500
Didecyldimethyl ammonium chlorides	1/63,000	1/84,000

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Table 13–16. *Hard Water Tolerances (PPM $CaCO_3$) of Dialkyldimethyl Quaternary Ammonium Compounds with Long-Carbon Chains (R_1 + R_2) that Total 19 to 22 Carbons*

Totals Carbons (R_1 + R_2)*	Hard Water Tolerance (ppm $CaCO_3$)						
19	700	800	500	550			
20	500	900	1200	1400	1100		
21	300	700	900	1300	1300		
22	400	550	750	900	800	650	R_1 carbon
	6	7	8	9	10	11	length

*R_2 carbon chain length obtained by subtracting R_1 carbon chain length from the R_1 + R_2 total carbon chain length.

Table 13–17. *Germicidal Activity of Cetyl Pyridinium Chloride Aqueous Solution*

Organism	No. Strains Tested	Average Critical Killing Dilution In Terms of Active Ingredients at 37°C, No serum
Staphylococcus aureus	5	1:83,000
Staphylococcus albus	1	1:73,000
Streptococcus viridans	1	1:42,500
Streptococcus hemolyticus	2	1:127,500
Neisseria catarrhalis	2	1:84,000
Diplococcus pneumoniae I	1	1:95,000
Pseudomonas aeruginosa	2	1:5,800
Klebsiella pneumoniae	2	1:49,000
Corynebacterium diphtheriae	1	1:64,000
Mycobacterium phlei	1	1:1,500
Eberthella typhosa	5	1:48,000
Escherichia coli	2	1:66,000
Proteus vulgaris	2	1:34,000
Shigella dysenteriae	1	1:60,000
Shigella paradysenteriae (Flexner)	2	1:52,000
Shigella paradysenteriae (Hiss)	1	1:49,000
Shigella sonne	2	1:68,000

2. Teratogenicity 2nd
3. Dermal absorption
4. 90-days neurotoxicity

C. *The third-tier studies:*
1. Chronic feeding, two species
2. Oncogenicity, two species
3. Reproduction
4. Metabolism to clarify issues concerning structure activity relationships

On February 26, 1988, the EPA clustered the quaternary ammonium compounds into four groups, so that the toxicity study would be facilitated by selecting one member from each group for testing.

Group I: The straight-chain alkyl or hydroxyalkyl quaternaries; the most common product in this group is the twin chain quats. The EPA will allow a full set of subchronic and chronic toxicity studies on didecyldimethyl ammonium chloride and teratology studies on alkyltrimethyl ammonium chloride.

Group II: The nonhalogenated substituted benzyl and alkyldimethylbenzyl ammonium compounds (ADBAC); namely benzalkonium chlorides. The EPA will allow a full set of subchronic and chronic studies on alkyl[C_{12}—40%, C_{14}—50%, C_{16}—10%] dimethylbenzyl ammonium chloride. The EPA notes that after evaluating the data it expects to receive on the Group I and II compounds, it may also require one or more studies on alkyl[C_{12}—68%, C_{14}—32%]dimethylethylbenzyl ammonium chloride, to determine whether the Group I and ADBAC data is representative of the toxicity of the ethylbenzyl quats.

Group III: The alkyl[di- and tri- chlorobenzyl]dimethyl ammonium compounds. The EPA will allow a full set of subchronic and chronic studies on alkyl[C_{12}—5%, C_{14}—90%, C_{16}—5%] dimethyl 3,4-dichlorobenzyl ammonium chloride.

Group IV: The heterocyclic ammonium compounds with unusual substituents. In this group the EPA was unable to reach a conclusion in testing a small subset of quats. Each chemical in this category must be tested separately, unless registrants can develop a testing scheme using representative compounds that is acceptable to the EPA. Representative compounds in *Group IV* are (a) alkyl[C_{12}/C_{16}] substituted Picolinium compounds; (b) alkyl[C_8/C_{18}] imidazolinium compounds; (c) alkyl[C_{14}/C_{18}] N-ethyl-morpholinium compounds; and (d)

Table 13–18. *Effect of Temperature and Serum Upon Germicidal Activity of Cetyl Pyridinium Chloride*

Organisms	Critical Killing Dilutions Expressed in Terms of Active Ingredients			
	37°C		20°C	
	No Serum	10% Bovine Serum	No Serum	10% Bovine Serum
Staphylococcus aureus	1:110,000	1:11,500	1:67,500	1:6,750
Eberthella typhosa	1:83,500	1:4,000	1:62,500	1:1,300

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Table 13–19. *Effects of pH on the Antiseptic Efficiency of a Quaternary Ammonium Germicide Against* Staphylococcus Aureus

Quaternary Ammonium Compound Conc.	Acid or Alkali per 100 ml	pH	Bacterial Growth after Minutes of Exposure		
			5	10	15
1:15,000	0.10 ml N/10 HCl	4.3	–	–	–
1:15,000	0.25 ml N/10 HCl	3.8	–	–	–
1:15,000	0.50 ml N/10 HCl	3.5	+	+	+
1:15,000	1.00 ml N/10 HCl	3.2	+	+	+
Control (no quaternary ammonium compound)	1.00 ml N/10 HCl	3.2	+	+	+
1:25,000	0	6.7	+	+	+
1:25,000	0.10 ml N/10 NaOH	9.4	+	+	+
1:25,000	0.25 ml N/10 NaOH	10.0	+	+	–
1:25,000	0.50 ml N/10 NaOH	10.2	+	+	–
1:25,000	1.00 ml N/10 NaOH	10.5	+	–	–
1:25,000	1.25 ml N/10 NaOH	10.6	+	–	–
Control (no quaternary ammonium compound)	1.25 ml N/10 NaOH	10.6	+	+	+

alkyl[C_{18}/C_{16}] isoquinolinium compounds and other heterogeneous structures.

A knowledge of the toxicologic properties of quaternary ammonium compounds is important so that the safety, health, and well-being of man and animals are not compromised by contact with these chemicals as they are manufactured, shipped, compounded, or used. Although these specific investigations are not germane, the studies to characterize the general toxicologic properties of antimicrobial quaternary ammonium compounds and the studies of the harmful consequences of these chemicals at concentrations simulating their use patterns are of primary importance. Toxicologic effect is measured at three levels: (1) at the amount necessary to bring about the response with 100% of the experimental animals, LD_{100}; (2) at the amount that will cause the response with 50% of the animals, LD_{50}; and (3) at the amount that does not produce the response with any of the animals, LD_0. The LD_{50} level is most frequently used to characterize toxicity. In addition, the methods of administering the chemical are also identified using descriptions such as oral, subcutaneous, intraperitoneal, intravenous, and dermal. Also, the toxicity is determined as an acute response following a single administration of the chemical, as a short-term or subacute response following a multiple, periodic administration of the chemical over a short period such as 30 or 90 days, and finally as a chronic response following a multiple, periodic administration over a long period, such as the animal's life span. The LD_{50} amounts of quaternary ammonium compound depend on the route of administration. A notable example of this is the work of Nelson and Lyster (1946), who examined myristal picolinium chloride and reported an LD_{50} of 250 mg/kg by oral administration, 200 mg/kg for subcutaneous injection, 7.5 mg/kg for intraperitoneal injection, and 30 mg/kg for intravenous injection. Using the acute oral LD_{50}, which is the toxicologic characteristic most frequently described, one may compare the toxicities of different quaternary compounds. When this is done with the work of Shelanski (1949) and Finnegan and Dienna (1954), we observe acute oral LD_{50} values in white rats of 445 mg/kg for alkyldimethylbenzyl ammonium chloride, 500 mg/kg for alkenyldimethylethyl ammonium bromide, 730 mg/kg for alkyldimethyl 3,4-dichlorobenzyl ammonium chloride, 420 mg/kg for diisobutylphenoxyethoxyethyldimethylbenzyl ammonium chloride, and 389 mg/kg for alkyltolymethyltrimethyl ammonium chloride. These and similar data obtained from studies with man and laboratory animals have led to the generalized conclusion that the antimicrobial quaternary ammonium compounds examined to date exhibit similar toxicologic and pharmacologic properties.

Table 13–20. *Critical Killing Dilution (10-Minute Contact Period) Required for Aqueous Dilutions of Quaternary Compounds to Kill Spores of* Trichophyton Interdigitalis; *Dilutions are Based on Anhydrous Germicide*

Chemical Structure	Avg. Killing Dil.
40% methyldodecylbenzyltrimethyl ammonium chloride, 10% methyldodecylxylylene bis (trimethyl ammonium chloride)	1/2000
25% *n*-alkyl (60%C_{14}, 30%C_{16}, 5%C_{12}, 5%C_{18}) dimethylbenzyl ammonium chloride, 25% *n*-alkyl (68%C_{12}, 32%C_{14}) dimethylethylbenzyl ammonium chlorides	1/800
Octyldodecyldimethyl ammonium bromide	1/5000
Didecyldimethyl ammonium chloride	1/4750

Dermal toxicity was also examined by Shelanski (1949) and Finnegan and Dienna (1954), who concluded from animal and human testing that 0.1% was the maximum concentration of the aforementioned compounds that would not produce primary irritation on intact skin or act as a sensitizer. The conjunctival mucous membrane obviously requires special consideration, which was undertaken by a number of investigators. Nelson and Lyster (1946) observed that a 1:3000 (333 ppm) solution of myr-

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istal picolinium chloride produced a slight irritation of the conjunctival mucosa of rabbits. Walter (1938) reported that a 1:1000 dilution (1000 ppm) of alkyldimethylbenzyl ammonium chloride instilled into the eyes of human subjects produced burning and stinging reactions, whereas a 1:5000 dilution (200 ppm) produced no unpleasant sensations. Whitehill (1945) determined that the maximum nonirritating concentrations of alkyldimethylbenzyl ammonium chloride and cetylpyridimium chloride in the eyes of rabbits were 1:3000 (333 ppm) and 1:2000 (500 ppm), respectively. Finnegan and Dienna (1954) observed comparable irritation levels for diisobutylphenoxyethoxyethyldimethyl benzyl ammonium chloride and for alkyltolymethyltrimethyl ammonium chloride (Shelanski, unpublished); in Shelanski's investigation of laurylisoquinolinium bromide, he observed that the compound at 0.5% produced moderate to severe corneal involvement of the rabbit eye, which cleared up in 5 days. However, rabbit eyes similarly exposed and washed at 2 or 4 seconds after instillation produced only a slight conjunctival involvement, which cleared up after 2 days. A most important toxicologic consideration arising from the widely encountered application area of sanitizing food contact surfaces is the cumulative effect in animals and man of the ingestion of food contacted by the small residues of the quaternary sanitizer. The laboratory study simulating this situation is the daily oral administration of quaternary ammonium compound in the food or drinking water of test animals over a considerable period of time, including the normal life span of the animal. Such studies were made early by Nelson and Lyster (1946), who determined that myristal picolinium chloride at 0.1% in the diet of rats did not interfere with their normal growth rate, but this concentration produced death when administered as a sole source of fluid and required reduction to 0.05% to permit a normal growth rate of the test animal.

Fitzhugh and Nelson (1948) summarized the results of a 2-year chronic feeding study of rats on a diet containing 0.5, 0.25, 0.125, or 0.063% alkyldimethylbenzyl chloride; they found that all the quaternary compound concentrations were toxic to rats, as evidenced by lower growth rates over an untreated control group and as determined by the histopathologic lesions present in the treated animals. Alfredson et al. (1951), however, using a significantly larger number of experimental animals and a more randomized statistical design, demonstrated that alkyldimethylbenzyl ammonium chloride at 0.25% in the diet of rats over a 2-year feeding period did not demonstrably affect the growth, food consumption, blood picture, or histopathology of the treated animals. Dogs that were fed over a 15-week period a diet containing 0.12% or less quaternary compound demonstrated similar nontoxic effects. Shelanski (1949) noted a minimal toxicologic effect, evidenced by weight loss, a depressed growth rate, and an abnormal blood or histopathology, for dogs that were allowed a 1:5000 aqueous dilution of either alkyldimethylbenzyl ammonium chloride, alkenyldimethylethyl ammonium bromide, laurylisoquinolinium bromide, or alkyldimethyl 3,4-dichlorobenzyl ammonium chloride as their sole source of drinking water over a 6-month period, for guinea pigs administered 25, 12.5, or 5 mg/kg of the aforementioned quaternary ammonium compounds daily for a 1-year period, and for rats similarly treated daily over a 2-year period. Finnegan and Dienna (1954) demonstrated that a concentration of 2500 ppm of alkyltolylmethyltrimethyl ammonium chloride or diisobutylphenoxyethoxyethyldimethyl benzyl ammonium chloride in the diet of rats produced minimal deleterious toxicologic effects over a 2-year period, whereas a concentration of 1000 ppm produced no undesirable toxicologic effects over the same period.

The concern of the Food and Drug Administration over the unsafe adulteration of food, especially of milk, with residues from quaternary sanitizers led to label statements for these products requiring a potable water rinse following the quaternary compound sanitization and reuse of the food equipment. Recently, a careful scrutiny by the FDA of additional chronic feeding studies supplied by manufacturers of quaternary ammonium compounds has concluded that the food additive regulations should be amended to provide for the safe use of a sanitizing solution on food processing equipment and utensils and on food contact surfaces in bars and restaurants if the solution does not exceed 200 ppm of quaternary compound and if the quaternary compound solution contains equal amounts of n-alkyl [C_{12}–C_{18}] benzyldimethyl ammonium chloride and n-alkyl [C_{12}–C_{14}]dimethylethylbenzyl ammonium chloride having an average molecular weight of 384. In this instance, safe use does not require a potable water rinse of treated food contact surfaces and equipment prior to reuse (Federal Register, 1969).

A short time later, the same statement was made for an aqueous solution containing n-alkyl [$C_{12}C_{18}$] benzyldimethyl ammonium chloride having an average molecular weight of between 351 and 380 and consisting principally of alkyl groups with 12 to 16 carbon atoms with or without, but not over, 1% each of groups with 8 and 10 carbon atoms (Federal Register, 1969).

Most recently, the same statement was made for a detergent-sanitizer solution containing the first sanitizing product described as a mixture of benzyl and ethylbenzyl quaternary compounds, with the optional adjuvant substances tetrasodium ethylenediaminetetraacetate or a(p-nonylphenyl)-w-hydroxypoly(oxyethylene) (or both) having an average poly(oxyethylene) content of 11 moles (Federal Register, 1974). Gleason (1969) points out that at least 10 human fatalities implicating quaternary ammonium compounds are medically recorded as resulting from alkyldimethylbenzyl ammonium chloride solutions of 10 or 15% that were introduced into the victims via oral ingestion, intramuscular or intravenous administration, or intrauterine instillation. The reader is referred to Gleason's work for a complete description of the medical and clinical symptoms of quaternary ammonium

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compound poisoning and also for the recommended treatment, including first aid, for the same. One symptom should be mentioned: namely, the ingestion of concentrated solution leads to immediate burning pain in the mouth, throat, and abdomen.

The toxicologic information presented is generalized as follows: quaternary ammonium compound germicides as concentrated solutions of 10% or more are toxic, causing death if taken internally and severe irritation to the skin and conjunctival mucosa when applied externally. With normal precautions and operating procedures, the probability of such contact is extremely remote, because the effective use-dilutions of these materials are well below the concentrated level causing death and severe injury. In addition, oral ingestion, the most likely route of accidental internal administration, is self-limiting for concentrated quaternary ammonium solutions, because the immediate burning in the mouth and throat acts as a signal. At the dilute solutions of the use levels of quaternary ammonium compound germicides, which range from several to about 1000 ppm, only the most deliberate distortions of normal operating procedures could offer acute toxicity problems. Finally, chronic toxicity, as from the cumulative effect of food adulterated with quaternary ammonium compound germicides via residues from dilute sanitizing solutions used on food processing equipment, is not a problem.

Aursnes (1982) studied the ototoxic effects of quaternary ammonium compounds (QAC), benzethonium chloride, and benzalkonium chloride, frequently used for skin disinfection. The disinfectant QACs, at concentrations of 0.1% in water solution or in 70% alcohol, were introduced into the tympanic cavity (middle ear) of guinea pigs for exposure times of 10, 30, or 60 minutes. The animals were sacrificed 2 or 9 weeks after the exposure, and the organ of Corti and vestibular neuroepithelia were studied as surface preparations with phase contrast microscopy. It was found that most of the ears exposed to the disinfectants had suffered damage, affecting both the vestibulum and the perilymphatic and endolymphatic spaces of the cochlea of the inner ear. The extent of the damage was related both to the duration of exposure and to the length of the animal's survival after the exposure. Furthermore it was found that the tympanic cavity and the perilymphatic spaces of vestibulum and cochlea were pathologically changed.

The final report on the safety assessment of benzethonium chloride and methylbenzethonium chloride has been issued by CTFA (1985) in the journal of the American College of Toxicology. It was concluded that both compounds are safe at concentrations of 0.5% in cosmetics applied to the skin. A maximum concentration of 0.02% is safe for cosmetics used in the eye area. Chronic and subchronic feeding studies indicated little or no toxic effects for both ingredients. In clinical studies, benzethonium chloride produced mild skin irritation at 5% but not at lower concentrations. Neither ingredient is considered to be a sensitizer.

APPLICATIONS

Following Domagk's publication in 1935, a large number of application areas were developed for the quaternary ammonium compounds. Initially, they were used as an adjunct to surgery, such as in preoperative patient skin treatment, degerming the hands of the surgical team preoperatively, and disinfection of surgical instruments. Wetzel, in 1935, described the superiority of 0.1% alkyldimethylbenzyl ammonium chloride over 60% alcohol for treatment of the surgeon's hands prior to surgery. Much additional evidence was provided for the treatment of the surgeon's hands with alkyldimethylbenzyl ammonium chloride by Caesar in 1935, Walter in 1938, Naegel in 1940, and Swan et al. in 1949. In 1938, both Walter and White et al. reported that the use of alkyldimethylbenzyl ammonium chloride as a skin preparation for a patient prior to surgery reduced the incidence of wound infection. Hagan et al., in 1946, compared the activity of cetylpyridinium chloride and alkydimethylbenzyl ammonium chloride to mercurials as skin preparation prior to surgery and found the quaternary compounds superior. However, Miller et al., in 1943, offered evidence that quaternary compounds applied to the skin formed a continuous residual sheet, beneath which bacteria survived.

Rahn (1946) proposed that the film is formed as an oriented adsorption of the quaternary compound on an organophilic surface, with the nontoxic ends directed toward the skin and the germicidally active ends directed toward the outside, which explained the favorable results obtained for quaternary compounds in many studies. Blank and Coolidge, in 1950, rejected the film concept and proposed instead that treatment with the quaternary compounds altered the charge of the skin to positive, which attracted and held the negatively charged bacteria, leading to the favorable, if inaccurate, results obtained in basin tests with quaternary compounds. In the same publication, they speculated that phospholipids present on the surface of the skin inhibited the antimicrobial activity of the quaternary compound.

These negative comments and the widespread popular use of hexachlorophene-based products should have seriously curtailed the use of quaternary compounds as skin degerming agents. Thus it is interesting to note that King and Zimmerman, in 1965, reporting on skin degerming practices in hospitals, did determine that 37% of hospitals were using quaternary compounds, compared to the 48% observed by Price in 1948. Walter, in 1965, described a hand degerming treatment for the surgeon as a mechanical soap hand scrubbing followed by application of a mixture of isopropyl alcohol, alkyldimethylbenzyl ammonium chloride, and cetyl alcohol. Verdon, in 1961, tested a number of skin degerming agents used in British hospitals and demonstrated the utility of quaternary compounds.

Caesar and Schmidt in 1935 and Walter in 1938 recommended the use of alkyldimethylbenzyl ammonium chloride for the disinfection of surgical instruments, Cae-

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sar specifying a 1:100 concentration of quaternary compound for a 10-minute contact period and Walter a 1:1000 concentration over a 30-minute contact period. Hornung (1935) recommended the addition of sodium carbonate to the quaternary solution to prevent rusting of the instruments; Post (1946) later proposed sodium nitrite for this purpose. The early investigators, with some few exceptions, did indeed subscribe to the idea of a total spectrum of germicidal activity for the quaternary compounds and thus endorsed them as surgical instrument sterilizers. Early exceptions were Zeissler and Gunther (1939), who pointed out that Zephirol would not kill spores from the soil.

Sommermeyer and Frobisher, in 1952, using glass rods inoculated with sputum containing tubercle bacilli to simulate contaminated thermometers, demonstrated that aqueous solutions of alkyldimethylbenzyl ammonium chloride and cetylpyridinium chloride at 1:1000 dilution did not kill the organism after a 10-minute contact period, whereas tinctures of the same compounds (50% alcoholic) did in fact kill the organism. The subsequent use of specific quaternary compound neutralizers as a requisite for testing has prevented additional instances of residual bacteriostasis that were interpreted as bactericidal activity, as in the case of tuberculocidal and sporicidal testing.

The inability of quaternary compounds to kill the tubercle bacillus or bacterial spores should seriously limit their use as surgical instrument sterilizers in the future. However, in some few special cases, they are still recommended for the disinfection of comparable devices (Kasper and Kirk, 1972).

The widespread use of quaternary ammonium compounds for environmental disinfection of floors, walls, and equipment surfaces in hospitals, nursing homes, public places, and gymnasia required the development of a detergent compatible with the quaternary compounds that would permit the combination of the two operations of cleaning and disinfection. Rahn and Eseltine (1947) pointed out the utility of such a system and indicated work in progress as described by Guiteras and Shapiro (1946), who combined cetyldimethylethyl ammonium bromide with the nonionic wetting agent alkylated aryl polyether alcohol and inorganic salts. The formulation prepared by Guiteras and Shapiro diluted as 1 oz/gal aqueous solution cleaned glass slides contaminated with heavy artificial soil inoculated with bacteria. When the contact period of the slide and diluted formulation was extended to 10 minutes, all the bacteria on the soiled slides were killed, as demonstrated by negative broth subcultures of the slides and by zero-count agar plates prepared from the broth subcultures.

The concept of a one-step operation to produce cleaning and antimicrobial activity, which made the treatment of large surface areas practical, met with success and furthered research to the present time toward the development of quaternary nonionic formulations with optimum cleaning and antimicrobial properties (Greene and Petrocci, 1980). Publications (Kundsin and Walter, 1961; Lenhart et al., 1961) of both in-vitro laboratory tests and of field tests under actual conditions attest to the utility of these products. Treatment of large areas of environmental surface is accomplished by a mop-and-bucket operation, by mechanical flooding and vacuum pick-up, by sponge or cloth hand-wipe operation, and by liquid spray. As an additional treatment procedure for disinfection of surfaces, there is available liquefied gas, quaternary ammonium compound germicidal formulation discharges from pressurized containers as an aerosol spray that, according to Fulton et al. (1948), killed both *Escherichia coli* and *Staphylococcus aureus* as air-borne suspensions and surface contaminations. The patent issued to Taylor and Prindle (1966) is an instance of popular and widely used surface disinfectant and space deodorant aerosol spray composition.

Recently, treatment of surfaces is reported via a mist of quaternary ammonium compound germicide generated from commercial fogging devices that produce droplets of approximately 50 μm in diameter by the shearing action of air pressure on the liquid germicide solution. This procedure is suggested as an adjunct for terminal disinfection of hospital areas. The germicidal results obtained with 0.2% active aqueous solution of a 50:50 mixture of alkyldimethylbenzyl ammonium chloride and alkyldimethylethylbenzyl ammonium chloride in field tests and in simulated in-use tests are described by Friedman et al. (1968) and Hauser et al. (1963).

Treatment of food contact surfaces, i.e., those found on equipment in the food processing industries and on food and beverage utensils in eating and drinking establishments, in order to obtain sanitary conditions that will prevent transmission of disease through the food, is defined as a sanitizing treatment or as sanitation of such surfaces. Practicable sanitizing requires that any treatment residual will not in itself be harmful; it does not require a destruction of all the bacteria present on the treated surface. The utility of quaternary ammonium compounds in sanitizing such surfaces was suggested by Krog and Marshall (1940), who experimentally contaminated the rims of glass drinking tumblers, allowed them to air dry, and treated them with a 1:5000 aqueous dilution of alkyldimethylbenzyl ammonium chloride for various contact periods before swabbing and plating procedures were employed to enumerate the viable bacteria present; this demonstrated a reduction to less than 100 bacteria per rim after only a 1 minute contact period. Two years later they reported that the use of alkyldimethylbenzyl ammonium chloride at a 1:6000 dilution in a dairy pasteurizing plant caused a 60 to 98% reduction of the bacteria count on the milk handling and processing equipment (Krog and Marshall, 1942). Frayer (1943), Mueller et al. (1946), DuBois and Dibblee (1946), Puhle (1950), and Speck and Lucas (1957) are several of the many investigators who determined the utility of quaternary ammonium compounds as germicides for the dairy farm and dairy plant. Similar reports to demon-

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strate the advantage of quaternary compounds as germicides in other food processing industries have appeared, such as those of Penniston and Hedrick (1945) in the egg processing industry, Tressler (1947) in the fishing industry, Lehn and Vignolo (1946) and Resuggan (1951) in the brewing industry, and Casey (1972) in the sugar refining industry.

The treatment of large amounts of water to destroy and prevent the proliferation of disease-producing microorganisms, of industrial process-interfering microorganisms, and finally of noxious, esthetically undesirable microorganisms is an important application area of the quaternary ammonium compounds. The destruction of cysts of *Entamoeba histolytica* was reported by Fair et al. (1945) and by Kessel and Moore (1946). Kessel and Moore offered their opinion that quaternary ammonium compounds should be considered for the emergency sterilization of drinking water; this opinion was based on the high germicidal levels observed. Sotier and Ward (1947) observed that a product containing 0.5% of Hyamine 1622 was an effective disinfectant of new water mains and of hemp used to prepare joints on the mains. Quaternary ammonium compounds have been recommended for treating process water in paper mills (Erskine et al., 1958; Shema, 1966) to prevent the proliferation of microorganisms on the surface of equipment contacting the pulp solution. These proliferations form microbial slimes that become detached and are carried along to become spots in the paper, often causing paper breaks during the manufacturing process that result in expensive, time-delaying machine shutdowns. Quaternary compounds are used in cooling water systems (Darragh and Stayner, 1954; Berneschot et al., 1964) to prevent proliferation of bacteria in the circulating cooling water system that would form microbial slimes that may reach the heat exchange units and impair their operational efficiency. Prusick and Gregory (1956) recommended the use of dicocodimethyl ammonium chloride to prevent proliferation of bacteria in injection water or brine used in secondary oil recovery operations. The injection water is pumped under pressure into injection wells drilled around the producing well, so that as it spreads through the subterranean sand structures containing residual oil, it will displace the residual oil in the direction of the producing well and increase the productivity of the well. If there is a proliferation of microorganisms in the injection waters, the subterranean sand structures will become plugged, requiring greater pressures to be applied to the injection waters until the operation cannot function. Steinberger (1963) described the ability of N-alkyl crude tar base quaternary ammonium compounds at 5 to 20 ppm to inhibit the proliferation of *Desulfovibrio desulfuricans*, which with pseudomonas species are primarily involved in plugging the sand formation.

Outdoor swimming pools are regularly treated with quaternary ammonium compounds to prohibit growth of algae, an esthetic rather than a public health problem. Palmer and Maloney (1955) determined that 2 ppm of several different quaternary ammonium compounds did effectively inhibit the growth of various algae species. Antonides and Tanner (1961) recommended a formulation containing trimethylalkyl ammonium compound at 4.5 ppm as a swimming-pool algicide and bactericide. Shay et al. (1964) demonstrated that alkyldimethylisopropylbenzyl ammonium chloride at 7 ppm killed both *Escherichia coli* and *Streptococcus faecalis* at a 30-second contact time by the AOAC test method prescribed for swimming pool bactericides.

The positively charged functional portion of the quaternary molecule is attracted to and substantive to negatively charged fabric; it may be applied to the fabric from a quaternary solution by rinsing, padding, or spraying. Taking advantage of this phenomenon, Benson et al. (1947, 1949) proposed that diapers be treated with [methylbenzethonium chloride]p-diisobutylcresoxyethoxyethyldimethylbenzyl ammonium chloride monohydrate in the final rinse of the laundry cycle. Treated diapers would prevent the formation of ammonia from urine by the action of ammonia-producing bacteria found in the infant's feces; thus, ammonia dermatitis leading to diaper rash could be prevented. Lawrence (1950) pointed out the need to apply chemical treatment between the laundry loads of different customers using public automatic clothes-washing machines.

Although large commercial laundries could provide sufficient volumes of hot water at disinfecting temperatures, the small automatic installations could not. Lawrence used alkyldimethylbenzyl ammonium chloride to treat laundry in the final rinse cycle of automatic machines in a coin-operated self-service laundry. He determined that there were high bacteria counts in the first soak and wash cycle water, which following treatment with the quaternary compound at 1:5000 (200 ppm) or 1:10,000 (100 ppm) dilution, were reduced to zero counts in the deep rinse cycle.

McNeil and Choper (1962) treated naturally soiled wash loads at the warm or hot water setting and in the wash or rinse cycle with alkyldimethylbenzyl ammonium chloride to provide 200 ppm of active quaternary compounds in the presence of anionic or nonionic laundry detergent. Compared to untreated wash loads, the quaternary compound used with the warm or hot water setting consistently reduced the numbers of bacteria in the wash and rinse waters and on fabric swatches attached to laundry items. This occurred with the quaternary compound added to the rinse cycle, and to the wash cycle when a nonionic detergent was used. Utilizing the AOAC antimicrobial laundry additive test procedure (Petrocci and Clarke, 1969), a simulated use test, Shay and Petrocci (1968) demonstrated sanitization of fabric during the laundry cycle following addition of a 50:50 mixture of alkyldimethylbenzyl ammonium chloride and alkyldimethylethylbenzyl ammonium chloride at a 200 ppm level based on the weight of dry laundry fabric. In addition, fabric similarly treated and allowed to air dry demonstrated residual bacteriostatic activity against

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