Chapter 2
Types of antimicrobial agents

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1 Introduction

Many different types of antimicrobial agents are now available and serve a variety of purposes in the medical, veterinary, dental and other fields (Russell *et al.*, 1984; Gorman & Scott, 1985; Gardner & Peel, 1986, 1991; Russell & Hugo, 1987; Russell, 1990a,b, 1991a,b; Russell & Gould, 1991a,b; Fleurette *et al.*, 1995; Merianos, 1995; Rossmore, 1995; Russell & Russell, 1995; Rutala, 1995a,b; Ascenzi, 1996a; Russell & Chopra, 1996). Subsequent chapters will discuss the factors influencing their activity and their role as disinfectants and antiseptics and as preservatives in a wide range of products or materials (Akers, 1984; Fels *et al.*, 1987; Eklund, 1989; Gould & Jones, 1989; Wilkins & Board, 1989; Russell & Gould, 1991a,b; Kabara & Eklund, 1991; Seiler & Russell, 1991). Lists of preservatives are provided by Denyer and Wallhäusser (1990) and by Hill (1995). Additional information is provided on their mechanism of action and on the ways in which microorganisms show resistance.

The present chapter will concentrate on the antimicrobial properties and uses of the various types of antimicrobial agents. Cross-references to other chapters are made where appropriate. A comprehensive summary of inhibitory concentrations, toxicity and uses is provided by Wallhäusser (1984).

2 Phenols

The historical introduction (Chapter 1) and the papers by Hugo (1979, 1991) and Marouchoc (1979) showed that phenol and natural-product distillates containing phenols shared, with chlorine and iodine, an early place in the armoury of antiseptics. Today they enjoy a wide use as general disinfectants and as preservatives for a variety of manufactured products (Freney, 1995). The main general restriction is that they should not be used where they can contaminate foods. As a result of their long history, a vast literature has accumulated dealing with phenol and its analogues and comprehensive review of these compounds can be found in Goddard and McCue (2001). Unfortunately, many different parameters have been used to express their biocidal and biostatic power but the phenol coefficient (Chapters 7.2 and 11) has probably been the most widely employed and serves as a reasonable cross-referencing cipher for the many hundreds of papers and reports written.

A reasonable assessment of the relationship between structure and activity in the phenol series was compiled by Suter (1941). The main conclusions from this survey were:

1 *para*-Substitutions of an alkyl chain up to six carbon atoms in length increases the antibacterial action of phenols, presumably by increasing the surface activity and ability to orientate at an interface. Activity falls off after this due to decreased water-solubility. Again, due to the conferment of polar properties, straight chain *para*-substituents confer greater activity than branched-chain substituents containing the same number of carbon atoms.

2 Halogenation increases the antibacterial activity of phenol. The combination of alkyl and halogen substitution which confers the greatest antibacterial activity is that where the alkyl group is *ortho* to the phenolic group and the halogen *para* to the phenolic group.

3 Nitration, while increasing the toxicity of phenol towards bacteria, also increases the systemic toxicity and confers specific biological properties on the molecule, enabling it to interfere with oxidative phosphorylation. This has now been shown to be due to the ability of nitrophenols to act as uncoupling agents. Studies (Hugo & Bowen, 1973) have shown that the nitro group is not a prerequisite for uncoupling, as ethylphenol is an uncoupler. Nitrophenols have now been largely superseded as plant protection chemicals, where at one time they enjoyed a large vogue, although 4-nitrophenol is still used as a preservative in the leather industry.

4 In the bisphenol series, activity is found with a direct bond between the two C_6H_5-groups or if they are separated by $–CH_2–$, –S– or –O–. If a –CO–, –SO– or –CH(OH)– group separates the phenyl groups, activity is low. In addition, maximum activity is found with the hydroxyl group at the 2,2′- position of the bisphenol. Halogenation

of the bisphenols confers additional biocidal activity.

2.1 Sources of phenols—the coal-tar industry

Most of the phenols that are used to manufacture disinfectants are obtained from the tar obtained as a by-product in the destructive distillation of coal. Coal is heated in the absence of air and the volatile products, one of which is tar, condensed. The tar is fractionated to yield a group of products, which include phenols (called tar acids), organic bases and neutral products, such as alkyl naphthalenes, which are known in the industry as neutral oils.

The cresols consist of a mixture of 2-, 3- and 4-cresol. The 'xylenols' consist of the six isomeric dimethylphenols plus ethylphenols. The combined fraction, cresols and xylenols, is also available as a commercial product, which is known as cresylic acid. High-boiling tar acids consist of higher alkyl homologues of phenol: e.g. the diethylphenols, tetramethylphenols, methylethylphenols, together with methylindanols, naphthols and methylresorcinols, the latter being known as dihydrics. There may be traces of 2-phenylphenol. The chemical constituents of some of the phenolic components are shown in Fig. 2.1.

Extended information on coal tars and their constituents is given in the *Coal Tar Data Book* (1965). As tar distillation is a commercial process, it should be realized that there will be some overlap between fractions. Phenol is obtained at 99% purity. Cresol of the *British Pharmacopoeia* (2002) (2-, 3- and 4-cresols) must contain less than 2% of phenol. A commercially mixed xylenol fraction contains no phenols or cresols but may contain 22 of the higher-boiling phenols. High-boiling tar acids may contain some of the higher-boiling xylenols, for example 3,4-xylenol (boiling-point (b.p.) 227 °C).

Mention must be made of the neutral oil fraction, which has an adjuvant action in some of the formulated disinfectants to be considered below. It is devoid of biocidal activity and consists mainly of hydrocarbons, such as methyl- and dimethylnaphthalenes, *n*-dodecane, naphthalene, tetramethylbenzene, dimethylindenes and tetrahydronaphthalene. Some tar distillers offer a neutral oil, boiling range 205–296 °C, for blending with phenolics destined for disinfectant manufacture (see also section 2.4.2).

2.2 Properties of phenolic fractions

The passage from phenol (b.p. 182 °C) to the higher-boiling phenols (b.p. up to 310 °C) is accompanied by a well-defined gradation in properties, as follows: water-solubility decreases, tissue trauma decreases, bactericidal activity increases, inactivation by organic matter increases. The ratio of activity against Gram-negative to activity against Gram-positive organisms, however, remains fairly constant, although in the case of pseudomonads, activity tends to decrease with decreasing water-solubility; see also Table 2.1.

2.3 Formulation of coal-tar disinfectants

It will be seen from the above data that the progressive increase in desirable biological properties of the coal-tar phenols with increasing boiling-point is accompanied by a decrease in water solubility. This presents formulation problems and part of the story of the evolution of the present-day products is found in the evolution of formulation devices.

The antiseptic and disinfectant properties of coal tar had been noted as early as 1815, and in 1844 a Frenchman called Bayard made an antiseptic powder of coal tar, plaster, ferrous sulphate and clay, an early carbolic powder. Other variations on this theme appeared during the first half of the nineteenth century. In 1850, a French pharmacist, Ferdinand Le Beuf, prepared an emulsion of coal tar using the bark of a South American tree, the quillaia. This bark contained a triterpenoid glycoside with soap-like properties belonging to the class of natural products called saponins. By emulsifying coal tar, Le Beuf made a usable liquid disinfectant, which proved a very valuable aid to surgery. A 'solution' of coal tar prepared with quillaia bark was described in the *Pharmaceutical Codex* (1979). Quillaia is replaced by polysorbate 80 in formulae for coal-tar 'solutions' in the *British Pharma-*

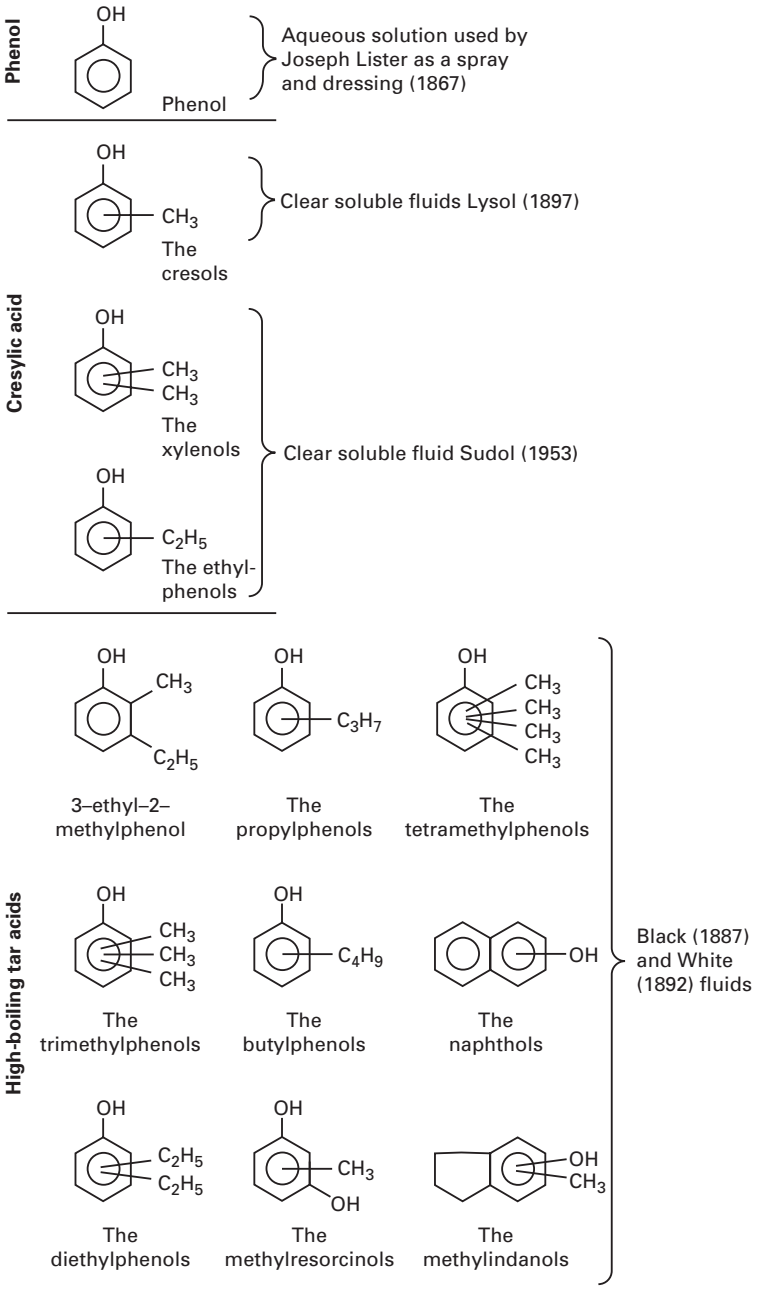


Figure 2.1 Phenol, cresols, xylenols, ethylphenols and high-boiling tar acids.

copoeia (2002). In 1887 the use of soap and coal tar was first promulgated, and in 1889 a German experimenter, T. Damman, patented a product which was prepared from coal tar, creosote and soap and which involved the principle of solubilization. Thus, between 1850 and 1887, the basis for the formulation of coal-tar disinfectants had been laid and subsequent discoveries were either rediscoveries or modifications of these two basic themes of emulsification and solubilization. Better-quality tar acid

Table 2.1 Phenol coefficients of coal-tar products against *Salmonella typhi* and *Staphylococcus aureus*.

	Phenol coefficient		
Product and m.p., m. range (°C)	S. typhi	Staph. aureus	*Water solubility (g/100 mL)*
Phenol			
182	1	1	6.6
Cresols			
190–203	2.5	2.0	2.0
4-Ethylphenol			
195	6	6	Slightly
Xylenols			
210–230	5	4.5	Slightly
High-boiling tar acids			
230–270	40	25	Insoluble
High-boiling tar acids			
250–275	60	40	Insoluble

fractions and products with clearer-cut properties aided the production of improved products. At the same time, John Jeyes of Northampton patented a coal-tar product, the well-known Jeyes fluid, by solubilizing coal-tar acids with a soap made from the resin of pine trees and alkali. In 1897, Engler and Pieckhoff in Germany prepared the first Lysol by solubilizing cresol with soap.

2.4 The modern range of solubilized and emulsified phenolic disinfectants

Black fluids are essential coal-tar fractions solubilized with soaps; white fluids are prepared by emulsifying tar fractions. Their composition as regards phenol content is shown in Fig. 2.1. The term 'clear soluble fluid' is also used to describe the solubilized products Lysol and Sudol.

2.4.1 Cresol and soap solution British Pharmacopoeia *(BP) 1963 (Lysol)*

This consists of cresol (a mixture of 2-, 3- and 4-cresols) solubilized with a soap prepared from linseed oil and potassium hydroxide. It forms a clear solution on dilution and is a broad spectrum disinfectant showing activity against vegetative bacteria, mycobacteria, fungi and viruses (British Association of Chemical Specialities, 1998). Most vegetative pathogens, including mycobacteria, are killed in 15 min by dilutions of Lysol ranging from 0.3 to 0.6%. Bacterial spores are much more resistant, and there are reports of the spores of *Bacillus subtilis* surviving in 2% Lysol for nearly 3 days. Even greater resistance has been encountered among clostridial spores. Lysol still retains the corrosive nature associated with the phenols and should be used with care. Both the method of manufacture and the nature of the soap used have been found to affect the biocidal properties of the product (Tilley & Schaffer, 1925; Berry & Stenlake, 1942). Rideal–Walker (RW) coefficients [British Standard (BS) 541: 1985] are of the order of 2.

2.4.2 Black fluids

These are defined in a British Standard (BS 2462: 1986) which has now been superceeded by specific European standard methods for products in medical, veterinary, industrial, domestic and institutional usage. They consist of a solubilized crude phenol fraction prepared from tar acids, of the boiling range 250–310 °C (Fig. 2.1).

The solubilizing agents used to prepare the black fluids of commerce include soaps prepared from the interaction of sodium hydroxide with resins (which contain resin acids) and with the sulphate and sulphonate mixture prepared by heating castor oil

with sulphuric acid (called sulphonated castor oil or Turkey red oil).

Additional stability is conferred by the presence of coal-tar hydrocarbon neutral oils. These have already been referred to in section 2.1 and comprise such products as the methyl naphthalenes, indenes and naphthalenes. The actual mechanism whereby they stabilize the black fluids has not been adequately explained; however, they do prevent crystallization of naphthalene present in the tar acid fraction. Klarmann and Shternov (1936) made a systematic study of the effect of the neutral oil fraction and also purified methyl- and dimethylnaphthalenes on the bactericidal efficiency of a coal-tar disinfectant. They prepared mixtures of cresol and soap solution (Lysol type) of the *United States Pharmacopeia* with varying concentrations of neutral oil. They found, using a phenol coefficient-type test and *Salmonella typhi* as test organism, that a product containing 30% cresols and 20% neutral oil was twice as active as a similar product containing 50% cresols alone. However, the replacement of cresol by neutral oil caused a progressive decrease in phenol coefficient when a haemolytic *Streptococcus* and *Mycobacterium tuberculosis* were used as test organisms. The results were further checked using a pure 2-methylnaphthalene in place of neutral oil and similar findings were obtained.

Depending on the phenol fraction used and its proportion of cresylic acids to high-boiling tar acid, black fluids of varying RW coefficients reaching as high as 30 can be produced; however, as shown in section 2.2, increasing biocidal activity is accompanied by an increasing sensitivity to inactivation by organic debris. To obtain satisfactory products, the method of manufacture is critical and a considerable expertise is required to produce active and reproducible batches.

Black fluids give either clear solutions or emulsions on dilution with water, those containing greater proportions of higher phenol homologues giving emulsions. They are partially inactivated by the presence of electrolytes.

2.4.3 White fluids

White fluids are also defined in BS 2462: 1986, which has since been superceeded by specific European standard methods. They differ from the foregoing formulations in being emulsified, as distinct from solubilized, phenolic compounds. The emulsifying agents used include animal glue, casein and the carbohydrate extractable from the seaweed called Irish moss. Products with a range of RW coefficients may be manufactured by the use of varying tar-acid constituents.

As they are already in the form of an oil-in-water emulsion, they are less liable to have their activity reduced on further dilution, as might happen with black fluids if dilution is carried out carelessly. They are much more stable in the presence of electrolytes. As might be expected from a metastable system—the emulsion—they are less stable on storage than the black fluids, which are solubilized systems. As with the black fluids, products of varying RW coefficients may be obtained by varying the composition of the phenol. Neutral oils from coal tar may be included in the formulation.

An interesting account of the methods and pitfalls of manufacture of black and white fluids is given by Finch (1958).

2.5 Non-coal-tar phenols

The coal-tar (and to a lesser extent the petrochemical) industry yields a large array of phenolic products; phenol itself, however, is now made in large quantities by a synthetic process, as are some of its derivatives. Three such phenols, which are used in a variety of roles, are 4-tertiary octylphenol, 2-phenylphenol and 4-hexylresorcinol (Fig. 2.2).

2.5.1 4-Tertiary octylphenol

This phenol (often referred to as octylphenol) is a white crystalline substance, melting-point (m.p.) 83 °C. The cardinal property in considering its application as a preservative is its insolubility in water, 1 in 60 000 (1.6×10^{-3}%). The sodium and potassium derivatives are more soluble. It is soluble in 1 in 1 of 95% ethanol and proportionally less soluble in ethanol containing varying proportions of water. It has been shown by animal-feeding experiments to be less toxic than phenol or cresol.

Alcoholic solutions of the phenol are 400–500 times as effective as phenol against Gram-positive

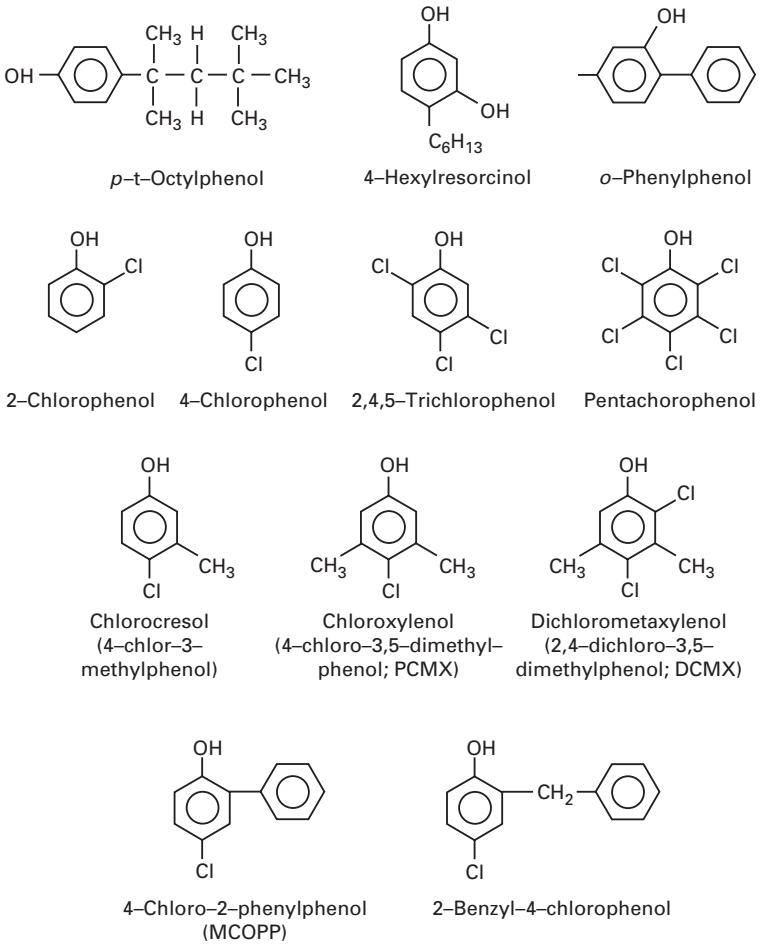


Figure 2.2 Examples of phenolic compounds.

organisms but against Gram-negative bacteria the factor is only one-fiftieth. Octylphenol is also fungistatic, and has been used as a preservative for proteinaceous products, such as glues and non-food gelatins. Its activity is reduced in the presence of some emulgents, a property that might render it unsuitable for the preservation of soaps and cutting oils.

2.5.2 2-Phenylphenol (2-phenylphenoxide)

This occurs as a white crystalline powder, melting at 57 °C. It is much more soluble than octylphenol, 1 part dissolving in 1000 parts of water, while the sodium salt is readily soluble in water. It is both antibacterial and antifungal and is used as a preservative, especially against fungi, in a wide variety of applications. Typical minimal inhibitory concentrations (MICs, μg/mL) for the sodium salt are: *Escherichia coli*, 32; *Staphylococcus aureus*, 32; *Bacillus subtilis*, 16; *Pseudomonas fluorescens*, 16; *Aspergillus niger*, 4; *Epidermophyton* spp., 4; *Myrothecium verrucaria*, 2; *Trichophyton interdigitale*, 8. Many strains of *P. aeruginosa* are more resistant requiring higher concentrations than those listed above for their inhibition.

Its main applications have been as ingredients in disinfectants of the pine type, as preservatives for cutting oils and as a general agricultural disinfectant. It has been particularly useful as a slimicide and fungicide in the paper and cardboard industry, and as an addition to paraffin wax in the prepara-

tion of waxed paper and liners for bottle and jar caps.

2.5.3 4-Hexylresorcinol

This occurs as white crystalline needles (m.p. 67 °C). It is soluble 0.5% in water but freely soluble in organic solvents, glycerol and glycerides (fixed oils). It is of low oral toxicity, having been used for the treatment of round- and whipworm infections in humans. It is used as a 0.1% solution in 30% glycerol as a skin disinfectant and in lozenges and medicated sweets for the treatment of throat infections.

2.6 Halo and nitrophenols

The general effect of halogenation (Fig. 2.2) upon the antimicrobial activity of phenols is to increase their activity, with the *para* position being more effective than the *ortho* position, but reduce their water solubility (section 2.1). There is also a tendency for them to be inactivated by organic matter. The work on substituted phenols dates from the early twentieth century and was pioneered by Ehrlich and studied extensively by Klarmann *et al.* (1929, 1932, 1933).

To illustrate the effect of chlorination on the biocidal activity of phenols, RW coefficients are as follows: 2-chlorophenol, 3.6; 4-chlorophenol, 4; 3-chlorophenol, 7.4; 2,4-dichlorophenol, 13; 2,4,6-trichlorophenol, 22; 4-chloro-3-methylphenol, 13; 4-chloro-3,5-dimethylphenol, 30.

Chlorophenols are made by the direct chlorination of the corresponding phenol or phenol mixture, using either chlorine or sulphuryl chloride.

2.6.1 2,4,6-Trichlorophenol

This is a white or off-white powder, which melts at 69.5 °C and boils at 246 °C. It is a stronger acid than phenol with a pK_a (negative logarithm of acidic ionization constant; see section 3.2) of 8.5 at 25 °C. It is almost insoluble in water but soluble in alkali and organic solvents. This phenol has been used as a bactericidal, fungicidal and insecticidal agent. It has found application in textile and wood preservation, as a preservative for cutting oils and as an ingredient in some antiseptic formulations. Its phenol coefficient against *S. typhi* is 22 and against *Staph. aureus* 25.

2.6.2 Pentachlorophenol (2-phenylphenoxide)

A white to cream-coloured powder, m.p. 174 °C, it can crystallize with a proportion of water, and is almost insoluble in water but soluble in organic solvents. Pentachlorophenol or its sodium derivative is used as a preservative for adhesives, textiles, wood, leather, paper and cardboard. It has been used for the in-can preservation of paints but it tends to discolour in sunlight. As with other phenols, the presence of iron in the products which it is meant to preserve can also cause discoloration.

2.6.3 4-Chloro-3-methylphenol (chlorocresol)

Chlorocresol is a colourless crystalline compound, which melts at 65 °C and is volatile in steam. It is soluble in water at 3.8 g/L and readily soluble in ethanol, ether and terpenes. It is also soluble in alkaline solutions. Its pK_a at 25 °C is 9.5. Chlorocresol is used as a preservative in pharmaceutical products and an adjunct in a former UK pharmacopoeial sterilization process called 'heating with a bactericide', in which a combination of heat (98–100 °C) and a chemical biocide enabled a sterilization process to be conducted at a lower temperature than the more usual 121 °C (see Chapter 3). Its RW coefficient in aqueous solution is 13 and nearly double this value when solubilized with castor oil soap. It has been used as a preservative for industrial products, such as glues, paints, sizes, cutting oils and drilling muds.

2.6.4 4-Chloro-3,5-dimethylphenol (chloroxylenol; **para-***chloro-***meta-***xylenol; PCMX)*

PCMX is a white crystalline substance, melting at 155 °C and has a pK_a of 9.7 at 25 °C. It is reasonably soluble in water (0.33 g/L at 20 °C) but is more soluble in alkaline solutions and organic solvents. To improve the solubility of PCMX and to achieve full antimicrobial potential, correct formulation is essential (Goddard & McCue, 2001). It is used chiefly

as a topical antiseptic and a disinfectant. To improve solubility PCMX is often solubilized in a suitable soap solution and often in conjunction with terpineol or pine oil. The *British Pharmacopoeia* (2002) contains a model antiseptic formulation for a chloroxylenol solution containing soap, terpineol and ethanol.

Phenol coefficients for the pure compound are: *S. typhi*, 30; *Staph. aureus*, 26; *Streptococcus pyogenes*, 28; *Trichophyton rosaceum*, 25; *P. aeruginosa*, 11. It is not sporicidal and has little activity against the tubercle bacillus. It is also inactivated in the presence of organic matter. Its properties have been re-evaluated (Bruch, 1996).

*2.6.5 2,4-Dichloro-3,5-dimethylphenol (dichloroxylenol; dichloro-*meta*-xylenol; DCMX)*

This is a white powder, melting at 94 °C. It is volatile in steam and soluble in water at 0.2 g/L at 20 °C. Although it is slightly less soluble than PCMX, it has similar properties and antimicrobial spectrum. It is used as an ingredient in pine-type disinfectants and in medicated soaps and hand scrubs.

*2.6.6 4-Chloro-3-methylphenol (*para*-chloro-*meta*-cresol; PCMC)*

PCMC is more water soluble than other phenols with a solubility of 4 g/L at 20 °C. It retains a reasonably broad spectrum of activity of antimicrobial activity over a wide pH range due to its solubility. This makes it suitable as an industrial preservative for products such as thickeners, adhesives and pigments (Goddard & McCue, 2001).

2.6.7 Monochloro-2-phenylphenol

This is obtained by the chlorination of 2-phenylphenol and the commercial product contains 80% of 4-chloro-2-phenylphenol and 20% of 6-chloro-2-phenylphenol. The mixture is a pale straw-coloured liquid, which boils over the range 250–300 °C. It is almost insoluble in water but may be used in the formulation of pine disinfectants, where solubilization is effected by means of a suitable soap.

2.6.8 2-Benzyl-4-chlorophenol (chlorphen; ortho*-benzyl-*para*-chlorophenol; OBPCP)*

This occurs as a white to pink powder, which melts at 49 °C. It has a slight phenolic odour and is almost insoluble in water (0.007 g/L at 20 °C) but like PCMX is more soluble in alkaline solution and organic solvents. Suitably formulated by solubilization with vegetable-oil soaps or selected anionic detergents, it has a wide biocidal spectrum, being active against Gram-positive and Gram-negative bacteria, viruses, protozoa and fungi. However, OBPCP is more commonly used in combination with other phenolics in disinfectant formulations (Goddard & McCue, 2001).

2.6.9 Mixed chlorinated xylenols

A mixed chlorinated xylenol preparation can be obtained for the manufacture of household disinfectants by chlorinating a mixed xylenol fraction from coal tar.

2.6.10 Other halophenols

Brominated and fluorinated monophenols have been made and tested but they have not found extensive application.

2.6.11 Nitrophenols

Nitrophenols in general are more toxic than the halophenols. 3,5-Dinitro-*o*-cresol was used as an ovicide in horticulture, but the nitrophenol most widely used today is 4-nitrophenol, which is amongst a group of preservatives used in the leather manufacturing industry at concentrations of 0.1–0.5%. For a general review on the use and mode of action of the nitrophenols, see Simon (1953).

2.6.12 Formulated disinfectants containing chlorophenols

Some formulation device, such as solubilization, might be used to prepare liquid antiseptics and disinfectants based on the good activity and the low level of systemic toxicity and of the likelihood of tissue damage shown by chlorinated cresols and

xylenols. Indeed, such a formula was patented in Germany in 1927, although the use of chlorinated phenols as adjuncts to the already existent coal-tar products had been mooted in England in the early 1920s.

In 1933, Rapps compared the RW coefficients of an aqueous solution and a castor-oil soap-solubilized system of chlorocresol and chloroxylenol and found the solubilized system to be superior by a factor of almost two. This particular disinfectant recipe received a major advance (also in 1933) when two gynaecologists, seeking a safe and effective product for midwifery and having felt that Lysol, one of the few disinfectants available to medicine at the time, was too caustic, made an extensive evaluation of the chloroxylenol–castor-oil product; their recipe also contained terpineol (Colebrook & Maxted, 1933). It was fortunate that this preparation was active against β-haemolytic streptococci, which are a hazard in childbirth, giving rise to puerperal fever. A chloroxylenol–terpineol–soap preparation is the subject of a monograph in the *British Pharmacopoeia* (2002).

The bacteriology of this formulation has turned out to be controversial; the original appraisal indicated good activity against β-haemolytic streptococci and *E. coli*, with retained activity in the presence of pus, but subsequent bacteriological examinations by experienced workers gave divergent results. Thus Colebrook in 1941 cast doubt upon the ability of solubilized chloroxylenolterpineol to destroy staphylococci on the skin, a finding which was refuted by Beath (1943). Ayliffe *et al.* (1966) indicated that the product was more active against *P. aeruginosa* than *Staph. aureus*. As so often happens, however, *P. aeruginosa* was subsequently shown to be resistant and Lowbury (1951) found that this organism would actually multiply in dilutions of chloroxylenol–soap.

Although still an opportunistic organism, *P. aeruginosa* was becoming a dangerous pathogen, especially as more and more patients received radiotherapy or radiomimetic drugs, and attempts were made to potentiate the disinfectant and to widen its spectrum so as to embrace the pseudomonads. It had been well known that ethylenediamine tetraacetic acid (EDTA) affected the permeability of pseudomonads and some enterobacteria to drugs to which they were normally resistant (Russell, 1971a; Russell & Chopra, 1996) and both Dankert & Schut (1976) and Russell & Furr (1977) were able to demonstrate that chloroxylenol solutions with EDTA were most active against pseudomonads. Hatch and Cooper (1948) had shown a similar potentiating effect with sodium hexametaphosphate. This phenomenon may be worth bearing in mind when formulating hospital disinfectants. However, it is worth noting that recently the German industry trade association have undertaken to eliminate EDTA in products released to the aquatic environment which would include disinfectant products.

2.6.13 Phenol

The parent compound C_6H_5OH (Fig. 2.1) is a white crystalline solid, m.p. 39–40 °C, which becomes pink and finally black on long standing. It is soluble in water 1:13 and is a weak acid, pK_a 10. Its biological activity resides in the undissociated molecule. Phenol is effective against both Gram-positive and Gram-negative vegetative bacteria but is only slowly effective towards bacterial spores and acid-fast bacteria.

It is the reference standard for the RW and Chick–Martin tests for disinfectant evaluation. It finds limited application in medicine today, but is used as a preservative in such products as animal glues.

Although first obtained from coal tar, it is now largely obtained by synthetic processes, which include the hydrolysis of chlorobenzene of the high-temperature interaction of benzene sulphonic acid and alkali.

2.7 Pine disinfectants

As long ago as 1876, Kingzett took out a patent in Germany for a disinfectant deodorant made from oil of turpentine and camphor and which had been allowed to undergo oxidation in the atmosphere. This was marketed under the trade name Sanitas. Later, Stevenson (1915) described a fluid made from pine oil solubilized by a soap solution.

The chief constituent of turpentine is the cyclic hydrocarbon pinene (Fig. 2.3), which has little or

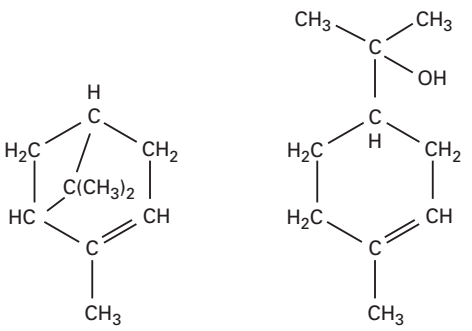


Figure 2.3 Pinene and terpineol.

no biocidal activity. The terpene alcohol terpineol (Fig. 2.3), which may be produced synthetically from pinene or turpentine via terpin hydrate, or in 80% purity by steam-distilling pine-wood fragments, is another ingredient of pine disinfectants and has already been exploited as an ingredient of the Colebrook and Maxted (1933) chloroxylenol formulation. Unlike pinene, it possesses antimicrobial activity in its own right and it shares with pinene the property of modifying the action of phenols in solubilized disinfectant formulations, although not in the same way for all microbial species. An interesting experiment by Moore and Walker (1939) showed how the inclusion of varying amounts of pine oil in a PCMX/soap formulation modified the phenol coefficient of the preparation, depending on the test organism used.

Pine oil concentrations of from 0 to 10% caused a steady increase in the phenol coefficient from 2.0 to 3.6 when the test organism was *S. typhi*. With *Staph. aureus* the value was 0% pine oil, 0.6; 2.5% pine oil, 0.75; thereafter the value fell, having a value of only 0.03 with 10% oil, a pine-oil concentration which gave the maximum *S. typhi* coefficient. In this respect, pinene and terpineol may be compared with the neutral oils used in the coal-tar phenol products (section 2.4.2), but it should be remembered that terpineol possesses intrinsic biocidal activity.

Terpineol is a colourless oil, which tends to darken on storing. It has a pleasant hyacinth odour and is used in perfumery, especially for soap products, as well as in disinfectant manufacture. A series of solubilized products has been marketed, with 'active' ingredients ranging from pine oil, pinene through terpineol to a mixture of pine oil and/or terpineol and a suitable phenol or chlorinated phenol. This gave rise to a range of products, extending from those which are really no more than deodorants to effective disinfectants.

Unfortunately there has been a tendency to ignore or be unaware of the above biocidal trends when labelling these varied products, and preparations containing a small amount of pine oil or pinene have been described as disinfectants. Attempts to remedy this situation were made through the publication of a British Standard entitled *Aromatic Disinfectant Fluids* (BS 5197: 1976). This standard has now been withdrawn and been replaced by specific European standard methods for products in medical, veterinary, industrial, domestic and institutional areas.

2.8 Theory of solubilized systems

Solubilization is achieved when anionic or cationic soaps aggregate in solution to form multiple particles of micelles, which may contain up to 300 molecules of the constituent species. These micelles are so arranged in an aqueous solution that the charged group is on the outside of the particle and the rest of the molecule is within the particle. It is in this part, often a hydrocarbon chain, that the phenols are dissolved, and hence solubilized, in an aqueous milieu.

The relationship between solubilization and antimicrobial activity was explored in detail by Bean & Berry (1950, 1951, 1953), who used a system consisting of 2-benzyl-4-chlorophenol (section 2.6.8) and potassium laurate, and of 2,4-dichloro-3,5-dimethylphenol (section 2.6.5) and potassium laurate. The advantage to a fundamental understanding of the system is that potassium laurate can be prepared in a pure state and its physical properties have been well documented. 2-Benzyl-4-chlorophenol is almost insoluble in water and the antimicrobial activity of a solubilized system containing it will be uncomplicated by a residual water-solubility. The concepts were then extended to chlorocresol.

A plot of weight of solubilized substance per unit weight of solubilizer against the concentration of

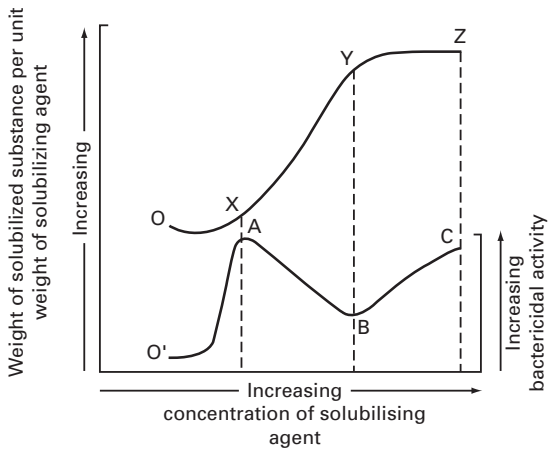


Figure 2.4 The relationship between solubilization and antibacterial activity in a system containing a constant ratio of solubilized substance to solubilizer and where the solubilized substance possesses low water-solubility. *Curve OXYZ*, weight of solubilized substance per unit weight of solubilizing agent plotted against the concentration of solubilizing agent. *Curve O′ABC*, bactericidal activity of the system.

solubilizer at a given ratio of solubilized substance to solubilizer usually shows the type of curve illustrated in Fig. 2.4, curve OXYZ. Above the line OXYZ a two-phase system is found; below the curve a one-phase system consequent upon solubilization is obtained. Upon this curve has been superimposed a curve (O′ABC) which illustrates the change in bactericidal activity of such a system which is found if the solubilized substance possesses antibacterial activity. Such data give some indication of the complex properties of solubilized systems, such as Lysol and Roxenol. Bactericidal activity at O′ is no more than that of the aqueous solution of the bactericide. The increase (O′–A) is due to potentiation of the action of the bactericide by unassociated soap molecules. At A, micelle formation and solubilization begin and thereafter (A–B) activity declines because, it has been suggested, the size of the micelle increases; the amount of drug per micelle decreases, and this is accompanied by a corresponding decrease in the toxicity of the system. However, at B an increase in activity is again found, reaching a maximum at C. This has been explained by the fact that at B, although increase in micellar size no longer occurs, increase in micellar number does, hence the gradual increase in activity.

The lethal event at cell level has been ascribed to an adsorption of the micelles by the bacterial cell and a passage of the bactericide from the micelle on to and into the bacterial cell. In short, this theory postulates that the bactericidal activity is a function of the concentration of the drug in the micelle and not its total concentration in solution. This was held to be the case for both the highly insoluble benzylchlorophenol and the more water-soluble chlorocresol (Bean & Berry, 1951, 1953). Alexander and Tomlinson (1949), albeit working with a different system, suggest a possible alternative interpretation. They agree that the increase, culminating at A, is due to the potentiation of the action of phenol by the solubilizing agent, which because it possesses detergent properties acts by disrupting the bacterial membrane, thereby permitting more easy access of the drug into the cell. The decline (A–B), however, was thought to be due to the removal of drug from the aqueous milieu into the micelles, thereby decreasing the amount available for reacting with the cell. They reject the notion that a drug-bearing micelle is lethal and capable itself of adsorption on the cell and passing its drug load to the cell, and declare that the activity of this system is a function of the concentration of bactericide in the aqueous phase. It must also be pointed out that high concentrations of soaps may themselves be bactericidal (reviewed by Kabara, 1978b) and that this property could explain the increase in activity noted between B and C.

The above is only an outline of one experimental system in a very complex family. For a very complete appraisal together with further patterns of interpretation of experimental data of the problem, the papers of Berry *et al.* (1956) and Berry and Briggs (1956) should be consulted. Opinion, however, seems to be settling in favour of the view that activity is a function of the concentration of the bactericide in the aqueous phase. Indeed, Mitchell (1964), studying the bactericidal activity of chloroxylenol in aqueous solutions of cetomacrogol, has shown that the bactericidal activity here is related to the amount of chloroxylenol in the aqueous phase of the system. Thus a solution which contained, as a result of adding cetomacrogol, 100 times as much

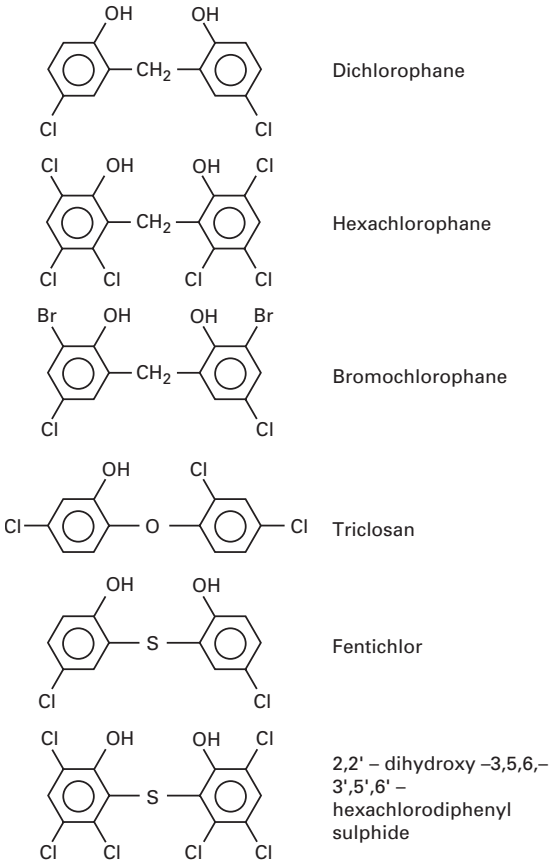


Figure 2.5 Bisphenols.

of the bactericide as a saturated aqueous solution was no more bactericidal than the saturated aqueous solution. Here again, this picture is complicated by the fact that non-ionic surface-active agents, of which cetomacrogol is an example, are known to inactivate phenols (Beckett & Robinson, 1958).

2.9 The bisphenols

Hydroxy halogenated derivatives (Fig. 2.5) of diphenyl methane, diphenyl ether and diphenyl sulphide have provided a number of useful biocides active against bacteria, fungi and algae. In common with other phenolics they all seem to have low activity against *P. aeruginosa*; they also have low water solubility and share the property of the monophenols in that they are inactivated by non-ionic surfactants.

Ehrlich and co-workers were the first to investigate the microbiological activity of the bisphenols and published their work in 1906. Klarmann and Dunning and colleagues described the preparation and properties of a number of these compounds (Klarmann & von Wowern, 1929; Dunning *et al.*, 1931). A useful summary of this early work has been made by Suter (1941). Later, Gump & Walter (1960, 1963, 1964) and Walter & Gump (1962) made an exhaustive study of the biocidal properties of many of these compounds, especially with a view to their use in cosmetic formulations.

2.9.1 Derivatives of dihydroxydiphenylmethane

Dichlorophen, G-4,5,5′-dichloro-2,2′-dihydroxydiphenylmethane (Panacide, Rotafix, Swansea, UK) is active to varying degrees against bacteria, fungi and algae. It is soluble in water at 30 μg/mL but more soluble (45–80 g/100 mL) in organic solvents. The pK_a values at 25 °C for the two hydroxyl groups are 7.6 and 11.6 and it forms a very alkaline solution when diluted. It is typically used as an algicide, fungicide and at a dilution of 1 in 20 as a surface biocide. It has found application as a preservative for toiletries, textiles and cutting oils and to prevent the growth of bacteria in water-cooling systems and humidifying plants. It is used as a slimicide in paper manufacture. It may be added to papers and other packing materials to prevent microbial growth and has been used to prevent algal growth in greenhouses.

Hexachlorophene, 2,2′-dihydroxy-3,5,6, 3′,5′, 6′-hexachlorodiphenylmethane, G11 is almost insoluble in water but soluble in ethanol, ether and acetone and in alkaline solutions. The pK_a values are 5.4 and 10.9. Its mode of action has been studied in detail by Gerhardt, Corner and colleagues (Corner *et al.*, 1971; Joswick *et al.*, 1971; Silvernale *et al.*, 1971; Frederick *et al.*, 1974; Lee & Corner, 1975). It is used mainly for its antibacterial activity but it is much more active against Gram-positive than Gram-negative organisms. Typical MICs (bacteriostatic) in μg/mL are: *Staph. aureus*, 0.9; *B. subtilis*, 0.2; *Proteus vulgaris*, 4; *E. coli*, 28; *P. aeruginosa*, 25. It has found chief application as an active ingredient in surgical scrubs and medicated soaps and has also been used to a limited ex-

tent as a preservative for cosmetics. Its use is limited by its insolubility in water, its somewhat narrow antibacterial spectrum and by the fact that in the UK it is restricted by a control order made in 1973. In general, this order restricted the use of this product to 0.1% in human medicines and 0.75% in animal medicines. Its toxicity has restricted its use in cosmetic products, and the maximum concentration allowed is 0.1%, with the stipulation that it is not to be used in products for children or personal hygiene products.

Bromochlorophane, 3,3′-dibromo-5,5′-dichlor-2,2′-dihydroxydiphenylmethane is soluble in water at 100 μg/mL and is markedly more active against Gram-positive organisms than bacteria. Strains of *Staph. aureus* are inhibited at from 8 to 11 μg/mL, whereas 100 times these concentrations are required for *E. coli* and *P. aeruginosa*. It has been used as the active ingredient in deodorant preparations and toothpastes.

2.9.2 Derivatives of hydroxydiphenylether

Triclosan, 2,4,4′-trichlor-2′-hydroxydiphenylether (Irgasan, registered Ciba Speciality Chemicals, Basle, Switzerland) is only sparingly soluble in water (10 mg/L) but soluble in solutions of dilute alkalis and organic solvents. Its activity is not compromised by soaps, most surfactants, organic solvents, acids or alkalis but ethoxylated surfactants such as polysorbate 80 (Tween 80) entrap triclosan within micelles thus preventing its action (Bhargava & Leonard, 1996). Triclosan is generally bacteriostatic against a broad range of Gram-positive and Gram-negative bacteria and also demonstrates some fungistatic activity. It inhibits staphylococci at concentrations ranging from 0.1 to 0.3 μg/mL. Paradoxically, a number of *E. coli* strains are inhibited over a similar concentration range. Most strains of *P. aeruginosa* require concentrations varying from 100 to 1000 μg/mL for inhibition. It inhibits the growth of several species of mould at from 1 to 30 μg/mL. Triclosan is commonly found in a wide range of personal care products such as toothpaste, handwashes, shower foams and deodorants. It is ideally suited to these applications as it has a low toxicity and irritancy and is substantive to the skin (Bhurgava & Leonard, 1996). More recently it has been used in a range of other applications such as incorporation in plastics and fabrics to confer antimicrobial activity. This, and the link made between triclosan-resistant bacteria and antibiotic resistance has led to concerns about its usage (McMurry *et al.*, 1998a,b; 1999). However, with the correct usage of this antimicrobial, there is no direct evidence to suggest a proliferation of antibiotic resistant bacteria will occur (Ochs, 1999).

2.9.3 Derivatives of diphenylsulphide

Fenticlor, 2,2′-dihydroxy-5,5′-dichlorodiphenylsulphide is a white powder, soluble in water at 30 μg/mL, but is much more soluble in organic solvents and oils. It shows more activity against Gram-positive organisms and a '*Pseudomonas* gap'. Typical inhibitory concentrations (μg/mL) are *Staph. aureus*, 2; *E. coli*, 100; *P. aeruginosa*, 1000. Typical inhibitory concentrations (μg/mL) for some fungi are: *Candida* spp., 12; *Epidermophyton interdigitale*, 0.4; *Trichophyton granulosum*, 0.4. Fenticlor has found chief application in the treatment of dermatophytic conditions. However, it can cause photosensitization and as such its use as a preservative is limited (Goddard & McCue, 2001). Its low water-solubility and narrow spectrum are further disadvantages, but it has potential as a fungicide. Its mode of action was described by Hugo & Bloomfield (1971a,b,c) and Bloomfield (1974).

The chlorinated analogue of fenticlor, 2,2′-dihydroxy-3,4,6,3′4′,6′-hexachlorodiphenylsulphide; 2,2′-thiobis(3,4,6-trichlorophenol) is almost insoluble in water. In a field test, it proved to be an effective inhibitor of microbial growth in cutting-oil emulsions.

An exhaustive study of the antifungal properties of hydroxydiphenylsulphides was made by Pflege *et al.* (1949).

3 Organic and inorganic acids: esters and salts

3.1 Introduction

A large family of organic acids (Fig. 2.6), both aromatic and aliphatic, and one or two inorganic acids have found application as preservatives, more espe-

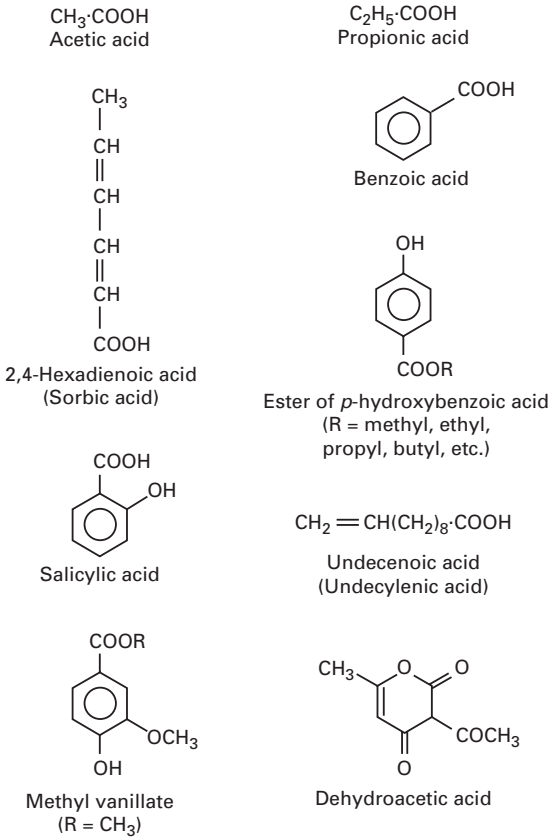


Figure 2.6 Organic acids and esters.

cially in the food industry. Some, for example benzoic acid, are also used in the preservation of pharmaceutical products; others (salicylic, undecylenic and again benzoic) have been used, suitably formulated, for the topical treatment of fungal infections of the skin.

Vinegar, containing acetic acid (ethanoic acid) has been found to act as a preservative. It was also used as a wound dressing. This application has been revived in the use of dilute solutions of acetic acid as a wound dressing where pseudomonal infections have occurred.

Hydrochloric and sulphuric acids are two mineral acids sometimes employed in veterinary disinfection. Hydrochloric acid at high concentrations is sporicidal and has been used for disinfecting hides and skin contaminated with anthrax spores. Sulphuric acid, even at high concentrations, is not sporicidal, but in some countries it is used, usually in combination with phenol, for the decontamination of floors, feed boxes and troughs (Russell & Hugo, 1987).

Table 2.2 pK_a values of acids and esters used as antimicrobial agents.

Acid or esters	pK_a
Acetic (ethanoic) acid	4.7
Propionic (propanoic acid)	4.8
Sorbic acid (2,4-hexadienoic acid)	4.8
Lactic acid	3.8
Benzoic acid	4.2
Salicylic acid	3.0
Dehydroacetic acid	5.4
Sulphurous acid	1.8, 6.9
Methyl-*p*-hydroxybenzoic acid	8.5
Propyl-*p*-hydroxybenzoic acid	8.1

Citric acid is an approved disinfectant against foot-and-mouth virus. It also appears, by virtue of its chelating properties, to increase the permeability of the outer membrane of Gram-negative bacteria (Shibasaki & Kato, 1978; Ayres *et al.*, 1993) when employed at alkaline pH. Malic acid and gluconic acid, but not tartaric acid, can also act as permeabilizers at alkaline pH (Ayres *et al.*, 1993); see also section 14.4.

3.2 Physical factors governing the antimicrobial activity of acids

If an acid is represented by the symbol AH, then its ionization will be represented by A^-H^+. Complete ionization, as seen in aqueous solutions of mineral acids, such as hydrogen chloride (where AH = ClH), is not found in the weaker organic acids and their solutions will contain three components: A^-, H^+ and AH. The ratio of the concentration of these three components is called the ionization constant of that acid, K_a, and $K_a = A^- \times H^+/AH$. By analogy with the mathematical device used to define the pH scale, if the negative logarithm of K_a is taken, a number is obtained, running from about 0 to about 14, called pK_a. Some typical pK_a values are shown in Table 2.2.

An inspection of the equation defining K_a shows that the ratio A^-/AH must depend on the pH of the solution in which it is dissolved, and Henderson and Hasselbalch derived a relationship between this ratio and pH as follows:

$$\log(A^-/AH) = pH - pK_a$$

An inspection of the formula will also show that at the pH value equal to the pK_a value the product is 50% ionized. These data enable an evaluation of the effect of pH on the toxicity of organic acids to be made. Typically it has been found that a marked toxic effect is seen only when the conditions of pH ensure the presence of the un-ionized molecular species AH. As the pH increases, the concentration of HA falls and the toxicity of the system falls; this may be indicated by a higher MIC, longer death time or higher mean single-survivor time, depending on the criterion of toxicity (i.e. antimicrobial activity) chosen.

An inspection of Fig. 2.7 would suggest that HA is more toxic than A^-. However, an altering pH can alter the intrinsic toxicity of the environment. This is due to H^+ alone, the ionization of the cell surface, the activity of transport and metabolizing enzymes and the degree of ionization of the cell surface and hence sorption of the ionic species on the cell.

Predictions for preservative ability of acids validated at one pH are rendered meaningless when such a preservative is added without further consideration to a formulation at a higher pH. The pK_a of the acid preservative should always be ascertained and any pH shift of 1.5 units or more on the alkaline side of this can be expected to cause progressive loss of activity quite sufficient to invalidate the originally determined performance. That pH modifies the antimicrobial effect of benzoic acid has been known for a long time (Cruess & Richert, 1929). For more detailed accounts of the effect of pH on the intensity of action of a large number of ionizable biocides, the papers of Simon and Blackman (1949) and Simon & Beeves (1952a,b) should be consulted.

3.3 Mode of action

The mode of action of acids used as food preservatives has been studied by Freese *et al.* (1973), Sheu *et al.* (1975), Krebs *et al.* (1983), Salmond *et al.* (1984), Eklund (1980, 1985, 1989), Sofos *et al.* (1986), Booth & Kroll (1989) Cherrington

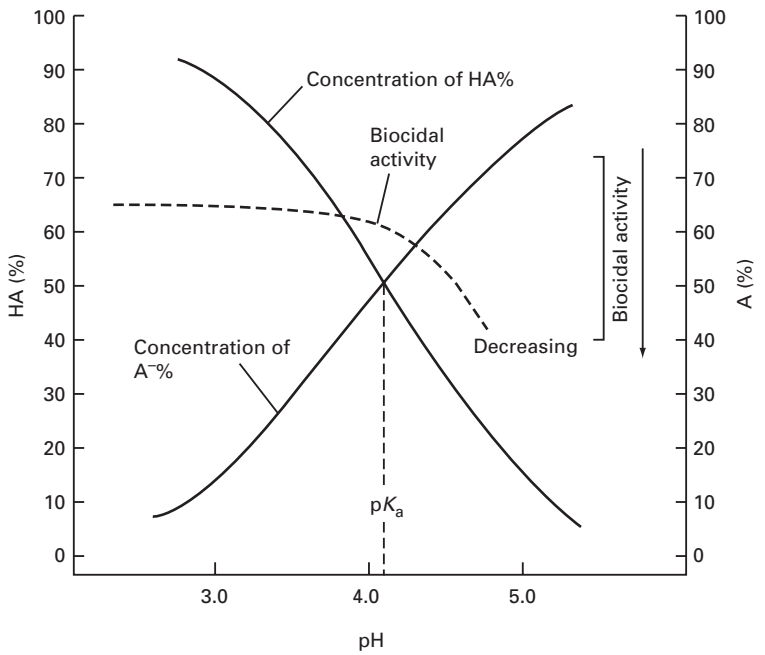


Figure 2.7 A generalized diagram of the effect of pH on the ionization and biocidal activity of an acid (HA) of pK_a 4.1.

et al. (1990, 1991) and Russell (1992). Convincing evidence has been produced that many acid preservatives act by preventing the uptake of substrates which depend on a proton-motive force for their entry into the cell, in other words they act as uncoupling agents (Chapter 5). In addition to acids such as benzoic, acetic and propionic, the esters of *p*-hydroxybenzoic acid (the parabens) were also included in some of the above studies; they too acted as uncoupling agents but also inhibited electron transport.

Equally interesting were experiments on the pH dependence of the substrate uptake effect. The intensity of uptake inhibition by propionate, sorbate and benzoate declined between pH 5 and 7, while that induced by propyl-*p*-hydroxybenzoic acid (pK_a 8.5) remained constant over the same pH range. The growth-inhibitory effect of ionizable biocides shows pH dependence and this, as might be expected, is applicable to a biochemical effect upon which growth in turn depends.

Organic acids, such as benzoic and sorbic, are deliberately used as preservatives. Acids such as acetic, citric and lactic are often employed as acidulants, i.e. to lower artificially the pH of foods. A low pK_a value is not the only significant feature of acidulants, however, since: (1) sorbate and acetate have similar pK_a values but the latter is a less potent preservative; (2) organic acids used as preservatives are more potent inhibitors than other weak acids of similar pH; and (3) weak organic acid preservatives are more effective inhibitors of pH homeostasis than other acids of similar structure.

3.4 Individual compounds

3.4.1 Acetic acid (ethanoic acid)

This acid, as a diluted petrochemically produced compound or as the natural product vinegar, is used primarily as a preservative for vegetables. The toxicity of vinegars and diluted acetic acid must rely to an extent on the inhibitory activity of the molecule itself, as solutions of comparable pH made from mineral acid do not exhibit the same preservative activity. A 5% solution of acetic acid contains 4.997% CH_3COOH and 0.003% H^+. As might be expected from the pK_a value, 4.7, the activity is rapidly lost at pH values above this value. This suggests that the acetate ion is less toxic than the undissociated molecule, although, as has been said, the concomitant reduction in hydrogen ion concentration must play some part in the reduction of toxicity. As has been stated, diluted 1–5% acetic acid has been used as a wound dressing where infection with *Pseudomonas* has occurred (Phillips *et al.*, 1968).

3.4.2 Propionic acid

This acid is employed almost exclusively as the sodium, and to a lesser extent the calcium, salt in the baking industry, where it is used to inhibit mould and bacterial growth in breads and cakes. It is particularly useful in inhibiting the growth of the spore-forming aerobe *Bacillus macerans*, which gives rise to an infestational phenomenon called ropy bread. Manufacturers give explicit directions as to the amount to be used in different products, but in general 0.15–0.4% is added to the flour before processing. Other products that have been successfully preserved with propionates include cheeses and malt extract. In addition to foods, wrapping materials for foods have also been protected from microbial damage with the propionates.

3.4.3 Undecanoic acid (undecylenic acid)

This has been used either as such or as the calcium or zinc salt in the treatment of superficial dermatophytoses. It is usually applied in ointment form at concentrations of 2–15%.

3.4.4 2,4-Hexadienoic acid (sorbic acid)

This unsaturated carboxylic acid is effective against a wide range of microorganisms (Bell *et al.*, 1959) and has been used as the acid itself, or its potassium salt, at concentrations of 0.01–0.1% to preserve bakery products, soft drinks, alcoholic beverages, cheeses, dried fruits, fish, pickles, wrapping materials and pharmaceutical products. As with all acids, there is a critical pH, in this case 6.5, above which activity begins to decline. Again it is the undissociated acid which is the active antimicrobial species (Beneke & Fabian, 1955; Gooding *et al.*, 1955).

Sorbic acid was believed to act by interfering with the functioning of the citric acid cycle (York & Vaughan, 1955; Palleroni & de Prinz, 1960).

Sorbic acid is known to interfere with the uptake of amino and oxo acids in *E. coli* and *B. subtilis*; it affects the proton-motive force in *E. coli* and accelerates the movement of H^+ ions from low media pH into the cytoplasm. It probably acts overall by dissipating ΔpH across the membrane and inhibiting solute transport. The membrane potential ($\Delta\psi$) is reduced but to a much smaller extent than ΔpH (Eklund, 1989; Cherrington *et al.*, 1991; Kabara & Eklund, 1991; Russell & Chopra, 1996). A combination of sorbic acid with monolaurin has been shown to be often more active than parabens or sorbic acid alone (Kabara, 1980).

3.4.5 Lactic acid

Lactic acid shares with some other hydroxyacids the interesting property of being able to destroy airborne microorganisms (Lovelock *et al.*, 1944; see also section 19). A careful study of hydroxy-acids, including lactic acid, as air disinfectants was made by Lovelock (1948). Lactic acid was found to be a cheap, efficient aerial bactericide when sprayed into the area to be sterilized. It has, however, a slight irritant action on the nasal mucosa, which tends to limit its use. It could be used in emergencies for sterilizing glove boxes or hoods if other means of sterilization are not provided (see also section 19).

Lactic acid in liquid form is less active than several other organic acids (Eklund, 1989) but nevertheless is used as an acidulant for low-pH foods and fruit juices (Russell & Gould, 1991a,b). It has been shown to be an effective permeabilizer (Alakomi *et al.*, 2001) and is discussed in more detail in section 14.4.

3.4.6 Benzoic acid

Benzoic acid, first shown to be antifungal in 1875, is a white crystalline powder, which is soluble 1:350 in water. It is used as a preservative for foods and pharmaceutical products, but is rapidly inactivated at pH values above 5.0 (Eklund, 1989; Kabara & Eklund, 1991; Russell & Gould, 1991b). As with other preservatives, its activity may also be modified by the milieu in which it acts (Anderson & Chow, 1967; Beveridge & Hope, 1967). Resistance may develop (Ingram, 1959) and the acid may be metabolized by a contaminant it is meant to inhibit (Stanier *et al.*, 1950; Hugo & Beveridge, 1964; Stanier & Orston, 1973). In addition to its use as a preservative, benzoic acid has been combined with other agents for the topical treatment of fungal infections. Benzoic acid, like many other compounds, inhibits swarming of *Bacillus* spp. (Thampuran & Surendran, 1996). Studies with benzoic acid derivatives have demonstrated that lipophilicity and pK_a are the two most important parameters influencing activity (Ramos-Nino *et al.*, 1996).

3.4.7 Salicylic acid

This is often used, in combination with benzoic acid and other antifungal agents, for the topical treatment of fungal infections. Salicylic acid has keratinolytic activity and in addition affects metabolic processes. For an account of the action of benzoic and salicylic acids on the metabolism of microorganisms, see Bosund (1962) and Freese *et al.* (1973).

3.4.8 Dehydroacetic acid (DHA)

Dehydroacetic acid is a white or light yellow, odourless, crystalline compound, which is soluble at less than 0.1% in water; the sodium salt is soluble to the extent of 33%. Typical inhibitory concentrations (%) of the latter for selected microorganisms are: *Aerobacter aerogenes*, 0.3; *B. cereus*, 0.3; *Lactobacillus plantarum*, 0.1; *Staph. aureus*, 0.3; *P. aeruginosa*, 0.4; *A. niger*, 0.05; *Penicillium expansum*, 0.01; *Rhizopus nigricans*, 0.05; *T. interdigitale*, 0.005; *Saccharomyces cerevisiae*, 0.1. Extensive toxicological studies have indicated that the product is acceptable as a preservative for foods, cosmetics and medicines. The pK_a value of DHA is 5.4 but an inspection of pH/activity data suggests that activity loss above the pK_a value is not as great as with other preservative acids (propionic, benzoic) and indeed, in Wolf's 1950 paper, the MIC against *Staph. aureus* remained at 0.3% from pH 5 to 9. Loss of activity at alkaline pH values was, however, noted by Bandelin (1950) in his detailed

study of the effect of pH on the activity of antifungal compounds, as would be predicted by the pK_a value. Little was known about its mode of action, although Seevers *et al.* (1950) produced evidence that DHA inhibited succinoxidase activity in mammalian tissue, while Wolf and Westveer (1950) showed that it did not react with microbial –SH enzymes.

3.4.9 Sulphur dioxide, sulphites, bisulphites

The fumes of burning sulphur, generating sulphur dioxide, have been used by the Greeks and Egyptians as fumigants for premises and food vessels to purify and deodorize. Lime sulphur, an aqueous suspension of elementary sulphur and calcium hydroxide, was introduced as a horticultural fungicide in 1803. Later, the salts, chiefly sodium, potassium and calcium, of sulphurous acid were used in wine and food preservation. In addition to their antimicrobial properties, members of this group also act as antioxidants helping to preserve the colour of food products, as enzyme inhibitors, as Maillard reaction inhibitors and as reducing agents (Gould & Russell, 1991).

A pH-dependent relationship exists in solution between the species SO_2, HSO_3^- and SO_3^{2-}. As the pH moves from acid to alkaline, the species predominance moves from SO_2, the toxic species, through HSO_3^- to SO_3^{2-}. Above pH 3.6, the concentration of SO_2 begins to fall, and with it the microbicidal power of the solution. It is postulated that SO_2 can penetrate cells much more readily than can the other two chemical species (Rose & Pilkington, 1989).

Yeasts and moulds can grow at low pH values, and hence the value of sulphites as inhibitors of fungal growth in acid environments, such as fruit juices. For reviews on the antimicrobial activity of sulphur dioxide, see Hammond and Carr (1976), Wedzicha (1984), Rose and Pilkington (1989) and Gould and Russell (1991).

3.4.10 Esters of p*-hydroxybenzoic acid (parabens)*

The marked pH-dependence of acids for their activity and the fact that the biocidal activity lay in the undissociated form led to the notion that esterification of an aromatic hydroxy carboxylic acid might give rise to compounds in which the phenolic group was less easily ionized. Sabalitschka (1924) prepared a series of alkyl esters of *p*-hydroxybenzoic acid and tested their antimicrobial activity (Sabalitschka & Dietrich, 1926; Sabalitschka *et al.*, 1926). This family of biocides, which may be regarded as either phenols or esters of aromatic hydroxy carboxylic acids, are among the most widely used group of preservatives (Richardson, 1981). The esters usually used are the methyl, ethyl, propyl, butyl and benzyl compounds and are active over a wider pH range (4–8) than acid preservatives (Sokol, 1952). They have low water-solubility, which decreases in the order methyl–benzyl (Table 2.3). A paper which gives extensive biocidal data is that of Aalto *et al.* (1953). Again it can be seen that activity increases from the methyl to the benzyl ester. The compounds show low systemic toxicity (Mathews *et al.*, 1956). Russell & Furr (1986a,b, 1987) and Russell *et al.* (1985, 1987) studied the effects of parabens against wild-type and envelope mutants of *E. coli* and *Salmonella typhimurium*, and found that, as the homologous series was ascended, solubility decreased but activity became more pronounced, especially against the deep rough strains.

In summary, it can be said that the parabens are generally more active against Gram-positive bacteria and fungi, including yeasts, than against Gram-negative bacteria, and in the latter *P. aeruginosa* is, as is so often seen, more resistant, especially to the higher homologues.

Hugo and Foster (1964) showed that a strain of *P. aeruginosa* isolated from a human eye lesion could metabolize the esters in dilute solution, 0.0343%, a solution strength originally proposed as a preservative vehicle for medicinal eye-drops. Beveridge and Hart (1970) verified that the esters could serve as a carbon source for a number of Gram-negative bacterial species. Rosen *et al.* (1977) studied the preservative action of a mixture of methyl (0.2%) and propyl (0.1%) *p*-hydroxybenzoic acid in a cosmetic lotion. Using a challenge test, they found that this concentration of esters failed to kill *P. aeruginosa*. It was part of their work indicating that these esters + imidazolindyl urea

Table 2.3 Chemical and microbiological properties of esters of *p*-hydroxybenzoic acid.

	Ester			
Property[a]	*Methyl*	*Ethyl*	*Propyl*	*Butyl*
Molecular weight	152	166	180	194
Solubility in water (g/100 g) at 15 °C	0.16	0.08	0.023	0.005
K_w^o (arachis oil)	2.4	13.4	38.1	239.6
Log *P* (octanol:water)	1.96	2.47	3.04	3.57
MIC values (molar basis)[b]				
Escherichia coli (wild type)	3.95×10^{-3}	2.7×10^{-3}	1.58×10^{-3}	1.03×10^{-3}
Escherichia coli (deep rough)	2.63×10^{-3}	1.2×10^{-3}	2.78×10^{-4}	1.03×10^{-4}
MIC values (μg/mL)[c]				
Escherichia coli	800	560	350	160
Pseudomonas aeruginosa	1000	700	350	150
Concentration (mmol/L) giving 50% inhibition of growth and uptake process in[d]				
Escherichia coli	5.5	2.2	1.1	0.4
Pseudomonas aeruginosa	3.6	2.8	>1.0	>1.0
Bacillus subtilis	4.3	1.3	0.9	0.46

[a] K_w^o, partition coefficient, oil:water; *P*, partition coefficient, octanol:water.
[b] Russell *et al.* (1985).
[c] El-Falaha et al, (1983).
[d] Eklund (1980).

(section 17.2.2) were ideal to provide a broad-spectrum preservative system, pseudomonads being successfully eliminated.

The rationale for the use of these esters in mixtures might be seen in the preservation of water-in-oil emulsion systems, where the more water-soluble methyl ester protected the aqueous phase while the propyl or butyl esters might preserve the oil phase (O'Neill *et al.*, 1979). The use of fennel oil in combination with methyl, ethyl, propyl and butyl parabens has been shown to be synergistic in terms of antimicrobial activity (Hodgson *et al.*, 1995). Another factor which must be borne in mind when using parabens is that they share the property found with other preservatives containing a phenolic group of being inactivated by non-ionic surface agents. Hydrogen bonding between the phenolic hydrogen atom and oxygen residues in polyoxyethylated non-ionic surfactants is believed to be responsible for the phenomenon. Experiments to support this inactivation are described by Patel & Kostenbauder (1958), Pisano & Kostenbauder (1959) and Blaug & Ahsan (1961). Various ways of quenching paraben activity, including the use of polysorbates, are considered by Sutton (1996).

The mode of action of the parabens has been studied by Furr & Russell (1972a,b,c), Freese *et al.* (1973), Freese & Levin (1978), Eklund (1980, 1985, 1989) and Kabara & Eklund (1991). Haag & Loncrini (1984) have produced a comprehensive report of their antimicrobial properties.

3.4.11 Vanillic acid esters

The methyl, ethyl, propyl and butyl esters of vanillic acid (4-hydroxy-3-methoxy benzoic acid) possess antifungal properties when used at concentrations of 0.1–0.2%. These esters are not very soluble in water and are inactivated above pH 8.0. The ethyl ester has been shown to be less toxic than sodium benzoate and it has been used in the preservation of foods and food-packing materials against fungal infestation.

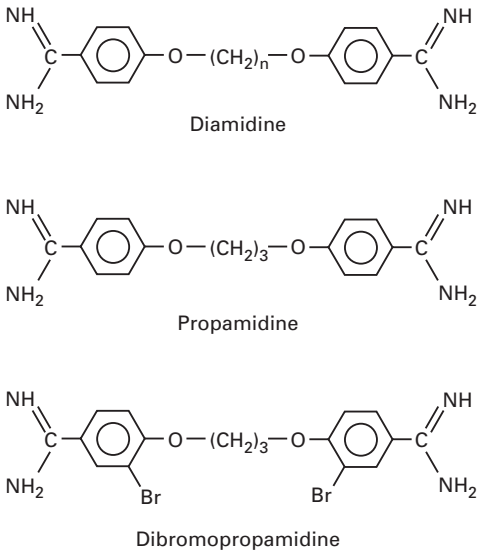


Figure 2.8 Typical structure of a diamidine; propamidine; dibromopropamidine.

4 Aromatic diamidines

Diamidines are a group of organic compounds of which a typical structure is shown in Fig. 2.8. They were first introduced into medicine in the 1920s as possible insulin substitutes, as they lowered blood-sugar levels in humans. Later, they were found to possess an intrinsic trypanocidal activity and from this arose an investigation into their antimicrobial activity (Thrower & Valentine, 1943; Wien *et al.*, 1948). From these studies two compounds, propamidine and dibromopropamidine, emerged as useful antimicrobial compounds, being active against both bacteria and fungi.

4.1 Propamidine

Propamidine is 4,4′-diamidinophenoxypropane is usually supplied as the di(2-hydroxyethanesulphate), the isethionate, to confer solubility on this molecule. This product is a white hygroscopic powder, which is soluble in water, 1 in 5. Antimicrobial activity and clinical applications are described by Thrower & Valentine (1943). A summary of its antibacterial and antifungal activity is given in Table 2.4. Its activity is reduced by serum, blood and by low pH values. Microorganisms exposed to propamidine quickly acquire a resistance to it by serial subculture in the presence of increasing doses. Methicillin-resistant *Staph. aureus* (MRSA) strains may show appreciable resistance to propamidine (Al-Mausaudi *et al.*, 1991). It is chiefly used in the form of a cream containing 0.15% as a topical application for wounds.

4.2 Dibromopropamidine

Dibromopropamidine (2,2′-dibromo-4,4′-diamidinodiphenoxypropane), usually supplied as the isethionate, occurs as white crystals which are readily soluble in water. Dibromopropamidine is active against Gram-positive, non-spore-forming organisms; it is less active against Gram-negative organisms and spore formers, but is active against fungi (Table 2.4). Resistance can be acquired by serial subculture, and resistant organisms also show some resistance to propamidine. Russell and Furr (1986b, 1987) found that Gram-negative bacteria present a permeability barrier to dibromopropamidine isethionate, and MRSA strains may be resistant to the diamidine. Its activity is reduced in acid environments and in the presence of blood and serum. It is usually administered as an oil-in-water cream emulsion containing 0.15% of the isethionate.

More detailed reviews on this group of compounds will be found in Hugo (1971) and Fleurette *et al.* (1995).

5 Biguanides

Various biguanides show antimicrobial activity, including chlorhexidine, alexidine and polymeric forms.

5.1 Chlorhexidine

Chlorhexidine (Fig. 2.9a) is one of a family of N^1, N^5-substituted biguanides which has emerged from extensive synthetic and screening studies (Curd & Rose, 1946; Davies *et al.*, 1954; Rose & Swain,

Table 2.4 Antimicrobial properties of propamidine and dibromopropamidine isethionates.

	MIC (μg/mL) of	
Microorganism	*Propamidine isethionate*[a]	*Dibromopropamidine isethionate*[b]
Staphylococcus aureus	1–16	1
Staphylococcus albus	6	
MRSA[c]	800/100	
MRSE[d]	250–800	
Streptococcus pyogenes	0.24–4	1
Streptococcus viridans	1–4	2
Streptococcus faecalis	25	
Pseudomonas aeruginosa	250–400	32 (64)
Proteus vulgaris	125–400	128 (256)
Escherichia coli	64–100	4 (32)
Clostridium perfringens	3–32	512
Clostridium histolyticum	256	256
Shigella flexneri	32	8
Salmonella enteriditis	256	65
Salmonella typhimurium	256	64
Actinomyces kimberi	100	10
Actinomyces madurae	100	50
Actinomyces hominis	1000	1000
Trichophyton tonsurans	100	25
Epidermophyton floccosum	250	
Achorion schoenleinii	3.5	
Blastomyces dermatitidis	3.5	
Geotrichum dermatitidis	3.5	200
Hormodendron langevonii		500

[a]Data from various sources, including Wien *et al.* (1948).
[b]Data from Wien *et al.* (1948).
[c]MRSA, methicillin-resistant *Staph. aureus* carrying *qac*A/*qac*B gene (data of Littlejohn *et al.*, 1992).
[d]MRSE, methicillin-resistant *Staph. epidermidis* (data of Leelaporn *et al.*, 1994).
Figures in parentheses denote bactericidal concentrations.

1956). It is available as a dihydrochloride, diacetate and gluconate. At 20 °C the solubilities of the dihydrochloride and diacetate are 0.06 and 1.9% w/v, respectively; the gluconate is freely soluble. Chlorhexidine and its salts occur as white or faintly cream-coloured powders and are available in a number of pharmaceutical formulations. It is widely used combined with cetyltrimethylammonium bromide as a topical antiseptic (Savlon, Novartis Consumer Health, Basle, Switzerland).

Chlorhexidine has a wide spectrum of antibacterial activity against both Gram-positive and Gram-negative bacteria. Some bacteria, notably strains of *Proteus* and *Providencia* spp., may be highly resistant to the biguanide (Stickler *et al.*, 1983; Ismaeel *et al.*, 1986a,b; Russell, 1986; Baillie, 1987). It is not sporicidal (Shaker *et al.*, 1986, 1988a,b; Russell, 1990a,b, 1991b; Russell & Day, 1993; Ranganathan, 1996; Russell & Chopra, 1996). Chlorhexidine is not lethal to acid-fast organisms, although it shows a high degree of bacteriostasis (Russell, 1995, 1996; Russell & Russell, 1995; Table 2.5). It is, however, tuberculocidal in ethanolic solutions and sporicidal at 98–100 °C. A range of bacteriostatic and bactericidal values against a variety of bacterial species is shown in Tables 2.5 and 2.6, respectively.

Activity is reduced in the presence of serum,

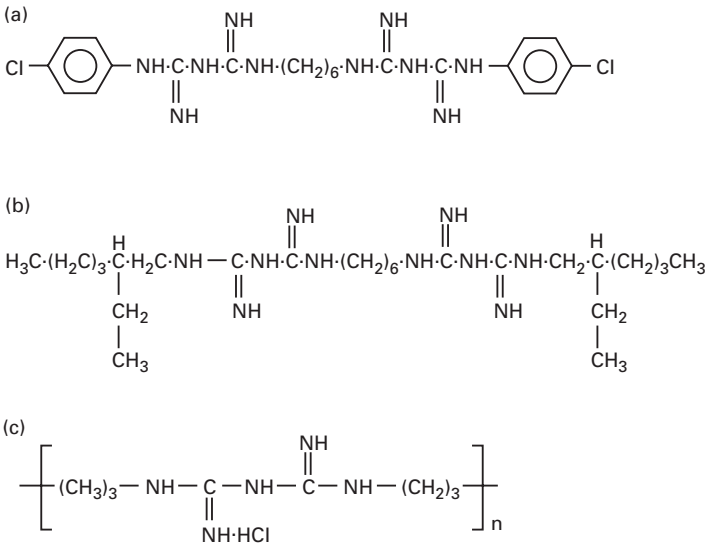


Figure 2.9 Chlorhexidine (a), alexidine (b) and Vantocil 1B, a polymeric biguanide (c), in which mean *n* is 5.5.

blood, pus and other organic matter. Because of its cationic nature, its activity is also reduced in the presence of soaps and other anionic compounds. Another cause of activity loss is due to the low solubility of the phosphate, borate, citrate, bicarbonate, carbonate or chloride salts.

Its main use is in medical and veterinary antisepsis (Holloway *et al.*, 1986). An alcoholic solution is a very effective skin disinfectant (Lowbury & Lilley, 1960). It is used in catheterization procedures (Traore *et al.*, 2000), in bladder irrigation and in obstetrics and gynaecology. It is one of the recommended bactericides for inclusion in eye-drops and is widely used in contact-lens solutions (Gavin *et al.*, 1996). In the veterinary context (Russell & Hugo, 1987), chlorhexidine fulfils the major function of the application of a disinfectant of cows' teats after milking and can also be used as an antiseptic wound application. Chlorhexidine is also widely employed in the dental field due to its broad spectrum of activity, substantivity and low toxicity (Gorman & Scott, 1985; Molinari, 1995; Cottone & Molinari, 1996; Gomes *et al.*, 2001). It has also been investigated in combination with sodium hypochlorite as an endodontic irrigant (Kuruvilla & Kamath, 1998).

Its mode of action has been studied by various authors (Hugo & Longworth, 1964a,b, 1965, 1966; Longworth, 1971; Hugo, 1978; Fitzgerald *et al.*, 1989, 1992a,b; Kuyyakanond & Quesnel, 1992; Barrett-Bee *et al.*, 1994; Russell & Day, 1996). ^{14}C-chlorhexidine gluconate is taken up very rapidly by bacterial (Fitzgerald *et al.*, 1989) and fungal (Hiom *et al.*, 1995a,b) cells. At lower concentrations, up to 200 μg/mL, it inhibits membrane enzymes and promotes leakage of cellular constituents, which is probably associated with bacteriostasis. As the concentration increases above this value, cytoplasmic constituents are coagulated and a bactericidal effect is seen (Chapter 5). Chlorhexidine has low oral toxicity and it may be administered for throat medication in the form of lozenges.

Extensive details on uses and application, together with relevant biocidal data, will be found in the booklet *Hibitane* (Imperial Chemical Industries, n.d.). Comprehensive surveys of its activity and uses have been published (Russell & Day, 1993; Reverdy, 1995a; Ranganathan, 1996).

5.2 Alexidine

Alexidine (Fig. 2.9b) is a bisbiguanide that possesses ethylhexyl end-groups as distinct from the chlorophenol end-groups found in chlorhexidine. Alexidine is considerably more active than

chlorhexidine in inducing cell leakage from *E. coli*, and concentrations of alexidine (but not of chlorhexidine) above the MIC induce cell lysis (Chawner & Gilbert, 1989a,b). Alexidine has been recommended for use as an oral antiseptic and antiplaque compound (Gjermo *et al.*, 1973).

Unlike chlorhexidine, both alexidine and polyhexamethylene biguanide (PHMB) (section 5.3) induce membrane lipid-phase separation and domain formation.

Table 2.5 Bacteriostatic activity of chlorhexidine against various bacterial species.

Organism	*Concentration of chlorhexidine (μg/mL) necessary for inhibition of growth*
Streptococcus lactis	0.5
Streptococcus pyogenes	0.5
Streptococcus pneumoniae	1.0
Streptococcus faecalis	1.0
Staphylococcus aureus	1.0
Corynebacterium diphtheriae	1.0
Salmonella typhi	1.67
Salmonella pullorum	3.3
Salmonella dublin	3.3
Salmonella typhimurium	5.0
Proteus vulgaris	5.0
Pseudomonas aeruginosa (1)	5.0
Pseudomonas aeruginosa (2)	5.0
Pseudomonas aeruginosa (3)	12.5
Enterobacter aerogenes	10
Escherichia coli	10[a]
Vibrio cholerae	3.3
Bacillus subtilis	0.5
Clostridium welchii	10
Mycobacterium tuberculosis	0.5
Candida albicans[b]	5.0

Inoculum: one loopful of 24-h broth culture per 10 mL Difco heart-brain infusion medium.
Incubation: 24 h at 37 °C.
[a]Much higher than normally recorded.
[b]Yeast.

5.3 Polymeric biguanides

A polymer of hexamethylene biguanide (Fig. 2.9c), with a molecular weight of approximately 3000 (weight average), has found particular use as a cleansing agent in the food industry. Its properties have been described by Davies *et al.* (1968) under the trade name Vantocil 1B. PHMB is soluble in water and is usually supplied as a 20% aqueous solution. It is also soluble in glycols and alcohols but is insoluble in non-polar solvents, such as petroleum ethers or toluene. It inhibits the growth of most bacteria at between 5 and 25 μg/mL but 100 μg/mL is required to inhibit *P. aeruginosa* while *P. vulgaris* requires 250 μg/mL. It is less active against fungi; for example, *Cladosporium resinae*, which has been implicated as a spoilage organism in pharmaceutical products, requires 1250 μg/mL to prevent growth.

PHMB is believed to gain access to Gram-negative bacteria by a mechanism of self-promotion through cation displacement from, predominantly, core lipopolysaccharide in the outer membrane (Gilbert *et al.*, 1990a). Antimicrobial activity of PHMB increases with increasing polymer length (Gilbert *et al.*, 1990b). It is a membrane-active agent (Broxton *et al.*, 1983, 1984a,b; Woodcock, 1988), inducing phospholipid phase separation (Ikeda *et al.*, 1984). A complete loss of membrane

Table 2.6 Bactericidal activity of chlorhexidine against various bacterial species.

	Concentration of chlorhexidine (μg/mL)		
Organism	*To effect 99% kill*	*To effect 99.9% kill*	*To effect 99.99% kill*
Staphylococcus aureus	8	14	25
Streptococcus pyogenes	–	–	50
Escherichia coli	6.25	10	20
P. aeruginosa	25	33	60
Salmonella typhi	5	–	8

Inoculum: 10^5 in distilled water. Contact time: 10 min at room temperature. Neutralizer: egg-yolk medium.

function ensues, with precipitation of intracellular constituents leading to a bactericidal effect.

Because of the residual positive charges on the polymer, PHMB is precipitated from aqueous solutions by anionic compounds, which include soaps and detergents based on alkyl sulphates. It is also precipitated by detergent constituents, such as sodium hexametaphosphate, and in a strongly alkaline environment.

It finds use as a general sterilizing agent in the food industry, provided the surfaces to which it is applied are free from occlusive debris, a stricture that applies in all disinfection procedures. Because it is not a surface-active agent, it can be used in the brewing industry, as it does not affect head retention on ales and beers. Contact should be avoided with one commonly used material in food manufacture, anionic caramel, as this will, like other anionic compounds, inactivate the polymer. It has also been used very successfully for the disinfection of swimming pools. Apart from copper, which it tarnishes, this polymeric biguanide has no deleterious effect on most materials it might encounter in use.

PHMB has activity against both the trophozite and the cyst forms of *Acanthamoeba castellanii* (Khunkitti *et al.*, 1996, 1997, 1998; see also Chapter 8.1). More recently PHMB has been shown to have a beneficial effect in inhibiting plaque when used in mouthwashes (Rosin *et al.*, 2002).

6 Surface-active agents

Surface-active agents (surfactants) have two regions in their molecular structure, one being a hydrocarbon water-repellent (hydrophobic) group and the other a water-attracting (hydrophilic or polar) group. Depending on the basis of the charge or absence of ionization of the hydrophilic group, surface-active agents are classified into anionic, cationic, non-ionic and ampholytic (amphoteric) compounds.

6.1 Cationic agents

Cationic surfactants possess strong bactericidal, but weak detergent, properties. The term 'cationic detergent' usually signifies a quaternary ammonium compound (QAC, quats, onium compound). Lawrence (1950), D'Arcy and Taylor (1962a,b), Merianos (1991), Joly (1995) and Reverdy (1995b) have reviewed the surface-active quaternary ammonium germicides, and useful data about their properties and activity are provided by Wallhäusser (1984) and about their uses by Gardner and Peel (1986,1991) and Denyer and Wallhäusser (1990). Early references to their use are found in Jacobs (1916), Jacobs *et al.* (1916a,b) and Domagk (1935).

6.1.1 Chemical aspects

The QACs may be considered as being organically substituted ammonium compounds, in which the nitrogen atom has a valency of five, and four of the substituent radicals (R^1–R^4) are alkyl or heterocyclic radicals and the fifth (X^-) is a small anion (Fig. 2.10: general structure). The sum of the carbon atoms in the four R groups is more than 10. For a QAC to have a high antimicrobial activity, at least one of the R groups must have a chain length in the range C_8 to C_{18} (Domagk, 1935). Three of the four covalent links may be satisfied by nitrogen in a pyridine ring, as in the pyridinium compounds, such as cetylpyridinium chloride. This and the other important QACs are listed in Fig. 2.10. The cationic onium group may be a simple aliphatic ammonium, a pyridimum or piperidinium or other heterocyclic group (D'Arcy & Taylor, 1962b).

Apart from the monoquaternary compounds, monoquaternary derivatives of 4-aminoquinaldine (e.g. laurolinium) are potent antimicrobial agents, as are the bisquaternary compounds, such as hedaquinium chloride and dequalinium. These are considered in more detail in section 10 (see also Figs 2.21 and 2.22).

In addition to the compounds mentioned above, polymeric QACs are used as industrial biocides. One such compound is poly(oxyethylene (dimethylimino)ethylene)dichloride. Organosilicon-substituted (silicon-bonded) QACs, organic amines or amine salts have been introduced recently. Compounds with antimicrobial activity in solution are also highly effective on surfaces. One such compound, 3-(trimethoxysily)propyloctade-

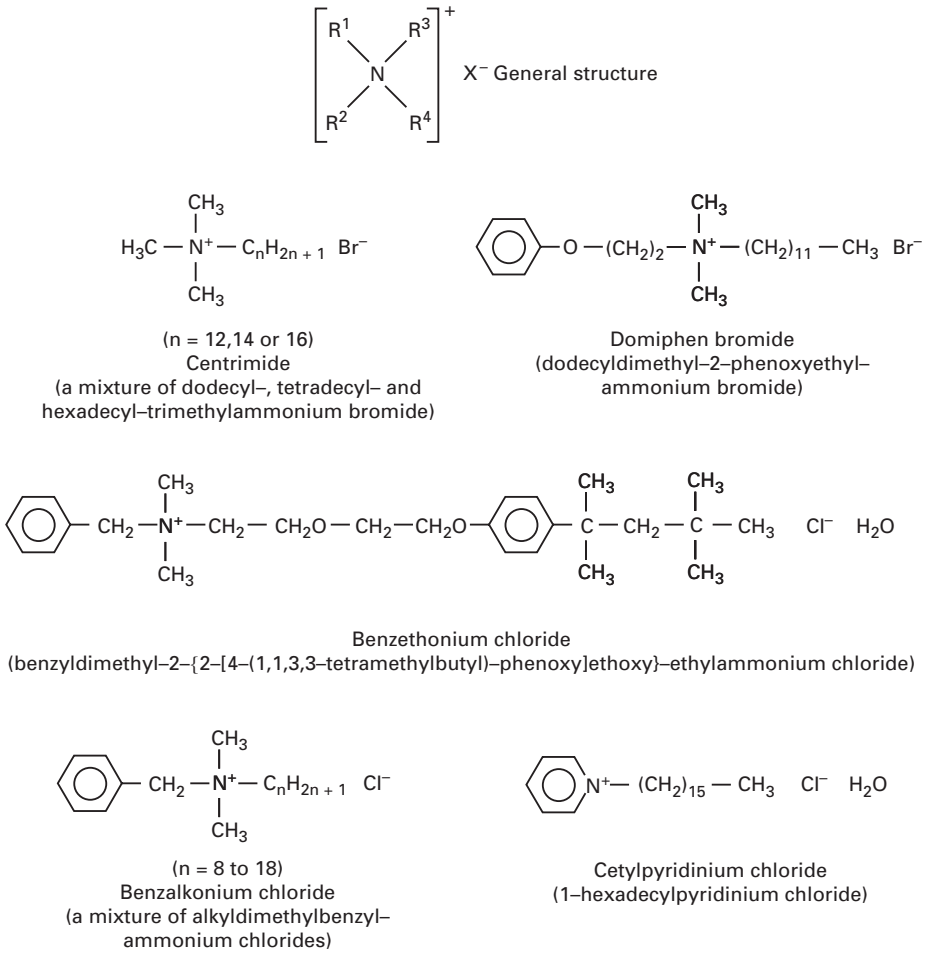


Figure 2.10 General structure and examples of quaternary ammonium compounds (QACs).

cyldimethyl ammonium chloride, demonstrates powerful antimicrobial activity while chemically bonded to a variety of surfaces (Malek & Speier, 1982; Speier & Malek, 1982). Schaeufele (1986) has pointed out that fatty alcohols and/or fatty acids, from both natural and synthetic sources, form the basis of the production of modern QACs, which have improved organic soil and increased hard-water tolerance.

6.1.2 Antimicrobial activity

The antimicrobial properties of the QACs were first recognized in 1916, but they did not attain prominence until the work of Domagk in 1935. Early workers claimed that the QACs were markedly sporicidal, but the fallacy of this hypothesis has been demonstrated by improved testing methods. Weber and Black (1948) had earlier recommended the use of lecithin as a neutralizer for QACs. Lawrence (1948) showed that soaps and anionic detergents failed to inactivate QACs, and suggested suramin sodium for this purpose. British Standard 6471 (1984), recommended lecithin (2%) solubilized with Lubrol W (3%), this standard has now been replaced by BS EN 1276 (1997). Lubrol W itself may be toxic to streptococci, a point discussed more fully by Russell *et al.* (1979) and Russell (1981).

Sutton (1996) describes appropriate neutralizing systems for QACs and other biocides; cyclodextrin (Simpson, 1992) may prove to be useful.

The QACs are primarily active against Gram-positive bacteria, with concentrations as low as 1 in 200 000 (0.0005%) being lethal; higher concentrations (*c.* 1 in 30 000 or 0.0033%) are lethal to Gram-negative bacteria (Hamilton, 1971), although *P. aeruginosa* tends to be highly resistant (Davis, 1962). Nevertheless, cells of this organism which are highly resistant to benzalkonium chloride (1 mg/mL, 0.1%) may still show ultrastructural changes when grown in its presence (Hoffman *et al.*, 1973). The QACs have a trypanocidal activity (reviewed by D'Arcy & Taylor, 1962b) but are not mycobactericidal (Sykes, 1965; Smith, 1968), presumably because of the lipid, waxy coat of these organisms. Gram-negative bacteria, such as *E. coli*, *P. aeruginosa* and *S. typhimurium*, exclude QACs, but deep rough mutants are sensitive (El-Falaha *et al.*, 1983; Russell & Furr, 1986a,b; Russell *et al.*, 1986; Russell & Chopra, 1996). Contamination of solutions of QACs with Gram-negative bacteria has often been reported (Frank & Schaffner, 1976; Kaslow *et al.*, 1976).

Viruses are more resistant than bacteria or fungi to the QACs. This is clearly shown in the excellent review of Grossgebauer (1970), who points out that the QACs have a high protein defect, and that, whereas they are active against lipophilic viruses (such as herpes simplex, vaccinia, influenza and adenoviruses), they have only a poor effect against viruses (enteroviruses, e.g. poliovirus, coxsackievirus and echovirus) that show hydrophilic properties. The viricidal properties of QACs and other biocides are considered in detail in Chapter 9.

The QACs possess antifungal properties, although they are fungistatic rather than fungicidal (for a review, see D'Arcy, 1971). This applies not only to the monoquaternary compounds, but also to the bisonium compounds, such as hedaquinium and dequalinium (section 10; see also Chapter 7).

The Ferguson principle stipulates that compounds with the same thermodynamic activity will exert equal effects on bacteria. Weiner *et al.* (1965) studied the activity of three QACs (dodecyltrimethylammonium chloride, dodecyldimethylammonium chloride and dodecylpyridinium chloride) against *E. coli*, *Staph. aureus* and *Candida albicans*, and correlated these results with the surface properties of these agents. A clear relationship was found between the thermodynamic activity (expressed as a ratio of the surface concentration produced by a solution and the surface concentration at the critical micelle concentration (CMC)) and antibacterial activity.

Because most QACs are mixtures of homologues, Laycock and Mulley (1970) studied the antibacterial activity of mono- and multicomponent solutions, using the homologous series *n*-dodecyl, *n*-tetradecyl and *n*-hexadecyl trimethylammonium bromides individually, binary systems containing C_{12}/C_{14} or C_{14}/C_{16} mixtures, and a ternary mixture (centrimide) of the $C_{12}/C_{14}/C_{16}$ compounds. Antibacterial activity was measured as the concentrations needed to produce survivor levels of 1.0 and 0.01%; CMC was measured by the surface-tension method. In almost every instance, the thermodynamic activity (CMC/concentration to produce a particular survivor level) producing an equivalent biological response was reasonably constant, thereby supporting the Ferguson principle for these micelle-forming QACs.

QACs are incompatible with a wide range of chemical agents, including anionic surfactants (Richardson & Woodford, 1964), non-ionic surfactants, such as lubrols and Tweens, and phospholipids, such as lecithin and other fat-containing substances. Benzalkonium chloride has been found to be incompatible with the ingredients of some commercial rubber mixes, but not with silicone rubber; this is important when benzalkonium chloride is employed as a preservative in multipledose eye-drop formulations (*Pharmaceutical Codex* 1994, *British Pharmacopoeia*, 2002).

Although non-ionic surfactants are stated above to inactivate QACs, presumably as a consequence of micellar formation (see Elworthy, 1976, for a useful description of micelles), nevertheless potentiation of the antibacterial activity of the QACs by means of low concentrations of non-ionic agents has been reported (Schmolka, 1973), possibly as a result of increased cellular permeability induced by the non-ionic surfactant (see Chapter 3 for a more detailed discussion).

The antimicrobial activity of the QACs is

affected greatly by organic matter, including milk, serum and faeces, which may limit their usefulness in practice. The uses of the QACs are considered below (section 6.1.3) and also in more general terms in section 20.1. They are more effective at alkaline and neutral pH than under acid conditions. The action of benzalkonium chloride on *P. aeruginosa* is potentiated by aromatic alcohols, especially 3-phenylpropanol (Richards & McBride, 1973).

6.1.3 Uses

The QACs have been recommended for use in food hygiene in hospitals (Kelsey & Maurer, 1972) and are frequently used in food processing industries. Resistance to benzalkonium chloride among food associated Gram-negative bacteria and *Enterococcus* spp is not common but if such disinfectants are used at sub-lethal concentrations resistance may occur (Singh *et al.*, 2002). Benzalkonium chloride has been employed for the preoperative disinfection of unbroken skin (0.1–0.2%), for application to mucous membranes (up to 0.1%) and for bladder and urethra irrigation (0.005%); creams are used in treating nappy (diaper) rash caused by ammonia-producing organisms, and lozenges for the treatment of superficial mouth and throat infections. In the UK, benzalkonium chloride (0.01%) is one of four antimicrobial agents officially recognized as being suitable preservatives for inclusion in eye-drop preparations (*Pharmaceutical Codex* 1994, *British Pharmacopoeia*, 2002). Benzalkonium chloride is also widely used (at a concentration of 0.001–0.01%) in hard contact lens soaking (disinfecting) solutions. The QAC is too irritant to be used with hydrophilic soft (hydrogel) contact lenses because it can bind to the lens surface, be held within the water present in hydrogels and then be released into the eye (Davies, 1980). Polyquad, a QAC used commercially in contact lens disinfectant solutions has been shown to be active against the microorganisms associated with eye infections (Codling *et al.*, 2003).

Benzethonium chloride is applied to wounds as an aqueous solution (0.1%) and as a solution (0.2%) in alcohol and acetone for preoperative skin disinfection and for controlling algal growth in swimming pools.

Cetrimide is used for cleaning and disinfecting burns and wounds and for preoperative cleansing of the skin. For general disinfecting purposes, a mixture (Savlon) of cetrimide with chlorhexidine is often employed. At pH 6, but not at pH 7.2, this product may be liable to contamination with *P. aeruginosa* (Bassett, 1971). Solutions containing 1–3% of cetrimide are employed as hair shampoos (e.g. Cetavlon P.C., a concentrate to be diluted with water before use) for seborrhoea capitis and seborrhoeic dermatitis.

Cetylpyridinium chloride is employed pharmaceutically, for skin disinfection and for antiseptic treatment of small wound surfaces (0.1–0.5% solutions), as an oral and pharyngeal antiseptic (e.g. lozenges containing 1–2 mg of the QAC) and as a preservative in emulsions. Cosmetically (see also Quack, 1976), it is used at a concentration of between 0.1 and 0.5% in hair preparations and in deodorants; lower concentrations (0.05–0.1%) are incorporated into face and shaving lotions.

In the veterinary context, the QACs have been used for the disinfection of automatic calf feeders and have been incorporated into sheep dips for controlling microbial growth in fleece and wool. They are not, however, widely used on farm sites because of the large amount of organic debris they are likely to encounter.

In general, then, the QACs are very useful disinfectants and pharmaceutical and cosmetic preservatives. Further information on their uses and antimicrobial properties is considered in Chapters 3 and 14; see also BS 6471: 1984, BS EN 1276: 1997, BS 6424: 1984 and Reverdy (1995b).

6.2 Anionic agents

Anionic surface-active agents are compounds which, in aqueous solution, dissociate into a large complex anion, responsible for the surface activity, and a smaller cation. Examples of anionic surfactants are the alkali-metal and metallic soaps, amine soaps, lauryl ether sulphates (e.g. sodium lauryl sulphate) and sulphated fatty alcohols.

Anionic surfactants have excellent detergent properties but have been generally considered to have little or no antibacterial action (Davis, 1960). This view, however, is at odds with the literature

which reported the antibacterial potential of anionic and non-ionic surfactants as far back as the 1930s. Cowles (1938) studied the bacteriostatic properties of a series of sodium alkyl sulphates and found that in general they inhibited the growth of Gram-positive bacteria but not Gram-negative bacteria. Similar findings were published by Birkeland and Steinhaus (1939) and Kabara (1978a). Baker *et al.* (1941) studied the bactericidal properties of a selection of anionic and cationic detergents and concluded that the anionics much less effective than the cationics and were only effective against the Gram-positive bacteria. Fatty acids have been shown to be active against Gram-positive but not Gram-negative bacteria (Galbraith *et al.*, 1971), Kabara (1984) has reported more recently on this topic.

The benefit of anionic detergents in use is their stability and their lack of corrosive action. They also have wetting qualities resulting in a uniform film forming over the surface to be disinfected thus producing a complete disinfecting action. Scales and Kemp (1941) investigated the germicidal properties of a range of anionic surfactants, including Triton No. 720; Aerosol OS; Aerosol OT; Aerosol DGA; and various sulphonated oils. They concluded that solutions of such commercial surfactants possessed excellent germicidal properties, particularly when the pH of the solution was acidic. Solutions of the surfactants at a pH of 4.0 possessed a germicidal action greater than that seen with sodium hypochlorite. At this pH they also found no difference in action of these anionic surfactants against Gram-positive and Gram-negative bacteria.

6.3 Non-ionic agents

These consist of a hydrocarbon chain attached to a non-polar water-attracting group, which is usually a chain of ethylene oxide units (e.g. cetomacrogols). The properties of non-ionic surfactants depend mainly on the proportions of hydrophilic and hydrophobic groups in the molecule. Other examples include the sorbitan derivatives, such as the polysorbates (Tweens).

The non-ionic surfactants are considered to have no antimicrobial properties. However, low concentrations of polysorbates are believed to affect the permeability of the outer envelopes of Gram-negative cells (Brown, 1975), which are thus rendered more sensitive to various antimicrobial agents. Non-ionic surfactants have also been shown to possess antifungal properties (Spotts & Cervantes, 1987). The non-ionic surfactant Ag-98, which is 80% octyl phenoxypolyethoxyethanol, inhibited spore germination, germ tube growth and mycelial growth of *Botrytis cinerea, Mucor piriformis* and *Penicillium expansum*. It was also observed that Ag-98 had a potentiating effect on the antifungal activity of chlorine. The effect of alcohol ethoxylates on the green algae, Chlamydomonas, has also been demonstrated (Ernst *et al.*, 1983).

Pluronic F68 (polyoxyethylene-polyoxypropylene block co-polymer) has been shown to have an effect on membrane permeabilization and the enzyme activity of a batch culture of *S. cerevisiae* (Laouar *et al.*, 1996). Similar results were also seen with Triton X-100. These effects occurred at concentrations in excess of the CMC of the surfactants, no measurable effect was seen below these concentrations. More detailed information regarding the antimicrobial activities of non-ionic (and anionic) surfactants, including structure function relationships can be found in Moore (1997).

High concentrations of Tweens overcome the activity of QACs, biguanides, parabens and phenolics. This is made use of in designing appropriate neutralizing agents (Russell *et al.*, 1979; Sutton, 1996) and is considered in more detail in Chapter 3.

6.4 Amphoteric (ampholytic) agents

Amphoteric agents are compounds of mixed anionic–cationic character. They combine the detergent properties of anionic compounds with the bactericidal properties of the cationic. Their bactericidal activity remains virtually constant over a wide pH range (Barrett, 1969) and they are less readily inactivated than QACs by proteins (Clegg, 1970). Examples of amphoteric agents are dodecyl-β-alanine, dodecyl-β-aminobutyric acid and dodecyl-di(aminoethyl)-glycine (Davis, 1960). The last-named belongs to the Tego series of compounds, the name Tego being a trade name (Goldschmidt, Essen).

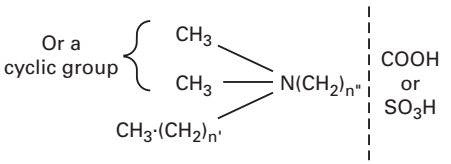


Figure 2.11 General structure of betaines ($n' = 14–16$, $n'' = 1$ or 2).

The Tego compounds are bactericidal to Gram-positive and Gram-negative bacteria, and, unlike the QACs and anionic and non-ionic agents, this includes the mycobacteria (James, 1965; Croshaw, 1971), although the rate of kill of these organisms is less than that of the others (Block, 1983). Compounds based on dodecyl-di(aminoethyl)glycine find use as disinfectants in the food industry (Kornfeld, 1966).

Betaines are a group of amphoteric surfactants, which have a similar structure to betaine or trimethylglycine itself, a natural constituent of beetroot and sugar beet obtained as a by-product of the sugar-beet industry. Such compounds are compatible with anionics and have a high water solubility (Ernst & Miller, 1982).

Analogues, in which one of the methyl groups is replaced by a long-chain alkyl residue (Fig. 2.11), find application as detergents and as a basis for solubilizing or emulsifying phenolic biocides. They have also been used in quaternary ammonium biocides (Moore & Hardwick, 1958) but are not considered as biocides *per se*.

Other chemical variants include the replacement of the –COOH group by $–SO_3H$ (Fig. 2.11) and of the two methyl groups by a ring system.

7 Aldehydes

Two aldehydes are currently of considerable importance as disinfectants, namely glutaraldehyde and formaldehyde, although others have been studied and shown to possess antimicrobial activity. Glyoxal (ethanedial), malonaldehyde (propanedial), succinaldehyde (butanedial) and adipaldehyde (hexanedial) all possess some sporicidal action, with aldehydes beyond adipaldehyde having virtually no sporicidal effects (Pepper & Chandler, 1963).

This section on aldehydes will deal mainly with glutaraldehyde and formaldehyde, although a 'newer' aldehyde, *o*-phthalaldehyde, will also be considered briefly.

7.1 Glutaraldehyde (pentanedial)

7.1.1 Chemical aspects

Glutaraldehyde is a saturated five-carbon dialdehyde with an empirical formula of $C_5H_8O_2$ and a molecular weight of 100.12. Its industrial production involves a two-step synthesis via an ethoxydihydropyran. Glutaraldehyde is usually obtained commercially as a 2, 25 or 50% solution of acidic pH, although for disinfecting purposes a 2% solution is normally supplied, which must be 'activated' (made alkaline) before use.

The two aldehyde groups may react singly or together to form bisulphite complexes, oximes, cyanohydrins, acetals and hydrazones. Polymerization of the glutaraldehyde molecule occurs by means of the following possible mechanisms:

1 The dialdehyde exists as a monomer, with an equilibrium between the open-chain molecule and the hydrated ring structure (Fig. 2.12a,b).

2 Ring formation occurs by an intramolecular mechanism, so that aqueous solutions of the aldehyde consist of free glutaraldehyde, the cyclic hemiacetal of its hydrate and oligomers of this is equilibrium (Fig. 2.12c).

3 Different types of polymers may be formed at different pH values, and it is considered that polymers in the alkaline range are unable to revert to the monomer, whereas those in the neutral and acid range revert easily (Boucher, 1974; Fig. 2.13).

Polymerization increases with a rise in pH, and above pH 9 there is an extensive loss of aldehyde groups. Glutaraldehyde is more stable at acid than alkaline pH; solutions at pH 8 and above generally lose activity within 4 weeks. Novel formulations have been produced, and continue to be designed, to overcome the problems of loss of stability (Babb *et al.*, 1980; Gorman *et al.*, 1980; Power, 1997).

7.1.2 Interactions of glutaraldehyde

Glutaraldehyde is a highly reactive molecule. It

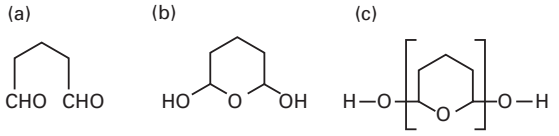


Figure 2.12 (a) Free glutaraldehyde; (b) hydrated ring structure (cyclic hemiacetal of its hydrate); (c) oligomer.

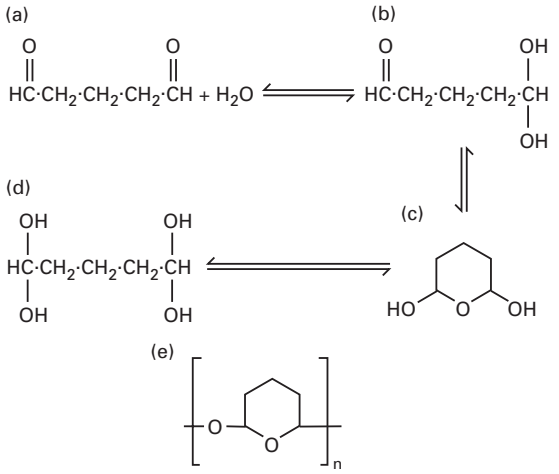


Figure 2.13 (a) Open-chain molecule of glutaraldehyde; (b), (c) and (d) formation of several more stable 'polymers' (hydrated) in aqueous alkaline solution; (e) polymer with an acetal-like structure, in neutral and acid ranges (after Boucher, 1974).

reacts with various enzymes (but does not sterically alter them to loss all activity) and with proteins; the rate of reaction is pH-dependent, increasing considerably over the pH range 4–9, and the reaction product is highly stable (Hopwood *et al.*, 1970). Glutaraldehyde prevents the dissociation of free ribosomes (Russell & Hopwood, 1976), but under the normal conditions of fixation (Hopwood, 1975) little reaction appears to occur between nucleic acids and glutaraldehyde. There is little published information on the possible reactions of glutaraldehyde and lipids (Russell & Hopwood, 1976).

7.1.3 Microbicidal activity

Glutaraldehyde possesses high microbicidal activity against bacteria and their spores, mycelial and spore forms of fungi and various types of viruses (Borick, 1968; Borick & Pepper, 1970). Although there was some doubt about its mycobactericidal potency, glutaraldehyde is now considered to be an effective antimycobacterial agent (Collins, 1986; Broadley *et al.*, 1991; Russell, 1994, 1996; Ascenzi, 1996c). The mechanism of action of glutaraldehyde involves a strong interaction with the outer cell walls of bacterial cells. This, and the mechanism of action of glutaraldehyde against viruses, spores and mycobacterium is dealt with in greater detail in McDonnell & Russell (1999). Additional information is provided in Chapter 6.4; see also Power (1997).

A summary of the antimicrobial efficacy of glutaraldehyde is presented in Table 2.7, which demonstrates the effect of pH on its activity. However, acid-based products are also available commercially which are claimed to be of equal activity to potentiated alkaline glutaraldehyde. Acid glutaraldehyde is itself an effective microbicide provided that long contact periods are used. The exact mechanism of action of the dialdehyde is unknown, but the fact that its rate of interaction with proteins and enzymes increases with increasing pH (Hopwood *et al.*, 1970; Russell & Munton, 1974) is undoubtedly of importance. The cross-linking mechanism is also influenced by time, concentration and temperature (Eager *et al.*, 1986; Bruch, 1991; Russell, 1994, 1996). Acid glutaraldehyde is a markedly inferior disinfectant to alkaline glutaraldehyde, but this discrepancy disappears with increasing temperature. Resistance development to glutaraldehyde is a late event in sporulation (Power *et al.*, 1988; Russell, 1990a,b, 1994, 1995; Knott *et al.*, 1995) and sodium hydroxide-induced revival of spores of *Bacillus* spp. has been demonstrated (Dancer *et al.*, 1989; Power *et al.*, 1989, 1990; Williams & Russell, 1992).

Organic matter is considered to have no effect on the antimicrobial activity of the aldehyde. In view of the interaction of glutaraldehyde with the amino groups in proteins, this would appear to be a rather unusual finding. It is, however, true to state that it retains a considerable degree of activity in the presence of high levels of organic matter, such as 20% serum (A.D. Russell, unpublished data).

Dried spores are considerably more resistant to

Table 2.7 Microbicidal activity of glutaraldehyde.[a]

Form of glutaraldehyde	*Approximate pH value*	*Fungicidal activity*[b]	*Viricidal activity*	*Bactericidal activity*[c]	*Sporicidal activity*
Acid	4–5	Low	Low to high	Low	Low to very high
Alkaline	8	High	High	High	Reasonable to very high

[a]See also Gorman *et al.* (1980), Russell (1994) and Favero (1995).

[b]Use of low dialdehyde concentrations (0.01–0.02%); 2 % solutions of acid and alkaline glutaraldehyde are both highly active against bacteria and probably viruses. A high-concentration (3.2%) glutaraldehyde solution is also available (Akamatsu *et al.*, 1997).

[c]Activity of acid glutaraldehyde increases markedly with temperature and at *c.* 37 °C its activity approaches that of alkaline glutaraldehyde. Acid glutaraldehyde may also be sporicidal at ambient temperatures, provided that long periods of time (*c.* 10 h) are used (Rutala *et al.*, 1993a,b).

chemical disinfectants than are spores in suspension, and it would appear that glutaraldehyde is no exception. The use of the Association of Official Analytical Chemists (AOAC) test with dried spores of *B. subtilis* has shown that 2% alkaline glutaradehyde may require up to 10 h to achieve sterilization at 20 °C (Rubbo *et al.*, 1967).

The antimicrobial activity of glutaraldehyde has been reviewed by Gorman *et al.* (1980), Bruch (1991), Russell (1994), Ascenzi (1996b) and Power (1997).

7.1.4 Uses of glutaraldehyde

Glutaraldehyde has been recommended for the disinfection/sterilization of certain types of medical equipment, notably cystoscopes and anaesthetic equipment. Favero and Bond (1991) have rightly drawn attention to the differences between physical methods of sterilization and liquid chemical germicides and point out that 2% alkaline glutaraldehyde is capable of acting as a sterilizing agent but only after prolonged periods of contact. Bearing this comment in mind, glutaraldehyde has long been used for the high-level disinfection of endoscopes, although problems have arisen because of its toxicity. Glutaraldehyde has also been employed for the disinfection of arthroscopes and laparoscopes (Loffer, 1990).

As pointed out, alkaline glutaraldehyde is more active, but less stable, than the acid form. However, 2% activated alkaline glutaraldehyde should not be used continuously to disinfect endoscopes for 14 days after activation, although it is effective over this period if not repeatedly reused (Babb, 1993; Babb & Bradley, 1995). These authors recommend reuse for endoscopes provided that the concentration does not fall appreciably below 1.5%.

Problems in reusing glutaraldehyde are associated with accumulation of organic matter, dilution of disinfectant, change in product pH and difficulties in accurately assaying residual concentrations (Mbithi *et al.*, 1993; Rutala & Weber, 1995; Springthorpe *et al.*, 1995). Colour indicators are not always satisfactory (Power & Russell, 1988). Glutaraldehyde has been employed in the veterinary field for the disinfection of utensils and of premises (Russell & Hugo, 1987), but its potential mutagenic and carcinogenic effects (Quinn, 1987) make these uses hazardous to personnel. The main advantages claimed for glutaraldehyde are as follows: it has a broad spectrum of activity with a rapid microbicidal action, and it is non-corrosive to metals, rubber and lenses. Its toxicity (see above) remains a problem and as such is no longer used in some countries.

Peracetic acid (discussed in further detail in section 12.2) is considered a suitable alternative disinfectant to glutaraldehyde as only oxygen and water are produced on decomposition and it has a broad spectrum of activity. As a very strong oxidizing agent, peracetic acid can be corrosive to some metals. However, commercial formulations have been shown to reduce this problem (Mannion,

1995). One such formulation is 'Nu-Cidex' (Johnson & Johnson, USA), a buffered peracetic acid solution, which has been demonstrated to be an effective mycobactericidal agent (Middleton *et al.*, 1997; Griffiths *et al.*, 1999).

Ortho-phthaladehyde is also a suitable alternative to glutaraldehyde. It has been demonstrated to be an effective bactericidal agent (Alfa & Sitter, 1994), with activity also demonstrated against mycobacteria (Walsh *et al.*, 2001). This agent is discussed in further detail in section 7.3.

7.2 Formaldehyde (methanal)

Formaldehyde is used as a disinfectant as a liquid or vapour. Gaseous formaldehyde is referred to briefly in section 18 and in more detail in Chapter 12.3. The liquid form will be considered mainly in this section.

The Health and Safety Executive of the UK has indicated that the inhalation of formaldehyde vapour may be presumed to pose a carcinogenic risk to humans. This indication must have considerable impact on the consideration of the role and use of formaldehyde and formaldehyde releasers in sterilization and disinfection processes.

7.2.1 Chemical aspects

Formaldehyde occurs as formaldehyde solution (formalin), an aqueous solution containing *c.* 34–38% w/w CH_2O. Methyl alcohol is present to delay polymerization. Formaldehyde displays many typical chemical reactions, combining with amines to give methylolamines, carboxylic acids to give esters of methylene glycol, phenols to give methylphenols and sulphides to produce thiomethylene glycols.

7.2.2 Interactions of formaldehyde

Formaldehyde interacts with protein molecules by attaching itself to the primary amide and amino groups, whereas phenolic moieties bind little of the aldehyde (Fraenkel-Conrat *et al.*, 1945). Subsequently, it was shown that formaldehyde gave an intermolecular cross-linkage of protein or amino groups with phenolic or indole residues.

In addition to interacting with many terminal groups in viral proteins, formaldehyde can also react extensively with the amino groups of nucleic acid bases, although it is much less reactive with deoxyribonucleic acid (DNA) than with ribonucleic acid (RNA) (Staehelin, 1958).

7.2.3 Microbicidal activity

Formaldehyde is a microbicidal agent, with lethal activity against bacteria and their spores, fungi and many viruses. Its first reported use as a disinfectant was in 1892. Its sporicidal action is, however, slower than that of glutaraldehyde (Rubbo *et al.*, 1967). Formaldehyde combines readily with proteins and is less effective in the presence of protein organic matter. Plasmid-mediated resistance to formaldehyde has been described, presumably due to aldehyde degradation (Heinzel, 1988). Formaldehyde vapour may be released by evaporating formalin solutions, by adding potassium permanganate to formalin or alternatively by heating, under controlled conditions, the polymer paraformaldehyde ($HO(CH_2O)_nH$), urea formaldehyde or melamine formaldehyde (Tulis, 1973). The activity of the vapour depends on aldehyde concentration, temperature and relative humidity (r.h.) (section 18.2).

7.2.4 Formaldehyde-releasing agents

Noxythiolin (oxymethylenethiourea; Fig. 2.14a) is a bactericidal agent (Kingston, 1965; Wright & McAllister, 1967; Browne & Stoller, 1970) that apparently owes its antibacterial activity to the release of formaldehyde (Kingston, 1965; Pickard, 1972; cf. Gucklhorn, 1970):

$$CH_3 \cdot NH \cdot CS \cdot NH \cdot CH_2OH$$
$$CH_3 \cdot NH \cdot CS \cdot NH_2H + CHO$$

Noxythiolin has been found to protect animals from lethal doses of endotoxin (Wright & McAllister, 1967; Haler, 1974) and is claimed to be active against all bacteria, including those resistant to other types of antibacterial agents (Browne & Stoller, 1970).

Noxythiolin has been widely used both topically and in accessible body cavities, notably as an irriga-

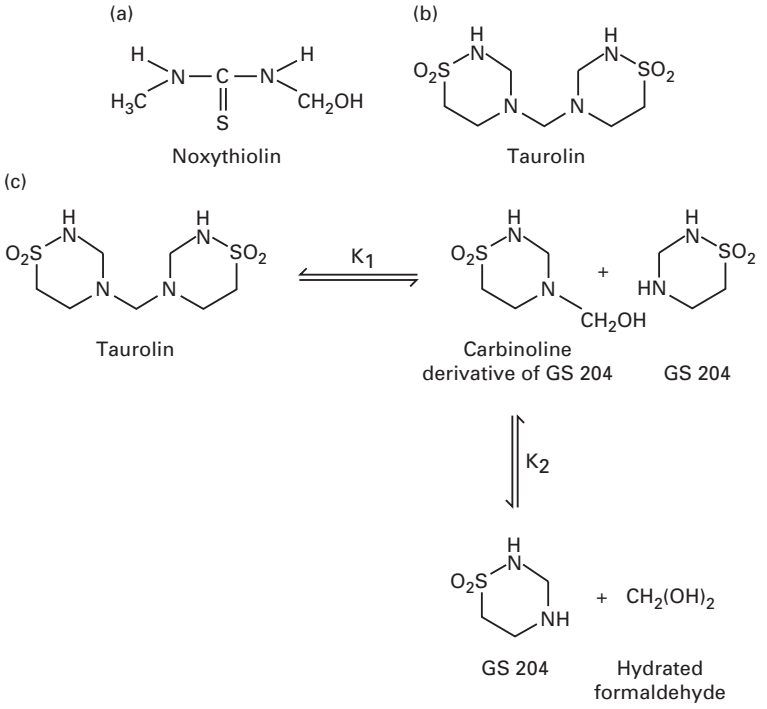


Figure 2.14 (a) Noxythiolin; (b) taurolin; (c) postulated equilibrium of taurolin in aqueous solution (after Myers *et al.*, 1980).

tion solution in the treatment of peritonitis (Pickard, 1972). Unfortunately, solutions are rather unstable (after preparation they should be stored at 10 °C and used within 7 days). Commercially, noxythiolin is available as Noxyflex S and Noxyflex (Geistlich Ltd., Chester, UK), the latter containing amethocaine hydrochloride as well as noxythiolin. Solutions of Noxyflex (containing 1 or 2.5% noxythiolin) are employed where local discomfort is experienced.

More recently, the amino acid taurine has been selected as the starting-point in the design of a new antibacterial agent, taurolin (Fig. 2.14b), which is a condensate of two molecules of taurine and three molecules of formaldehyde. Taurolin (bis-(1, 1-dioxoperhydro-1,2,4-thiazinyl-4)methane) is water-soluble and is stable in aqueous solution. It has a wide spectrum of antimicrobial activity *in vitro* and *in vivo* (Reeves & Schweitzer, 1973; Browne *et al.*, 1976, 1977, 1978).

Taurine is considered to act as a non-toxic formaldehyde carrier, donating methylol groups to bacterial protein and endotoxin (Browne *et al.*, 1976). According to these authors, taurine has a lower affinity for formaldehyde than bacterial protein, but a greater affinity than animal protein, the consequence of which is a selective lethal effect. Taurolin has been shown to protect experimental animals from the lethal effects of *E. coli* and *Bacteroides fragilis* endotoxin (Pfirrman & Leslie, 1979).

This viewpoint that the activity of taurolin results from a release of formaldehyde which is adsorbed by bacterial cells is, however, no longer tenable. When taurolin is dissolved in water (Myers *et al.*, 1980), an equilibrium is established (Fig. 2.14c) to release two molecules of the monomer (1,1-dioxoperhydro-1,2,4-thiadizine (GS 204)) and its carbinolamine derivative. The antibacterial activity of taurolin is considerably greater than that of free formaldehyde (Myers *et al.*, 1980; Allwood & Myers, 1981) and these authors thus concluded that the activity of taurolin was not due entirely to bacterial adsorption of free formaldehyde but also to a reaction with a masked (or latent) formaldehyde. Since GS 204 has only a low antibacterial effect, then the carbinolamine must obviously play an important role.

Clinically, the intraperitoneal administration of taurolin has been shown to bring about a significant reduction of morbidity in peritonitis (Browne *et al.*, 1978).

A third formaldehyde-releasing agent is hexamine (methenamine); hexamine itself is inactive but it breaks down by acid hydrolysis to release formaldehyde. It has been reviewed by Allwood and Myers (1981). Derivatives of hexamine are considered in section 17.4 and other formaldehyde-releasing agents in sections 17.2 (imidazole derivatives), 17.5 (triazines) and 17.6 (oxazolo-oxazoles). Table 2.11 should also be consulted, as well as section 18.2 (which deals with release of gaseous formaldehyde) and Paulus (1976).

7.2.5 Uses of formaldehyde

Formaldehyde is employed as a disinfectant in both the liquid and gaseous states. Vapour-phase formaldehyde is used in the disinfection of sealed rooms; the vapour can be produced as described above, or alternatively an equal volume of industrial methylated spirits (IMS) can be added to formaldehyde and the mixture used as a spray. Other uses of formaldehyde vapour have been summarized by Russell (1976). These include the following: low-temperature steam plus formaldehyde vapour (LTSF) for the disinfection/sterilization of heat-sensitive medical materials (see also Chapter 12.1); hospital bedding and blankets; and fumigation of poultry houses, of considerable importance in hatchery hygiene (Anon., 1970).

Aerobic spores exposed to liquid formaldehyde can be revived by a sublethal post-heat treatment (Spicher & Peters, 1976, 1981). Revival of LTSF-treated *B. stearothermophilus* spores can also be accomplished by such means (Wright *et al.*, 1996), which casts considerable doubt on the efficacy of LTSF as a potential sterilizing process.

Formaldehyde in liquid form has been used as a viricidal agent in the production of certain types of viral vaccines, such as polio (inactivated) vaccine. Formaldehyde solution has also been employed for the treatment of warts, as an antiseptic mouthwash, for the disinfection of membranes in dialysis equipment and as a preservative in hair shampoos. Formaldehyde-releasing agents were considered in section 7.2.4. Formaldehyde and formaldehyde condensates have been reviewed in depth by Rossmore and Sondossi (1988).

7.3 *Ortho*-phthalaldehyde

o-Phthalaldehyde (Fig. 2.15) is an aromatic aldehyde. It has been shown to have potent bactericidal and viricidal activity (Alfa & Sitter, 1994; Gregory *et al.*, 1999, Walsh *et al.*, 1999a,b, 2001; Rutala *et al.*, 2001). Studies have been carried out in an attempt to elucidate the possible mechanisms of action of *o*-phthalaldehyde against Gram-positive and Gram-negative bacteria (Walsh *et al.*, 1999b) and mycobacteria (Walsh *et al.*, 2001). *o*-Phthalaldehyde has been shown to interact with amino acids, proteins and microorganisms although not as effectively as glutaraldehyde. However, *o*-phthalaldehyde is lipophilic aiding uptake through the cell walls of Gram-negative bacteria and mycobacteria compensating for its lower cross-linking ability compared with glutaraldehyde (Simons *et al.*, 2000).

Some sporicidal activity has also been reported for this agent but activity is not as great as that seen against vegetative cells. The spore coat appears to be a significant factor in this reduced activity but is not the only factor as *o*-phthalaldehyde appears to demonstrate sporicidal activity by blocking the spore germination process (Cabrera-Martinez *et al.*, 2002).

7.4 Other aldehydes

Other aldehydes have been studied but results have sometimes been conflicting and they have thus been reinvestigated (Power & Russell, 1990). Sporidicin, used undiluted and containing 2% glutaraldehyde plus 7% phenol and 1.2% phenate, is slightly more active against spores than is 2% activated, alkaline glutaraldehyde. Gigasept, containing butan-1,4-dial, dimethoxytetrahydrofuran and formalde-

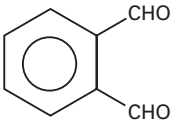


Figure 2.15 *Ortho*-phthalaldehyde (OPA).

hyde, and used at 5% and 10% v/v dilutions, is considerably less active (Power & Russell, 1990). Glyoxal (2%) is weakly sporicidal, and butyraldehyde has no activity. It is essential that adequate procedures are employed to remove residual glutaraldehyde (and phenol/phenate, if present) or other aldehyde in determining survivor levels. This has not always been appreciated (Pepper, 1980; Leach, 1981; Isenberg, 1985).

The properties and uses of various aldehydes have been reviewed by Bartoli and Dusseau (1995).

8 Antimicrobial dyes

There are three main groups of dyes, which find application as antimicrobial agents: the acridines, the triphenylmethane group and the quinones. Halogenated fluorescein (hydroxyxanthene) dyes have also been demonstrated to possess antimicrobial activity against *Staph. aureus* (Rasooly & Weisz, 2002). This study demonstrated that activity against the test organism increases with increasing number of substituted halogens in the hydroxanthene moiety.

8.1 Acridines

8.1.1 Chemistry

The acridines (Fig. 2.16) are heterocyclic compounds that have proved to be of some value as antimicrobial agents. Acridine itself is feebly basic, but two of the five possible monoaminoacridines are strong bases, and these (3-aminoacridine and 9-aminoacridine) exist as the resonance hybrid of two canonical formulae. Both these monoacridines are well ionized as the cation at pH 7.3, and this has an important bearing on their antimicrobial activity (see below and Table 2.8). Further information on the chemistry of the acridines can be found in Albert's excellent book (Albert, 1966) and Wainright's comprehensive review (Wainwright, 2001).

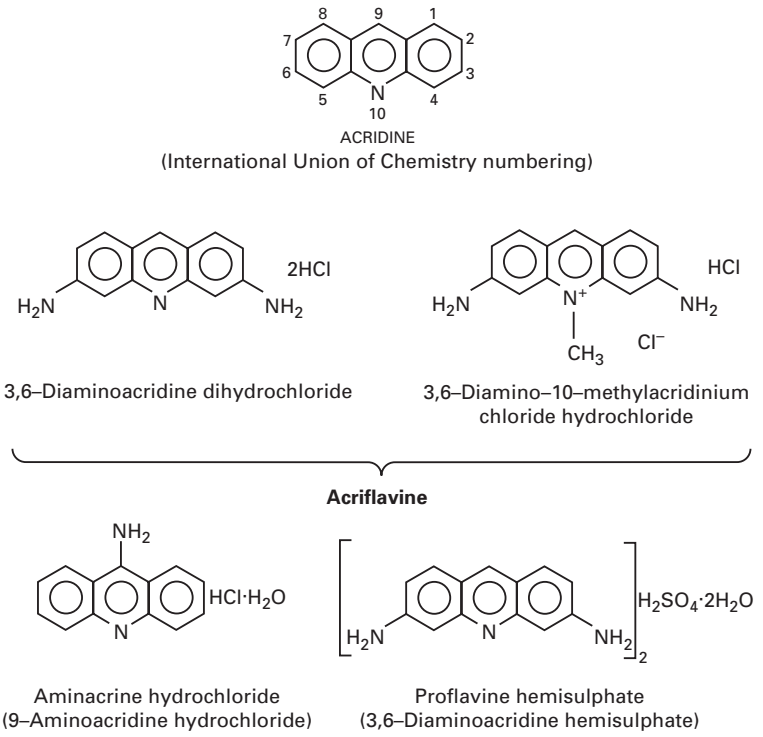


Figure 2.16 Acridine compounds.

Table 2.8 Dependence of antibacterial activity of acridines on cationic ionization (based on the work of Albert and his colleagues (see Albert, 1966)).

Substance	*Predominant type (and percentage) of ionization at pH 3 and 37 °C*	*Inhibitory activity*
9-Aminoacridine	Cation (99%)	High
9-Aminoacridine-2-carboxylic acid	Zwitterion (99.8%)	Low
Acridine	Neutral molecule (99.7%)	Low
Acridine-9-carboxylic acid	Anion (99.3%)	Low

8.1.2 Antimicrobial activity

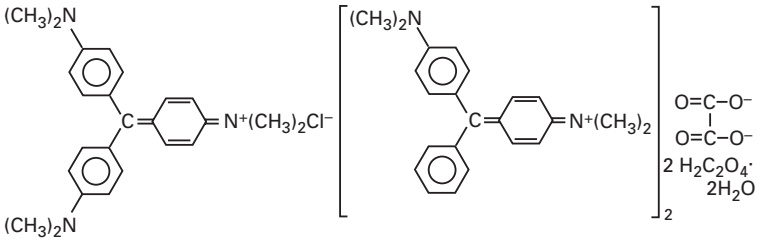
The acridines are of considerable interest because they illustrate how small changes in the chemical structure of the molecule cause significant changes in antibacterial activity. The most important limiting factor governing this activity is the degree of ionization, although this must be cationic in nature (Table 2.8). Acridine derivatives that form anions or zwitterions are only poorly antibacterial in comparison with those that form cations. In general terms, if the degree of ionization is less than 33% there is only feeble antibacterial activity, whereas above about 50% there is little further increase in activity (Albert, 1966). Acridines do not display a selective action against Gram-positive organisms, nor are they inactivated by serum. Acridines compete with H^+ ions for anionic sites on the bacterial cell and are more effective at alkaline than acid pH (Browning *et al.*, 1919–20). They are relatively slow in their action and are not sporicidal (Foster & Russell, 1971). Resistance to the acridines develops as a result of mutation and indirect selection (Thornley & Yudkin, 1959a,b). Interestingly, acridines can eliminate ('cure') resistance in R^+ strains (see Watanabe, 1963, for an early review). Viljanen and Boratynski (1991) provide more recent information about plasmid curing. The antimicrobial activity of aminoacridines has been shown to be increased on illumination with low power white light (Wainwright *et al.*, 1997, 1998). However, attempts at increasing the degree of bacterial DNA intercalation and hence antimicrobial activity by synthesis of dimeric bis(aminoacridines) did not lead to increased activity (Wainwright *et al.*, 1998). MRSA and methicillin-resistant *Staphylococcus epidermidis* (MRSE) strains are more resistance to acridines than are antibiotic-sensitive strains, although this resistance depends on the presence of *qac* genes, especially *qac*A or *qac*B (Littlejohn *et al.*, 1992; Leelaporn *et al.*, 1994).

8.1.3 Uses

For many years, the acridines held a valuable place in medicine. However, with the advent of antibiotics and other chemotherapeutic agents, they are now used infrequently. Their major use has been the treatment of infected wounds. The first compound to be used medically was acriflavine (a mixture of 3,6-diaminoacridine hydrochloride and 3,6-diamino-10-methylacridinium hydrochloride, the former component being better known as proflavine). Proflavine hemisulphate and 9-aminoacridine (aminacrine) have found use in treating wounds; aminacrine is particularly useful as it is non-staining.

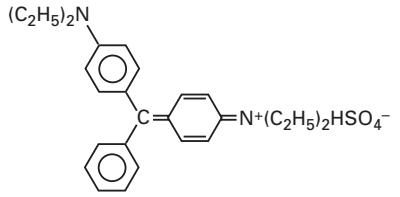
8.2 Triphenylmethane dyes

The most important members of this group are crystal violet, brilliant green and malachite green (Fig. 2.17). These were used as local antiseptics for application to wounds and burns, but were limited in being effective against Gram-positive bacteria (inhibitory concentrations 1 in 750 000 to 1 in 5 000 000) but much less so against Gram-negative organisms, and in suffering a serious decrease in activity in the presence of serum. Their selective activity against Gram-positive bacteria has a practical application in the formulation of selective media for diagnostic purposes, e.g. crystal violet lactose broth in water filtration-control work.



Crystal violet
(methyl violet; gentian violet)

Malachite green



Brilliant green

Figure 2.17 Triphenylmethane dyes.

The activity of the triphenylmethane dyes is a property of the pseudobase, the formation which is established by equilibrium between the cation and the base; thus, both the ionization and the equilibrium constants will affect the activity (Albert, 1966). Antimicrobial potency depends on external pH, being more pronounced at alkaline values (Moats & Maddox, 1978).

MRSA and MRSE strains containing *qac* genes are more resistant to crystal violet than are plasmidless strains of *Staph. aureus* and *Staph. epidermidis*, respectively (Littlejohn *et al.*, 1992; Leelaporn *et al.*, 1994). This is believed to be the result of an efficient efflux system in the resistant strains (Paulsen *et al.*, 1996a,b). However, crystal violet finds little, if any, use nowadays as an antibacterial agent, and the clinical relevance of this finding thus remains uncertain (Russell & Chopra, 1996; Russell, 1997).

8.3 Quinones

Quinones are natural dyes, which give colour to many forms of plant and animal life. Chemically (Fig. 2.18), they are diketocyclohexadienes; the simplest member is 1,4-benzoquinone. In terms of toxicity to bacteria, moulds and yeast, naphthaquinones are the most toxic, followed (in this order) by phenanthrenequinones, benzoquinones and anthraquinones.

Antimicrobial activity is increased by halogenation and two powerful agricultural fungicides are chloranil (tetrachloro-1,4-benzoquinone) and dichlone (2,3-dichloro-1,4-naphthaquinone); see D'Arcy (1971) and Owens (1969).

9 Halogens

The most important microbicidal halogens are iodine compounds, chlorine compounds and bromine. Fluorine is far too toxic, irritant and corrosive for use as a disinfectant (Trueman, 1971), although, interestingly, fluoride ions have been shown to induce bacterial lysis (Lesher *et al.*, 1977). This section will deal predominantly with iodine, iodophors and chlorine-releasing compounds (those which are bactericidal by virtue of 'available chlorine'), but bromine, iodoform and chloroform will be considered briefly. A more detailed treatment of the chemistry, antibacterial activity and uses of chlorine and chlorine based biocides can be found in Khanna and Naidu (2000).

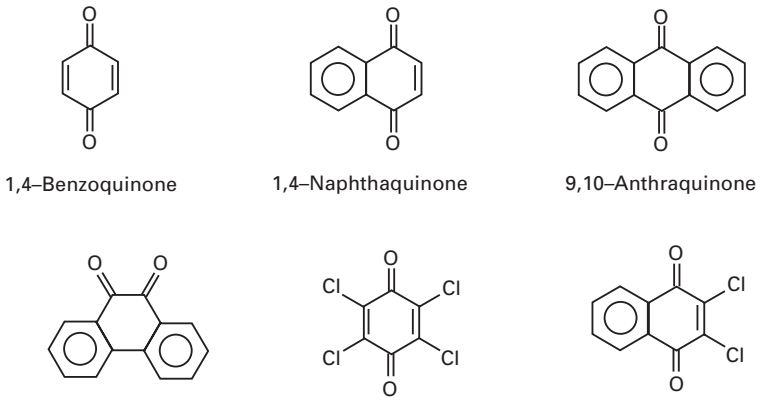


Figure 2.18 Quinones.

Table 2.9 Effect of pH on the antimicrobial activity of iodine compounds (based on Trueman, 1971).

pH	*Active form*	*Comment*
Acid and neutral	I_2(diatomic iodine)	Highly bactericidal
	Hypo-iodous acid	Less bactericidal
Alkaline	Hypo-iodide ion	Even less bactericidal
	Iodate (IO_3^-), iodide (I^-) and tri-iodide (I_3^-) ions	All inactive

9.1 Iodine compounds

9.1.1 Free iodine

Iodine was first employed in the treatment of wounds some 140 years ago and has been shown to be an efficient microbicidal agent with rapid lethal effects against bacteria and their spores, moulds, yeasts and viruses (Gershenfeld, 1956; Anon., 1965; Sykes, 1965; Russell, 1971b; Kelsey & Maurer, 1972). It is normally used in aqueous or alcoholic solution; it is only sparingly soluble in cold water but solutions can be made with potassium iodide. Iodine is less reactive chemically than chlorine, and is less affected by the presence of organic matter than is the latter; however, it must be added that, whereas the activity of high concentrations of iodine is little affected by organic matter, that, of low concentrations is significantly lowered. The activity of iodine is greater at acid than at alkaline pH; see Table 2.9. Unfortunately, iodine solutions stain fabric and tend to be toxic.

The antimicrobial activity of iodine incorporated in an enzyme-based disinfectant have been reported by Duan *et al.* (1999). This disinfectant is a powder concentrate composed of sodium iodide, horseradish peroxidase, citric acid and calcium peroxide. Horseradish peroxidase catalyses the oxidation of iodide to molecular iodine in the presence of water:

$$2H^+ + 2I^- + 2H_2O_2 \rightarrow I_2 + 2H_2O$$

This system is able to reoxidize reduced iodine giving the advantage of a controlled and continuous release of active iodine and demonstrates rapid bactericidal, fungicidal and virucidal activity.

9.1.2 Iodophors

Certain surface-active agents can solubilize iodine

to form compounds (the iodophors) that retain the germicidal action but not the undesirable properties of iodine. The uses of the iodophors as detergent-sterilizers have been described by Blatt and Maloney (1961) and Davis (1962). It must be noted that different concentrations of iodophors are used for antiseptic and disinfectant purposes, and that the lower concentrations employed in antisepsis are not claimed to be sporicidal (Favero, 1985, 1995).

Gershenfeld (1962) has shown that povidone–iodine is sporicidal, and Lowbury *et al.* (1964) found that povidone–iodine compresses reduced the numbers of viable spores of *Bacillus globigii* on the skin by >99% in 1 h, suggesting that this iodophor had a part to play in removing transient sporing organisms from operation sites. The importance of povidone–iodine in preventing wound infection was re-emphasized as a result of the studies of Galland *et al.* (1977) and Lacey (1979). Povidone–iodine has been shown to be effective against a range of MRSA, *Chlamydia,* Herpes simplex, adenoviruses and enteroviruses (Reimer *et al.*, 2002) and produced significant reductions in skin microflora, demonstrating its suitability as a pre-surgical hand treatment (Darwish, 2002). More in-depth information regarding the physical properties and antimicrobial activity of povidone–iodine can be found in Barabas and Brittain (1998).

The concentration of free iodine in aqueous or alcoholic iodine solutions is responsible for microbicidal activity. Likewise, the concentration of free iodine in an iodophor is responsible for its activity (Allawala & Riegelman, 1953).

In most iodophor preparations, the carrier is usually a non-ionic surfactant, in which the iodine is present as micellar aggregates. When an iodophor is diluted with water, dispersion of the micelles occurs and most (80–90%) of the iodine is slowly liberated. Dilution below the CMC of the non-ionic surface-active agent results in iodine being in simple aqueous solution. A paradoxical effect of dilution on the activity of povidone–iodine has been observed (Gottardi, 1985; Rackur, 1985). As the degree of dilution increases, then beyond a certain point bactericidal activity also increases. An explanation of this arises from consideration of physicochemical studies, which demonstrate that, starting from a 10% commercially available povidone–iodine solution, the concentration of non-complexed iodine (I_2) initially increases as dilution increases. This reaches a maximum value at about 0.1% and then falls. In contrast, the content of other iodine species (e.g. I^- and I_3^-) decreases continuously. These properties affect the sporicidal activity of iodine solutions (Williams & Russell, 1991).

The iodophors, as stated above, are microbicidal, with activity over a wide pH range. The presence of a surface-active agent as carrier improves the wetting capacity. Iodophors may be used in the dairy industry (when employed in the cleansing of dairy plant it is important to keep the pH on the acid side to ensure adequate removal of milkstone) and for skin and wound disinfection. Iodophors, such as Betadine, in the form of alcoholic solutions are widely used in the USA for disinfection of hands before invasive procedures such as operations and obstetrics (see also Chapter 18). Leung *et al.* (2002) demonstrated that warming povidone–iodine for use before amniocentesis to increase patient comfort still gives the desired level of antimicrobial efficacy. Pseudobacteraemia (false-positive blood cultures) has been found to result from the use of contaminated antiseptics. Craven *et al.* (1981) have described such an outbreak of pseudobacteraemia caused by a 10% povidone–iodine solution contaminated with *Burkholderia cepacia*.

The properties, antimicrobial activity, mechanisms of action and uses of iodine and its compounds have been described by Rutala (1990), Favero & Bond (1991), Banner (1995), Favero (1995) and Bloomfield (1996). Information about the revival of iodine-treated spores of *B. subtilis* is provided by Williams & Russell (1992, 1993a,b,c).

9.1.3 Iodoform

When applied to tissues, iodoform (CHI_3) slowly releases elemental iodine. It thus has some weak antimicrobial activity. It is not often used in practice, and thus will not be considered further.

9.2 Chlorine compounds

9.2.1 Chlorine-releasing compounds

Until the development of chlorinated soda solution,

surgical (Dakin's solution), in 1916, the commercial chlorine-releasing disinfectants then in use were not of constant composition and contained free alkali and sometimes free chlorine. The stability of free available chlorine in solution is dependent on a number of factors, especially (1) chlorine concentration, (2) pH of organic matter and (3) light (Dychdala, 1983).

The types of chlorine compounds that are most frequently used are the hypochlorites and *N*-chloro compounds (Trueman, 1971; Dychdala, 1983; Gardner & Peel, 1986, 1991; Favero & Bond, 1991; Bloomfield & Arthur, 1994; Banner 1995; Favero, 1995; Bloomfield, 1996). Chlorine compounds are commonly used as sanitizing agents in the food industry due to their high antimicrobial efficacy, low toxicity to humans, range of applications and low cost but suffer the disadvantages of being irritant and corrosive. The organochlorines are less irritating and corrosive than inorganic chlorines and have a greater stability but are slower acting in terms of bactericidal efficacy by comparison (Khanna & Naidu, 2000).

Hypochlorites have a wide antibacterial spectrum, although they are less active against spores than against non-sporulating bacteria and have been stated to be of low activity against mycobacteria (Anon., 1965; Croshaw, 1971). Recent studies have suggested that chlorine compounds are among the most potent sporicidal agents (Kelsey *et al.*, 1974; Coates & Death, 1978: Death & Coates, 1979; Coates & Hutchinson. 1994). The hypochlorites show activity against lipid and non-lipid viruses (Morris & Darlow 1971; Favero, 1995; Bloomfield, 1996; see Chapter 9).

Two factors that can affect quite markedly their antimicrobial action are organic matter, since chlorine is a highly reactive chemical, and pH, the hypochlorites being more active at acid than at alkaline pH (Table 2.10); acid pH promotes the hydrolysis of HOCl (Khanna & Naidu, 2000). The former problem can, to some extent, be overcome by increasing the hypochlorite concentration, and it has been shown that the sporicidal activity of sodium hypochlorite (200 parts/10^6 available chlorine) can be potentiated by 1.5–4% sodium hydroxide, notwithstanding the above comment about pH (Russell, 1971b, 1982). The sporicidal activity can also be potentiated by low concentrations of ammonia (Weber & Levine, 1944) and in the presence of bromine (Farkas-Himsley, 1964); chlorine-resistant bacteria have been found to be unaffected by bromine but to be readily killed by chlorine-bromine solutions (Farkas-Himsley, 1964). Such mixtures could be of value in the disinfection of natural waters.

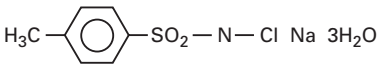
Organic chlorine compounds (*N*-chloro compounds), which contain the =N–Cl group, show microbicidal activity. Examples of such compounds, the chemical structures of which are shown in Fig. 2.19, are chloramine-T, dichloramine-T, halazone, halane, dichloroisocyanuric acid, sodium and potassium dichloroisocyanurates and trichloroisocyanuric acid. All appear to hydrolyse in water to produce an imino (=NH) group. Their action is claimed to be slower than that of the hypochlorites,

Table 2.10 Factors influencing activity of hypochlorites.

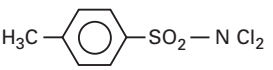
Factor	*Result*
pH	Activity decreased by increasing pH (see text and use of NaOH also)
Concentration of hypochlorite (pH constant)	Activity depends on concentration of available chlorine
Organic matter	Antimicrobial activity reduced considerably
Other agents	Potentiation may be achieved by: (1) addition of ammonia (2) 1.5–4% sodium hydroxide[a] (3) addition of small amounts of bromide[b]

[a]Cousins & Allan (1967).

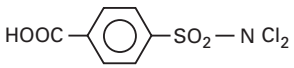
[b]In the presence of bromide, hypochlorite also has an enhanced effect in bleaching cellulosic fibres.



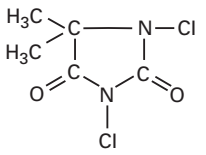
Chloramine T
(sodium-*p*-toluene-sulphonchloramide)



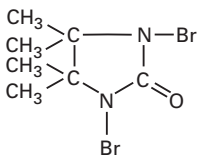
Dichloramine T
(*p*-toluene-sulphondichloramide)



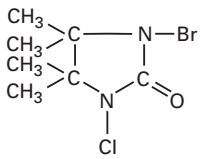
Halazone
(*p*-sulphondichloramide benzoic acid)



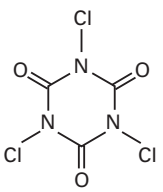
Halane



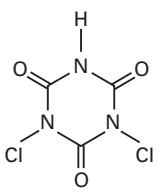
1,3-dibromo-4,4,5,5-
tetramethyl-2-imidazoldinone



1-bromo-3-chloro-4,4,5,5-
tetramethyl-2-imidazolidinone



Trichloroisocyanuric acid



Dichloroisocyanuric acid

Figure 2.19 Organic chlorine compounds.

although this can be increased under acidic conditions (Cousins & Allan, 1967). A series of imidazolidinone *N′*,*N′*-dihalamine disinfectants has been described (Williams *et al.*, 1987, 1988; Worley *et al.*, 1987). The dibromo compound (Fig. 2.19) was the most rapidly acting bactericide, particularly under halogen demand-free conditions, with the mixed bromo–chloro compound (Fig. 2.19) occupying an intermediate position. However, when stability of the compounds in the series was also taken into account, it was concluded that the mixed product was the most useful as an aqueous disinfectant solution.

Coates (1985) found that solutions of sodium hypochlorite (NaOCl) and sodium dichloroisocyanurate (NaDCC) containing the same levels of available chlorine had similar bactericidal activity despite significant differences in their pH. Solutions of NaDCC are less susceptible than NaOCl to inactivation by organic matter (Bloomfield & Miles, 1979a,b; Bloomfield & Uso, 1985; Coates, 1985, 1988).

9.2.2 Uses of chlorine-releasing compounds

Chlorinated soda solution (Dakin's solution), which contains 0.5–0.55% (5000–5500 p.p.m.) available chlorine, and chlorinated lime and boric acid solution (Eusol), which contains 0.25% (2500 p.p.m.) available chlorine, are chlorine disinfectants that contain chlorinated lime and boric acid. Dakin's solution is used as a wound disinfectant or, when appropriately diluted, as an irrigation solution for bladder and vaginal infections. Eusol is used as a wound disinfectant, but Morgan (1989) has suggested that chlorinated solutions delay wound healing.

Chlorine gas has been employed to disinfect pub-

lic water-supplies. Sodium hypochlorite is normally used for the disinfection of swimming-pools.

Blood spillages containing human immunodeficiency virus (HIV) or hepatitis B virus can be disinfected with NaOCl solutions containing 10 000 p.p.m. available chlorine (Working Party, 1985). Added directly to the spillage as powder or granules, NaDCC is also effective, may give a larger margin of safety because a higher concentration of available chlorine is achieved and is also less susceptible to inactivation by organic matter, as pointed out above (Coates, 1988). Furthermore, only a very short contact time (2–3 min) is necessary before the spill can be removed safely (Coates & Wilson, 1989). Chlorine-releasing powder formulations with high available chlorine concentrations are particularly useful for this purpose (Bloomfield & Miller, 1989; Bloomfield *et al.*, 1990).

Chlorine dioxide, an alternative to sodium hypochlorite, retains its biocidal activity over a wide pH range (Simpson *et al.*, 2001) and in the presence of organic matter and is more environmentally satisfactory (BS 7152, 1991). Oxine (Biocide International Inc., USA) is a sodium chlorite solution which when acidified generates chlorine dioxide, giving a final solution which is a mixture of chlorite and chlorine dioxide. This product is an Environmental Protection Agency (EPA) registered disinfection compound and is more efficacious in controlling the growth of pathogenic bacteria compared with chlorine dioxide alone (Lin *et al.*, 1996). It has been shown to reduce bacterial populations on food products such as potatoes (Tsai *et al.*, 2001) and seafood (Kim *et al.*, 1999a).

Chlorine-releasing agents continue to be widely studied. Their sporicidal activity has been described by Te Giffel *et al.* (1996) and Coates (1996), their antiviral efficacy by Bellamy (1995), van Bueren (1995), Bond (1995) and Hérnandez *et al.* (1996, 1997) and their usefulness in dental practice by Molinari (1995), Cottone and Molinari (1996) and Gurevich *et al.* (1996).

9.2.3 Chloroform

Chloroform ($CHCl_3$) has been used as a preservative in many pharmaceutical products intended for internal use, for more than a century. In recent years, with the object of minimizing microbial contamination, this use has been extended. Various authors, notably Westwood and Pin-Lim (1972) and Lynch *et al.* (1977), have shown chloroform to be a bactericidal agent, although it is not sporicidal and its high volatility means that a fall in concentration could result in microbial growth. For details of its antibacterial activity in aqueous solutions and in mixtures containing insoluble powders and the losses, through volatilization, under 'in-use' conditions, the paper by Lynch *et al.* (1977) should certainly be consulted.

Chloroform does not appear as an approved material in the latest version of *Cosmetics Directive* (2002) but is still listed in the *British Pharmacopoeia* (2002) as a general anaesthetic and a preservative. It is totally banned in the USA.

9.3 Bromine

The antimicrobial activity of bromine was first observed in the 1930s, but it was not until the 1960s that it was used commercially in water disinfection. The most commonly used oxidizing biocide in recirculating waters is chlorine, but bromine has been put forward as an alternative (Elsmore, 1993).

Elemental bromine is not itself employed commercially. The two available methods (Elsmore, 1995) are: (1) activated bromide produced by reacting sodium bromide with a strong oxidizing agent, such as sodium hypochlorite or gaseous chlorine; and (2) organic bromine-releasing agents, such as *N*-bromo-*N*-chlorodimethylhydantoin (BCDMH; Fig. 2.20a). When BCDMH hydrolyses in water, it liberates the biocidal agents hypobromous acid (HOBr) and hypochlorous acid (HOCl), together with the carrier, dimethylhydantoin (DMH; Fig. 2.20b).

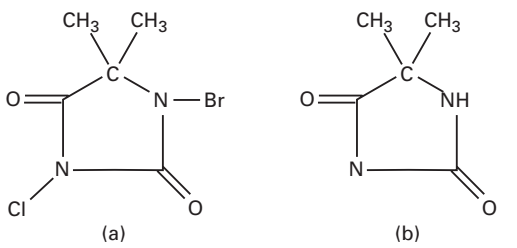


Figure 2.20 (a) Bromochlorodimethylhydantoin (BCDMH); (b) dimethylhydantoin (DMH).

Both HOBr and HOCl would appear to contribute towards the overall germicidal activity of BCDMH. However, Elsmore (1993, 1995) has pointed out that the primary agent present in water is HOBr. Hypochlorous acid is used up in regenerating 'spent bromine' produced when HOBr reacts with organic materials and microorganisms:

$$HOCl + Br^- \rightarrow HOBr + Cl^-$$

Bromine is claimed to have a greater bactericidal activity than chlorine. It is effective against *Legionella pneumophila* in the laboratory and in field studies (McCoy & Wireman, 1989). The pK_a for HOBr (8.69) is higher than that for HOCl (7.48) and thus, at the normal alkaline pH values found in cooling towers, there is a significantly higher amount of active biocide present with HOBr than with HOCl.

10 Quinoline and isoquinoline derivatives

There are three main groups of derivatives: 8-hydroxyquinoline derivatives, 4-aminoquinaldinium derivatives and isoquinoline derivatives. They are described in Figs. 2.21 and 2.22.

However, new quinoline derivatives such as hydrazinoquinolines (Naik *et al.*, 1998), pyridazinoquinoline and spiroindoquinoline (El-Ahl *et al.*, 1996) have been shown to possess antimicrobial activity.

10.1 8-Hydroxyquinoline derivatives

8-Hydroxyquinoline (oxine) possesses antibacterial activity against Gram-positive bacteria, but much less against Gram-negative organisms. It also has antifungal activity, although this occurs at a slower rate. Other useful compounds are depicted in Fig. 2.21b). Like oxine, clioquinol, chlorquinandol and halquinol have very low water solubilities, and are generally employed as applications to the skin. An interesting feature of their activity is the fact that they are chelating agents, which are active only in the presence of certain metal ions.

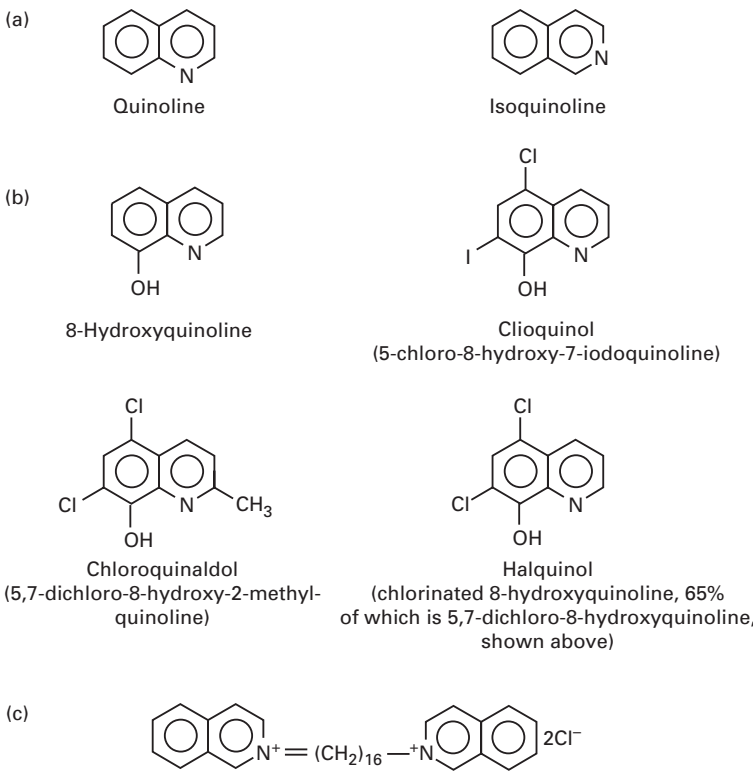
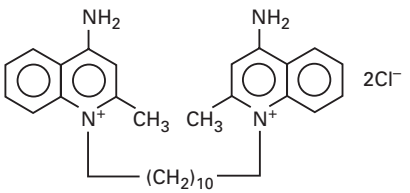


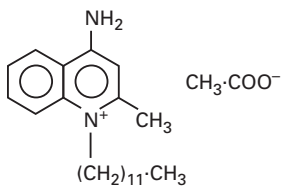
Figure 2.21 (a) Structures of quinoline and isoquinoline; (b) 8-hydroxyquinoline derivatives with antimicrobial properties; (c) hedaquinium chloride.

10.2 4-Aminoquinaldinium derivatives

These are QACs (see Fig. 2.22), which also fall into this grouping. The most important members are laurolinium acetate and dequalinium chloride (a bis-QAC). Both compounds possess antibacterial activity, especially against Gram-positive bacteria (Collier *et al.*, 1959; Cox & D'Arcy, 1962), as well as significant activity against many species of yeasts and fungi (Frier, 1971; D'Arcy, 1971). Dequalinium chloride is used for the treatment of vaginal infections and has been shown to have a broad spectrum of antimicrobial activity against relevant organisms (Della Casa *et al.*, 2002). It is also used as lozenges or paint in the treatment of infections of the mouth and throat. Laurolinium has been used as a preoperative skin disinfectant, although this was never widely adopted. The activity of both agents is decreased in the presence of lecithin; serum decreases the effectiveness of laurolinium but not of dequalinium.



Dequalinium chloride



Laurolinium acetate

Figure 2.22 4-Aminoquinaldinium derivatives with antimicrobial properties.

10.3 Isoquinoline derivatives

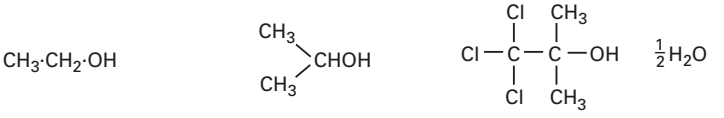
The most important isoquinoline derivative is hedaquinium chloride (Fig. 2.21c), another hisquaternary salt. This possesses antibacterial and antifungal activity (Collier *et al.*, 1959; D'Arcy, 1971), and is regarded as one of the most active antifungal QAC agents (D'Arcy, 1971).

11 Alcohols

Several alcohols have been shown to possess antimicrobial properties. Generally, the alcohols have rapid bactericidal activity (Morton, 1950), including acid-fast bacilli, but are not sporicidal; they have low activity against some viruses, but are viricidal towards others. Their chemical structures are shown in Fig. 2.23.

11.1 Ethyl alcohol (ethanol)

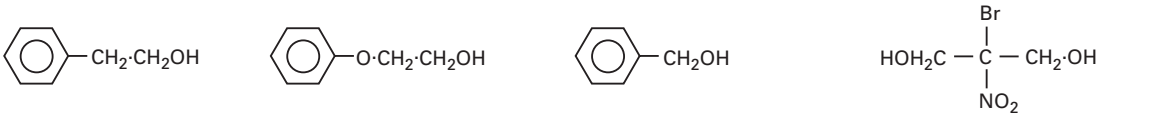
Ethanol is rapidly lethal to non-sporulating bacteria and destroys mycobacteria (Croshaw, 1971) but is ineffective at all concentrations against bacterial



Ethanol

Isopropanol
(Propan-2-ol)

Chlorobutanol
(1,1,1-trichloro-2-methyl-propan-2-ol)



2-Phenylethanol

2-Phenoxyethanol

Benzyl alcohol
(Phenylmethanol)

Bronopol
(2-bromo-2-nitropropan-1,3-diol)

Figure 2.23 Alcohols.

spores (Russell, 1971b). The recent work of Setlow *et al.*, (2002) has shown that a reduction in *B. subtilis* spores can be achieved by treatment with ethanol at 65°C. The proposed mechanism of action of this killing is a disruption to the spore permeability barrier. The presence of water is essential for its activity, but concentrations below 30% have little action. Activity, in fact, drops sharply below 50% (Rutala, 1990).

The most effective concentration is about 60–70% (Price, 1950; see also Croshaw, 1977; Morton, 1977; Scott & Gorman, 1987). Solutions of iodine or chlorhexidine in 70% alcohol may be employed for the preoperative disinfection of the skin. Ethanol is the alcohol of choice in cosmetic products because of its relative lack of odour and irritation (Bandelin, 1977). Alcohol-based hand rubs are becoming increasingly popular for sanitizing hands. Hand hygiene is of particular importance in the healthcare professions to prevent nosocomial infections caused by cross transmission of microorganisms. Compliance with a hand sanitizing regime has been shown to be improved by the introduction of an alcohol-based hand rub compared with soap and water (Bischoff *et al*, 2000). However, there is some debate about the effectiveness of such products. Whilst some studies report alcohol hand rubs to be more effective than handwashing with antiseptic soap and water (Zaragoza *et al.*, 1999; Girou *et al.*, 2002) other investigators have reported such products to be less effective (Moadab *et al.*, 2001).

Some variable results have been reported about the effects of ethanol on HIV. Tjøtta *et al.* (1991) showed that 70% ethanol in the presence of 2.5% serum produced a 3-log/mL reduction in virus titre after a 10-min contact period, as determined by plaque assay or immunofluorescence. In contrast, using a tissue culture infective dose 50% ($TCID_{50}$) assay, Resnick *et al.* (1986) found that 70% alcohol after 1 min and in the presence of 50% plasma yielded a 7-log reduction in $TCID_{50}$/mL, again in a suspension test. Van Bueren *et al.* (1994) also described a rapid inactivation of HIV-1 in suspension, irrespective of the protein load. The rate of inactivation decreased when high protein levels were present when a carrier test was employed. A notable feature of the experiments carried out by van Bueren *et al.* (1994) was the care taken to ensure that residual alcohol was neutralized to prevent toxicity to the cell line employed in detecting uninactivated virus. The non-enveloped poliovirus is more resistant to biocides in general than the herpesvirus, and ethanol caused no inactivation of poliovirus in a suspension test (Tyler *et al.*, 1990). Further information on the viricidal activity of ethanol can be found in Chapter 9.

11.2 Methyl alcohol (methanol)

Methyl alcohol has poor antibacterial activity and is not sporicidal (Russell, 1971b; Bandelin, 1977; Coates & Death, 1978; Death & Coates, 1979). Furthermore, it is potentially toxic, and is thus little used. However, freshly prepared mixtures of alcohols (especially methanol) and sodium hypochlorite are highly sporicidal (Coates & Death, 1978). Although it was then considered that methanol was potentiating the activity of hypochlorites, it is, in fact, more likely that hypochlorites, by virtue of their effects on the outer spore layers (Bloomfield & Arthur, 1994), are aiding the penetration of methanol into the spore.

11.3 Isopropyl alcohol (isopropanol)

Isopropyl and *n*-propyl alcohols are more effective bactericides than ethanol (Anon., 1965; Kelsey & Maurer, 1972), but are not sporicidal. They are miscible with water in all proportions, but isopropanol has a less objectionable odour than *n*-propanol and is considered as a suitable alternative to ethanol in various cosmetic products, either as a solvent or as a preservative (Bandelin, 1977; Hill, 1995). Isopropanol has viricidal activity, but not towards 'hydrophilic' (non-lipid-enveloped) viruses (Rutala, 1990). Van Bueren *et al.* (1994) have demonstrated inactivation of HIV type 1 by isopropanol. For further information, the papers by Tyler *et al.* (1990) and Sattar & Springthorpe (1991) should be consulted (see also Chapter 9).

11.4 Benzyl alcohol

In addition to having antimicrobial properties, benzyl alcohol is a weak local anaesthetic. It has

activity against Gram-positive and Gram-negative bacteria and against moulds (D'Arcy, 1971). Benzyl alcohol is incompatible with oxidizing agents and is inactivated by non-ionic surfactants; it is stable to autoclaving and is normally used at a concentration of 1% v/v (Denyer & Wallhäusser, 1990).

11.5 Phenylethanol (phenylethyl alcohol)

Phenylethyl alcohol is an antimicrobial agent with selective activity against various bacteria [especially Gram-negative (Lilley & Brewer, 1953)] and has been recommended for use as a preservative in ophthalmic solutions, often in conjunction with another microbicide. Because of its higher activity against Gram-negative bacteria, phenylethyl alcohol may be incorporated into culture media for isolating Gram-positive bacteria from mixed flora, e.g. phenylethyl alcohol agar.

Phenylethanol is commonly used at a concentration of 0.3–0.5% v/v; it shows poor stability with oxidants and is partially inactivated by non-ionic surfactants (Denyer & Wallhäusser, 1990).

11.6 Bronopol

Bronopol, 2-bromo-2-nitropropan-1,3-diol, is an aliphatic halogenonitro compound with potent antibacterial activity but limited activity against fungi (Guthrie, 1999). Its activity is reduced somewhat by 10% serum and to a greater extent by sulphydryl compounds, but is unaffected by 1% polysorbate or 0.1% lecithin. It has a half-life of about 96 days at pH 8 and 25 °C (Toler, 1985).

Bronopol is most stable under acid conditions; the initial decomposition appears to involve the liberation of formaldehyde and the formulation of bromonitroethanol (Fig. 2.24a). A second-order reaction involving bronopol and formaldehyde occurs simultaneously to produce 2-hydroxymethyl-2-nitro-1,3-propanediol (Fig. 2.24b), which itself decomposes with the loss of formaldehyde.

Bronopol has been employed extensively as a preservative for pharmaceutical and cosmetic products. However, its use to preserve products containing secondary amines should be avoided as the by-product of this reaction is nitrosoamine which is

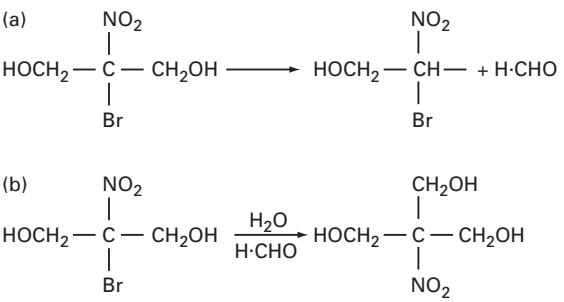


Figure 2.24 (a) Initial process in the decomposition of bronopol; (b) second-order reaction involving bronopol and formaldehyde.

carcinogenic. Details of the microbiological activity, chemical stability, toxicology and uses of bronopol are documented by Bryce *et al.* (1978), Croshaw & Holland (1984), Toler (1985) and Rossmore and Sondossi (1988). Denyer and Wallhäusser (1990) have provided useful information about bronopol, the typical in-use concentration of which is 0.01–0.1% w/v. Sulphhydryl compounds act as appropriate neutralizers in preservative efficacy tests.

11.7 Phenoxyethanol (phenoxetol)

The antimicrobial activity of phenoxyethanol and other preservatives has been reviewed by Gucklhorn (1970, 1971). Phenoxyethanol was shown by Berry (1944) to possess significant activity against *P. aeruginosa*, but it has less activity against other Gram-negative organisms or against Gram-positive bacteria. Phenoxyethanol is stable to autoclaving and is compatible with anionic and cationic surfactants, but it shows reduced activity in the presence of polysorbate 80. It is used as a preservative, typical concentration 1% (Denyer & Wallhäusser, 1990).

11.8 Chlorbutanol (chlorbutol)

Chlorbutol is an antibacterial and antifungal agent. It has been used, at a concentration of 0.5% w/v, as a bactericide in injections. One drawback to its employment is its instability, since at acid pH it decomposes at the high temperature used in sterilization

processes into hydrochloric acid, and at alkaline pH it is unstable at room temperature.

Chlorbutanol is incompatible with some non-ionic surfactants. Its typical in-use concentration as a pharmaceutical preservative is 0.3–0.5% w/v (Denyer & Wallhäusser, 1990).

11.9 2,4-Dichlorobenzyl alcohol

This substance is a white powder, soluble in water to 1% and readily soluble in alcohols. Its ionization is negligible for all practical purposes and it is thus active over a wide pH range. It has a broad spectrum of activity, but both pseudomonads and *Staph. aureus* show some resistance to it (Toler, 1985).

12 Peroxygens

12.1 Hydrogen peroxide

Hydrogen peroxide (H_2O_2) is a familiar household antiseptic. It was discovered in 1818 and was early recognized as possessing antibacterial properties. These were extensively investigated in 1893 by Traugott. Hydrogen peroxide is available as a solution designated as 20- or 10-volume, a means of indicating its strength by describing the volume (20 or 10, respectively) of oxygen evolved from 1 volume of the peroxide solution. Strengths for industrial use of 35, 50 or 90% are available. Hydrogen peroxide solutions are unstable, and benzoic acid or another suitable substance is added as a stabilizer.

Hydrogen peroxide solutions possess disinfectant, antiseptic and deodorant properties. When in contact with living tissue and many metals they decompose, evolving oxygen. Hydrogen peroxide its bactericidal and sporicidal (Russell, 1982, 1990a,b, 1991a,b; Baldry, 1983; Baldry & Fraser, 1988). It is believed to act as a generator of free hydroxyl radicals, which can cause DNA strand breakage in growing bacteria but the mechanism of action of hydrogen peroxide on spores is not the same. The current hypothesis is that hydrogen peroxide treatment results in spores which cannot swell properly during spore germination (Melly *et al.*, 2002). It is an oxidizing agent and reacts with oxidizable material, for example alkali nitrites used in anticorrosion solutions. It is environmentally friendly because its decomposition products are oxygen and water (Miller, 1996) and has been investigated as a potential sanitizing agent in the food industry (Shin *et al.*, 2001; Melly *et al.*, 2002).

Hydrogen peroxide has been used in aseptic packaging technology and also for disinfecting contact lenses as it has been shown to be effective against the opportunistic pathogen *Acanthameoba*, the causative agent of *Acanthameoba* keratitis. This is a potentially blinding infection which contact lens users are more susceptible to (Hughes & Kilvington, 2001). The use of hydrogen peroxide as a contact-lens disinfectant has been reviewed (Miller, 1996) and is further described in Chapter 8.1.

Microbial inactivation is more rapid with liquid peroxide than with vapour generated from that liquid acting at the same temperature (Sintim-Damoa, 1993). However, the vapour can be used for the purposes of sterilization, where, at a concentration of 1–5 mg/L, it generally shows good penetration.

Attention has recently been devoted to developing a plasma-activated peroxide vapour process, in which radio waves produce the plasma. This is believed to be microbicidal by virtue of the hydroxyl ions and other free radicals that are generated (Groschel, 1995; Lever & Sutton, 1996).

12.2 Peracetic acid

Peracetic acid, $CH_3{\cdot}COOOH$, was introduced as an antibacterial agent in 1955. It is available commercially as a 15% aqueous solution, in which an equilibrium exists between peracetic acid and its decomposition products acetic acid (CH3·COOH) and hydrogen peroxide.

Peracetic acid solution has a broad spectrum of activity, including bacteria and their spores, moulds, yeasts, algae and viruses. It finds extensive use in the food industry and for disinfecting sewage sludge. It is a powerful oxidizing agent and in certain situations can be corrosive. The great advantage of peracetic acid is that its final decomposition products, oxygen and water, are innocuous. More comprehensive data on peracetic acid are provided by Baldry (1983), Fraser (1986), Baldry & Fraser (1988), Coates (1996) and Russell & Chopra (1996).

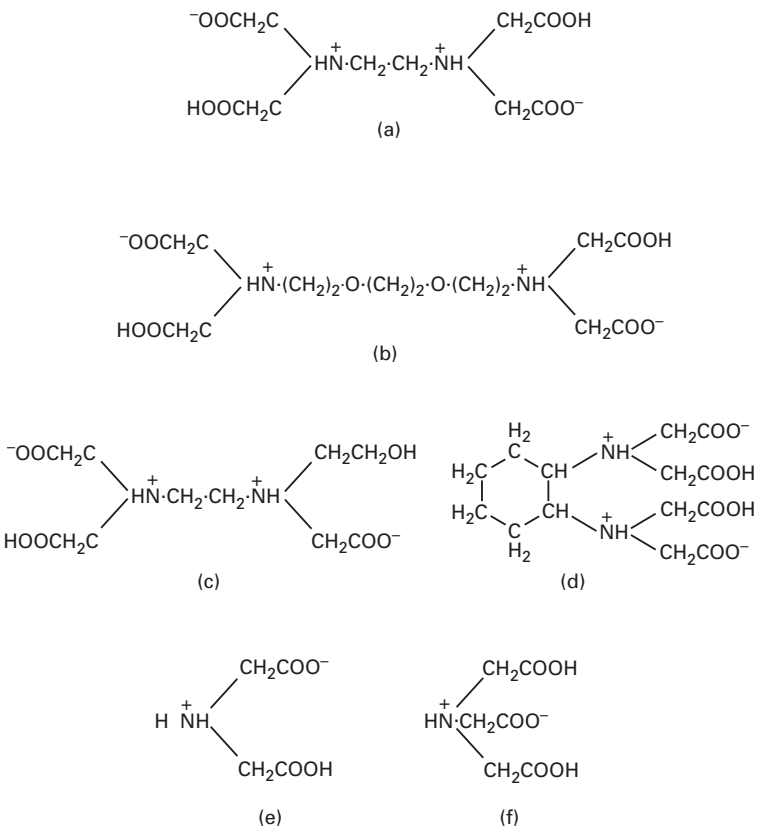


Figure 2.25 Chelating agents. (a) Ethylenediamine tetraacetic acid (EDTA); (b) ethylenedioxybis (ethyliminodi(acetic acid)) (EGTA); (c) *N*-hydroxyethylenediamine-*NN′N′*-triacetic acid (HDTA); (d) *trans*-1,2-diaminocyclohexane-*NNN′N′*-tetraacetic acid (CDTA); (c) iminodiacetic acid (IDA); (f) nitrilotriacetic acid (NTA).

13 Chelating agents

This section will deal briefly with chelating agents based on EDTA. Ethylenediamine tetraacetic acid has been the subject of intensive investigation for many years, and its antibacterial activity has been reviewed by Russell (1971a), Leive (1974) and Wilkinson (1975). The chemical nature of its complexation with metals has been well considered by West (1969).

The chemical structures of EDTA, ethylenedioxybis(ethyliminodi(acetic acid)) (EGTA), *N*-hydroxyethylethylenediamine-*NN′N′*-triacetic acid (HDTA), *trans*-1,2-diaminocyclohexane-*NNN′N′*-tetraacetic acid (CDTA), iminodiacetic acid (IDA) and nitrilotriacetic acid (NTA) are provided in Fig. 2.25. Table 2.11 lists their chelating and antibacterial activities.

13.1 Ethylendiamine tetraacetic acid

In medicine, EDTA is commonly employed as the sodium or calcium-sodium salts. Sodium calcium edetate is used in the treatment of chronic lead poisoning, and the sodium salts are used clinically to chelate calcium ions, thereby decreasing serum calcium. Also EDTA is used as a stabilizing agent in certain injections and eye-drop preparations (Russell *et al.*, 1967).

The most important early findings, in a microbiological context, were made by Repaske (1956, 1958), who showed that certain Gram-negative bacteria became sensitive to the enzyme lysozyme in the presence of EDTA in tris buffer and that EDTA alone induced lysis of *P. aeruginosa*. The importance of tris itself has also been recognized (Leive & Kollin, 1967; Neu, 1969), since it appears to affect the permeability of the wall of various Gram-negative bacteria, as well as the nucleotide pool and RNA, which may be degraded. A lysozyme–tris–EDTA system in the presence of sucrose is a standard technique for producing spheroplasts/protoplasts in Gram-negative bacteria (McQuillen, 1960). During this conversion, several enzymes are

Table 2.11 Properties of chelating agents.

Property	*EDTA*	*EGTA*	*HDTA*	*CDTA*	*IDA*	*NTA*
Log stability constant[a]						
Ba	7.76	8.41	5.54	7.99	1.67	4.82
Ca	10.70	11.0	8.0	12.5	2.59	6.41
Mg	8.69	5.21	5.2	10.32	2.94	5.41
Zn	16.26	14.5	14.5	18.67	7.03	10.45
Antibacterial activity[b]						
Alone	Good	Good	Good	Low	Low	–
As a potentiating agent for disinfectants	Yes	—	Yes	Yes	Somewhat	Somewhat

[a]Abstracted from the information supplied by West (1969).
[b]Based on the activity against *P. aeruginosa* described by Roberts *et al.* (1970) and Haque & Russell (1974a,b).

released into the surrounding medium. A technique known as 'cold shock', which involves treating *E. coli* with EDTA + tris in hypertonic sucrose, followed by rapid dispersion in cold magnesium chloride—thus producing a sudden osmotic shift—again results in the release of enzymes, but without destroying the viability of the cells.

In the context of disinfection, EDTA is most important in that it will potentiate the activity of many antibacterial agents against many types of Gram-negative but not Gram-positive bacteria. This was clearly shown by Gray and Wilkinson (1965) and has since been confirmed and extended (Russell, 1971a; Wilkinson, 1975). EDTA induces a non-specific increase in the permeability of the outer envelope of Gram-negative cells (Leive, 1974), thereby allowing more penetration of non-related agents. Ayres *et al.* (1993) reported on the permeabilizing activity of EDTA and other agents against *P. aeruginosa* in a rapid test method, the principle of which was the rapid lysis induced in this organism on exposure to the presumed permeabilizing agent plus lysozyme, an enzyme normally excluded in whole cells from its peptidoglycan target.

13.2 Other chelating agents

Chelating agents other than EDTA are described chemically in Fig. 2.25, and some of their properties (based in part on the excellent book of West, 1969) are listed in Table 2.11. While EGTA forms a stronger complex with Ca than does EDTA, for most other metals, except Ba and Hg, it is a weaker complexing agent than EDTA. Notably, there is a divergency of 5.79 log *K* units between the stability constants of the Ca and Mg complexes with EGTA (West, 1969). Compared with EDTA, CDTA has superior complexing powers and it is better than all the other chelating agents listed in complexing Mg^{2+} ions. From a microbiological point of view, CDTA was found by Roberts *et al.* (1970) and Haque & Russell (1974a,b) to be the most toxic compound to *P. aeruginosa* and other Gram-negative bacteria in terms of leakage, lysis and loss of viability and in extracting metal ions from isolated cell envelopes (Haque & Russell, 1976).

The chelating agent HDTA corresponds to EDTA, one acetic acid of the latter molecule being replaced by a hydroxyethyl group. Its complexes are invariably less stable than those of EDTA. In a microbiological context, HDTA was found (Haque & Russell, 1976) to be rather less effective than EDTA. Metal chelation of EDTA has also been shown to reduce its antimicrobial activity (Bergen, Klaveness & Aasen, 2001).

EHPG (*N*,*N*′- ethylenebis[2-(2-hydroxyphenyl)-glycine]) a chelating agent containing two phenyl groups has been shown to exhibit antimicrobial activity against a range of bacteria and fungi and was shown to be more active than EDTA (Bergan et al., 2001).

Iminodiacetic acid forms weak complexes with most metal ions, whereas NTA is more reactive. Both have little activity against *P. aeruginosa*, although both, to some extent, potentiate the activity of other agents (disinfectants) against this organism.

14 Permeabilizers

Permeabilizers (permeabilizing agents) are chemicals that increase bacterial permeability to biocides (Vaara, 1992). Such chemicals include chelating agents, described above in section 13, polycations, lactoferrin, transferrin and the salts of certain acids.

14.1 Polycations

Polycations such as poly-L-lysine (lysine$_{20}$; PLL) induce lipopolysaccharide (LPS) release from the outer membrane of Gram-negative bacteria. Organisms treated with PLL show greatly increased sensitivity to hydrophobic antibiotics (Vaara & Vaara, 1983a,b; Viljanen, 1987) but responses to biocides do not appear to have been studied.

14.2 Lactoferrin

Lactoferrin is an iron-binding protein that acts as a chelator, inducing partial LPS loss from the outer membrane of Gram-negative bacteria (Ellison *et al.*, 1988). The resulting permeability alteration increases the susceptibility of bacteria to lysozyme (Leitch & Willocx, 1998) and antibiotics such as penicillin (Diarra *et al.*, 2002) resulting in synergistic combinations. Lactoferricin B is a peptide produced by gastric peptic digestion of bovine lactoferrin. It is a much more potent agent than lactoferrin, binds rapidly to the bacterial cell surface and damages the outer membrane but has reduced activity in the presence of divalent cations (Jones *et al.*, 1994). Further information regarding lactoferrin can be found in the reviews of Chierici (2001) and Weinberg (2001).

14.3 Transferrin

This iron-binding protein is believed to have a similar effect to lactoferrin (Ellison *et al.*, 1988). All are worthy of further studies as potentially important permeabilizers.

14.4 Citric and other acids

Used at alkaline pH, citric, gluconic and malic acids all act as permeabilizers (Ayres *et al.*, 1993). They perform as chelating agents and activity is reduced in the presence of divalent cations.

Lactic acid has also been demonstrated to permeabilize the outer membrane of Gram-negative bacteria but at low pH. The proposed mechanism of action for this agent is not a chelator like citric, gluconic and malic acids but as a protonator of anionic components such as phosphate and carbonyl groups resulting in weakening of the molecular interactions between outer membrane components (Alakomi *et al.*, 2000).

15 Heavy-metal derivatives

The historical introduction (Chapter 1) has already described the early use of high concentrations of salt employed empirically in the salting process as a preservative for meat, and the use of copper and silver vessels to prevent water from becoming fouled by microbial growth. Salting is still used in some parts of the world as a meat preservative and salts of heavy metals, especially silver, mercury, copper and, more recently, organotin, are still used as antimicrobial agents. The metal derivatives of copper, mercury, silver and tin, which find use as antiseptics and preservatives, will be discussed in this chapter. Kushner (1971) has reviewed the action of solutes other than heavy metal derivatives on microorganisms.

In addition to possessing antimicrobial activity in their own right, many metal ions are necessary for the activity of other drugs. A typical example is 8-hydroxyquinoline (section 10.1), which needs Fe^{2+} for activity. The interesting relationship between antimicrobial compounds and metal cations has been reviewed by Weinberg (1957).

15.1 Copper compounds

Although the pharmacopoeias list a number of recipes containing copper salts (sulphate, actetate, citrate) as ingredients of antiseptic astringent lotions, the main antimicrobial use of copper derivatives is in algicides and fungicides. The copper(II) ion Cu^{2+} is pre-eminently an algicidal ion and at a final concentration of 0.5–2.9 μg/mL, as copper sulphate, it has been used to keep

swimming-pools free from algae. Copper is thought to act by the poisoning effect of the copper(II) ion on thiol enzymes and possibly other thiol groups in microbial cells.

Copper ions have been shown to potentiate the antimicrobial activity of two commonly used disinfectants, cetylpyridinium chloride and povidone–iodine against hospital isolates of *Staph. aureus, P. aeruginosa* and *Candida albicans* (Zeelie & McCarthy, 1998).

Copper sulphate and copper sulphate mixed with lime, Bordeaux mixture, introduced in 1885, are used as fungicides in plant protection. The latter formulation proved especially efficacious, as it formed a slow-release copper complex which was not easily washed from foliage. It was said to be first used as a deterrent to human predators of the grape crop and its antifungal properties emerged later. Copper metal, in powder form, finds an interesting application as an additive to cements and concretes. Its function is to inhibit microbial attack on the ingredients of these artificial products. The uses of copper metal here, and as vessels for drinking-water in the ancient world, illustrate a phenomenon which has been called the oligodynamic action of metals (Langwell, 1932). Metals are slightly soluble in water, and in the case of copper, and also silver (q.v.), a sufficient concentration of ions in solution is achieved to inhibit microbial growth. Copper complexes, such as copper naphthenate and copper-7-hydroxyquinolate, have been particularly successful in the preservation of cotton fabrics. More recently polyester fibres coated with copper sulphides have been demonstrated to possess some antimicrobial activity (Grzybowski & Trafny, 1999). Copper compounds are mainly used in the wood, paper and paint industry as preservatives, but have little, if any, use in the pharmaceutical and cosmetic industry.

15.2 Silver compounds

Silver and its compounds have found a place in antimicrobial application from ancient times to the present day (Weber & Rutala, 1995). Apart from the use of silver vessels to maintain water in a potable state, the first systematic use of a silver compound in medicine was its use in the prophylaxis of ophthalmia neonatorum by the installation of silver nitrate solution into the eyes of newborn infants. Silver compounds have been used in recent years in the prevention of infection in burns, but are not very effective in treatment. An organism frequently associated with such infections as *P. aeruginosa*, and Brown and Anderson (1968) have discussed the effectiveness of Ag^+ in the killing of this organism. Among the Enterobacteriaceae, plasmids may carry genes specifying resistance to antibiotics and to metals. Plasmid-mediated resistance to silver salts is of particular importance in the hospital environment, because silver nitrate and silver sulphadiazine (AgSu) may be used topically for preventing infections in severe burns (Russell, 1985).

As might be imagined, silver nitrate is a somewhat astringent compound, below 10^{-4} mol/L a protein precipitant, and attempts to reduce this undesirable propensity while maintaining antimicrobial potency have been made. A device much used in pharmaceutical formulation to promote slow release of a potent substance is to combine it with a high-molecular-weight polymer. By mixing silver oxide or silver nitrate with gelatin or albumen, a water-soluble adduct is obtained, which slowly releases silver ions but lacks the caustic astringency of silver nitrate. A similar slow-release compound has been prepared by combining silver with disodiumdinaphthylmethane disulphate (Goldberg *et al.*, 1950). Silver nitrate has also been investigated as a treatment for peridontitis. A concentration of 0.5 µg/mL was sufficient to produce a minimum of 3 log reductions against a range of peridontal pathogens. However increasing the concentration of silver nitrate by 100-fold was not sufficient to kill oral streptococci (Spacciapoli, 2001).

Sustained release of silver nitrate from a subgingival drug delivery system has also shown to be active against a range of peridontal microorganisms over a period of 21 days (Bromberg *et al.*, 2000). The inclusion of silver ions in other surfaces with the aim of producing an antimicrobial effect has been investigated further. Kim *et al.* (1998) demonstrated the antimicrobial effect of a ceramic composed of hydroxapatite and silver nitrate against *E. coli,* whilst the inclusion of silver and zinc ions on stainless steel pins has been shown to have anti-

microbial activity against *Staph. aureus* (Bright, Gerba & Rusin, 2002) and *Legionella pneumophilia* (Rusin, Bright & Gerba, 2003).

The oligodynamic action of silver (Langwell, 1932), already referred to in the historical introduction (Chapter 1) and above, has been exploited in a water purification system employing what is called katadyn silver. Here, metallic silver is coated on to sand used in filters for water purification. Silver-coated charcoal has been used in a similar fashion (Bigger & Griffiths, 1933; Gribbard, 1933; Brandes, 1934; Moiseev, 1934). The activity of a silver-releasing surgical dressing has been described by Furr *et al.* (1944), who used a neutralization system to demonstrate that Ag^+ ions released were responsible for its antibacterial effects.

Russell and Hugo (1994) have reviewed the antimicrobial activity and action of silver compounds. At a concentration of 10^{-9} to 10^{-6} mol/L, Ag^+ is an extremely active biocide. Originally considered to act as a 'general protoplasmic poison', it is now increasingly seen that this description is an oversimplification. It reacts strongly with structural and functional thiol groups in microbial cells, induces cytological changes and interacts with the bases in DNA.

Silver sulphadiazine is essentially a combination of two antibacterial agents, Ag^+ and sulphadiazine. It has a broad spectrum of activity, produces surface and membrane blebs and binds to various cell components, especially DNA (reviewed by Russell & Hugo, 1994), although its precise mode of action has yet to be elucidated. Silver sulphadiozine has been reinvestigated by Hamilton-Miller *et al.* (1993).

15.3 Mercury compounds

Mercury, long a fascination for early technologists (alchemists, medical practitioners, etc.), was used in medicine by the Arabian physicians. In the 1850s, mercury salts comprised, with phenol, the hypochlorites and iodine, the complement of topical antimicrobial drugs at the physician's disposal. Mercuric chloride was used and evaluated by Robert Koch and by Geppert. Nowadays its use in medicine has decreased, although a number of organic derivatives of mercury (Fig. 2.26) are used as bacteriostatic and fungistatic agents and as preservatives and bactericides in injections; examples include mercurochrome, nitromersol, thiomersal and phenylmercuric nitrate (Fig. 2.26). Salts such as the stearate, oleate and naphthenate were, until much more recently, extensively employed in the preservation of wood, textiles, paints and leather. With the advent of a major health disaster in Japan due to mercury waste, feeling is hardening all over the world against the use of mercury in any form where it might pollute the environment, and it is unlikely that the inclusion of mercury in any product where environmental pollution may ensue will be countenanced by regulatory authorities.

Mercury resistance is inducible and is not the result of training or tolerance. Plasmids conferring resistance are of two types: (1) 'narrow-spectrum', encoding resistance to Hg(II) and to a few specified organomercurials; and (2) 'broad-spectrum', encoding resistance to those in (1) plus other organomercury compounds (Foster, 1983). In (1) there is enzymatic reduction of mercury to Hg metal and its vaporization, and in (2) there is enzymatic hydrolysis of an organomercurial to inorganic

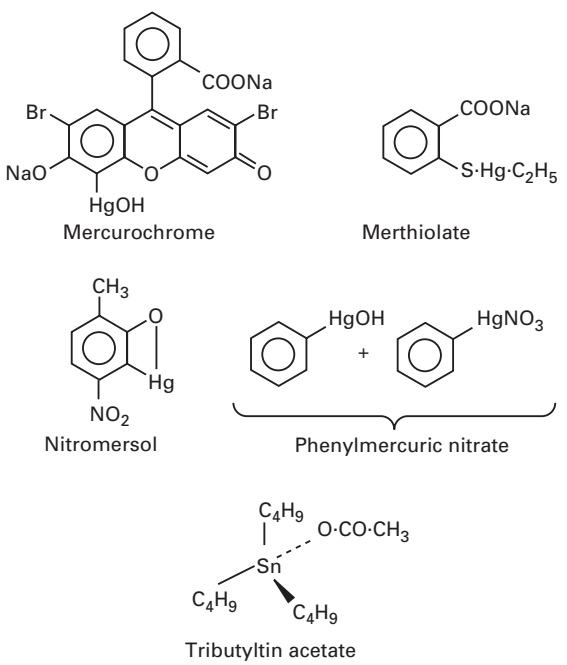


Figure 2.26 Mercurochrome, merthiolate (thiomersal, sodium ethylmercurithiosalicylate), nitromersol, phenylmercuric nitrate and tributyltin acetate.

mercury and its subsequent reduction as in (1) (Silver & Misra, 1988). Further details are provided in Chapter 6.2 and by Russell & Chopra (1996).

Mercury is an environmental pollutant of considerable concern because it is very toxic to living cells. Ono *et al.* (1988) showed that the yeast cell wall acted as an adsorption filter for Hg^+. Later (Ono *et al.*, 1991) they demonstrated that methylmercury-resistant mutants of *S. cerevisiae* overproduced hydrogen sulphide, with an accumulation of hydrosulphide (HS^-) ions intracellularly, which was responsible for detoxification of methylmercury.

15.3.1 Mercurochrome (disodium-2,7-dibromo-4-hydroxymercurifluorescein)

This is now only of historical interest; it was the first organic mercurial to be used in medicine and an aqueous solution enjoyed a vogue as a substitute for iodine solutions as a skin disinfectant.

15.3.2 Nitromersol (anhydro-2-hydroxymercuri-6-methyl-3-nitrophenol)

A yellow powder, it is not very soluble in water or organic solvents but will dissolve in aqueous alkali, and is used as a solution of the sodium salt. It is active against vegetative microorganisms but ineffective against spores and acid-fast bacteria. It is mostly used in the USA.

*15.3.3 Thiomersal (merthiolate; sodium-*o*-(ethylmercurithio)-benzoate)*

This derivative was used as a skin disinfectant, and is now employed as a fungicide and as a preservative (0.01–0.02%) for biological products, for example, bacterial and viral vaccines. It possesses antifungal properties but is without action on spores.

Solutions are stable when autoclaved but less stable when exposed to light or to alkaline conditions, and they are incompatible with various chemicals, including heavy-metal salts (Denyer & Wallhäusser, 1990).

15.3.4 Phenylmercuric nitrate (PMN)

This organic derivative is used as a preservative in multidose containers of parenteral injections and eye-drops at a concentration of 0.001% and 0.002% w/v, respectively (Brown & Anderson, 1968). It was formerly employed in the UK as an adjunct to heat in the now-discarded process of 'heating with a bactericide'.

Phenylmercuric nitrate is incompatible with various compounds, including metals. Its activity is reduced by anionic emulsifying and suspending agents (Denyer & Wallhäusser, 1990). Sulphydryl agents are used as neutralizers in bactericidal studies and in sterility testing (Russell *et al.*, 1979; Sutton, 1996). Phenylmercuric nitrate is a useful preservative and is also employed as a spermicide.

Phenylmercuric nitrate solutions at room temperature are ineffective against bacterial spores, but they possess antifungal activity and are used as antifungal agents in the preservation of paper, textiles and leather. Voge (1947) has discussed PMN in a short review. An interesting formulation of PMN with sodium dinaphthylmethanedisulphonate has been described, in which enhanced activity and greater skin penetration is claimed (Goldberg *et al.*, 1950).

15.3.5 Phenylmercuric acetate (PMA)

This has the same activity, properties and general uses as PMN (Denyer & Wallhäusser, 1990) and finds application as a preservative in pharmaceutical and other fields.

15.4 Tin and its compounds (organotins)

Tin, stannic or tin(IV) oxide was at one time used as an oral medicament in the treatment of superficial staphylococcal infections. Tin was claimed to be excreted via sebaceous glands and thus concentrated at sites of infection. More recently, organic tin derivatives (Fig. 2.26) have been used as fungicides and bactericides and as textile and wood preservatives (Smith & Smith, 1975).

The organotin compounds which find use as biocides are derivatives of tin (IV). They have the general structure R_3SnX where R is butyl or phenyl and X is acetate, benzoate, fluoride, oxide or hydroxide. In structure-activity studies, activity has been shown to reside in the R group; the nature of X

determines physical properties such as solubility and volatility (Van der Kerk & Luijten, 1954; Rose & Lock, 1970). The R_3SnX compounds, with R = butyl or phenyl, combine high biocidal activity with low mammalian toxicity. These compounds are used as biocides in the paper and paint industry, and in agriculture as fungicides and pesticides. Tributyltin benzoate ($(C_4H_9)_3$ $SnOCOC_6H_5$) is used as a germicide when combined with formaldehyde or a QAC and triphenyltin hydroxide ($(C_6H_5)_3SnOH$) as a disinfectant (as well as an agricultural pesticide). Examples of MIC of tributyltin oxide are shown in Table 2.12. Tin differs significantly from copper, silver and mercury salts in being intrinsically much less toxic. It is used to coat cans and vessels used to prepare food or boil water. Organotin compounds have some effect on oxidative phosphorylation (Aldridge & Threlfall, 1961) and act as ionophores for anions. Possible environmental toxicity should be borne in mind when tin compounds are used.

Table 2.12 Minimum inhibitory concentrations (MICs) of tributyltin oxide towards a range of microorganisms.

Organism	*MIC (μg/mL)*
Aspergillus niger	0.5
Chaetomium globosum	1.0
Penicillium expansum	1.0
Aureobasidium pullulans	0.5
Trichoderma viride	1.0
Candida albicans	1.0
Bacillus mycoides	0.1
Staphylococcus aureus	1.0
Bacterium ammoniagenes	1.0
Pseudomonas aeruginosa	>500
Enterobacter aerogenes	>500

15.5 Titanium

The use of titanium as an antimicrobial agent in the oral cavity has been investigated. Granules of titanium were examined for activity against oral bacteria but showed very low antibacterial activity (Leonhardt & Dahlén, 1995). Titanium also has found use in medical implants including dental implants. Titanium surfaces implanted with fluorine ions demonstrated antimicrobial activity but this was not found to be due to fluorine ion release but hypothesized to be due to formation of a titanium-fluorine complex on the implant surface (Yoshinari *et al.*, 2001).

16 Anilides

Anilides (Fig. 2.27) have the general structure $C_6H_5{\cdot}NH{\cdot}COR$. Two derivatives—salicylanilide, where R = C_6H_4OH, and diphenylurea (carbanilide), where R = $C_6H_5{\cdot}NH$– have formed the basis for antimicrobial compounds.

16.1 Salicylanilide

The parent compound, salicylanilide, was introduced in 1930 as a fungistat for use on textiles (Fargher *et al.*, 1930). It occurs as white or slightly pink crystals, m.p. 137 °C, which are soluble in water and organic solvents. It has also been used in

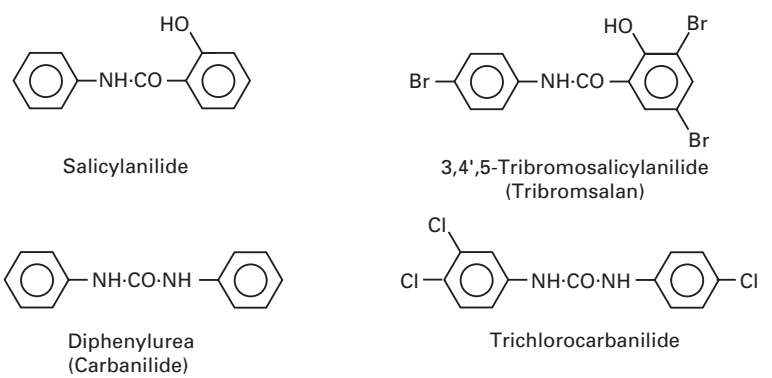


Figure 2.27 Anilides.

ointment form for the treatment of ringworm, but concentrations above 5% should not be used in medicinal products because of skin irritancy. Minimum inhibitory concentrations (μg/mL) for a number of fungi were: *Trichophyton mentagrophytes*, 12; *Trichophyton tonsurans*, 6; *Trichophyton rubrum*, 3; *Epidermophyton floccosum*, 6; *Microsporum audovinii*, 1.5. Despite the effectiveness of the parent compound, attempts were made to improve on its performance by the usual device of adding substituents, notably halogens, to the benzene residues; these are considered below.

Lemaire *et al.* (1961) investigated 92 derivatives of salicylanilide and related compounds, i.e. benzanilides and salicylaldehydes. The intrinsic antimicrobial activity was obtained from literature values and was usefully summarized as follows. One ring substituent would give a MIC value for *Staph. aureus* of 2 μg/mL, but this value could be decreased to 1 μg/mL if substitution occurred in both rings.

The researchers were particularly interested in the role of these compounds as antiseptics for addition to soaps, and went on to evaluate them in this role. They were also interested to find to what extent they remained on the skin (skin substantivity) after washing with soaps containing them. They found that di- to pentachlorination or bromination with more or less equal distribution of the substituent halogen in both rings gave the best results both for antimicrobial activity and skin substantivity. However, it was also found that skin photosensitization was caused by some analogues.

Of the many compounds tested, the 3,4′,5-tribromo, 2,3,5,3′- and 3,5,3′,4′ tetrachlorosalicylanilides have been the most widely used as antimicrobial agents; however, their photosensitizing properties have tended to restrict their use in any situation where they may come in contact with human skin.

Over and above this, many workers who have investigated germicidal soaps, i.e. ordinary soap products with the addition of a halogenated salicylanilide, carbanilide, or for that matter phenolic compounds such as hexachlorophene (2.9.1) or DCMX (2.6.5), have doubted their value in this role, although some may act as deodorants by destroying skin organisms which react with sweat to produce body odour.

16.2 Diphenylureas (carbanilides)

From an extensive study by Beaver *et al.* (1957), 1 3,4,4′-trichlorocarbanilide (TCC, triclocarban) emerged as one of the most potent of this family of biocides. It inhibits the growth of many Gram-positive bacteria including MRSA and vancomycin-resistant enterococcus (VRE) (Suller & Russell, 1999) but is not active against Gram-negative organisms (Walsh *et al.*, 2003). Typical growth inhibitory concentrations for Gram-positive organisms range from 0.1 to 1.0 μg/mL. Fungi were found to be more resistant, since 1000 μg/mL failed to inhibit *Aspergillus niger*, *Penicillium notatum*, *C. albicans* and *Fusarium oxysporium*. *Trichophyton gypseum* and *Trichophyton inguinale* were inhibited at 50 μg/mL.

It occurs as a white powder, m.p. 250 °C and is very slightly soluble in water. Like the salicylanilides, it has not found favour in products likely to come in contact with human skin, despite the fact that it had been extensively evaluated as the active ingredient of some disinfectant soaps.

16.3 Mode of action

The mode of action of salicylanilides and carbanilides (diphenylureas) has been studied in detail by Woodroffe and Wilkinson (1966a,b) and Hamilton (1968). The compounds almost certainly owe their bacteriostatic action to their ability to discharge part of the proton-motive force, thereby inhibiting processes dependent upon it, i.e. active transport and energy metabolism. Further general details will be found by consulting Russell and Chopra (1996).

17 Miscellaneous preservatives

Included in this section are those chemicals which are useful preservatives but which do not form part of the biocidal groups already discussed.

17.1 Derivatives of 1,3-dioxane

17.1.1 2,6-dimethyl-1,3-dioxan-4-ol acetate (dimethoxane)

Dimethoxane (Fig. 2.28) is a liquid, colourless

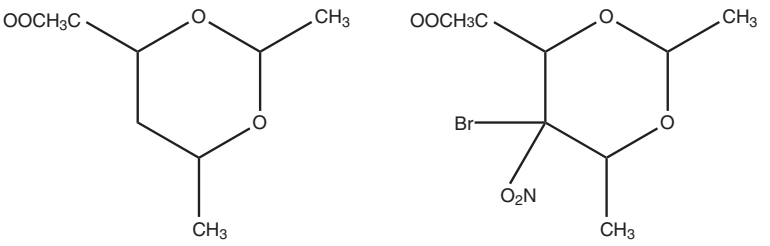


Figure 2.28 Dioxanes: dimethoxane and bronidox.

when pure and soluble in water and organic solvents. It has a characteristic odour.

Dimethoxane is not affected by changes in pH but it is slowly hydrolysed in aqueous solution, producing ethanal (acetaldehyde). It is compatible with non-ionic surface-active agents (Anon., 1962) but may cause discoloration in formulations that contain amines or amides.

Dimethoxane finds application as a preservative for emulsions, water based industrial processes, emulsion paints and cutting oils. In a bacteriological study, Woolfson (1977) attributed the action of the commercial product partially to its aldehyde content and partially to the 1,3-dioxane components.

17.1.2 5-Bromo-5-nitro-1,3-dioxane (Bronidox: Care Chemicals)

This nitro-bromo derivative of dioxane is available as a 10% solution in propylene glycol as Bronidox L. It is used as a preservative for toiletries and has been described in some detail by Potokar *et al.* (1976) and Lorenz (1977). Its stability at various pH values is tabulated by Croshaw (1977).

It is active against bacteria and fungi and does not show a *Pseudomonas* gap. Minimum inhibitory concentrations of the active ingredient (μg/mL) were: *E. coli*, 50; *P. aeruginosa*, 50; *P. vulgaris*, 50; *P. fluorescens*, 50; *S. typhi*, 50; *Serratia marcescens*, 25; *Staph. aureus*, 75; *Strep. faecalis*, 75; *C. albicans*, 25; *S. cerevisiae*, 10; *Aspergillus niger*, 10.

Its activity is not affected between pH 5 and 9 and it probably acts as an oxidizing agent, oxidizing –SH to –S–S– groups in essential enzymes. It does not act as a formaldehyde releaser.

It is suitable for the preservation of surfactant preparations, which are rinsed off after application and do not contain secondary amines.

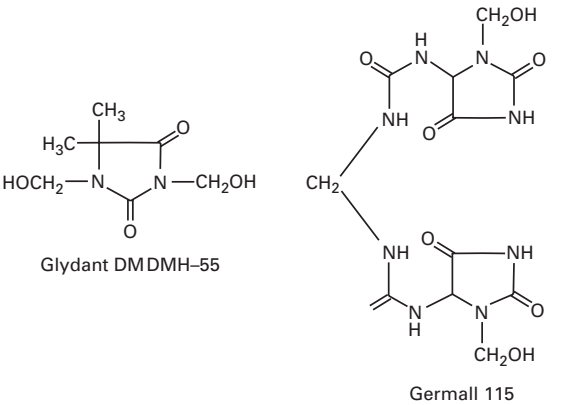


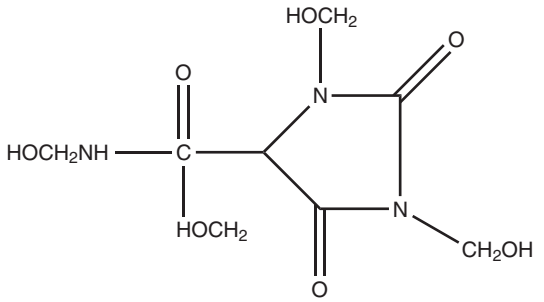
Figure 2.29 Dantoin or Glydant DMDMH-55 and Germall 115.

17.2 Derivatives of imidazole

Imidazolines (Fig. 2.29) are 2,3-dihydroimidazoles; 2-heptadecyl-2-imidazoline was introduced as an agricultural fungicide as far back as 1946. Other derivatives containing the imidazole ring have recently found successful application as preservatives. Two are derivatives of 2,4-dioxotetrahydroimidazole, the imidazolidones; the parent diketone is hydantoin.

17.2.1 1,3-Di(hydroxymethyl)-5,5-dimethyl-2,4-dioxoimidazole; 1,3-di-hydroxymethyl)-5,5-dimethylhydantoin (Dantoin)

A 55% solution of this compound (Fig. 2.29) is available commercially as Glydant (Lonza U.K. Ltd., Cheltenham, U.K.). This product is water-soluble, stable and non-corrosive, with a slight odour of formaldehyde. It is active over a wide range of pH and is compatible with most ingredients used in cosmetics. It has a wide spectrum of activity against bacteria and fungi, being active at concentrations of



Diazolidinyl urea

Figure 2.30 Diazolidinyl urea.

between 250 and 500 μg/mL. The moulds *Microsporum gypseum* and *Trichophyton asteroides*, however, are particularly susceptible, being inhibited at 32 μg/mL. Its mode of action is attributed to its ability to release formaldehyde, the rate of release of which is more rapid at high pH values, 9–10.5, than low, 3–5. Its optimum stability lies in the range pH 6–8. It has an acceptable level of toxicity and can be used as a preservative over a wide field of products. It has been evaluated by Schanno *et al.* (1980).

Glydant 2000, based on a patented process, is an ultra-low (<0.1%) free formaldehyde hydantoin recently launched for use in the personal care industry.

17.2.2 N,N″-methylene-bis-[5′[1-hydroxymethyl]-2,5-dioxo-4-imidazolidinyl urea] (Germall 115: ISP, Wayne, New Jersey, USA)

In 1970 a family of imidazolidinyl ureas for use as preservatives was described (Berke & Rosen, 1970). One of these, under the name Germall 115, has been studied extensively (Rosen & Berke, 1973; Berke & Rosen, 1978). Germall 115 is a white powder very soluble in water, and hence tends to remain in the aqueous phase of emulsions. It is non-toxic, non-irritating and non-sensitizing. It is compatible with emulsion ingredients and with proteins.

A claimed property of Germall 115 has been its ability to act synergistically with other preservatives (Jacobs *et al.*, 1975; Rosen *et al.*, 1977; Berke & Rosen, 1980). Intrinsically it is more active against bacteria than fungi. Most of the microbiological data are based on challenge tests in cosmetic formulations, data which are of great value to the cosmetic microbiologist. An investigation of its activity against a series of *Pseudomonas* species and strains (Berke & Rosen, 1978) showed that in a challenge test 0.3% of the compound cleared all species but *P. putida* and *P. aureofaciens* in 24 h. The latter species were killed between 3 and 7 days. In an agar cup-plate test, 1% solution gave the following size inhibition zones (mm): *Staph. aureus*, 7,6; *Staph. aureus*, penicillin sensitive, 15.5; *Staph. albus*, 9.0; *B. subtilis*, 15.0; *Corynebacterium acne*, 5.0; *E. coli*, 3.6; *P. ovale*, 2.0.

17.2.3 Diazolidinyl urea

Diazolidinyl urea (Fig 2.30) is a heterocyclic substituted urea produced by the reaction of allantoin and formaldehyde in a different ratio than that for imidazolidinyl urea. It is available as a powder commercially as Germall II (ISP, Wayne, New Jersey, USA) and when used at 0.1–0.3% is stable at pH 2–9 providing broad spectrum antibacterial activity with some activity against moulds. It is twice as active as imidazolidinyl urea. It is often used in conjunction with methyl and propyl paraben to provide additional activity against mould. When combined with 3-iodo-2-propynylbutylcarbamate a synergistic action is achieved.

17.3 Isothiazolones

Ponci *et al.* (1964) studied the antifungal activity of a series of 5-nitro-1,2-dibenzisothiazolones and found many of them to possess high activity. Since this publication a number of isothiazolones (Fig. 2.31) have emerged as antimicrobial preservatives. They are available commercially, usually as suspensions rather than as pure compounds, and find use in a variety of industrial situations. Nicoletti *et al.* (1993) have described their activity.

17.3.1 5-Chloro-2-methyl-4-isothiazolin-3-one (CMIT) and 2-methyl-4-isothiazolin-3-one (MIT)

A mixture of these two derivatives (3 parts CMIT to 1 part MIT), known as Kathon 886 MW (Rohm and Haas (UK) Ltd., Croydon, CR9 3NB, UK), con-

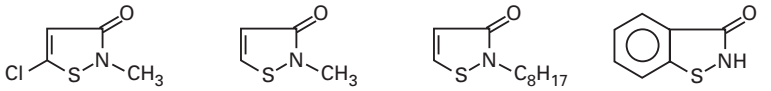


Figure 2.31 Isothiazolones. From left to right: 5-chloro-2-methyl-4-isothiazolin-3-one (CMIT), 2-methyl-4-isothiazolin-3-one (MIT), 2-*n*-octyl-4-isothiazolin-3-one and 1,2-benzisothiazolin-3-one (BIT).

taining about 14% of active ingredients is available as a preservative for cutting oils and as an in-can preservative for emulsion paints. This mixture is active at concentrations of 2.25–9 μg/mL active ingredients against a wide range of bacteria and fungi and does not show a *Pseudomonas* gap. It is also a potent algistat.

Kathon CG, containing 1.5% active ingredients and magnesium salts has been widely used in cosmetic products. The level of activity to be included in cosmetic rinse-off products is restricted to 15 p.p.m. and for leave-on products 7.5 p.p.m. because of irritancy issues primarily due to the CMIT element. It possesses the additional advantage of being biodegradable to non-toxic metabolites, water-soluble and compatible with most emulgents. The stability of Kathon 886 at various pH values is described by Croshaw (1977).

Methylisothiazolinone alone as 9.5% in water, commercially available as Neolane M-10 (Rohm & Haas), is stable over a wide range of pH and temperature conditions and is compatible with a variety of surfactants. It has broad spectrum activity and is said to be particularly useful for replacing formaldehyde in a wide range of applications at levels of 50–150 p.p.m. active ingredient (Diehl, 2002).

From August 2001, in the EC, any product containing CMIT/MIT in the ratio 3:1 in excess of 15 p.p.m. must display an appropriate R phrase warning.

*17.3.2 2-*n*-Octyl-4-isothiazolin-3-one (Skane: Rohm & Haas)*

This is available as a 45%, solution in propylene glycol and is active against bacteria over a range of 400–500 μg/mL active ingredient. To inhibit the growth of one strain of *P. aeruginosa* required 500 μg/mL. Fungistatic activity was shown against a wide number of species over the range 0.3–8.0 μg/mL. It is also effective at preventing algal growth at concentrations of 0.5–5.0 μg/mL. It is biodegradable but shows skin and eye irritancy. As might be expected from its *n*-octyl side-chain, it is not soluble in water.

17.3.3 1,2-Benzisothiazolin-3-one (BIT)

This is available commercially in various formulations and is recommended as a preservative for industrial emulsions, adhesives, polishes, glues, household products and paper products. It possesses low mammalian toxicity but is not a permitted preservative for cosmetics.

17.3.4 Mechanism of action

At growth-inhibitory concentrations, BIT has little effect on the membrane integrity of *Staph. aureus*, but significantly inhibits active transport and oxidation of glucose and has a marked effect on thiol-containing enzymes.

Thiol-containing compounds quench the activity of BIT, CMIT and MIT against *E. coli*, which suggests that these isothiazolones interact strongly with –SH groups. The activity of CMIT is also overcome by non-thiol amino acids, so that this compound might thus react with amines as well as with essential thiol groups (Collier *et al.*, 1990a,b).

17.4 Derivatives of hexamine

Hexamine (hexamethylene tetramine; 1,3,5,7-triaza-1-azonia-adamantane) has been used as a urinary antiseptic since 1894. Its activity is attributed to a slow release of formaldehyde. Other formaldehyde-releasing compounds are considered in sections 7.2.4, 17.2, 17.5 and 17.6. Wohl in 1886 was the first to quaternize hexamine, and in 1915–16 Jacobs and co-workers attempted to extend the antimicrobial range of hexamine by quaternizing one of its nitrogen atoms with halo-hydrocarbons (Jacobs & Heidelberger, 1915a,b; Jacobs *et al.*, 1916a,b). These workers did not consider that their

Table 2.13 Inhibitory concentrations for hexamine quaternized with $-CH_2Cl=CHCl$ compared with values for hexamine and formaldehyde.

	MIC[a] *against*					
Inhibitor	Staph. aureus	S. typhi	K. aerogenes	P. aeruginosa	B. subtilis	D. desulfuricans
Hexamine quaternized with:						
$-CH_2$-CH=CHCl	4×10^{-4} (100)	2×10^{-4} (50)	2×10^{-4} (50)	2×10^{-3} (500)	4×10^{-4} (100)	2.9×10^{-2} (7250)
Hexamine	3.5×10^{-2} (5000)	3.5×10^{-3} (500)	—	—	—	5.3×10^{-2} (7500)
Formaldehyde	1.6×10^{-3} (50)	3.3×10^{-3} (100)	1.6×10^{-3} (50)	—	—	–

[a]Molar values (in parentheses μg/mL).

compounds acted as formaldehyde releasers but that activity resided in the whole molecule.

The topic was taken up again by Scott and Wolf (1962). These workers re-examined quaternized hexamine derivatives with a view to using them as preservatives for toiletries, cutting oils and other products. They looked at 31 such compounds and compared their activity also with hexamine and formaldehyde. As well as determining their inhibitory activity towards a staphylococci, enterobacteria and a pseudomonad, they also assessed inhibitory activity towards *Desulfovibrio desulfuricans*, a common contaminant of cutting oils.

Polarographic and spectroscopic studies of formaldehyde release were made on some of the derivatives; this release varied with the substituent used in forming the quaternary salt. A typical set of data for the antimicrobial activity (MIC) of one derivative compared with hexamine and formaldehyde is shown in Table 2.13. In general, the quaternized compounds were found to be more active w/w than hexamine but less active than formaldehyde. Although chemically they contain a quaternized nitrogen atom, unlike the more familiar antimicrobial quaternized compounds (section 6.1), they are not inactivated by lecithin or protein. The compounds are not as surface-active as conventional QACs. Thus an average figure for the surface tension, dyne/cm, for 0.1% solutions of the quaternized hexamines was 54; that for 0.1% cetrimide (section 6.1) was 34.

One of these derivatives of hexamine – that quaternized with *cis*-1,3-dichloropropene – is being used as a preservative under the name Dowicil 200

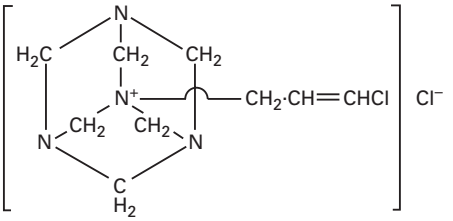


Figure 2.32 Dowicil 200 (*N*-(3-*cis*-chloroallyl)hexamine).

(Dow Chemical Company). *Cis*-1-(3-cis-chloroallyl)-3,5,7-triaza-1-azonia-admantane chloride *N*-(3-chloroallyl) hexamine (Dowicil 200; Fig. 2.32) is a highly water-soluble hygroscopic white powder; it has a low oil solubility. It is active against bacteria and fungi. Typical MIC (μg/mL) were: *E. coli*, 400; *P. vulgaris*, 100; *S. typhi*, 50; *Alcaligenes faecalis*, 50; *P. aeruginosa*, 600; *Staph. aureus*, 200; *B. subtilis*, 200; *Aspergillus niger*, 1200; *T. interdigitale*, 50.

It is recommended for use as a preservative for cosmetic preparations at concentrations of from 0.05 to 0.2%. Because of its high solubility, it does not tend to concentrate in the oil phase of these products, but remains in the aqueous phase, where contamination is likely to arise. It is not inactivated by the usual ingredients used in cosmetic manufacture. Its activity is not affected over the usual pH ranges found in cosmetic or cutting oil formulations. For further information, see Rossmore & Sondossi (1988).

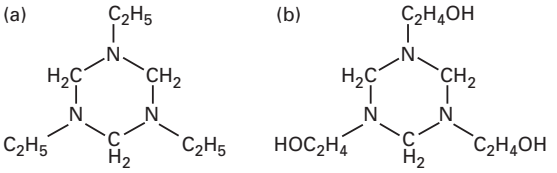


Figure 2.33 (a) Hexahydro-1,3,5-triethyl-*s*-triazine: (b) 1,3,5-tris(2-hydroxyethyl)-*s*-triazine (Grotan).

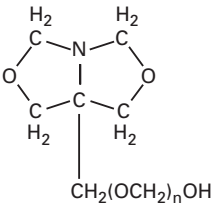


Figure 2.34 Nuosept 95 ($n = 0–5$).

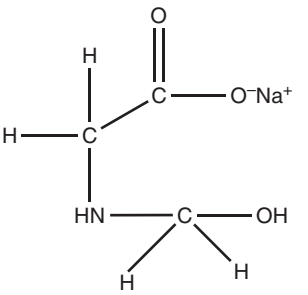


Figure 2.35 Sodium hydroxymethylglycinate.

17.5 Triazines

The product, theoretically from the condensation of three molecules of ethylamine with three of formaldehyde, is hexahydro-1,3,5-triethyl-*s*-triazine ; Fig. 2.33a). This is a clear white or slightly yellow viscous liquid, readily soluble in water, acetone, ethanol and ether. It is bactericidal and fungicidal and inhibits most bacteria, including *P. aeruginosa* and *D. desulfuricans* at concentrations of 0.3 mg/mL. Fungi, such as *A. niger*, *Penicillium glaucum* and *P. notatum* are inhibited at 0.1 mg/mL, and *S. cerevisiae* at 0.05 mg/mL. It owes its activity to a release of formaldehyde. It has been used as a preservative for cutting oils, for the 'in-can' preservation of emulsion paints for proteinaceous adhesives and to control slime in paper and cardboard manufacture, and to prevent the growth of microorganisms in water-cooling systems. It has a low intrinsic toxicity and at use dilutions is not irritant to the skin.

If formaldehyde is reacted with ethanolamine, the compound 1,3,5-tris(2-hydroxyethyl)-*s*-triazine can be formed (Grotan: Troy Corporation, New Jersey, USA; Fig. 2.33b). This has both antibacterial and antifungal activity and is recommended as a preservative for cutting oils. Despite the figures for fungal inhibition, it is often found, in practical preservation situations, that, although this triazine will inhibit microbial growth, a fungal superinfection is often established; a total preservation system which includes a triazine might well have to contain an additional antifungal compound (Rossmore *et al.*, 1972; Paulus, 1976). This situation may be compared with that found with imidazole derivatives (section 17.2).

Rossmore (1979) has discussed the uses of heterocyclic compounds as industrial biocides, and Rossmore and Sondossi (1988) have reviewed formaldehyde condensates in general.

17.6 Oxazolo-oxazoles

By reacting formaldehyde with tris(hydroxymethyl)-methylamine, a series of derivatives is obtained. The commercial product (Nuosept 95: ISP, Wayne, New Jersey, USA; Fig. 2.34) contains the molecule species: 5-hydroxymethoxymethyl-1-aza-3,7-dioxabicyclo (3.3.0) octane, 24.5%; 5-hydroxymethyl-1-aza-3,7-dioxabicyclo (3.3.0) octane, 17.7%; 5-hydroxypolymethylenoxy (74% C_2, 21% C_3, 4% C_4, 1% C_5) methyl-1-aza-3, 7-dioxabicyclo (3.3.0) octane, 7.8%, and acts as a biostat by virtue of being a formaldehyde releaser.

It is obtained as a clear, pale-yellow liquid, which is miscible with water, methanol, ethanol, chloroform and acetone in all proportions, and is recommended as a preservative for cutting oils, water treatment, plants, emulsion (latex) paints, industrial slurries and starch- and cellulose-based products. It is slightly irritant to intact and abraded skin and is a severe eye irritant.

17.7 Sodium hydroxymethylglycinate

A 50% aqueous solution of this compound (Fig.

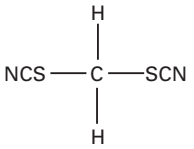


Figure 2.36 Methylene bisthiocyanate.

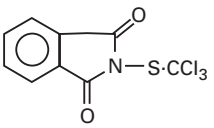


Figure 2.37 Captan.

2.35) is available commercially as Suttocide A (ISP, Wayne, New Jersey, USA). The solution is a clear alkaline liquid with a mild characteristic odour. It is active over a pH range of 3.5–12.0 and has a broad spectrum of activity against Gram-positive and -negative bacteria, yeasts and mould at concentrations between 0.05 and 0.25%.

17.8 Methylene bisthiocyanate

This is available commercially as a 10% solution and is recommended for the control of slime in paper manufacture, where it provides a useful alternative to mercurials. The compound (Fig. 2.36) is a skin and eye irritant and thus care is required in its use. Its toxicity is low enough to enable it to be used in the manufacture of papers destined for the packaging of food. At in-use dilutions, it is unlikely to cause corrosion of materials used in the construction of paper-manufacturing equipment.

17.9 Captan

Captan is *N*-(trichloromethylthio)cyclohex-4-ene-1,2-dicarboximide (Fig. 2.37). It is a white crystalline solid, insoluble in water and only slightly soluble in organic solvents. It is decomposed in alkaline solution. Despite its low solubility, it can be shown to be an active biocide, being active against both Gram-negative and Gram-positive bacteria, yeasts and moulds. It has been used as an agricultural fungicide, being primarily employed against diseases of fruit trees. It has also been used to prevent spoilage of stored fruit and in the treatment of skin infections due to fungi in humans and animals.

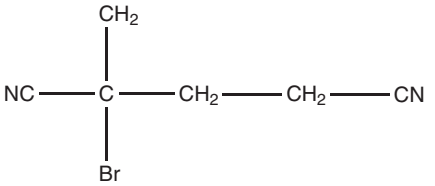


Figure 2.38 1,2-dibromo-2,4-dicyanobutane (Tektamer 38).

17.10 1,2-dibromo-2,4-dicyanobutane (Tektamer 38)

A halogenated aliphatic nitrile, 1,2-dibromo-2,4-dicyanobutane (Fig 2.38) is an off-white powder with a mildly pungent odour. It is available commercially as Tektamer 38 (Nalco Chemical Company, Illinois). It is a broad spectrum antimicrobial being most active over the pH range 2.0–9.5, at an effective concentration of 0.025–0.15%. It is used in paints, joint cements, adhesives, pigments and metal working fluids.

17.11 Essential oils

Essential oils have been used empirically throughout history as preservatives. Their re-examination as antimicrobial agents has received attention from many workers, as their use as natural preservatives has contemporary appeal.

Melaleuca alternifolia (tea tree) oil has been included increasingly in consumer products as an antimicrobial agent. Studies have shown that Tea tree oil is an effective antimicrobial agent demonstrating activity against methicillin-resistant *Staph. aureus* (Carson *et al.*, 1995; Elsom & Hide, 1999; May *et al.*, 2000), yeasts (Hammer *et al.,* 1998; (Hammer *et al.,* 2000; Mondello *et al.*, 2003) and Herpes simplex virus (Carson *et al.*, 2001).

Thymol and carvacrol (an isomer of thymol) are believed to be the active ingredient of several essential oils and are found in plants such as thyme and oregano (Nakatsu *et al.*, 2000). Oregano and thyme essential oils have been shown to be strongly microbiocidal against Gram-positive and Gram-negative bacteria and fungi (Nakatsu *et al.,* 2000) and are very effective against *E. coli* O157:H7 (Burt &

Reinders, 2003). Carvacrol has been hypothesized to exert its antimicrobial activity by destabilizing the cytoplasmic membrane and acting as a proton exchanger (Ultee *et al.,* 2002*)*. Thymol has also found use as an active ingredient in mouthwashes. Listerine® Antiseptic which contains thymol, menthol and eucalyptol essential oils demonstrated antimicrobial activity against oral micro-organisms in dental plaque (Fine *et al.*, 2000, 2001; Pan *et al.*, 2000).

Essential oils have also been shown to act synergistically with other antimicrobial agents (Hodgson *et al.*, 1995; Shin & Kang, 2003) and physical conditions (Skandamis & Nychas, 2000; Karatzas *et al.*, 2001).

Many other essential oils have been isolated and investigated with varying degrees of antimicrobial activity demonstrated. Activity has been shown to be influenced by factors such as the vapour activity of the oils (Inouye *et al.*, 2001) and the test method employed (Suhr & Neilson, 2003). The antibacterial properties of essential oils have been reviewed by Deans and Ritchie (1987) and Nakatsu *et al.*, 2000).

17.12 General statement

Much of the information concerning the compounds properties and uses is found in the manufacturers' information brochures. Any person wishing to explore their use should consult the manufacturers. An ever-present problem with cosmetics preservation is that of contact sensitization. This is discussed in some detail by Marzulli and Maibach (1973) and is a point which must be carefully checked before a preservative is committed to a product. Another hazard which may arise is that of an induced change in the skin microflora during continuous use of products containing antimicrobial preservatives; this is discussed by Marples (1971).

18 Vapour-phase disinfectants

Gaseous sterilization is the subject of Chapter 12.3, and thus only a few comments will be made here. It is only comparatively recently that a scientific basis for using gases as sterilizing or disinfecting agents has been established. Factors influencing the activity of gaseous formaldehyde were described by Nordgren (1939) and later by a Committee on Formaldehyde Disinfection (Anon., 1958). The possible uses of gaseous formaldehyde in the disinfection of hospital bedding and blankets and, in conjunction with low-temperature steam, for disinfection of heat-sensitive material, are considered in section 18.2 (see also Chapter 12.3).

Phillips & Kaye (1949) reviewed the earlier work which had taken place with ethylene oxide, which has bactericidal, mycobactericidal, sporicidal, fungicidal and viricidal activity (Ernst, 1974). A later review is by Richards *et al.* (1984).

Other gases of possible value include propylene oxide, ozone, methyl bromide and glycidaldehyde (Russell, 1976). Physical and chemical properties of these and the two most important ones (ethylene oxide and formaldehyde) are listed in Table 2.14 and their chemical structures are given in Fig. 2.39.

18.1 Ethylene oxide

This is discussed in detail later (Chapter 12.3) and will not be considered here in detail. Its antimicrobial activity is affected by concentration, temperature, relative humidity and the water content of microorganisms. It acts, by virtue of its alkylating properties, on proteins and nucleic acids. A consideration of its antimicrobial activity with compounds of a similar chemical structure (Figs 2.39 and 2.40) demonstrates that cyclopropane, which is not an alkylating agent, is not antimicrobial whereas those that have the ability to alkylate are potent antimicrobials.

Useful reviews are those by Hoffman (1971), Phillips (1977), Richards *et al.* (1984), Burgess and

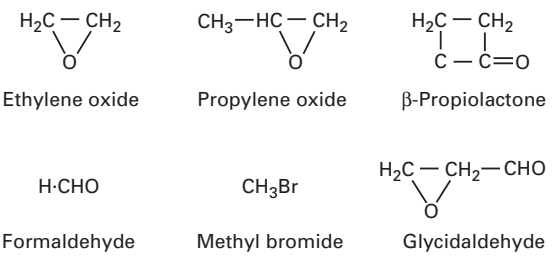


Figure 2.39 Chemical structures of gaseous disinfectants.

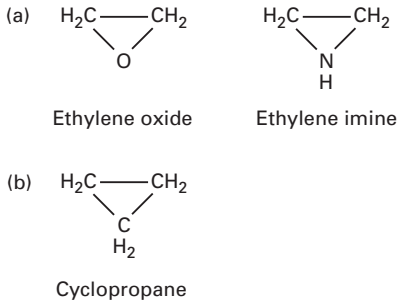


Figure 2.40 Compounds similar to ethylene oxide: (a) alkylating and antimicrobial compounds; (b) non-alkylating, non-antimicrobial agent.

Reich (1993), Jorkasky (1993), Page (1993) and Sintim-Damoa (1993).

18.2 Formaldehyde-releasing agents

Paraformaldehyde ($HO(CH_2O)_n \cdot H$, where n = 8–100) is a polymer of formaldehyde and is produced by evaporating aqueous solutions of formaldehyde. Although it was considered originally to be of little practical use (Nordgren, 1939) paraformaldehyde has since been shown to depolymerize rapidly when heated, to produce formaldehyde (Taylor *et al.*, 1969). Paraformaldehyde is considered by Tulis (1973) to be an excellent source of monomeric formaldehyde gas, because it can be produced in a temperature-controlled reaction, and there are no contaminating residues (methanol and formic acid) produced during evaporation of formalin solutions, in contrast to the method of evaporating formalin solutions containing 10% methanol to prevent polymerization.

Other formaldehyde-releasing agents are melamine formaldehyde and urea formaldehyde (Fig. 2.41). The former is produced from formaldehyde and melamine under alkaline conditions and the latter is a mixture of monomethyloyl urea and dimethyloyl urea. When exposed to elevated temperatures these agents release potentially sterilizing amounts of gaseous formaldehyde, the rate of release being a function of time and temperature. These formaldehyde-releasing agents are, however, much less effective as disinfecting or sterilizing sources than paraformaldehyde. The reason for this is that there is a much greater release of formaldehyde from paraformaldehyde than from the resins at various temperatures, and the microbicidal process is strictly a function of the available formaldehyde gas.

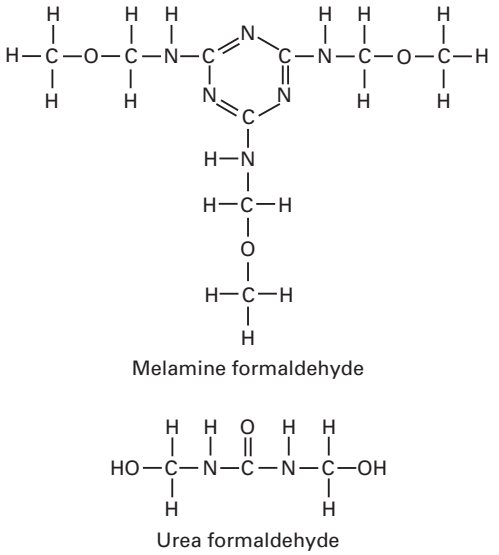


Figure 2.41 Melamine formaldehyde and urea formaldehyde.

Applications and mode of action of formaldehyde-condensate biocides have been reviewed by Rossmore and Sondossi (1988) and Rossmore (1995).

Formaldehyde vapour has found use as a disinfectant in the following situations (Russell, 1976):
1 in combination with low-temperature steam (70–90 °C) as a method for disinfecting heat-sensitive materials (Alder *et al.*, 1971, 1990). This will be discussed later (Chapter 12.1); however, some studies (Wright *et al.*, 1996) have cast doubt on the efficacy of this process as a sterilization method because it has been possible by means of a post-heating shock to revive some treated spores;
2 rarely, in the disinfection of hospital bedding and blankets, when formaldehyde solutions are used in the penultimate rinse of laundering blankets to give a residual bactericidal activity because of the slow evolution of formaldehyde vapour (Dickinson & Wagg, 1967; Alder *et al.*, 1971, 1990);
3 in the terminal disinfection of premises, although this is considered to be of limited value (Kelsey, 1967);

Table 2.14 Properties of the most commonly used gaseous disinfectants.

Gaseous disinfectant	*Molecular weight*	*Boiling point (°C)*	*Solubility in water*	*Sterilizing concn (mg/L)*	*Relative humidity requirements (%)*	*Penetration of materials*	*Microbidical activity*[a]	*Best application as gaseous disinfectant*[b]
Ethylene oxide	44	10.4	Complete	400–1000	Non-desiccated 30–50; large load 60	Moderate	Moderate	Sterilization of plastic medical supplies
Propylene oxide	58	34	Good	800–2000	Non-desiccated 30–60	Fair	Fair	Decontamination
Formaldehyde	30	90 °C/ Formalin[c]	Good	3.10	75	Poor (surface sterilant)	Excellent	Surface sterilant for rooms
Methyl bromide	95	4.6	Slight	3500	30–50	Excellent	Poor	Decontamination

[a]Based on an equimolar comparison.
[b]See later also, Chapter 12.3.
[c]Formalin contains formaldehyde plus methanol.

4 as a fumigant in poultry houses after emptying and before new stock is introduced (Nicholls *et al.*, 1967; Anon., 1970) and in the hatchery to prevent bacterial contamination of shell eggs (Harry, 1963); **5** in the disinfection of safety cabinets.

18.3 Propylene oxide

Propylene oxide requires only mild heating to produce the vapour form and has a fair penetration of materials (Table 2.14). It hydrolyses slowly in the presence of only a small amount of moisture to give the non-toxic propylene glycol (Kereluk, 1971) and there is no need to remove it from exposed materials (Sykes, 1965). Antibacterial activity decreases with an increase in r.h. (Bruch & Koesterer, 1961), although with desiccated organisms the reverse applies (Himmelfarb *et al.*, 1962). Propylene oxide has been shown to be suitable for treating powdered or flaked foods (Bruch & Koesterer, 1961).

18.4 Methyl bromide

Methyl bromide is a gas at normal temperatures. It is considerably less active as an antibacterial agent than ethylene oxide (Kelsey, 1967; Kereluk, 1971) or propylene oxide (Kelsey, 1967) but has good penetrative ability (Table 2.14). Methyl bromide is listed by Kereluk (1971) as being suitable for some types of fumigation.

18.5 Ozone

Ozone, O_3, is an allotropic form of oxygen. It has powerful oxidizing properties, inhibits bacterial growth (Ingram & Haines, 1949; Baird-Parker & Holbrook, 1971) and is bactericidal, viricidal and sporicidal, although spores are 10–15 times more resistant than non-sporing bacteria (Gurley, 1985; Rickloff, 1985). Gaseous ozone reacts with amino acids, RNA and DNA. It is unstable chemically in water, but activity persists because of the production of free radicals, including HO·. Synergistic effects have been shown with the simultaneous use of sonication (Burleson *et al.*, 1975), and negative air ions (Fan *et al.*, 2002). The use of ozone for enhancing food safety and quality has been reviewed by Kim *et al.*, (1999) and Khadre *et al.* (2001).

18.6 Carbon dioxide

Carbon dioxide in soft drinks inhibits the development of various types of bacteria (Dunn, 1968). The growth of psychrotolerant, slime-producing bacteria is markedly inhibited by CO_2 gas in the atmosphere (Clark & Lentz, 1969).

18.7 Mechanism of action

Only a few brief comments will be made, and the interested reader is directed to the reviews of Bruch

and Bruch (1970), Hoffman (1971), Russell (1976), Richards *et al.* (1984) and Russell and Chopra (1996) for further information. As noted above (section 18.1, Figs. 2.39 and 2.40), there is strong evidence that ethylene oxide acts by virtue of its alkylating properties; this gaseous agent reacts with proteins and amino acids, and with nucleic acid guanine (to give 7-(2′-hydroxyethyl) guanine), with alkylation of phosphated guanine possibly being responsible for its activity (Michael & Stumbo, 1970). Formaldehyde is an extremely reactive chemical, which interacts with cell protein, RNA and DNA (Russell & Hopwood, 1976).

19 Aerial disinfectants

An early procedure for aerial disinfection was the employment of sulphur dioxide, obtained by burning sulphur, or of chlorine for fumigating sickrooms.

An effective aerial disinfectant should be capable of being dispersed in the air so that complete and rapid mixing of infected air and disinfectant ensues. Additionally, an effective concentration should be maintained in the air, and the disinfectant must be highly and rapidly effective against airborne microorganisms at different relative humidities. To these microbiological properties must be added the property of no toxicity or irritancy.

The most important means of using aerial disinfectants is by aerosol production. Aerosols consist of a very fine dispersed liquid phase in a gaseous (air) disperse phase. The lethal action of aerosols is believed to be due to condensation of the disinfectant on to the microbial cell (Sykes, 1965). Thus, the disinfectant must be nebulized in a fine spray to enable it to remain airborne and thereby come into contact, by random collision, with any microorganisms present in the air. Aerosol droplets of <1 mm tend to be the accepted standard. Relative humidity has an important bearing on activity and at low r.h. inadequate condensation of disinfectant on to the microbial cell occurs. This means that dust-borne organisms are less susceptible to aerial disinfectants than are those enclosed in droplets; the optimum r.h. is usually 40–60%. In practice, chemical aerosols may be generated by spraying liquid chemicals into the air from an atomizer; solids may be vaporized by heat from a thermostatically controlled hotplate or dissolved in an appropriate solid and atomized.

Various chemicals have been employed for disinfecting air, including the following:

1 Hexylresorcinol: this phenolic substance is active against a wide range of bacteria, but not spores, in air. It is vaporized from a thermostatically controlled hotplate, and the vapour is odourless and non-toxic.

2 Lactic acid: this is an effective bactericidal aerial agent, but is unfortunately irritant at high concentrations.

3 Propylene glycol: this may be employed as a solvent for dissolving a solid disinfectant prior to atomization, but is also a fairly effective and non-irritating antimicrobial agent in its own right (Baird-Parker & Holbrook, 1971).

4 Formaldehyde: in summary of previous information, formaldehyde gas may be generated by:

(a) evaporating commercial formaldehyde solution (formalin);

(b) adding formalin to potassium permanganate;

(c) volatilizing paraformaldehyde (Taylor *et al.*, 1969);

(d) exposing certain organic resins or polymers, such as melamine formaldehyde or urea formaldehyde, to elevated temperatures (Tulis, 1973; see Russell, 1976).

Fumigation by formaldehyde has found considerable use in poultry science (Anon., 1970).

20 Other uses of antimicrobial agents

Antimicrobial agents are used widely as disinfectants and antiseptics in the hospital and domestic environments, as preservatives or bactericides in sterile or non-sterile pharmaceutical or cosmetic products (Hodges & Denyer, 1996), and as preservatives in certain foodstuffs. Additionally, they are employed in certain specialized areas, such as cutting oils, fuels, paper, wood, paint, textiles and the construction industry.

20.1 Disinfectants in the food, dairy, pharmaceutical and cosmetic industries

The effectiveness of many disinfectants is reduced in the presence of organic matter in its various forms, such as blood, serum pus, dirt, earth, milkstone, food residues and faecal material (Chapter 3). This decreased activity has an important bearing on disinfectant use in the cosmetic (Davis, 1972a), pharmaceutical (Bean, 1967), food (Kornfeld, 1966; Goldenberg & Reif, 1967; Olivant & Shapton, 1970; Banner, 1995) and dairy (Clegg, 1967, 1970; Davis, 1972b; Anon., 1977) industries. The principles in all cases are the same, namely either adequate precleaning before use of the disinfectant or a combination of the disinfectant with a suitable detergent.

Organic matter may reduce activity either as a result of a chemical reaction between it and the compound, thus leaving a smaller antimicrobial concentration for attacking microorganisms, or through a protection of the organisms from attack (Sykes, 1965). Phospholipids in serum, milk and faeces will reduce the antimicrobial activity of QACs.

20.2 Disinfectants in recreational waters

The growing popularity of public and private swimming-pools has led to the inevitable problems of maintaining adequate hygienic standards, notably in relation to the possible transmission of infective microorganisms from one person to another. At the same time, control measures must ensure that the swimming-pool water has no toxic or irritant effects on the users of the pool. Various microorganisms have been associated with infections arising from hydrotherapy pools, swimming-pools and whirlpools, but the most frequently implicated organism is *P. aeruginosa*, the source of which is often the pool pumps (Friend & Newsom, 1986; Aspinall & Graham, 1989). Disinfection of recreational, hydrotherapy pools and other pools in health care, is considered further in Chapter 20.3. Chlorine disinfectants are commonly used as a sanitary control measure. Iodine has been mooted as a potential swimming pool disinfectant but although it is cheaper and more stable than chlorine, unlike chlorine it is not active against algae rendering it unsuitable for this application (Black *et al.*, 1970a,b). Another useful agent used for the disinfection of swimming-pools is the polymeric biguanide, Baquacil SB (Avecia, Blackley, Manchester, UK). The properties of this type of compound have been described in section 5.3.

Warren *et al.* (1981) have published a comparative assessment of swimming-pool disinfectants. Problems arising from the increasing use of whirlpools are referred to in Report (1989).

21 Which antimicrobial agent?

21.1 Regulatory requirements

The Federal Drug Administration (FDA) in the USA, the EU for the European community and most other countries publish information on the permitted use and concentration of preservatives. Current regulations should be consulted and complied with when manufacturing in these countries and exporting to them.

Cosmetic preservatives allowed in the EU are prescribed in Annex VI of the Cosmetics Directive which includes details of concentration limits and restrictions for certain product types. In the UK, the Food Standards Agency publishes information on food additives and E-numbers.

21.2 Which preservative?

Because of the many variables which affect the activity of antimicrobial agents, it is almost impossible from a mere scrutiny of the literature to select a preservative that will be optimal in a particular product. Legislation passed in the USA by the FDA requires the manufacturers of cosmetics to declare the ingredients in their products and to state their function or purpose.

As regards combinations, an appraisal of the literature seems to suggest that a combination of one of the more water-soluble esters of *p*-hydroxybenzoic acid, probably the methyl ester, together with one of the water-soluble urea derivatives or a sulphydryl reactive compound, might be a good combination to start with. Denyer *et al.* (1985) have discussed synergy in preservative combinations.

If the product is a water-in-oil emulsion, and it is felt that the oily phase needs protection, especially from mould infestation, then a third component, one of the oil-soluble esters of *p*-hydroxybenzoic acid, e.g. the butyl ester, or an oil-soluble phenol, such as *o*-phenylphenol, might well be added. Over and above this, there remains the question-begging proviso 'providing other criteria such as compatibility, stability, toxicity and regulatory requirements are satisfied'.

21.3 New concepts

In recent years, 'natural antimicrobial agents' have increasingly been considered by food microbiologists as potential preservatives for food products. These agents may be associated with immune systems and have been examined in mammals, insects and amphibians. As pointed out by Board (1995), an agent active against prokaryotic but not mammalian cells is of obvious interest. Although Board (1995) was discussing natural antimicrobials from animals as potential food preservatives, it is clear that their possible use in other areas should also be investigated.

Likewise, the potential of natural food ingredients for the inhibition of growth of microorganisms has been investigated (Beales, 2002). Such ingredients include plant extracts, essential oils (covered in greater depth in section 17.11), citrus fruits such as grapefruit peel extracts (Negi & Jayaprakasha, 2001) and honey, shown to be active against Gram-positive cocci (Cooper *et al.*, 2002).

Bacteria such as lactic acid bacteria produce peptides which have been shown to have antimicrobial activity. These peptides are termed bacteriocins. Cleveland *et al.*, (2001) has reviewed the bacteriocins produced by lactic acid bacteria such as Nisin and Pediocin and has shown them to be safe and have potential as natural food preservatives. Whilst these agents themselves are not new, consumer focus is increasingly moving towards 'natural' or 'naturally produced' food additives. Further information about their antimicrobial spectrum, mode of action and physiochemical properties can be found in Ennahar *et al.*, (1999), Nes and Holo (2000), Cintas *et al.* (2001) and Cleveland *et al.* (2001).

Antimicrobial peptides can also be isolated from plants, insects and mammals and have been shown to have antifungal activity (Müller *et al.*, 1999; Lupetti *et al.*, 2002).

The use of light activated biocides (or photodynamic therapy) has received a lot of recent attention. This approach uses compounds which, when activated by a light source, will generate free radicals and reactive oxygen species and damage the target cells. Applications such as dentistry, for the treatment of periodontal disease, require the target cells to be killed without causing damage to human tissue. Poly-L-lysine-chlorin *e6* activated by red light have been demonstrated to be effective at killing oral bacteria without any adverse effects to epithelial cells (Soukos *et al.*, 1998). This technology can also be applied to wound sites. Griffiths and co-workers (1997) have demonstrated that aluminium disulphonated phthalocyanine when activated by red light killed a range of strains of methicillin-resistant *Staph. aureus*.

Titanium dioxide has been shown to possess bactericidal properties when irradiated with near UV light (Matsunaga *et al.*, 1985). The mechanism of action of this system has been investigated using *E. coli* as a model organism. Damage is proposed to occur initially at the cell envelope followed by progressive damage to the cytoplasmic membrane (Maness *et al.*,1999; Huang *et al.*,2000). Light activated titanium dioxide systems may also have applications in water sanitization although activity is reduced in the presence of organic material and inorganic-radical scavengers (Ireland *et al.*, 1993). In addition to antibacterial activity, photocatalysed titianium dioxide has also been demonstrated to have activity against endotoxin (Sunada *et al.*, 1998) and viruses (Lee *et al.*, 1998; Kashige *et al.*, 2001). Lee and co-workers (1998), using bacteriophage Q, as a model virus, proposed the mechanism of virucidal action was due to nucleic acid damage generated by photocatalysis.

Other photosensitive compounds and wavelengths of light have been investigated for use as photodynamic therapy systems and are discussed in the comprehensive review of Wainwright (1998).

Another future avenue for biocides may lie, not with new agents, but with novel delivery systems to ensure that the biocide reaches its target. One such

delivery system is the use of biodegradable lactic acid polymers for delivery of antibiotics in chronic bone infections (Kanellakopoulou *et al*., 1999). The aim of the delivery system is to obtain high levels of the antibiotic at the site of the infection. The use of pH-sensitive liposomes to deliver gentamicin has the same rationale. Gentamicin has a poor penetration through biological membranes and use of this delivery system was shown to increase gentamicin accumulation to the disease site (Lutwyche *et al.,* 1998; Cordeiro *et al.,* 2000). It is foreseeable that such techniques will be used for the delivery of biocides in the future.

22 The future

With the introduction of the Biocidal Products Directive in Europe (1998), the cost to manufacturers of registering even existing biocides has resulted in some being removed from the market, and the incentive to research and develop new biocides is severely restricted. New combinations of exisiting biocides are likely to be the focus of attention.

With the emergence of 'new' pathogenic entities, such as the prions, glycopeptide-resistant enterococci and multidrug-resistant mycobacteria, as well as biocide-resistant mycobacteria, it is clear that better usage of existing biocides is necessary. This has been discussed by Russell & Russell (1995) and Russell & Chopra (1996). In brief, future policies might well be to examine combinations of biocides, or of a biocide with a permeabilizer, to re-evaluate older, perhaps discarded, molecules, to consider whether physical procedures can enhance antimicrobial activity and, where relevant, to determine how natural antimicrobial systems can be better utilized.

A long-term goal should be the achievement of a better understanding of the ways in which microorganisms are inactivated and of the mechanisms whereby they circumvent the action of a biocide.

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