

13 Preservation of cosmetics and toiletries

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13.1 Historical background to the modern concept of preservation

With the manufacture of cosmetics and toiletries on an industrial scale it became from the commercial point of view very important to maintain the integrity of the products released for sale. Manufacturers, therefore, feared microbiological contamination problems merely for their adverse effects on product stability. It was seen as the objective of preservation to safeguard susceptible cosmetics from microbial spoilage during storage and shipment. The decision whether a newly developed formula would require preservative protection was mostly taken on the basis of experience with previously manufactured products of similar composition. It was not common to perform confirmatory tests for the efficacy of the preservative in the formulation concerned. The absence of signs indicating spoilage in stored products was considered as sufficient proof of adequate preservation.

This empirical approach towards preservation, prevailing in the industry until well past the middle of this century, had been encouraged by the experience with cosmetics manufactured prior to the 1950s. Their composition often provided an environment unfavourable for microbial growth. This was, for emulsions, attributable to the mildly inhibitory properties of the soaps, predominantly used as emulsifiers in those days, and the alkaline pH of the water phase in systems stabilized with soaps or other anionic surfactants. In addition, preference was frequently given to the formulation of water-in-oil (W/O) emulsions which are generally more stable against microbial attack. The preservation of these products, if need occurred, had traditionally been accomplished by the use of the esters of *p*-hydroxybenzoic acid (parabens), introduced in 1924.

13.1.1 Factors necessitating a new approach

13.1.1.1 Formulatory changes. After the Second World War a large number of nonionic surfactants became available. They were increasingly used as

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stabilizing agents in cosmetic emulsions because of their many advantages over soaps and other anionic emulsifiers.

However, the parabens as well as other preservatives commonly used in cosmetics, frequently failed in these new systems. Investigations into the causes of this problem provided the evidence for the efficacy-reducing effects of surface-active nonionics on those preservatives which were either lipophilic (e.g. phenolics) or contained a substantial lipophilic moiety in their molecules (e.g. quaternary ammonium compounds). The mechanism of inactivation was attributed to the complexation and solubilization of these preservatives within micelles formed by non-ionic surfactants in aqueous solution above a certain concentration (critical micelle concentration or CMC). For anionic and cationic surfactants it was already well documented in the literature that in high concentrations they diminished the activity of lipophilic biocides. However, preservation problems rarely occurred in cosmetics containing ionic surfactants. The antimicrobial properties of the latter (in particular of the cationics) often compensated for the activity losses of preservatives sufficiently to maintain product stability. Nonionics, on the other hand, were found not to possess any significant antimicrobial properties of their own, making the necessity for adequate preservation of systems containing them much greater. This became a difficult task additionally complicated by the fact that a number of other formulational factors were found to adversely modify the antimicrobial activity of preservatives available in those days.

In order to assist formulators in identifying the causes of product spoilage and in developing suitable preservation strategies, Wedderburn (1964) published a comprehensive review of the literature which had accumulated on these subjects. The author outlined a new approach to product preservation which can be summarized in the following steps:

- (i) The examination of each new formula for its growth-promoting or growth-inhibiting potential at the early stage of development in order to determine whether and to what extent the addition of preservatives will be needed.
- (ii) The selection of a preservative (or preservative combination) that meets the requirements of an individual formulation, is non-toxic, compatible with the packaging material and retains its activity during the shelf-life of a product and during product use.
- (iii) The verification of the efficacy of the chosen preservative(s) in the finished formulation by means of microbial challenge.

The modern concept of preservation has fully adopted this approach.

13.1.1.2 Health hazards from contamination. Traditionally, the microbiological criterion for accepting a finished cosmetic product as fit for its intended use was only based on the requirement of stability. In the 1960s, the watershed publications by Kallings *et al.* (1966) drew for the first time serious attention

to those microorganisms which survive in products without adversely affecting them, but can constitute a real health hazard if they are pathogenic and present in substantial numbers.

These reports, although exclusively dealing with non-sterile pharmaceuticals, alerted the cosmetic industry to the potential for similar problems. A number of independent and official surveys into the microbiological status of retail cosmetics and toiletries demonstrated a varying frequency of contamination. Additionally, several types of products had been reported in the literature as being the causes of severe infections by virtue of their pathogenic bioburden (Spooner, 1988). These incidents, although remaining low in number, indicated that individuals who were compromised in some way might be at risk of acquiring infections from contaminated cosmetics. This also included neonates because of their non-fully developed skin barrier and cutaneous microbial flora. Furthermore, ocular infections transmitted from cosmetics underlined the high susceptibility of the eye to microbial insult, especially at pre-existing lesions.

To ensure the safety of cosmetics and toiletries for any potential user it became evident that microbiological contamination in these products must be controlled. To provide member manufacturers with rational microbiological standards and the means of achieving them, the Cosmetic, Toiletry and Fragrance Association in the USA and the Cosmetic, Toiletry and Perfumery Association in the UK published sets of guidelines. These introduced microbiological limit criteria of different stringency for the various classes of cosmetic products (Anon, 1973, 1976a). They emphasized that the requisite quality could only be achieved by strict control of all sources contributing to product contamination as described in the relevant guidelines concerning good manufacturing practices. This ruled out the use of preservatives in lieu of good production hygiene. Their function was defined as to maintain the pre-determined microbiological standards for cosmetics subsequent to manufacture. For verifying the efficacy of preservatives in these products the guidelines of both trade associations included test methods.

13.1.1.3 Legislative actions. The concern about consumer safety also led to relevant legislations. In 1976 the European Economic Community Cosmetics Directive (76/768 EEC) (Anon, 1976b) came into force. Article 2 laid down that 'cosmetic products put on the market within the Community must not be liable to cause damage to human health when they are applied under normal conditions of use'. Thus the responsibility for the safety of products was placed on the manufacturer. Originating from the text of Article 8, proposals for a Commission Directive on the microbiological purity of cosmetics were made in 1978 and 1982 but failed to be accepted. Several member states rejected the 1982 proposal on the grounds that latest surveys of the microbiological quality of marketed cosmetics had not indicated the necessity to take specific statutory powers. Since then no further initiative

has been undertaken to impose official microbiological limits on cosmetics.

To protect the consumer against possible toxicological risks arising from preservatives, the need was foreseen in 1976 for a list of preservatives proven to be safe. Because of the preparatory work required, it was only in 1982 that such a list was added to the basic Directive as Annex VI. It subdivided preservatives into definitively and provisionally allowed substances, defined the maximum authorized concentration and specified the limitations on use as well as obligatory printed label warnings. Since 1982, several Commission Directives have adapted Annex VI to technical progress.

The legislative developments in the United States have followed a similar path. Since 1938, cosmetics have been regulated under the authority of the Federal Food, Drug, and Cosmetics Act. Amendments made to the Act in 1976 prohibited the marketing of adulterated cosmetics in interstate commerce (Anon, 1976c). A product was deemed to be adulterated (Section 601a, 21 U.S.C. 361) 'if it bears or contains any poisonous or deleterious substance which may render it injurious to users under the conditions of use described in the labeling thereof, or under such conditions of use as are customary or usual'. This section has been interpreted to include as adulteration the presence of harmful microorganisms (Madden and Jackson, 1981). However, as in Europe, there are no statutory limits covering cosmetics at present.

In 1973 the Food and Drug Administration (FDA) instituted a voluntary registration programme for cosmetic products. It required classification of active ingredients by their intended function in a formulation. The information obtained from participating companies on the use of substances designated as preservatives was first published in 1977 in the form of a frequency of use list, which has been updated about every two years. In view of the willingness of the cosmetic industry to self-regulate and to participate in voluntary programmes such as the Cosmetic Ingredient Review and the Voluntary Reporting Program it has been considered unnecessary to take specific enforcement measures to ensure the protection of public health.

13.2 Practical application of the concept

13.2.1 Microbiological limit criteria

It follows from the definition of the function of preservatives (see 13.1.1.2) that microbiological acceptance criteria need to be set for each individual formulation. Such criteria can be either developed in-house based on risk assessment or be adopted from the guidelines of the cosmetic trade associations.

In the past decade several countries have followed the examples of the American Cosmetic, Toiletry and Fragrance Association (CTFA) and the British Cosmetic, Toiletry and Perfumery Association (CTPA) by introducing

Table 13.1 Microbiological criteria for cosmetics and toiletries proposed by trade associations

Product category	Microbiological limit (cfu ^a g ⁻¹ or cfu ^a ml ⁻¹)	
	CTPA ^b (UK)	CTFA ^c (US)
Baby products	100	500
Products used about the eye	100	500
Products for general use	1000	1000

^a cfu: colony forming units.

^b Whenever substantial numbers of colonies are observed, the presence of harmful microorganisms should be excluded.

Undesirable microorganisms: *Clostridium* spp, *Pseudomonas* spp, *Staphylococcus aureus* and *Candida albicans*.

^c In addition to the microbial limits specified above, no product shall have a microbial content recognized as harmful to the user as determined by standard plate count procedures.

Specific microorganisms: *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

microbiological limit guidelines for cosmetics and toiletries. These all contain the same key elements: numerical limits for total viable counts (i.e. aerobic bacteria, yeasts and moulds) in finished products, stricter numerical limits for the risk classes of baby and eye products and absence criteria for specified objectionable microorganisms. However, the list of objectionable microorganisms may vary from country to country and the stringency of numerical limits applied to baby and eye products depends on whether the CTFA or CTPA approach has been adopted. The microbiological limit criteria proposed by these two trade associations are summarized in Table 13.1. While those of the CTFA have remained unchanged since their first publication (Anon, 1973), the CTPA introduced in later revisions in-house warning limits, recommended to be set ten times lower than the maximum tolerated numerical limits for finished products (Anon, 1990). These limits which are intended for application to products at the time of manufacture, are based on the assumption that the microorganisms present in the product do not proliferate after testing. The introductory sections of the guidelines, therefore, underline the responsibility of the manufacturers to produce adequately preserved products and to verify the efficacy of the preservatives used by proper test methods.

13.2.1.1 Objectionable microorganisms. The designation of a microorganism as objectionable for a particular category of non-serile products depends on its established pathogenic potential and ability to cause infections or diseases via the application route determined by the intended use of the product. Cosmetics and toiletries are, per definition, intended for external application and thus come into contact with the skin as well as with the mucous membranes of the eye, buccal cavity and genital/anal area. Consequently, those microorganisms which are able to infect the skin and mucosal tissues

represent the prime candidates considered to be objectionable in cosmetics and toiletries. On the other hand, there is always the risk of accidentally swallowing small amounts of products applied to the oral cavity. Therefore, the presence of enteric pathogens in this product type should additionally be seen as undesirable.

The microbiological limit guidelines contain only a small selection of potentially harmful microorganisms, headed by the classic skin pathogens *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. A more comprehensive listing of objectionable microorganisms according to product classes has been recently published by Orth (1990). Further information on this issue can be obtained from The Microbiological Update (Anon, March 1989, April 1990, August 1991, July 1992 and June 1994).

Until recently, mycotoxin-producing fungi have been regarded as a problem mainly restricted to the food industry. The publication of Meyer *et al.* (1992), however, suggests that they might have relevance to cosmetics as well. The authors concentrated their investigations in particular on trichothecenes. These mycotoxins, produced by the genus *Fusarium*, are not only orally toxic but also strong skin irritants (type A trichothecenes).

In the light of new experiences, the list of potentially harmful microorganisms continually expands. It is necessary to keep abreast of the developments in this area and to revise existing in-house microbiological specifications to reflect current knowledge.

Important note. One of the most recent changes in legislation and of far-reaching consequences for the cosmetic industry is the 6th Amendment to the EU Cosmetic Directive 76/768 (Anon, 1993). Article 7a lays down that the manufacturer (or other persons specified in this article) shall for control purposes keep the following information readily accessible to the competent authorities of the Member State concerned:

- The microbiological specifications of the raw materials and the finished product
- The purity and microbiological control criteria of the product
- The method of manufacture complying with GMP

In the interest of a unified approach in the Member States, it is very likely that the EU will promulgate microbiological criteria for cosmetics and the methods for testing product compliance with the criteria, as foreseen in Article 8 of the Directive 76/768.

13.2.2 Factors determining the need for preservation

The steadily increasing complexity of cosmetics has made the task of adequately preserving them ever more difficult. Negative consumer perceptions about the use of chemical preservatives have, on the other hand, put pressure

on cosmetic companies, particularly in Germany, to develop preservative-free products. While this has proven feasible for some, others will always need the protection of chemical preservatives.

The potential of these agents to cause adverse reactions is well documented in the literature (de Groot, 1988; Smith, 1993). All existing possibilities to increase the antimicrobial resistance of a formulation (without altering its functional attributes) should, therefore, be exploited so that the concentration of preservatives can be lowered to a level which is still effective in ensuring product integrity but minimizes the risk of undesirable responses.

The various factors involved in determining the microbiological behaviour of a developmental product and thus its preservation needs, will be addressed in this section. A method for rating these factors and calculating the need for supplemental preservation is described in the Weighted-Value Technique of the CTFA (Anon, 1986).

13.2.2.1 Moisture content. For microorganisms to grow and reproduce the presence of unbound water in the environment is absolutely necessary. Although cosmetics and toiletries often contain considerable amounts of water, its availability to microorganisms varies, depending on the nature and concentration of solutes.

To allow substrates to be compared as to their available water, the parameter water activity (a_w) is commonly used. It has been defined as the ratio of the vapour pressure of the solution (substrate) to that of the solvent (pure water) at the same temperature, with pure water having an a_w value of 1.000. The limiting water activity values for some bacteria, moulds and yeasts are given in Table 13.2. More detailed information on this subject, including methods for measuring the water activity, can be obtained from the literature (Troller, 1984; Curry, 1985; Blair *et al.*, 1988).

Knowledge of the water activity of a developmental product is useful in two respects:

Firstly, steps can be taken in time to reduce the available water in formulations rated as being highly susceptible to microbial growth ($>0.98 a_w$).

Table 13.2 Water activity limits and pH requirements for microbial growth

a_w Limits	Organisms	pH Range
0.96	<i>Pseudomonas</i> spp.	6.0–8.0
0.95	Coliforms	6.0–8.0
0.90	<i>Staphylococcus</i> spp.	5.0–8.0
0.90	Sporeformers	5.0–8.0
0.87	Common yeasts	3.5–5.0
0.80	Common moulds	3.5–5.0
0.60	Cut-off point for microbial growth	

This can be achieved by adding water-binding substances or by increasing the concentration of those which are already present in the formulation concerned. Examples for a_w depressants are: sodium chloride, glycerol, carboxymethyl cellulose, glucose, sucrose, ethanol, urea. Although the possibilities for such alterations may be limited by the product concept, it needs to be borne in mind that even small changes in the water activity may significantly affect microbial proliferation.

Secondly, the a_w value of any formula can be utilized to rank it for its low, moderate or high need for preservation. Water-free systems ($a_w < 0.6$) do not need to be preserved.

13.2.2.2 pH. Although the pH tolerance limits for bacteria, moulds and yeasts may vary considerably, the pH range required for their optimal growth is narrower and specific for the different types of microorganisms (Table 13.2). As a rule of thumb, acidic conditions are encouraging to moulds and yeasts, whereas the majority of bacteria thrive best near neutral pH. Preservation by means of extreme pH values may be achieved in some specialist preparations such as perming lotions or depilatories. For cosmetics and toiletries, predominantly formulated in the range of pH 5.5–7.5, the option of drastically altering the pH is not available for reasons of skin incompatibility.

13.2.2.3 *Ingredients stimulating microbial growth.* A wide variety of cosmetic ingredients (e.g. sugars, fats, proteins) can be metabolized by microorganisms contaminating a product. The recent increase in the use of natural raw materials of animal or plant origin has brought a set of new problems, as these materials frequently have a high bioburden which may additionally contain pathogenic microorganisms.

Commercially, these products are offered as powders, or alcoholic or aqueous extracts. Dry powders do not present microbiological stability problems but can swamp the formulation to which they are added with high numbers of spores unless preventive measures are taken (microbial limit specifications imposed on the vendor). For alcoholic extracts to be accepted as self-preserving it needs to be ensured that the concentration of the solvent is sufficient (see 13.2.2.4.1). Aqueous extracts, most prone to biodegradation, must be either well preserved or decontaminated by appropriate procedures.

In using all of these materials, the need for supplemental preservation is usually high. Furthermore, it is often required to choose preservatives with a high specific spectrum of activity. While, for example, Gram-negative bacteria represent the main problem in preparations containing aloe vera extracts, a shift in the contamination pattern towards the more fastidious Gram-positive types should be expected in those formulations which contain biologics or complex plant extracts.

13.2.2.4 Ingredients restricting microbial growth. In this section a number of raw materials, commonly used in cosmetics and toiletries, will be described whose antimicrobial properties may render a formulation self-preserving or may enhance the efficacy of preservatives, thus allowing lower concentrations to be used.

13.2.2.4.1 Alcohols. Ethanol, *n*-propanol and iso-propanol are among the alcohols most frequently used in cosmetic preparations. Their antimicrobial efficacy increases with the molecular weight and chain length. The concentration in which they are present in a product determines whether they will kill or merely inhibit microorganisms. In low concentrations of c. 1%, alcohols may serve as metabolizable substrates for organisms. Data in the literature indicate that in the range of 10–20% the microbistatic effect of alcohols is sufficiently high to allow a reduction in the concentration of preservatives. However, to achieve adequate preservation by means of alcohol only, concentrations in excess of 30% are required. In this context, attention should be drawn to the fact that alcohols can contain high numbers of spores. Although these do not germinate in products with an alcohol level above 10%, they can increase the overall bioburden beyond acceptable limits.

Formulating personal care products with higher alcohol contents has been, however, severely hampered by the recently introduced volatile organic compound (VOC) regulations in California. Other states and the US Environmental Protection Agency (EPA) are considering similar regulations. In Europe, particularly in Germany, pressures by environmentalists on the industry have already resulted in companies introducing alcohol-free products (colognes, deocolognes) into the market. Furthermore, mouthwashes with 25% or more of alcohol, have been reported to expose users to a higher risk of acquiring mouth cancer (Microbiological Update July 1993, January 1994). As a consequence of this, many alcohol-based products will need to be reformulated (Boulden, 1992), presumably often at the cost of an increased microbiological instability. A new multifunctional raw material, SENSIVA SC50 (Schülcke & Mayr GmbH), could provide a solution to the problem. The test results of the supplier indicate that microbiological ineffective alcohol concentrations of 5–10% were rendered microbicidal in the presence of SENSIVA SC50.

13.2.2.4.2 Glycols. Propylene glycol and butylene glycol have a significant antimicrobial activity when present at concentrations above 10%. In addition, they are frequently used as solvents for preservatives of low water solubility (e.g. parabens), thus increasing their bioavailability in the water phase of a formulation.

13.2.2.4.3 Humectants. Typical representatives are glycerin, sorbitol and propylene glycol. Apart from the latter (see 13.2.2.4.2), these substances have

no inherent antimicrobial effects and may even be utilized at low concentrations (below 5%) as substrates for growth. In high concentrations, however, they will exert an indirect inhibitory effect by reducing the water activity (see 13.2.2.1).

13.2.2.4.4 Antioxidants. Among the reducing agents used, only phenolic antioxidants have been found to possess pronounced antimicrobial properties (Zeelle and McCarthy, 1983; Kabara, 1984; Post and Davidson, 1986). Although these may not be sufficient to afford adequate preservation of complex cosmetic system, the proven potentiating effects of these compounds on Bronidox L, Bronopol, Germall 115, Glydant and Kathon CG can be utilized to reduce the concentrations of these preservatives to lower levels. 2-*tert*-Butylhydroquinone (TBHQ) appears to be the most suitable antioxidant candidate (due to its low O/W partition coefficient) for such combinations, followed by propyl gallate (PG) and butylated hydroxyanisole (BHA).

13.2.2.4.5 Chelating agents. In addition to their usefulness in cosmetic formulations, chelating agents have become increasingly appreciated during the last decade as antimicrobials in their own right, but more importantly as synergists in preservative combinations (Hart, 1984). Chelating agents exhibit pronounced activities against Gram-negative bacteria but not against Gram-positives. This is attributed to the fundamental differences between the cell envelope structures of the two organism groups (Gilbert, 1988). As to their effectiveness against *Pseudomonas aeruginosa*, chelating agents have been ranked in the following decreasing order (Hart, 1984): cyclohexanediaminetetraacetic acid > ethylenediaminetetraacetic acid (EDTA) > diethylenetriaminepentaacetic acid > hydroxyethylethylenediaminetriacetic acid.

While the lack of broad-spectrum activity drastically limits the use of chelators as sole preservatives, they have proven utility in eliminating the resistance of Gram-negative bacteria against various preservatives (e.g. quaternary ammonium compounds) and in potentiating the effectiveness of broad-spectrum preservatives (e.g. Bronopol) so that lower concentrations can be used. In this context it must be emphasized that only the uncomplexed chelating agent is capable of exerting this action. As EDTA is the type of compound preferably used in combination with preservatives, Table 13.3 lists those EU-approved preservatives (see Appendices A and B) whose action is potentiated by this substance.

It should be noted that chelating agents have recently become an environmental issue in Europe because of their perceived role in elevating metal concentration in rivers.

13.2.2.4.6 Fragrances. A large number of aroma chemicals and essential oils possess antimicrobial properties (Morris *et al.*, 1979; Kabara, 1984). Their presence in the fragrance materials used in cosmetics and toiletries may contribute to the preservation of a formulation. The preference of consumers

Table 13.3 EU-approved preservatives potentiated by EDTA

Preservative	EEC No.
Annex VI, Part 1	
Salicylic acid	3
Sorbic acid	4
Potassium sorbate	4
Sodium <i>o</i> -phenylphenate	7
Parabens	12
Bronopol	21
Triclosan	25
Chloroxylenol	26
Imidazolidinyl urea	27
Quaternium-15	31
DMDM hydantoin	33
Chlorohexidine	42
Cetrimonium bromide	44
Annex VI, Part 2	
Benzethonium chloride	15
Benzalkonium chloride	16

for naturally preserved products has, however, increased the interest in fragrance compositions whose ingredients have been specifically selected for antimicrobial activity and thus can serve as a replacement for chemical preservatives. Some such recent examples are the heliotropin-based Conarom-HP (Biochema Schwaben, Germany), the Perfume H26 (Blakeway, 1990) and the fragrance blend described in the US Patent 4966754 (Aveda Corp., published Oct. 30, 1990).

The use of aroma preservatives is, however, fraught with problems (Kabara, 1984; de Groot, 1988; Blakeway, 1990; Diehl *et al.*, 1992; Smith, 1993). Note should be taken in particular of the following points.

Spectrum of activity: many materials show efficacy gaps against Gram-negative bacteria. A wide spectrum of activity can mostly be achieved by a combination of suitable fragrance ingredients, requiring extensive microbiological screening assays.

Use concentration: predominantly being in the range of 1–2%, it exceeds the maximum concentrations used in the fragrancing of leave on or rinse off products, as given in the monographs of the US Research Institute for Fragrance Materials (RIFM).

Safety in use: as fragrances have been identified as the most frequent cause of allergic reactions (Adams and Maibach, 1985; de Groot, 1988) the restrictive list of fragrance ingredients, published by the International Fragrance Association (IFRA) in Annex 2 of the Code of Practice for the Fragrance Industry, should be consulted in the selection of these materials. Furthermore,

it must be remembered that choosing any fragrance ingredient for which no in-use experience exists, necessitates a careful risk evaluation according to the Standards in Annex 1 of the Code. The same is true for well established materials used in higher concentrations than required for fragrancing.

Effect of high concentrations on product: the inherent colour of certain fragrance ingredients, particularly of essential oils, often results in product discoloration.

Olfactory acceptability: may be impaired by the intense and distinct odour of these materials, especially if used in high concentrations.

Interaction with formulatary ingredients: depending on the chemical structure of fragrance components, interactions with cosmetic ingredients in a formulation may occur. Gelatine and polysorbates have been reported to antagonize the activity of Perfume H26.

Partition coefficient: fragrances of limited water solubility may be insufficiently active to protect the water phase of emulsion systems against microbial proliferation.

Effect of packaging materials: low density polyethylenes are permeable to fragrance materials. Similar effects have been observed with oils unmodified polystyrenes. Acrylonitrile and butadiene copolymers appear to be compatible with fragrances. Glass containers may encourage isomerisation of fragrances when exposed to light. Addition of UV adsorbers is recommended as a preventive measure.

Long-term stability: may be questionable due to the volatility of fragrances. Stability tests are necessary to ensure the adequate preservation of a product during its shelf life and expected use life.

Fragrances and VOC regulations: the latest proposed CARB (California Air Resources Board) regulation (January 9, 1992) consider fragrances which exceed the maximum vapour pressure of 2.0 mm as VOCs. For calculating the vapour pressure of fragrance mixtures refer to the data base of the RIFM.

Costs: Last but not least the high price of fragrances needs to be mentioned as a further limiting factor for using these materials in high concentrations.

13.2.2.4.7 Surfactants. Surface-active agents are utilized in a wide variety of cosmetic products in which they primarily function as detergents, foaming or wetting agents, emulsifiers, solubilizers or dispersants.

Depending on the chemical structure, the use concentration and, where applicable, on the pH of the formulation, surfactants can exhibit a pronounced antimicrobial activity. This is attributed to the ability of these membrane-active compounds to increase the permeability of the cell envelope of microorganisms by adsorption to and disruption of the cytoplasmic membrane. Besides damaging the cell in its own right, this can facilitate the access of other antimicrobial agents to targets which were previously blocked off (synergistic effect). The proliferation of Gram-negative microorganisms in unpreserved cleansing preparations for hair and skin, points however to the limited spectrum of activity of surfactants. The cell envelope structure of these organisms, acts as a strong diffusion barrier for surface-active compounds unless destabilized by the addition of EDTA (see section 13.2.2.4.5).

In the following, examples of multifunctional surfactants with antimicrobial properties will be described. Although the class of nonionics is usually regarded as being ineffective in this respect, there are exceptions to the rule. Information on the chemical/physical properties, product application and suppliers of these materials is provided in the Surfactant Encyclopedia published in *Cosmetics and Toiletries* (Anon, 1989).

Anionic surfactants: examples of antimicrobial substances are the salts of the fatty acids (soaps), the alkyl ether sulfates and alkyl sulphates. The structure–function relationships of fatty acids has been reviewed by Kabara (1984a). The data indicate that lauric acid (C_{12}) is the most active of the saturated fatty acids, while mono-unsaturated and poly-unsaturated fatty acids need for the same effect 16 and 18 C atoms respectively. Anionics are most active in the acid pH range.

Cationic surfactants: examples of these potent antimicrobial substances are the alkylamines (active chain length C_{12-16}), and the group of quaternaries comprising the alkylbenzyltrimethyl ammonium salts (e.g. benzalkonium chloride, benzethonium chloride), the aromatic heterocyclics (e.g. cetylpyridinium chloride) and the tetra-alkyl ammonium salts (e.g. cetrimonium bromide). For a QAC to have a high antimicrobial activity, at least one of the four radical groups must have a chain length in the range C_8 to C_{18} (Domagk, 1935). These substances are more effective at alkaline and neutral pH than in acid conditions. Among the substances inactivating QACs, the nonionics (e.g. polysorbate 80) have been regularly quoted since their effect was first described in the 1940s (Wedderburn, 1964). While this is true at concentrations exceeding the CMC of nonionics due to the micellar solubilization of quaternaries, potentiating effects on these cationic compounds have been reported below the CMC (Schmolka, 1973).

The following QACs are included in the EU preservative list:

Cetrimonium bromide and chloride EEC No. 44 (Appendix A), Benzethonium chloride EEC No. 15 (Appendix B), Benzalkonium chloride, bromide, saccharinate EEC No. 16 (Appendix B).

Amphoteric surfactants: the most prominent examples in this group are the alkyl betaines with cocoamidopropyl betaine being the derivative widely used in shampoos and various other cleansing preparations. Due to their structural similarity with QACs these materials exhibit antimicrobial properties. Unlike ionic surfactants betaines remain constantly active over a wide pH range (Garrand, 1985).

Nonionic surfactants: examples are the monoglyceride derivatives with a fatty acid chain length of C_{12} (most active against Gram-positive bacteria). Those which are shorter in chain length (C_{10} , C_{11}) exhibit optimum activity against yeasts and moulds (Kabara, 1984b). The addition of antioxidants and chelators is required to overcome the efficacy gap against Gram-negative bacteria. The use of glyceryl monolaurate at high concentration (1–10%) may in combination with EDTA render a system self-preserving. Monolaurin (Lauricidin) is defined as GRAS (Generally Recognized as Safe)—FDA (21 CFR GRAS 182.4505).

Other nonionics reported to possess antimicrobial properties are the members of the polyethoxyalkyl phenol group (Lamikanra and Allwood, 1976). Like the previously mentioned polysorbates (see cationic surfactants), they potentiate in low concentrations the effectiveness of chemical preservatives.

13.2.2.4.8 Preservatives Preservatives can be utilized in formulations for purposes other than preservation. They find applications as deodorants (e.g. bromchlorophene, QACs, triclosan, triclocarbon), anti-dandruff agents (e.g. zinc pyrithione, salicylic acid), anti-plaque additives in oral hygiene (e.g. hexetidine, cetylpyridinium chloride) or antiseptics (e.g. triclosan, triclocarbon). In all these cases they are required to exert antimicrobial activity against specific microorganisms at the site of use. Nevertheless, preservatives functioning as active ingredients can significantly contribute to the preservation of a formulation as their use concentrations may exceed those laid down in Annex VI (see Appendices, preamble: point 2). For information on maximum use concentrations applying for anti-perspirants, deodorants and anti-dandruff agents consult the relevant lists of the Council of Europe; Cosmetic Products and their Ingredients, Second Edition (Document P-SG (84)43).

13.2.2.5 Complexity of formulation. Developing an in-house classification of cosmetic raw materials with respect to their growth-supporting and growth-restricting potential can be extremely helpful for designing a new formula. However, the overall response resulting from the interplay of

ingredients in a product remains unpredictable and, therefore, requires to be established by appropriate testing (see section 13.2.4).

13.2.2.6 Type of emulsion. Water-in-oil emulsions have always been known to be much less prone to microbial spoilage than those of the oil-in-water type. This is attributable to two causes: firstly, the continuous oil phase of the former prevents the access of environmental contaminants to the water phase. Secondly, the small size of the water droplets (discontinuous phase) severely restricts the proliferation of any background contaminants, provided that coalescence of the droplets does not occur. With the emulsification techniques nowadays available, particle sizes of the dispersed phase can be achieved which match the average cell diameter of coccoid bacteria (1 µm) or may even fall far below that as in microemulsions. W/O emulsions are, therefore, most suitable candidates for formulation without the inclusion of preservatives, reflected in the increasing number of such products on the market.

Manufacturing W/O emulsions without preservatives has, however, various consequences. All sources contributing to microbial contamination must be strictly controlled to keep the product bioburden to a minimum. The emulsion characteristics should be clearly defined (e.g. particle size distribution range). The manufacturing process should be appropriately designed and closely monitored (e.g. temperature) to ensure that each batch complies with the set criteria. The same applies to the conditions of storage.

Growth of moulds, which may occasionally occur on the surface of W/O emulsions stored in bulk containers, does not necessarily indicate product instability but can be caused by water film formation. This can be avoided by minimizing head space or by covering the product with sterile impervious foil.

13.2.2.7 Primary packaging design. A further important point in evaluating the need of a developmental product for supplemental preservation is the configuration of the primary packaging in regard to its potential for protecting the product from in-use microbial contamination.

Wide-mouthed jars offer the least protection as they allow direct contact between user and product and, in addition, present a large surface area for the access of environmental contaminants. The same applies to wide-necked bottles with screw-cap closures. Products distributed in this form should be either well preserved or possess a high intrinsic antimicrobial resistance due to the formulatory design. Tubes (aluminium or plastic) minimize the contact area and thus the risk of in-use contamination. Additional product protection can be achieved by selecting the right type of dispensing closure. Examples are the slit-cap, flip-cap or pump-top closures (Brannan and Dille, 1990). The highest security, however, is afforded to products which are supplied as aerosols. The delivery system can be non-gas pressurized (Paino, 1990) or air-pressurized (EP Spray Systems, Switzerland). In both cases the product must not support microbial proliferation. On the other hand, propellant

combinations of butane and dimethylether, carbon dioxide and dimethylether, and combinations of the three compounds at minimum total concentrations of 10% have been reported to act as preservative agents for cosmetic products (Ibrahim and Sonntag, 1991).

13.2.3 *Selecting the preservatives*

Having established that a new formula needs supplemental preservation, the next step is to decide which preservatives will ensure maximum product protection. This requires consideration of their individual characteristics as well as the nature of the formulation. Experience with preserved products that are already being manufactured and are similar in their composition to that under development may provide some assistance in the selection process. However, it should be borne in mind that one formulation never totally equates to another. Even minor differences can produce completely different responses. It is, therefore, imperative to test in all cases the efficacy of the chosen preservative system in the formulation (see section 13.2.4).

This section deals with the main criteria influencing the selection of preservatives. In addition, aspects of cost effectiveness and safe handling of these materials will be briefly addressed. For a description of the antimicrobial and physical/chemical properties of the EU-approved preservatives, the reader is referred to the Appendices. Information on a wider range of antimicrobials can be found in the monographs compiled by Wallhäußer (1988).

13.2.3.1 *Regulatory status of preservatives.* Any preservative considered for use in cosmetics and toiletries must comply with the regulations of the country where the product is going to be marketed. The regulatory status of preservatives worldwide varies substantially.

However the parabens (methyl, ethyl, propyl, butyl), phenoxyethanol, Kathon CG and sorbic acid are enjoying widespread approval (US, EU, Japan).

13.2.3.2 *pH.* One of the most important criteria determining the selection of preservatives is the pH of the aqueous phase.

For most ionizable preservatives, such as the organic acids, the antimicrobial activity and the degree of dissociation are closely linked and both are strongly influenced by pH. Table 13.4 shows the percentages of organic acids undissociated at various pHs. The parabens and other phenolics, whose effective pH range extends to *c.* 8, behave in a similar way. In alkaline formulations, the cationic preservatives are most effective; adsorption to the cell envelope is enhanced due to the negative charge on the microorganisms. Other preservatives may become less active because of chemical instability in an inappropriate pH environment (e.g. Bronopol at high pH).

Table 13.4 Percentage of organic acid preservatives undissociated in relation to pH

Preservative (EEC No.)	pH			
	4	5	6	7
Benzoic acid (1)	60	13	1.5	0.15
Propionic acid (2)	88	42	6.7	0.71
Salicylic acid (3)	8.6	0.94	0.094	0.0094
Sorbic acid (4)	86	37	6.0	0.6
Dehydroacetic acid (13)	95	65	15.8	1.9

13.2.3.3 Spectrum of antimicrobial activity. In general, a broad spectrum of activity will be required and in some cases increased specific activity may be desirable. This occurs when formulatary characteristics are selective for certain groups of microorganisms (see sections 13.2.2.1, 13.2.2.2). A single preservative rarely has this capability due to efficacy gaps or such low activity against certain organisms that an unacceptably high concentration would be required. Of particular concern is the *Pseudomonas* gap of certain preservatives. This has led to the widespread use of preservative combinations which can offer the following advantages:

- Broadened spectrum of activity (e.g. Bronopol with 2,4-dichlorobenzyl alcohol)
- Use of lower concentrations of individual preservatives, thus minimizing irritancy potential
- Reduced risk of the development of microbial resistance
- Possibility of synergy, giving greater than the anticipated simple additive effect (Denyer and Wallhäußer, 1990)
- Potentiation: activity of an antimicrobial agent is enhanced by combination with a microbiologically inactive or weakly active agent such as EDTA or Monolaurin (sections 13.2.2.4.5 and 13.2.2.4.7)
- Improved long-term stability of product by combining a labile, strongly biocidal preservative with a stable longer-acting agent (e.g. Bronopol with parabens)

Preservative combinations can be developed in-house or obtained commercially. Comprehensive information on 31 preservative mixtures (including their regulatory status in US, EU, Japan) has been published in *Cosmetics and Toiletries* (Anon, 1993).

13.2.3.4 Use concentration. The data sheets or preservative suppliers mostly specify minimum inhibitory concentrations (MIC) of the substance and often in addition the minimum microbicidal concentrations (MMC). The latter are only of value if information on the neutralizer used in the recovery medium is given and validated. With the knowledge of the pH (section 13.2.2.2) and the water activity (section 13.2.2.1) a judgement can be made

of which groups of microorganisms will possibly proliferate in the formulation. The MIC and MMC data for the most resistant organism in the relevant group will provide some guidance in the selection of a starting-point concentration. Bearing in mind that such data rarely correlate with the preservative activity in the formulation, a range of concentrations can be prepared and tested to identify the concentration exhibiting the required efficacy. Where maximum authorized concentrations exist, these must not be exceeded.

13.2.3.4.1 Microbial adaptation. It has been well documented in the literature that microorganisms which are repeatedly exposed to sublethal concentrations of a preservative, selectively develop resistance to that compound. Some examples of such adaptation are:

- *P. cepacia* to formaldehyde and benzoic acid (Borovian, 1983)
- *P. aeruginosa*, *P. cepacia*, *P. putida* to formaldehyde and imidazolidinyl urea (Schnittger, 1991)
- *P. cepacia* to chlorhexidine (Hugo *et al.*, 1986)
- *S. aureus*, *P. aeruginosa* and *E. coli* to methylchloroisothiazolinone (and methylisothiazolinone and DMDM hydantoin (Orth, 1985)

The ways in which microorganisms acquire resistance have been described by Gilbert and Wright (1987), Gilbert (1988) and Russell (1992).

Increasing the use concentration of a preservative to combat this problem does not provide the proper solution as it will unavoidably lead to levels which are unacceptably high. Instead, measures should be taken to eliminate all sources in the manufacturing process known to encourage adaptation (dilute product remaining in mixing vessels, hoses, pipes, etc.). Furthermore, it should be ensured that the activity of a preservative does not decrease in a formulation as a result of partitioning into the oil phase (emulsions), complexation with product ingredients, adsorption onto primary packaging material or adverse pH influences. In addition, it is recommended that preservatives with a low dilution coefficient (η), also known as concentration exponent (Denyer and Wallhäußer, 1990), are used.

13.2.3.5 Solubility. For a preservative to be effective it must be bioavailable in the aqueous phase in sufficient concentration.

Lipophilic preservatives, such as the parabens, are more soluble in oil than in water (solubility decreases with increasing chain length). As a result of this, they partition in emulsions between oil and water. A knowledge of the partition coefficient and the phase volume ratio is essential in estimating the amount of preservative remaining in the water phase. However, the type of oil strongly influences partitioning, in that vegetable oils have a greater effect than mineral oil (McCarthy, 1984). It is, therefore, important that measurements are performed in systems representative of the formulation.

A further factor affecting the bio-availability of preservatives is their solubilization by surface-active agents (detergents, emulsifiers).

Selection of preservatives with low oil-water partition coefficients is one strategy to overcome these problems. Another is adding to the water phase substances in which the lipophilic preservatives are preferentially soluble. Examples are glycols, glycerol and sorbitol.

13.2.3.6 Compatibility with ingredients. In view of the number of ingredients used in cosmetic formulations the potential for interactions with preservatives is considerable. Decrease in activity may occur through adsorption of the preservative onto suspended solid particles, depending on the surface area they present, the electrical charge at the surface, the polarity of the preservative and the pH of the system or through complexation with hydrocolloids (McCarthy, 1984). The inactivating effect of proteins on formaldehyde, formaldehyde-releasing agents, phenolics and QACs is well known. Lecithins and saponins neutralize the efficacy of membrane-active antimicrobials and they are therefore widely used as neutralizers in preservative efficacy testing.

Conversely the preservative should not affect the physical characteristics of the product.

13.2.3.7 Stability. The preservative system is expected to maintain its efficacy over the declared shelf life of the product and during the foreseeable consumer use. Preservative activity can be impaired through hydrolysis, oxidation or photodegradation. In establishing stability testing programmes to identify such deficiencies, consideration should also be given to the temperature changes a product might experience in the country of use.

13.2.3.8 Compatibility with packaging. For maintaining the preservative activity the selection of a suitable container material is of prime importance.

Antimicrobials can be adsorbed and absorbed by plastics, depending on the lipophilicity of the preservative, the type of formulation and the plastic used (McCarthy, 1984; Allwood, 1990).

On the other hand, the container itself should not release materials (antioxidants from polyethylene, plasticisers from PVC) which might interfere with the preservative system in the product. N.B. sorbic acid benefits from the presence of antioxidants as the latter prevent discoloration (Lück, 1993).

Furthermore, the inner surface of the container should not encourage adhesion of product contaminants and biofilm formation as this can result in failure of the preservative system.

Finally, packaging must offer good light protection for those preservatives prone to photodegradation.

13.2.3.9 Safety. Two aspects need to be considered separately. The first concerns the safe handling of preservatives in the manufacturing plant.

In Europe suppliers of chemicals, including preservatives, are obliged to provide safety information and handling instructions for each material in accordance with the European Directive on the Classification, Packaging and Labelling of Dangerous Preparations (1988).

The second aspect is the effect of preservatives in the finished formulation on the consumer. Using a single approved preservative up to the maximum authorized concentration is considered as safe. A restriction applies to preservatives which are nitrosating agents (e.g. Bronopol, Bronidox). They must not be used in the presence of secondary amines because of the potential formation of nitrosodiethanolamine (NDELA) unless inhibitors (e.g. ascorbic acid, butylated hydroxytoluene, alpha-tocopherol) are added to the formulation. As for preservative combinations, a total maximum permissible concentration is specified for paraben mixtures (EU: 0.8%, Japan: 1%).

13.2.3.10 Cost effectiveness. In selecting a preservative the previously addressed points should take priority over the consideration of cost. Only if two or more antimicrobials have all the desirable attributes can cost effectiveness become the determining selection criterion.

13.2.4 Preservative efficacy testing

The efficacy of a preservative system chosen for a developmental product cannot be predicted and has, therefore, to be tested in the formulation by microbial challenge. The method used should be adequately validated and take into account the type of product. Only a trained and accredited person should perform the test. General aseptic and safety procedures should be followed.

In this section, the available preservative efficacy test (PET) methods will be named, some areas of special concern will be addressed and recommendations on when to use the PET will be given. In recent years, in-use testing of cosmetic products has increased in importance. The rationale for such tests and ways of performing them will be briefly described.

13.2.4.1 Available PET documents. The test guidelines developed by the cosmetic trade associations in the US and UK and the test protocols of the leading Pharmacopoeias (Ph. Eur. II and USP XXII) as well as those of other organizations are presented in Table 13.5. The Japanese Pharmacopoeia (Ph.Jap.) does not contain a test Protocol.

In 1990, the CTFA Microbiology Committee (Anon, 1990) surveyed cosmetic companies (US) on the test methods they employ. The results indicated that 78% used a modified CTFA or in-house method. Modification of the CTFA protocol mainly included additional microorganisms, multiple challenges, higher inoculum concentration and stricter acceptance criteria. Only 3% used the method as published. The USP method was used by 19%, but no company applied the ASTM test.

Table 13.5 Preservative efficacy test documents in US and Europe

Country	Document	Comment
USA	CTFA M-3: The determination of preservation adequacy of water-miscible cosmetics and toiletry formulations.	M-3/M-4 incorporated into CTFA technical guidelines.
	CTFA M-4: Method for preservation testing of eye area cosmetics.	
	ASTM E-640-78: Standard test method for preservatives in water-containing cosmetics (American Society for Testing and Materials).	Intended for use by preservative suppliers.
	United States Pharmacopoeia (USP XXII) 1990: Antimicrobial Preservatives-Effectiveness <51>.	Only applicable for parenteral, otic, nasal and ophthalmic dosage forms.
	USP Convention 1992, Antimicrobial Effectiveness Testing, Pharmacopoeial Forum 18.	Proposed.
	AOAC Challenge Test: Determination of adequacy of preservation of water-miscible, non-eye area cosmetics by challenge test (Association of Official Analytical Chemists).	Proposed. Based on published methods (USP XXII, ASTM E-640, CTFA M-3).
UK	CTPA: Guidelines for preservation efficacy testing.	
EU	European Pharmacopoeia (Ph.Eur.II) 1992: Efficacy of antimicrobial preservation, Section VIII, 14.	Only applicable for parenteral, ophthalmic and oral preparations. Complete protocol including criteria for typical products is due to be published 01.01.95. Test is not intended for routine control purposes.

13.2.4.1.1 Alternative PET methods. To complete this overview, some other procedures for establishing the adequacy of preservation should be mentioned. These are:

The capacity test (Barnes and Denton, 1969): multiple challenges until extinction of preservative activity is achieved.

Advantage: detects marginally preserved and over-preserved formulas.

Disadvantage: large number of challenges, long test time, extensive dilution of product and overloading with organic substances which may interfere with some preservatives.

The single-inoculation test described by Olsen (1967): based on challenging product samples at the same time with a range of inoculum levels (e.g. 10^5 – 10^9 ml⁻¹).

The preservative system that destroys the highest inoculum level is rated as giving the best long-term protection for the product.

The double membrane test (Ahearn *et al.*, 1978): conceptionally differs from previously mentioned methods; based on diffusion of antimicrobial through a double membrane whose surface has been inoculated with a challenge organism.

Advantages: very small sample sizes can be tested; dilution effect on product is minimized; allows multiple or sequential challenges of single sample with different test organisms; gives results in a relatively short time.

The linear regression method (Orth, 1991): product sample is inoculated with a test organism and the number of viable cells are determined at intervals until aerobic plate count is lower than 10 per gramme or ml; a survival curve for each test organism is constructed by plotting the log number of survivors as a function of the time elapsed since inoculation. From these data, the D-value can be calculated, which is the time required for killing 90% (1 log) of the population of the test organism. Orth considers products as satisfactorily preserved if they meet the following acceptance criteria:

D-value of ≤ 4 h for pathogens

D-value of ≤ 28 h for non-pathogenic bacteria, yeasts and moulds

Problem: utility of the D-value depends on whether the inactivation (survival) curve follows first order kinetics (Sutton *et al.*, 1991).

Rapid methods: from a comprehensive study (Connolly *et al.*, 1993) of the direct epifluorescence technique (DEFT-MEM), ATP bioluminescence (ATP-B) and impedance for applicability to PET it was concluded that impedance represents a potential alternative method for PET.

Problem observed: correlation for preservative-treated microorganisms appeared to be different from that for untreated organisms; it may be necessary to obtain individual correlation curves for each different preservative system.

13.2.4.2 Some problem areas in PET. All conventional PETs share the same basic principles:

- Selection of the challenge microorganisms
- Inoculation of the preserved product with the defined organism at a defined level
- Sampling of the inoculated product at prescribed intervals
- Testing the sample for survivors
- Evaluation of the test results

While this suggests a simple procedure, there are in fact many factors involved which influence the outcome of the test. They are not comprehensively addressed in the various protocols. The need for a standardized PET protocol does not only exist in the pharmaceutical sector but also in the cosmetic one. In the former, the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH 2)

pursues the aim to achieve a single standard which will be applied in the registration of pharmaceutical preparations world wide. Whether the 6th amendment of the EEC Cosmetic Directive 76/768 will lead to the development of a standard PET protocol for the cosmetic industry, or not, remains to be seen. In the following some areas of concern in PET will be addressed. For a comprehensive discussion of the factors which influence preservative test results, the reader is referred to the *Handbook of Cosmetic Microbiology* by D.S. Orth, published in 1993 (Marcel Dekker Inc., New York).

13.2.4.2.1 Test organisms. Table 13.6 provides the information on the range of microorganisms used in various PETs. Table 13.7 lists the specified

Table 13.6 Challenge microorganisms in preservative efficacy testing

Microorganism	CTFA	CTPA	AOAC	USP	EP
Gram-positive cocci					
<i>Staphylococcus aureus</i>	+ ¹	+	+	+	+
<i>Staphylococcus epidermidis</i>	+ ¹	+	+		
Fermentative Gram-negative rods					
<i>Escherichia coli</i>	+ ²	+	+	+	+ ⁷
<i>Enterobacter gergoviae</i>	+ ²	+	+		
<i>Enterobacter cloacae</i>	+ ²				
<i>Klebsiella pneumoniae</i>	+ ²	+	+		
<i>Proteus</i> species	+ ²				
Non-fermentative Gram-negative rods					
<i>Pseudomonas aeruginosa</i>	+	+	+	+	+
<i>Pseudomonas cepacia</i>	+ ³	+	+		
<i>Pseudomonas fluorescens</i>	+ ³				
<i>Pseudomonas putida</i>	+ ³				
<i>Flavobacterium</i> species	+ ³				
<i>Acinetobacter</i> species	+ ³	+	+		
Yeasts					
<i>Candida albicans</i>	+ ⁴	+	+	+	+
<i>Candida parapsilosis</i>	+ ⁴				
<i>Zygosaccharomyces rouxii</i>					+ ⁸
Moulds					
<i>Aspergillus niger</i>	+ ⁵	+	+	+	+
<i>Penicillium luteum</i>	+ ⁵				
Spore forming bacteria					
<i>Bacillus subtilis</i>	+ ⁶				
Environmental/product isolates	+	+	+	+	+

¹ At least one of these

² At least two of these

³ At least one of these

⁴ At least one of these

⁵ At least one of these

⁶ Optional

⁷ For oral preparations

⁸ For oral preparations containing a high concentration of sugar

Table 13.7 ATCC strains used

Microorganism	ATCC No.	CTPA	AOAC	USP	EP
<i>Staphylococcus aureus</i>	6538	+	+	+	+
<i>Staphylococcus epidermidis</i>	12228	+	+		
<i>Escherichia coli</i>	8739	+	+	+	+ ¹
<i>Enterobacter gergoviae</i>	33028	+	+		
<i>Klebsiella pneumoniae</i>	10031	+	+		
<i>Pseudomonas aeruginosa</i>	9027	+ ²	+	+	+
<i>Pseudomonas cepacia</i>	25416	+	+		
<i>Acinetobacter calcoaceticus</i> var. <i>anitratus</i>	19606	+	+		
<i>Candida albicans</i>	10231	+	+	+	+
<i>Aspergillus niger</i>	16404	+	+	+	+

¹ For oral products² Additionally recommended ATCC 15442

reference organisms and Table 13.8 gives the sources of equivalents.

In using reference strains it needs to be borne in mind that each strain reacts similarly but not identically in the test. This can be observed with *P. aeruginosa* (CIP, NCIMB, ATCC). The variations in the stability of *Staph. aureus* ATCC 6538 are well known. For the PET it can have the implication that different criteria are met, dependent on the strain used. Standardization of culture conditions, strain management and quality control of growth media is, therefore, particularly important. For standardizing the sensitivity of test organisms, the AOAC Phenol Coefficient Test has been added to the proposed USP-AET (Antimicrobial Effectiveness Testing) which also includes the requirements given in the USP Sterility Test for ATCC cultures.

No cosmetic PET has so far addressed this problem.

13.2.4.2.2 Product challenge. The PETS often do not stress sufficiently the necessity of including an unpreserved sample in the test. This however should always be done for the following reasons:

- Provides information on whether the formulation itself is susceptible to microbial contamination and to which type of microorganisms.
- As all samples used in the challenge test should be submitted to a purity check before being inoculated, background contamination found in the unpreserved sample should itself be seen as a challenge and tested for the potential of proliferation. If this occurs, these contaminants need to be included in the range of test organisms selected for the challenge of the preserved product.
- All products to be tested should preferably be sterile (total viable count $\leq 10 \text{ g}^{-1}$) or not exceed a TVC of 100 g^{-1} . Another point frequently ignored is to surface inoculate solid W/O emulsions, preferably with mould spores. Preservatives may not be sufficiently available at the

Table 13.8 ATCC strains and equivalents (Ph.Eur.2)

Microorganisms	ATCC ¹	NCIMB ²	CIP ³	NCPF ⁴	IP ⁵	IMI ⁶	NCTC ⁷	NCYC ⁸
<i>Staph. aureus</i>	6538	9518	4.83				10788	
<i>P. aeruginosa</i>	9027	8626	82.118					
<i>E. coli</i>	8739	8545	53.126					
<i>Cand. albicans</i>	10231			3179	48.72			381
<i>Zygosacch. rouxii</i>					8021.92			
<i>Asp. niger</i>	16404				1431.83	149007		

¹ American Type Culture Collection, Rockville, Maryland, USA² National Collection of Industrial and Marine Bacteria Ltd, Aberdeen, UK³ Collection Institute Pasteur, Paris, France⁴ National Collection of Pathogenic Fungi, Mycological Reference Laboratory, London, UK⁵ Department of Insect Pathology, Institute of Entomology, Prague, Czech Republic⁶ International Mycological Institute, Egham, UK⁷ National Collection of Type Cultures, Central Public Health Laboratory, London, UK⁸ National Collection of Yeast Cultures, Norwich, UK

product/air interface and this encourages mould growth, particularly if water films form.

Protocols do not specify the required microbiological quality of test formulations designated for PET. As a high intrinsic bioburden can interfere with the test organisms as well as reduce the preservative activity, it should be as small as possible (e.g. total viable count of not more than 100 cfu g^{-1} or ml^{-1} is desirable).

The method by which the inoculum is distributed in the product should be validated to ensure homogeneous distribution and it should not affect the viability of the test organisms.

13.2.4.2.3 Sampling procedure. Attention should be drawn to the diluents, solubilizers and neutralizers used in the preparation of the sampled product for the determination of the TVC.

Diluents, solubilizers: they must be suitable to ensure a homogeneous distribution of the product (heterogeneity influences the reproducibility of TVC) and must not interfere with the test organisms. The latter for example applies to Triton-X 100 (generic) whose toxic properties are well known. Similar problems may occur with isopropylmyristate due to the content of free fatty acids. The proposed USP-AET suggests as a solubilization method for W/O emulsions and ointments the use of polysorbate and lecithin.

Neutralization; it is not recommended to use so-called all-purpose neutralizer combinations without proper validation. A comprehensive list of neutralizers for a wide range of preservatives is provided by Singer (1987). It should be noted that the commonly used Dey-Engley broth does not sufficiently neutralize H_2O_2 which is necessary for the recovery of H_2O_2 injured microorganisms.

Finally the mechanical method used for the homogenization of samples should not affect the viability of survivors and should be validated.

13.2.4.2.4 Evaluation of test results. Due to the lack of universally recognized acceptance standards, PET results may not predict the resistance of a preparation to microbial contamination in use (see 13.2.4.4).

13.2.4.3 Application of PET. The areas of application for the PET and the rationale are given in Table 13.9. In addition, this test is applied to aqueous concentrates which are used in the manufacturing of products to determine whether they will need supplemental preservation.

13.2.4.4 Consumer use testing. As previously mentioned there are as yet no universally recognized acceptance standards due to the lack of evidence that the test results of the PET relate to preservative performance in-use.

Table 13.9 Application of PET

Area	Product	Rationale
R & D	New formula unpreserved	Determination of need for supplemental preservation.
	preserved preserved (final packaging)	Determination of adequate preservation Determination of: compatibility with packaging long-term stability
	preservative free (final packaging)	Determination of: antimicrobial resistance long-term stability
	Redeveloped product: minor changes major changes	Confirmation of preservative efficacy Determination of: adequacy of preservation long-term stability
	Repackaged product	Determination of: compatibility with packaging long-term stability
Manufacture	At least first two batches	Stability of preservative system under manufacturing conditions

For a more objective setting of criteria, the comparison of PET performance of products with their in-use contamination has proved useful. The survey of the cosmetic industry carried out by the CTFA Microbiology Committee (Anon, 1990) showed that companies employ *in vitro*/in-use testing programmes. Although these programmes vary among individual companies, they are designed to:

- (i) Validate the results of *in vitro* challenge testing; and
- (ii) predict the microbiological stability of a product under conditions of normal use.

The in-use test must be carried out with the product in its final packaging. An example for the design and application of the in-use test is given by Lindstrom and Hawthorne (1986).

13.3 Alternatives to chemical preservatives

There is substantial interest in the development of preservative systems which do not rely on conventional chemical agents.

Myavert C (Boots Co. plc) is a product based on natural components in which hydrogen peroxide is generated from glucose and glucose oxidase. The hydrogen peroxide combines with anions present, usually thiocyanate and/or iodide anions, forming active anti-microbial agents such as hypothiocyanite anions; this combination is catalysed by a lacto-peroxidase enzyme component.

Variations in components are possible and Guthrie (1992) reports that a broad spectrum of activity can be achieved over an acceptable product life.

Other 'natural' antimicrobial systems are being researched, particularly iron binding proteins (lactoferrin, transferrin, ovotransferrin), peptide antibiotics (nisin, Pep 5), and the cell wall degrading enzyme, lysozyme.

The patent EP 0369 185 A2 (Singer) states that preservation of products can be achieved in containers pressurized with gas mixtures such as butane/butane plus carbon dioxide or dimethylether/dimethylether plus carbon dioxide. A long term efficacy and wide spectrum of activity are claimed.

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Appendices — Cosmetic preservatives allowed in the EU

The European Economic Community Cosmetics Directive 76/768 EEC (as amended) contains in Annex VI a list of preservatives which are allowed (Part 1) or, provisionally allowed (Part 2) for use in cosmetic products.

The preamble of Annex VI specifies:

1. Preservatives are substances which may be added to cosmetic products for the primary purpose of inhibiting the development of microorganisms in such products.
2. The substances marked with the symbol (+) may also be added to cosmetic products in concentrations other than those laid down in this Annex for other specific purposes apparent from the presentation of the products, e.g. as deodorants in soaps or as antidandruff agents in shampoos.
3. Other substances used in the formulation of cosmetic products may also have antimicrobial properties and thus help in the preservation of the products, as, for instance, many essential oils and some alcohols. These substances are not included in this Annex.
4. For the purpose of this list
 - 'Salts' is taken to mean: salts of the cations sodium, potassium, calcium, magnesium, ammonium and ethanolamines; salts of the anions chloride, bromide, sulphate, acetate.
 - 'Esters' is taken to mean: esters of ethyl, propyl, isopropyl, butyl, isobutyl, phenyl.
5. All finished products containing formaldehyde or substances in this Annex and which release formaldehyde must be labelled with the warning 'contains formaldehyde' where the concentration of formaldehyde in the finished product exceeds 0.05%.

Annex VI (Parts 1 and 2) specifies:

- The maximum authorized concentration for each substance
- The limitations and requirements applying for certain substances
- The conditions of use and warnings printed on the label (where applicable)
- The deadlines for the provisionally allowed preservatives.

Appendix A provides a profile of the preservatives listed in Part 1 of Annex VI, as amended by the 15th Commission Directive (92/86/EEC).

The order of the preservatives described corresponds to the EU list. Further quoted for each substance are:

- Colipa (Comité de Liaison des Syndicats Européens de l'Industrie de la Parfumerie et des Cosmétiques) number
- CAS (Chemical Abstracts Service) number
- CTFA (Cosmetic, Toiletry and Fragrance Association) adopted name.

The regulatory requirements (Point 1) correspond to the wording in Annex VI.

The information given on the properties of the preservatives (Point 2) concentrates exclusively on those criteria which primarily influence the selection of an antimicrobial agent. The profile has been compiled from the literature and the suppliers' data sheets. It is designed to be read in conjunction with section 13.2.3. In consideration of the enormous variations in the composition of cosmetic products, which can enhance or diminish the efficacy of preservatives, any information on minimal inhibitory concentrations has been deliberately excluded from the profile. Also excluded are data on the toxicity of the substances as the listing recognizes that they are safe if used in accordance with the regulatory requirements.

In the United States, cosmetic manufacturers may voluntarily register their product formulations with the Food and Drug Administration (FDA) in accordance with the regulations codified in Title 21, Code of Federal Regulations, Part 720. Since 1977 lists of substances designated by participants as 'preservatives' have been published at regular intervals quoting them in order of frequency of use in product formulations. The recently issued list (Frequency of Preservative Use in Cosmetic Formulas as Disclosed to FDA—1990, *Cosmetics & Toiletries*, **105**, March 1990) is based on 20 035 product entries. The calculated percentage frequency of use of EU approved preservatives in US cosmetic formulations is given in Point 3.

In Point 4 a list of international preservative suppliers is provided which does not claim to be exhaustive.

Appendix B contains a profile of the provisionally allowed preservatives which are listed in Part 2 of Annex VI, including the amendments of the 15th EEC Commission Directive.

Appendix A—Profiles of allowed preservatives**BENZOIC ACID**, its salts and esters (+)

EEC No.: 1
 Colipa No.: P 2
 CAS No.: 65-85-0; sodium benzoate 532-32-1
 CTFA Adopted Name: Benzoic acid, sodium benzoate

1. Regulatory requirements

Maximum concentration: 0.5% (acid).

2. Properties

Antimicrobial spectrum: More active against moulds and yeasts than bacteria.
 Optimum pH range: Below 5.

Solubility: In water (20°C) *c.* 0.3% (w/v) free acid; *c.* 56% (w/v) sodium salt; in emulsions, acid partitions into oil phase.

Incompatibility/inactivation: Nonionic and cationic surfactants; protein and glycerol reduce efficacy; acid can lose activity through adsorption onto particulate matter.

Stability: Stable at low pH.

3. Percentage of use in US cosmetic formulations

Benzoic acid 0.66%; sodium benzoate 0.34%.

4. Suppliers

Ashland Chemical Inc.; International Sourcing Inc.; Kalama Chemical Inc.; Pfizer, Inc.; Tri-K Industries, Inc.

PROPIONIC ACID and its salts (+)

EEC No.: 2
 Colipa No.: P 13
 CAS No.: 79-09-4; sodium propionate 137-40-6
 CTFA Adopted Name: Propionic acid, calcium propionate

1. Regulatory requirements

Maximum concentration: 2% (acid).

2. Properties

Antimicrobial spectrum: Primarily effective against moulds; however, tolerance may occur among *Penicillium* spp.; some yeasts metabolize acid.

Optimum pH Range: below 6.

Solubility: Most soluble as salts; acid freely miscible with water, therefore, hardly any migration into oil phase of emulsions.

Incompatibility/inactivation: —

Stability: Unstable at elevated pH and temperature.

3. *Percentage of use in US cosmetic formulations*

—

4. *Suppliers*

Aldrich Chemical; BASF; Union Carbide Chemicals and Plastics Company, Inc.

SALICYLIC ACID and its salts (+)

EEC No.: 3

Colipa No.: P 14

CAS No.: 69-72-7

CTFA Adopted Name: Salicylic acid

1. *Regulatory requirements*

Maximum concentration: 0.5% (acid).

Limitations on use: Not to be used in preparations for children under 3 years of age, except for shampoos.

Printed label warning: Not to be used for children under 3 years of age (solely for products which might be used for children under 3 years of age and which remain in prolonged contact with the skin).

2. *Properties*

Antimicrobial spectrum: More active against moulds and yeasts than bacteria.

Optimum pH range: Below 4.

Solubility: In water (20°C) c. 0.2% (w/v) acid; in emulsions, acid partitions into oil phase.

Incompatibility/inactivation: Iron salts.

Stability: Discoloration in the presence of iron salts and by exposure to light, unstable above 60°C.

3. *Percentage of use in US cosmetic formulations*

0.11%.

4. *Suppliers*

Aldrich Chemical; J.T. Baker Inc.; Bayer AG; Kalama Chemical Inc.; Merck & Co., Inc.; Riedel-de Haën AG; Rhone Poulenc, Inc.

SORBIC ACID (hexa-2,4-dienoic acid) and its salts (+)

EEC No.: 4

Colipa No.: P 15

CAS No.: 110-44-1; potassium sorbate 590-00-1

CTFA Adopted Name: Sorbic acid, potassium sorbate

1. Regulatory requirements

Maximum concentration: 0.6% (acid).

2. Properties

Antimicrobial spectrum: Moulds and yeasts primarily.

Optimum pH range: 3–6.

Solubility: In water (20°C) 0.16% (w/v) acid; potassium sorbate is water soluble. In emulsions, acid partitions into the oil phase.

Incompatibility/inactivation: Nonionic surfactants (slight).

Stability: Sensitive to light; oxidizes in solution unless stabilized with antioxidants (recommended: citric acid, BHT, propylgallate, α -tocopherol); solutions should be stored at refrigeration temperatures.

3. Percentage of use in US cosmetic formulations

Sorbic acid 0.89%; potassium sorbate 0.39%.

4. Suppliers

Ashland Chemical Inc.; Hoechst AG; International Sourcing Inc.; Merck & Co., Inc.; Pfizer, Inc.; Tri-K Industries, Inc.

FORMALDEHYDE and paraformaldehyde (+)

EEC No.: 1

Colipa No.: P 39

CAS No.: 50-00-0; paraformaldehyde 30525-89-4

CTFA Adopted Name: Formaldehyde, paraformaldehyde

1. Regulatory requirements

Maximum concentration: 0.2% (except for products for oral hygiene); 0.1% (products for oral hygiene), expressed as free formaldehyde.

Limitations on use: Prohibited in aerosol dispensers (sprays).

2. Properties

Antimicrobial spectrum: Broad spectrum (bactericide and fungicide), however, resistance has been demonstrated for *Pseudomonas* spp.

Optimum pH range: 3–10.

Solubility: Soluble in water.

Incompatibility/inactivation: Salts of heavy metals, iron, ammonia, alkali, hydrogen peroxide, iodine; inactivation by proteins and gelatine.

Stability: Highly reactive and volatile, oxidizes to formic acid at air exposure; paraformaldehyde depolymerizes when heated or under alkaline conditions.

3. Percentage of use in US cosmetic formulations

Formaldehyde 2.2%; paraformaldehyde 0.11%.

4. Suppliers

BASF AG; J.T. Baker Inc.; Merck & Co., Inc.

BIPHENYL-2-OL (*o*-phenylphenol) and its salts (+)

EEC No.: 7
Colipa No.: P 47
CAS No.: 90-43-7; sodium *o*-phenylphenate 132-27-4
CTFA Adopted Name: *o*-Phenylphenol; sodium *o*-phenylphenate

1. Regulatory requirements

Maximum concentration: 0.2%, expressed as the phenol.

2. Properties

Antimicrobial spectrum: Broad spectrum, particularly active against moulds and yeasts.

Optimum pH range: 6–10.

Solubility: In water (25 °C) 0.08% (w/v); soluble in alkali; should only be used in alkaline formulations (pK = 10) to avoid partitioning into the oil phase.

Incompatibility/inactivation: Nonionic, cationic and amphoteric surfactants; carboxymethylcellulose and proteins.

Stability: Good.

3. Percentage of use in US cosmetic formulations

o-Phenylphenol 0.05%; sodium *o*-phenylphenate 0.02%.

4. Suppliers

Ashland Chemical Inc.; Bayer AG; The Dow Chemical Company.

PYRITHIONE ZINC (INN) (+)

EEC No.: 81
Colipa No.: P 81
CAS No.: 13463-41-7
CTFA Adopted Name: Zinc pyrithione

1. Regulatory requirements

Maximum concentration: 0.5%.

Limitations on use: Authorized in products rinsed off, forbidden in products for oral hygiene.

2. Properties

Antimicrobial spectrum: Moulds, yeasts and bacteria.

Optimum pH range: 4–8.

Solubility: In water (20°C) 0.002% (w/v).

Incompatibility/inactivation: Slight inactivation by nonionic surfactants; incompatible with EDTA; chelation by heavy metals.

Stability: Unstable in light (consider addition of UV-absorbers), in the presence of oxidizing agents and at higher temperatures.

3. *Percentage of use in US cosmetic formulations*

0.14%.

4. *Suppliers*

Olin Chemical; Ruetgers-Nease Chemical Co.; Weyl GmbH.

INORGANIC SULPHITES and hydrogensulphites (+)

EEC No.: 9

Colipa No.: P 51

CAS No.: 10102-15-5 sodium sulphite; sodium metabisulphite 7681-57-4

CTFA Adopted Name: —

1. *Regulatory requirements*

Maximum concentration: 0.2%, expressed as free SO₂.

2. *Properties*

Antimicrobial spectrum: Moulds, yeasts and bacteria.

Optimum pH range: Below 4.

Solubility: Water soluble.

Stability: Unstable in solutions.

3. *Percentage of use in US cosmetic formulations*

Sodium sulphite 0.32%; sodium bisulphite 0.26%; sodium metabisulphite 0.01%.

4. *Suppliers*

Mallinckrodt, Inc.; Riedel-de-Haën AG.

SODIUM IODATE

EEC No.: 10

Colipa No.: P 66

CAS No.: 7681-55-2

CTFA Adopted Name: Sodium iodate

1. *Regulatory requirements*

Maximum concentration: 0.1%.

Limitations on use: Rinse-off products only.

4. Suppliers

Riedel-de Haën AG; Schulke & Mayr GmbH.

CHLOROBUTANOL (INN)

EEC No.: 11
Colipa No.: P 68
CAS No.: 57-15-8
CTFA Adopted Name: Chlorobutanol

1. Regulatory requirements

Maximum concentration: 0.5%.

Limitations on use: Prohibited in aerosol dispensers (sprays).

Printed label warning: Contains chlorobutanol.

2. Properties

Antimicrobial spectrum: Moulds and bacteria.

Optimum pH range: Below 4.

Solubility: 0.8% (w/v) in water.

Incompatibility/inactivation: Some nonionic surfactants; gum tragacanth; adsorbed by polyethylene and polypropylene.

Stability: Sensitive to excessive light exposure; volatile; increasingly unstable above pH 4.

3. Percentage of use in US cosmetic formulations

0.005%.

4. Suppliers

Aldrich Chemical; Merck & Co., Inc.

4-HYDROXYBENZOIC ACID and its salts and esters (+)

EEC No.: 12
Colipa No.: P 82
CAS No.: Methyl ester 99-76-3; ethyl ester 120-47-8; propyl ester 94-13-3; butyl ester 94-26-8.
CTFA Adopted Name: Parabens (various) esters.

1. Regulatory requirements

Maximum concentration: 0.4% (acid) for one ester; 0.8% (acid) for mixtures of esters.

2. *Properties*

Antimicrobial spectrum: Mostly esters (parabens) are used; more active against moulds and yeasts than bacteria (poor efficacy against pseudomonads); activity improves with increasing chain lengths of esters; usual combination 0.18% methylparaben + 0.02% propylparaben; used in combination with other preservatives, EDTA and polyalcohols to potentiate activity.

Optimum pH range: 4–8.

Solubility: In water (25°C) 0.25% (w/v) methyl ester; 0.11% (w/v) ethyl ester; 0.04% (w/v) propyl ester; 0.02% (w/v) butyl ester; partition coefficient in oils and waxes is high except for mineral oils.

Incompatibility/inactivation: Reduced activity in presence of nonionic and anionic surfactants; some gelling agents, proteins.

Stability: Degradation above pH 8 and at elevated temperatures.

3. *Percentage of use in US cosmetic formulations*

Methyl paraben 38.70%; ethyl paraben 4.04%, propyl paraben 31.66%; butyl paraben 5.99%.

4. *Suppliers*

Ashland Chemical Inc.; Chemag Aktiengesellschaft; Costec Inc.; Inolex Chemical Company; International Sourcing Inc.; Kalama Chemical Inc.; Napp Chemicals, Inc.; Nipa Laboratories, Inc.; Protameen Chemicals, Inc.; Tri-K Industrial Inc.; Van Dyk Division Mallinckrodt, Inc.

3-ACETYL-6-METHYLPYRAN-2,4(3H)-DIONE (dehydroacetic acid) and its salts

EEC No.: 13

Colipa No.: P 5

CAS No.: 520-45-6 (acid); 4418-26-2 (sodium salt)

CTFA Adopted Name: Dehydroacetic acid, sodium dehydroacetate

1. *Regulatory requirements*

Maximum concentration: 0.6% (acid).

Limitation on use: Prohibited in aerosol dispensers (sprays).

2. *Properties*

Antimicrobial spectrum: More active against moulds and yeasts than bacteria.

Optimum pH range: 5–6.5.

Solubility: In water (20°C) 0.1% (w/v) acid; sodium salt 33% (w/v).

Incompatibility/inactivation: Nonionic surfactants, iron compounds (discoloration).

Stability: Degradation above pH 6.

3. *Percentage of use in US cosmetic formulations*

Acid 0.80%; sodium dehydroacetate 1.11%.

4. *Suppliers*

Bayer AG; International Sourcing Inc.; Tri-K Industries, Inc.

FORMIC ACID (+)

EEC No.: 14
 Colipa No.: P 6
 CAS No.: 64-18-6
 CTFA Adopted Name: Formic Acid

1. *Regulatory requirements*

Maximum concentration: 0.5% (acid).

2. *Properties*

Antimicrobial spectrum: Moulds, yeasts and bacteria.
 Optimum pH range: Below 4.
 Solubility: Miscible with water, hardly any migration into oil phase of emulsions.
 Stability: Degradation above pH 4.

3. *Percentage of use in US cosmetic formulations*

—

4. *Suppliers*

BASF, Chemische Werke Hüls; Merck & Co., Inc.; Riedel-de Haën AG.

3,3'-DIBROMO-4,4'-HEXAMETHYLENE-DIOXYDIBENZAMIDINE (dibromohexamidine) and its salts (including isethionate)

EEC No.: 15
 Colipa No.: P 9
 CAS No.: 93856-82-7 Benzencarboximidamide,4,4'-[1,6-hexanediylbis-(oxy)]bis[3-bromo-
 93856-83-8 Ethanesulfonic acid,
 2-hydroxy-, compd. with 4,4'-[1,6-hexanediylbis(oxy)]
 bis[3-bromobenzenecarboximidamide] (2:1)

1. *Regulatory requirements*

Maximum concentration: 0.1%.

2. Properties

Antimicrobial spectrum: Bacteria, moderate activity against moulds and yeasts.

Optimum pH range: 5–8.

Solubility: Water soluble.

Incompatibility: Anionic surfactants, proteins.

4. Suppliers

May and Baker.

THIOMERSAL (INN)

EEC No.: 16

Colipa No.: P 12

CAS No.: Acid 148-61-8; Sodium salt 54-64-8

CTFA Adopted Name: Thiomersal

1. Regulatory requirements

Maximum concentration: 0.007% (of Hg); if mixed with other mercurial compounds authorized by this Directive, the maximum concentration of Hg remains fixed at 0.007%.

Limitations on use: For eye make-up and eye make-up remover only.

Printed label warning: Contains thiomersal.

2. Properties

Antimicrobial spectrum: Very active against moulds, yeasts and bacteria including pseudomonads.

Optimum pH range: 6–8.

Solubility: Water soluble.

Incompatibility/inactivation: Nonionic, cationics, lecithin, protein, heavy metal salts, thioglycollate; absorption to polyethylene, polypropylene, rubber.

Stability: Light sensitive; increased heat-lability in presence of Cu, Fe or Zn ions.

3. Percentage of use in US cosmetic formulations

0.10%.

Comments: As stated in the Inspection Operations Manual section on cosmetics (FDA), mercurial compounds should be limited to eye area cosmetics and used only if no other effective and safe preservative is available.

4. Suppliers

Eli Lilly.

PHENYLMERCURIC SALTS (including borate)

EEC No.: 17
Colipa No.: P 48
CAS No.: 8017-88-7 (phenylmercuric borate), 62-38-4
(phenylmercuric acetate)
CTFA Adopted Name: Phenylmercuric borate, phenylmercuric acetate

1. Regulatory requirements

Maximum concentration: 0.007% (of Hg); if mixed with other mercurial compounds authorized by this Directive, the maximum concentration of Hg remains fixed at 0.007%.

Limitations on use: For eye make-up and eye make-up remover only.

Printed label warning: Contains phenylmercuric compounds.

2. Properties

Antimicrobial spectrum: very effective against moulds, yeasts, gram-positive and gram-negative bacteria.

Optimum pH range: neutral to slightly alkaline.

Solubility: poorly soluble in water.

Incompatibility/inactivation: Anionic and nonionic surfactants; halogens, sulfhydryl compounds, thioglycollates; adsorption to polyethylene, polypropylene and rubber.

Stability: Sensitive to excessive light.

3. Percentage of use in US cosmetic formulations

Phenylmercuric acetate 0.17%.

Comment: As stated in the Inspection Operations Manual section on cosmetics (FDA), mercurial compounds should be limited to eye area cosmetics and used only if no other effective and safe preservative is available.

4. Suppliers

Centerchem, Inc.; Riedel-de Haën AG.

UNDEC-10-ENOIC ACID and salts (+)

EEC No.: 18
Colipa No.: P 16
CAS No.: 112-38-9
CTFA Adopted Name: Undecylenic acid

1. Regulatory requirements

Maximum concentration: 0.2% (acid).

2. *Properties*

Antimicrobial spectrum: Very effective against moulds including dermatophytes.

Optimum pH range: 4.5–6.

Solubility: Insoluble in water.

3. *Percentage of use in US cosmetic formulations*

–

4. *Suppliers*

Rewo Chem. Werke GmbH; Riedel-de Haën AG.

HEXETIDINE (INN) (+)

EEC No.: 19

Colipa No.: P 20

CAS No.: 141-94-6

CTFA Adopted Name: Hexetidine

1. *Regulatory requirements*

Maximum concentration: 0.1%.

2. *Properties*

Antimicrobial spectrum: Most active against moulds, yeasts and gram-positive bacteria.

Optimum pH range: 4–7.

Solubility: 0.01 g in 100 g water.

Incompatibility/inactivation: Acids, soaps.

Stability: Unstable at high temperatures.

3. *Percentage of use in US cosmetic formulations*

–

4. *Suppliers*

ANGUS Chemical Company; Chemische Fabrik Aubing GmbH; Goedecke.

5-BROMO-5-NITRO-1,3 DIOXANE

EEC No.: 20

Colipa No.: P 23

CAS No.: 30007-47-7

CTFA Adopted Name: 5-Bromo-5-nitro-1,3-dioxane

1. *Regulatory requirements*

Maximum concentration: 0.1%.

Limitations on use: Rinse-off products only; avoid formation of nitrosamines.

2. Properties

Antimicrobial spectrum: Good efficacy against moulds, yeasts and bacteria.

Optimum pH range: 5–8.

Solubility: 0.46 g in 100 g water.

Incompatibility/inactivation: Slight inactivation by proteins and reducing agents.

Stability: Stable at pH 5–8 below 40°C (no formaldehyde release).

3. Percentage of use in US cosmetic formulations

0.29%.

4. Suppliers

Henkel KGaA.

BRONOPOL (INN) (+)

EEC No.: 21

Colipa No.: P 24

CAS No.: 52-51-7

CTFA Adopted Name: 2-Bromo-2-nitropropane-1,3-diol

1. Regulatory requirements

Maximum concentration: 0.1%.

Limitation on use: Avoid formation of nitrosamines.

2. Properties

Antimicrobial spectrum: Good activity against bacteria; weaker against moulds and yeasts.

Optimum pH range: 5–7.

Solubility: 25% (w/v) in water at 22°C.

Incompatibility/inactivation: Sodium thiosulphate, sodium metabisulphite, sulphhydryl compounds, dimercaprol (BAL)

Stability: In aqueous solution, stable at low pH; decomposition is accelerated by increasing the pH or the temperature of the solution. Formaldehyde, one of the decomposition products was reported to be less than 15 ppm after three months storage at 45°C. Discoloration on exposure to light, especially under alkaline conditions. Corrosion and discoloration when in contact with iron and aluminium. Nitrite which is also a decomposition product may react with secondary and tertiary amines to form carcinogenic nitrosamines. This process can be retarded by the use of antioxidants (e.g. 0.05% BHT).

3. Percentage of use in US cosmetic formulations

1.60%.

4. Suppliers

ANGUS Chemical Company; The Boots Company plc; Costec, Inc.; Inolex Chemical Company; Tri-K Industries, Inc.

2,4-DICHLOROBENZYL ALCOHOL (+)

EEC No.: 22
Colipa No.: P 74
CAS No.: 1777-82-8
CTFA Adopted Name: Dichlorobenzyl alcohol

1. Regulatory requirements:

Maximum concentration: 0.15%.

2. Properties

Antimicrobial spectrum: Broad spectrum; most active against moulds and yeasts including dermatophytes.

Optimum pH range: 4–6.

Solubility: In water (20°C) 0.1% (w/v).

Incompatibility/inactivation: Reduced activity in the presence of nonionic and anionic surfactants.

Stability: Stable at temperatures normally experienced in the manufacture and storage of cosmetic formulations.

3. Percentage of use in US cosmetic formulations

0.06%.

4. Suppliers

The Boots Company plc; Induchem Ltd.; Inolex Chemical Company.

TRICLOCARBAN (INN) (+)

EEC No.: 23
Colipa No.: P 29
CAS No.: 101-20-2
CTFA Adopted Name: Triclocarban

1. Regulatory requirements

Maximum concentration: 0.2%.

Limitations on use: Purity Criteria

3,3',4,4'-tetrachloroazobenzene less than 1 ppm;

3,3',4,4'-tetrachloroazoxybenzene less than 1 ppm.

2. Properties

Antimicrobial spectrum: Very active against gram-positive and gram-negative bacteria.

Optimum pH range: 4–8.

Solubility: Poorly soluble in water.

Incompatibility/inactivation: Proteins, phosphatides.

Stability: Stable.

3. Percentage of use in US cosmetic formulations

0.02%.

4. Suppliers

Monsanto.

4-CHLORO-M-CRESOL (+)

EEC No.: 24

Colipa No.: P 30

CAS No.: 59-50-7

CTFA Adopted Name: p-Chloro-m-cresol

1. Regulatory requirements

Maximum concentration: 0.2%.

Limitation on use: Prohibited in products intended to come into contact with mucous membranes.

2. Properties

Antimicrobial spectrum: Broad spectrum activity.

Optimum pH range: 4–6, less active at alkaline pH.

Solubility: In water (20°C) 0.4% (w/v).

Incompatibility/inactivation: Cationic surfactants; inactivated by some nonionics and anionics at higher concentrations; loss of activity in the presence of proteins; discoloration with iron salts; migrates into the oil phase of emulsions; adsorbed onto polyethylene, in particular low and medium density grade, process is accelerated at elevated temperature (45°C).

Stability: Yellow discoloration of aqueous solutions in the presence of light and oxygen.

3. Percentage of use in US cosmetic formulations

—

4. Suppliers

Bayer AG; Chemag AG.

TRICLOSAN (INN) (+)

EEC No.: 25
 Colipa No.: P 32
 CAS No.: 3380-34-5
 CTFA Adopted Name: Triclosan

1. Regulatory requirements

Maximum concentration: 0.3%.

2. Properties

Antimicrobial spectrum: High efficacy against *staphylococci*; good to moderate against Gram-negative bacteria (except *Pseudomonas* spp.), moulds and yeasts.
 Optimum pH range: 4–8.
 Solubility: Poorly soluble in water.
 Incompatibility/inactivation: Cationic surfactants; Tween 80; lecithin.
 Stability: Good stability against acids and alkali.

3. Percentage of use in US cosmetic formulations

0.9%.

4. Suppliers

Ciba-Geigy Corporation.

4-CHLORO-3,5-XYLENOL (+)

EEC No.: 26
 Colipa No.: P 37
 CAS No.: 88-04-0
 CTFA Adopted Name: Chloroxlenol

1. Regulatory requirements

Maximum concentration: 0.5%.

2. Properties

Antimicrobial spectrum: Broad spectrum activity; resistance has been reported for Gram-negative bacteria, notably *Pseudomonas aeruginosa*.
 Optimum pH range: 4–9.
 Solubility: In water (20°C) 0.03% (w/v).
 Incompatibility/inactivation: Cationic and nonionic surfactants; loss of activity in the presence of proteins; migrates into the oil phase of emulsions; adsorbed onto polyethylene.
 Stability: Stable.

3. Percentage of use in US cosmetic formulations

0.27%.

4. Suppliers

Ferro Corporation; Nipa Laboratories, Inc.; Chemag AG.

3,3'-BIS (1-HYDROXYMETHYL-2,5-DIOXOIMIDAZOLIDIN-4-YL)-1,1'-METHYLENEDIUREA ('Imidazolidinyl urea') (+)

EEC No.: 27

Colipa No.: P 43

CAS No.: 39236-46-9

CTFA Adopted Name: Imidazolidinyl urea

1. Regulatory requirements

Maximum concentration: 0.6%.

2. Properties

Antimicrobial spectrum: Primarily effective against bacteria; for a broader spectrum activity combinations with other preservatives are required such as parabens, sorbic acid, dehydroacetic acid, quaternary ammonium compounds, phenoxyethanol and Kathon CG; certain surfactants (e.g. sodium lauryl sulphate, sodium lauryl ether sulphate) increase efficacy; adaptation of *Pseudomonas* spp. to preservative has been demonstrated.

Optimum pH range: 3–9.

Solubility: 200 g in 100 g water (20°C).

Incompatibility/inactivation: None known.

Stability: Stable.

3. Percentage of use in US cosmetic formulations

13.72%.

4. Suppliers

Ashland Chemical Inc.; Biophil Chimica SAS; Chemag AG; Costec, Inc.; Induchem, Ltd; International Sourcing Inc.; Lipo Chemical Inc.; Sutton Laboratories, Inc.; Tri-K Industries, Inc.

POLY(1-HEXAMETHYLENEBIGUANIDE) HYDROCHLORIDE (+)

EEC No.: 28

Colipa No.: P 52

CAS No.: 32289-58-0

CTFA Adopted Name: Polyaminopropyl biguanide

1. *Regulatory requirements*

Maximum concentration: 0.3%.

2. *Properties*

Antimicrobial spectrum: More active against bacteria than moulds and yeasts.

Optimum pH range: 5–7.

Solubility: Water soluble.

Incompatibility/inactivation: Precipitated by anionic surfactants, strong alkalis and complex phosphates.

Stability: Stable below 80°C.

3. *Percentage of use in US cosmetic formulations*

—

4. *Suppliers*

ICI Americas Inc.; Atlas Chemie.

2-PHENOXYETHANOL (+)

EEC No.: 29

Colipa No.: P 53

CAS No.: 122-99-6

CTFA Adopted Name: Phenoxyethanol

1. *Regulatory requirements*

Maximum concentration: 1%.

2. *Properties*

Antimicrobial spectrum: Moderate activity, preferably against Gram-negative bacteria.

Optimum pH range: 4–9.

Solubility: 2.6% in water (20°C).

Incompatibility/inactivation: Slight inactivation by some nonionic and cationic surfactants; some loss of activity in polyethylene containers.

Stability: Good.

3. *Percentage of use in US cosmetic formulations*

1.87%.

4. *Suppliers*

Haarmann & Reimer Corporation; Henkel Corporation (Emery Group); Induchem, Ltd.; Lipo Chemical Inc.; Nipa Laboratories, Inc.; Tri-K Industries, Inc.

HEXAMETHYLENETETRAMIN (+) (methanamine) (INN)

EEC No.: 30
Colipa No.: P 55
CAS No.: 100-97-0
CTFA Adopted Name: Methenamine

1. Regulatory requirements

Maximum concentration: 0.15%.

2. Properties

Antimicrobial spectrum: Efficacy is due to formaldehyde release under acidic conditions; the antimicrobial spectrum is the same as for formaldehyde.

Optimum pH range: 4–6.

Solubility: Soluble in water.

Incompatibility/inactivation: Complexes with metal ions.

Stability: Formaldehyde release under acidic conditions and at elevated temperatures.

3. Percentage of use in US cosmetic formulations

0.005%.

4. Suppliers

Degussa AG; Merck & Co., Inc.

METHENAMINE 3-CHLOROALLYLOCHLORIDE (INN)

EEC No.: 31
Colipa No.: P 63
CAS No.: 51229-78-8
CTFA Adopted Name: Quaternium-15

1. Regulatory requirements

Maximum concentration: 0.2%.

2. Properties

Antimicrobial spectrum: Good efficacy against Gram-positive and Gram-negative bacteria; moderately active against moulds and yeasts; adaptation of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* to the preservative has been demonstrated.

Optimum pH range: 4–10.

Solubility: Water soluble (127.2 g/100 g water at 25°C).

Incompatibility/inactivation: None known.

Stability: Unstable at pH below 4 and above 10. Concentrated (> 1%) aqueous solutions should not be exposed to temperatures above 50°C as this

reduces activity; use concentrations in cosmetics are not affected by short time heating up to 80°C. Discoloration (yellowing) of creams has been observed, addition of 0.05–0.1% sodium borate or sodium sulphite is recommended as preventive measure.

3. *Percentage of use in US cosmetic formulations*

3.52%.

4. *Suppliers*

Bayer AG; Costec, Inc.; The Dow Chemical Company.

1-(4-CHLOROPHENOXY)-1-(IMIDAZOL-1-YL) 3,3-DIMETHYLBUTAN-2-ONE (+)

EEC No.: 32
Colipa No.: P 64
CAS No.: 38083-17-9
CTFA Adopted Name: Climbazole

1. *Regulatory requirements*

Maximum concentration: 0.5%.

2. *Properties*

Antimicrobial spectrum: Mainly moulds.
Optimum pH range: 4–7.
Solubility: Poorly soluble in water.
Incompatibility/inactivation: Unstable in alcoholic solutions.
Comment: Application in antidandruff formulations.

3. *Percentage of use in US cosmetic formulations*

—

4. *Suppliers*

Bayer AG.

1,3-BIS(HYDROXYMETHYL)-5,5-DIMETHYLIMIDAZOLIDINE-2,4-DIONE (+)

EEC No.: 33
Colipa No.: P 65
CAS No.: 6440-58-0
CTFA Adopted Name: DMDM Hydantoin

1. *Regulatory requirements*

Maximum concentration: 0.6%.

2. Properties

Antimicrobial spectrum: Broad spectrum; less effective against moulds and yeasts; adaptation of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* has been reported.

Optimum pH range: 4–9.

Solubility: Soluble in water.

Incompatibility/inactivation: None known.

Stability: Stable over wide pH range; increased formaldehyde release above pH 9; tolerates temperatures up to 80°C.

3. Percentage of use in US cosmetic formulations

2.75%.

4. Suppliers

Glyco, Inc., Lonza Inc.; McIntyre Group; Naarden International France.

BENZYL ALCOHOL (+)

EEC No.: 34

Colipa No.: P 67

CAS No.: 100-51-6

CTFA Adopted Name: Benzyl alcohol

1. Regulatory requirements:

Maximum concentration: 1%.

2. Properties

Antimicrobial spectrum: Broad spectrum at maximum concentration.

Optimum pH range: 4–5.

Solubility: 1 g/25 g water.

Incompatibility/inactivation: Tween 80; oxidizing agents; adsorption onto polyethylene, particularly at elevated temperatures (45°C).

Stability: Oxidizes to benzaldehyde.

3. Percentage of use in US cosmetic formulations

0.32%.

4. Suppliers

Ashland Chemical Inc.; Bayer AG; Haarmann & Reimer Corporation; Kalama Chemical Inc.; Merck & Co., Inc.; Quest International Fragrances; Riedel-de-Haën AG.

1-HYDROXY-4-METHYL-6 (2,4,4-TRIMETHYLPENTYL) 2-PYRIDON and its monoethanolamine salt (+)

EEC No.: 35
 Colipa No.: P 59
 CAS No.: 68890-66-4
 CTFA Adopted Name: Piroctone olamine

1. *Regulatory requirements*

Maximum concentration: 1%; 0.5%.
 Limitations on use: 1% for rinse-off products; 0.5% for other products.

2. *Properties*

Antimicrobial spectrum: Very effective against bacteria (except *Ps. aeruginosa*) and yeasts.
 Optimum pH range: 5–9.
 Solubility: In water c. 0.05%.
 Incompatibility/inactivation: Some fragrances.
 Stability: Stable at pH 5–9 and temperature up to 80°C.

3. *Percentage of use in US cosmetic formulations*

–

4. *Suppliers*

Hoechst AG.

1,2-DIBROMO-2,4-DICYANOBUTANE

EEC No.: 36
 Colipa No.: P 77
 CAS No.: 35691-65-7
 CTFA Adopted Name: Methyldibromo glutaronitrile

1. *Regulatory requirements*

Maximum concentration: 0.1%.
 Limitation on use: Not to be used in cosmetic sunscreen products at a concentration exceeding 0.025%.

2. *Properties*

Antimicrobial spectrum: Bacteria, moulds and yeasts.
 Optimum pH range: 4–8.
 Solubility: Poorly water soluble.
 Incompatibility/inactivation: Interaction with pigments, in particular with iron oxide pigments (formulations containing them require higher concentrations of the preservative); occurrence of discoloration in combination with nitrogen-containing surfactants, depending on type and concentration of the surfactant (compatibility tests should precede the use of the preservative in

such preparations); yellow discoloration of products in presence of iron ions (addition of chelators, such as EDTA is recommended).

Stability: Unstable in acidic and neutral systems under prolonged heat stresses above 60°C; highly temperature-sensitive in alkaline formulations (avoid any heating at pH above 8).

3. *Percentage of use in US cosmetic formulations*

—

4. *Suppliers*

Calgon Corporation; Merck & Co., Inc.; Schülke & Mayr GmbH.

**6,6-DIBROMO-4,4-DICHLORO-2,2'-METHYLENE-DIPHENOL
(BROMOCHLOROPHEN) (+)**

EEC No.: 37
Colipa No.: P 25
CAS No.: 15435-29-7
CTFA Adopted Name: Bromochlorophene

1. *Regulatory requirements*

Maximum concentration: 0.1%.

2. *Properties*

Antimicrobial spectrum: Primarily effective against Gram-positive bacteria, in particular against cocci.

Optimum pH range: 5–6.

Solubility: Less than 0.1% in water.

Incompatibility/inactivation: Nonionic and cationic surfactants; proteins.

Stability: Unstable in light.

3. *Percentage of use in US cosmetic formulations*

—

4. *Suppliers*

ICI Americas Inc.; Merck & Co., Inc.

4-ISOPROPYL-M-CRESOL

EEC No.: 38
Colipa No.: P 44
CAS No.: 3228-20-2
CTFA Adopted Name: 0-Cymen-5-ol

1. Regulatory requirement

Maximum concentration: 0.1%.

2. Properties

Antimicrobial spectrum: Effective against bacteria, moulds and yeasts.

Optimum pH range: 4–9.

Solubility: 0.03–0.07% in water.

Incompatibility/inactivation: Nonionic and cationic surfactants.

Stability: No instabilities known.

3. Percentage of use in US cosmetic formulations

0.04%.

4. Suppliers

Naarden International France; Osaka Kasei Company, Ltd.

MIXTURE OF 5-CHLORO-2-METHYLISOTHIAZOL-3(2H)-ONE AND 2-METHYL-ISOTHIAZOL-3(2H)-ONE WITH MAGNESIUM CHLORIDE AND MAGNESIUM NITRATE

EEC No.: 39

Colipa No.: P 56

CAS No.: 26172-55-4 5-Chloro-2-methyl-3(2H)-isothiazolone
2682-20-4 2-Methyl-3(2H)-isothiazolone

CTFA Adopted Name: Methylchloroisothiazolinone and
methylisothiazolinone

1. Regulatory requirement

Maximum concentration: 0.0015% (of a mixture in the ratio 3:1 of 5-chloro-2-methylisothiazol-3(2H)-one and 2-methylisothiazol-3(2H)-one).

Comment: The Expert Panel of the Cosmetics Ingredients Review (C.I.R.), in the United States on February 13th 1990, ruled Kathon CG as safe to use at recommended use levels of 15 ppm active ingredient in rinse-off products and 7.5 ppm active ingredient in leave-on applications. Permitted in Japan for rinse-off products at 15 ppm of active ingredient.

2. Properties

Antimicrobial spectrum: Bacteria, moulds and yeasts; adaptation of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* to the preservative has been demonstrated.

Optimum pH range: Up to 8.

Solubility: Miscible with water.

Incompatibility/inactivation: Degradation of the preservative (in particular the chlorinated isothiazolone) in the presence of free amines, reducing agents (e.g. bisulphite), proteins (including its derivatives) and allantoin, negligible or suppressed at acidic pH. For detailed information on formulational techniques for the preservation of these systems with isothiazolones consult the suppliers' brochures.

Stability: Slight activity loss of the chlorinated component in aqueous solution at pH 6 to 7, accelerated degradation at rising pH and temperature. Stability problems are minimized by formulating products below pH 7 and incorporating the preservative into the product at temperatures not exceeding 40°C. Stability tests of the finished formulation should be performed.

3. *Percentage of use in US formulations*

3.55%.

4. *Suppliers*

Riedel-de Haën AG; Rohm and Haas; Schülke & Mayr GmbH.

2-BENZYL-4-CHLOROPHENOL (Chlorophene)

EEC No.: 40
Colipa No.: P 22
CAS No.: 120-32-1
CTFA Adopted Name: Chlorophene

1. *Regulatory requirement*

Maximum concentration: 0.2%.

2. *Properties*

Antimicrobial spectrum: Mainly Gram-positive bacteria.

Optimum pH range: 3–10.

Solubility: Insoluble in water (0.007% at 25°C).

Incompatibility/inactivation: Quaternary compounds, nonionic surfactants and proteins.

Stability: Stable below 50°C.

3. *Percentage of use in US formulations*

—

4. *Suppliers*

Bayer AG.

2-CHLOROACETAMIDE

EEC No.: 41
Colipa No.: P 27

CAS No.: 79-07-2
CTFA Adopted Name: Chloroacetamide

1. *Regulatory requirement*

Maximum concentration: 0.3%.
Printed label warning: Contains chloroacetamide.

2. *Properties*

Antimicrobial spectrum: Moderately active against bacteria, moulds and yeasts.
Optimum pH range: 4–8.
Solubility: c. 5% in 100 g water (20°C).
Incompatibility/inactivation: Strong acids and alkali.

3. *Percentage of use in US formulations*

0.41%.

4. *Suppliers*

Aldrich Chemical; Riedel-de Haën AG.

CHLORHEXIDINE (INN) and its digluconate, diacetate and dihydrochloride (+)

EEC No.: 42
Colipa No.: P 35
CAS No.: 55-56-1
CTFA Adopted Name: Chlorhexidine digluconate, chlorhexidine dihydrochloride

1. *Regulatory requirement*

Maximum concentration: 0.3% expressed as chlorhexidine.

2. *Properties*

Antimicrobial spectrum: Gram-positive and Gram-negative bacteria, low activity against moulds; contamination of aqueous preservative solutions with *Pseudomonas* spp. and *Serratia marcescens* has been reported in the literature.

Optimum pH range: 5–8, preferably at neutral pH.

Solubility: 0.008% of the free base in water; chlorhexidin digluconate > 70% water soluble, chlorhexidin dihydrochloride 0.6% soluble in water (20°C).

Incompatibility/inactivation: Soaps and other anionic surfactants; inactivated by Tween 80 at higher concentrations; formation of insoluble salts with phosphates, borates, citrates, carbonates, bicarbonates, sulphates, and chlorides; incompatible with sodium alginate and gums; strong adsorption onto particulate matter.

Stability: Unstable at high temperatures and alkaline pH.

3. *Percentage of use in US formulations*

Chlorhexidine acetate 0.01%; chlorhexidine digluconate 0.09%; chlorhexidine dihydrochloride 0.02%.

4. *Suppliers*

Accurate Chemical & Scientific Corp.; Atlas Chemie; ICI Americas Inc.

1-PHENOXYPROPAN-2-OL

EEC No.: 43
Colipa No.: P 54
CAS No.: 4169-04-4
CTFA Adopted Name: Phenoxyisopropanol

1. *Regulatory requirements*

Maximum concentration: 1%.
Limitation on use: Only for rinse-off products.

2. *Properties*

Antimicrobial spectrum: Broad spectrum activity.
Optimum pH range: 4–8.
Solubility: Soluble in water at 1%, heating and stirring is recommended.
Incompatibility/inactivation: Loss of activity in polyethylene containers.
Stability: Good.

3. *Percentage of use in US formulations*

0.02%.

4. *Suppliers*

Nipa Laboratories, Inc.

ALKYL (C12-C22) TRIMETHYL AMMONIUM bromide and chloride (+)

EEC No.: 44
Colipa No.: P 72
CAS No.: 57-09-0
CTFA Adopted Name: Cetrimonium bromide, Cetrimonium chloride

1. Regulatory requirement

Maximum concentration: 0.1%.

2. Properties

Antimicrobial spectrum: Very active against gram-positive bacteria; higher concentrations required for gram-negative bacteria, moulds and yeasts.

Optimum pH range: 4–10; increased activity at higher pH.

Solubility: Soluble in water.

Incompatibility/inactivation: Soaps and other anionic surfactants; heavy metals; oxidizing agents; proteins, phospholipids; nitrates; rubber.

Stability: Stable under acidic conditions.

3. Percentage of use in US formulations

Cetrimonium bromide 0.03%.

4. Suppliers

Akzo Chemie; Henkel KGaA; Hoechst AG.

4,4-DIMETHYL-1,3-OXAZOLIDINE

EEC No.: 45

Colipa No.: P 75

CAS No.: 51200-87-4

CTFA Adopted Name: Dimethyl oxazolidine

1. Regulatory requirement

Maximum concentration: 0.1%.

Limitation on use: The pH of the finished product must not be lower than 6.

2. Properties

Antimicrobial spectrum: Activity against bacteria, moulds and yeasts.

Optimum pH range: 6–11.

Solubility: Soluble in water.

Incompatibility/inactivation: None known.

Stability: Unstable below pH 5.

3. Percentage of use in US formulations

—

4. Suppliers

ANGUS Chemical Company.

N-(HYDROXYMETHYL)-N-(DIHYDROXYMETHYL-1,3-DIOXO-2,5-IMIDAZOLIDINYL-4)-N¹-(HYDROXYMETHYL) UREA

EEC No.: 46
 Colipa No.: P 79
 CAS No.: 78491-02-8
 CTFA Adopted Name: Diazolidinyl urea

1. *Regulatory requirement*

Maximum concentration: 0.5%.

2. *Properties*

Antimicrobial spectrum: More effective than Germall 115; the broad activity spectrum also includes resistant pseudomonads, such as *Pseudomonas cepacia*.
 Optimum pH range: Effective over a wide pH range.
 Solubility: 230 g/100 g water.
 Incompatibility/inactivation: None known.
 Stability: Should be incorporated into products at temperatures below 60°C.

2. *Percentage of use in US formulations*

1.40%.

4. *Suppliers*

Chemag AG; Sutton Laboratories, Inc.

HEXAMIDINE (INN) and its salts (including isethionate and *p*-hydroxybenzoate) (+)

EEC No.: 47
 Colipa No.: P 8
 CAS No.: 3811-75-4 Benzenecarboximidamide,
 4,4' [1,6-hexanediylbis(oxy)]bis-659-40-5
 Ethanesulfonic acid 2-hydroxy-compd. with
 4,4' [1,6-hexanediylbis(oxy)]bis
 [benzenecarboximidamide] (2:1)
 CTFA Adopted Name: Hexamidine diisethionate

1. *Regulatory requirement*

Maximum concentration: 0.1%.

2. *Properties*

Antimicrobial spectrum: More effective against bacteria (in particular Gram-positive bacteria) than moulds and yeasts.
 Optimum pH range: 5–9.
 Solubility: Water soluble.

Incompatibility/inactivation: Chloride and sulphate ions; proteins.

Stability: Thermostable below 100°C.

3. *Percentage of use in US cosmetic formulations*

0.02%.

4. *Suppliers*

Rhône-Poulenc.

Appendix B—Profiles of preservatives provisionally allowed**CHLORPHENESIN (INN)**

EEC No.: 2
Colipa No.: P 4
CAS No.: 104-29-0
CTFA Adopted Name: Chlorphenesin

1. Regulatory requirement

Maximum concentration 0.3%.

2. Properties

Antimicrobial spectrum: Moderately effective against Gram-positive bacteria, moulds and yeasts

Optimum pH range: 4–6.

Solubility: In water c. 0.5%.

Incompatibility/inactivation: None known.

Stability: Thermostable below 100°C.

3. Percentage of use in US cosmetic formulations

—

4. Suppliers

Lentia GmbH.

BENZETHONIUM CHLORIDE

EEC No.: 15
Colipa No.: P 70
CAS No.: 121-54-0
CTFA Adopted Name: Benzethonium chloride

1. Regulatory requirement

Maximum concentration: 0.1%.

Limitation on use: Only for deodorants, hair care products and aftershave products. Not permitted in products intended to come into contact with mucous membranes.

2. Properties

Antimicrobial spectrum: Bacteria (primarily Gram-positive), moulds and yeasts.

Optimum pH range: 4–10, more effective at higher pH.

Solubility: Soluble in water.

Incompatibility/inactivation: Soaps and other anionic surfactants; proteins; precipitation with mineral acids and some salts.

Stability: Stable to melting point.

3. *Percentage of use in US cosmetic formulations*

0.11%.

4. *Suppliers*

Aldrich Chemical; Lonza Inc.

BENZALKONIUM CHLORIDE, bromide and saccharinate (INN) (+)

EEC No.: 16

Colipa No.: P 71

CAS No.: 139-07-1

CTFA Adopted Name: Benzalkonium chloride

1. *Regulatory requirement*

Maximum concentration: 0.25%.

2. *Properties*

Antimicrobial spectrum: Bacteria (preferably Gram-positive), moulds and yeasts; pseudomonas resistance has been reported.

Optimum pH range: 4–10, more effective at higher pH.

Solubility: Water soluble.

Incompatibility/inactivation: Soaps and other anionic surfactants; strong oxidants; proteins; heavy metals; salts (citrates, nitrates, phosphates); absorbed by rubbers and some plastic materials.

Stability: good.

3. *Percentage of use in US cosmetic formulations*

0.23%.

4. *Suppliers*

Hoechst AG; Henkel KGaA; Lonza Inc.; Rohm and Haas.

BENZYLHEMIFORMAL

EEC No.: 21

Colipa No.: P 21

CAS No.: 14548-60-8 Methanol, (phenylmethoxy)-
35445-70-6

Methanol, [(phenylmethoxy)methoxy]-
CTFA Adopted Name: Benzylhemiformal

1. *Regulatory requirement*

Maximum concentration: 0.2%.

2. Properties

Antimicrobial spectrum: Bacteria; moulds and yeasts require higher concentrations.

Optimum pH range: 3–10.

Solubility: 2.5% in water at 20°C.

Incompatibility/inactivation: Proteins; activity losses in the presence of ammonium salts or ammonia at alkaline pH.

Stability: Thermostable below 50°C.

3. Percentage of use in US cosmetic formulations

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4. Suppliers

Bayer AG.

GLUTARALDEHYDE

EEC No.: 26

Colipa No.: P 76

CAS No.: 111-30-8

CTFA Adopted Name: Glutaral

1. Regulatory requirement

Maximum concentration: 0.1%.

Limitation on use: Prohibited in aerosols (sprays).

Printed label warning: Contains glutaraldehyde where the glutaraldehyde exceeds 0.05%.

2. Properties

Antimicrobial spectrum: Bacteria, moulds and yeasts.

Optimum pH range: 4–9, bactericidal at alkaline pH.

Solubility: Water soluble.

Incompatibility/inactivation: Primary amines or ammonia at neutral and alkaline pH; sodium bisulphite.

Stability: Instable at elevated temperatures.

3. Percentage of use in US cosmetic formulations

0.20%.

4. Suppliers

Union Carbide Chemicals and Plastics Company, Inc.

3-DECYLOXY-2-HYDROXY-1-AMINOPROPANE-HYDRO-CHLORIDE (Decominol)
(INN)

EEC No.: 27
Colipa No.: P 87

1. Regulatory requirement

Maximum concentration: 0.5%.

2. Percentage of use in US cosmetic formulations

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7-ETHYLBICYCLOOXAZOLIDINE

EEC No.: 28
Colipa No.: P 90
CAS No.: 7747-35-5
CTFA Adopted Name: 7-Ethyl bicyclooxazolidine

1. Regulatory requirement

Maximum concentration: 0.3%.

Limitations on use: Prohibited in dental hygiene products and in products intended to come into contact with mucous membrane.

2. Properties

Antimicrobial spectrum: Bacteria, moulds and yeasts.

Optimum pH range: 6–11.

Solubility: Water soluble; can partition into oil phase of emulsions.

3. Percentage of use in US cosmetic formulations

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4. Suppliers

ANGUS Chemical Company.

3-iodo-2-propynyl-butyl carbamate

EEC No.: 29
Colipa No.: P 91
CAS No.: 55406-53-6
CTFA Adopted Name: Iodopropynyl butylcarbamate

1. Regulatory requirement

Maximum concentration: 0.1%.

2. *Properties*

Antimicrobial spectrum: Wide spectrum activity; particularly effective against moulds and yeasts.

Optimum pH range: 3–9.

Solubility: Readily soluble in the presence of water and a polar organic compound (surfactants, alcohols, glycols, glycolethers).

Incompatibility/inactivation: None known.

Stability: Thermostable up to 80°C for extended periods.

3. *Percentage of use in US cosmetic formulations*

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4. *Suppliers*

Lonza Inc.

SODIUM HYDROXYMETHYL AMINOACETATE

EEC No.: 30

Colipa No.: P 84

CAS No.: 70161-44-3

CTFA Adopted Name: Sodium hydroxymethylglycinate

1. *Regulatory requirement*

Maximum concentration: 0.1%.

2. *Properties*

Antimicrobial spectrum: Bacteria, moulds, yeasts.

Optimum pH range: 3–12.

Solubility: Water soluble.

Incompatibility/inactivation: None known.

Stability: Unstable at elevated temperatures.

3. *Percentage of use in US cosmetic formulations*

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4. *Suppliers*

Sutton Laboratories, Inc.

Addendum to Point 3 (Appendices A and B)

After compilation of the preservative profiles two up-dated lists on the frequency of preservative use in US products were published in 1992 (Cosmetics & Toiletries, Vol. 107, June 1992) and 1993 (Cosmetics &

Toiletries, Vol. 108, October 1993). Both contain as new entries the following preservatives: methyldibromo glutaronitrile (EC No. 36, Annex VI, Part 1) and sodium hydroxymethylglycinate (EC No. 30, Annex VI, Part 2).

As the 1992 and 1993 databases of the FDA no longer distinguish ingredients by their function in products, the figures in these lists do not truly correspond to earlier reports on the frequency of preservative use.