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Preface

Microbial quality assurance in all types of manufactured products is a topic which continues to cause concern amongst scientists. In April 1987, a postgraduate school was held at the Chelsea Department of Pharmacy, King's College London, University of London. The aim of the school was to provide scientists working in the pharmaceutical, cosmetics and toiletries industries, and in the hospital pharmacy, with a comprehensive review of the approaches and methods currently adopted to control microbial contamination in non-sterile pharmaceutical products and in cosmetic and toiletry products.

The papers presented at this conference have been used as the basis for this book. Some of the chapters are written by academic scientists and some by industrial scientists and hospital pharmacists and, as a result, the book represents a 'blend' of both practical information and the scientific principles on which the subject is based.

Although some of the information has been published elsewhere, much of it is now out of date and is 'scattered' in a variety of different journals. This book represents a unique attempt to gather together, to update and to review this information as a single comprehensive text.

The book reviews all aspects of microbial quality assurance related to non-sterile pharmaceutical, cosmetic and toiletry products, including control of microbial contamination (good manufacturing practice) and the formulation and preservation of products to ensure that microbial quality is maintained during storage and use. Guidelines, etc., both official and unofficial, for microbial quality are also comprehensively reviewed.

It is intended that the book will provide a valuable reference source for all scientists (chemists, pharmacists, etc., as well as microbiologists) working in research and development and quality control laboratories concerned with the production of pharmaceuticals, cosmetics and toiletries.

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Sally F. Bloomfield

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Control of microbial contamination in non-sterile pharmaceuticals, cosmetics and toiletries

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In the manufacture of pharmaceuticals, cosmetics and toiletries, quality assurance represents a major consideration. This book is particularly concerned with one aspect of quality, namely the assurance that products are not contaminated with organisms which might affect their safety, efficacy or acceptability to the patient or consumer.

One obvious approach to this problem is to ensure that all products are manufactured either as sterile products or as 'single-use' packs. For cosmetics and toiletries and many pharmaceuticals, this is neither appropriate nor commercially viable and alternative means of microbial quality assurance are therefore sought. During product manufacture, microbial contamination is mainly controlled by the application of good manufacturing practice (GMP) whilst the maintenance of quality during storage and use (also the responsibility of the manufacturer) is achieved largely by the inclusion of preservatives but also by product design and by product formulation and packaging.

In practice, the presence of micro-organisms in pharmaceuticals, cosmetics and toiletries constitutes a potential hazard for two reasons. These aspects are reviewed in chapter 2. Firstly, it may result in spoilage of the product — the metabolic versatility of micro-organisms is such that almost any formulation ingredient may undergo degradation in the presence of a suitable micro-organism. Alternatively, it may constitute an infection hazard to the consumer or patient, although here we have to bear in mind that the degree of hazard will vary considerably from one situation to another according to the intended use of the product (oral, topical, application to the eye, etc.) by the patient or consumer.

In attempting to define acceptable standards or limits for microbial contamination for non-sterile products, it is therefore impossible to establish what levels and types of contamination represent a hazard and what can be considered as safe.

If the problem is examined from a historical point of view it is found that, prior to about 1960, although products were manufactured under hygienic conditions with the inclusion of a preservative, there was little evidence of the rigorous approach which is currently adopted. During the 1960s, as a result of the growing numbers of reports of infection outbreaks associated with contaminated products (see chapter 2), there was a general realization of the need for improvements in microbial quality assurance for all types of products.

In order to assess how this might be achieved, a number of studies were initiated to obtain a general picture of the quality of products being manufactured at that time. Some of the studies were official or national studies as in Sweden and Denmark, whilst others were independent surveys. These investigations included cosmetics and toiletries as well as pharmaceuticals. In the UK, two official studies of pharmaceuticals were initiated, one by the Public Health Laboratory Service to study hospital medicaments, the other by the Pharmaceutical Society of Great Britain (PSGB) to study all types of products. The results of all these surveys are reviewed in chapter 5.

Having established the nature and extent of the problem, the next step was to try to control it; the various official bodies had to decide the extent to which manufacturers should be asked to limit microbial contamination in their products and how the standards should be applied. Inevitably, it is found that the limits applied vary from one country and one situation to another. Accepted limits may be 'in-house' limits or, for pharmaceuticals, may be applied via the pharmacopoeia or licensing systems. The various official, unofficial and 'in-house' standards which have been developed over the last 20 or so years are outlined in chapter 5.

In examining the methods which are currently adopted for controlling microbial contamination in pharmaceuticals, cosmetics and toiletries, it can be seen that in general these have been developed from specific recommendations laid out in the original PSGB working party report and the report of a joint working party representing the Toiletry Goods Association and the Society of Cosmetic Chemists. The recommendations of the PSGB have since been further developed and incorporated into the *Guide to Good Manufacturing Practice,* the most recent edition being published in 1983.

If we refer to these recommendations, we find that they divide into three groups: recommendations related to GMP, recommendations related to formulation of the product, and recommendations which relate to the inclusion of preservatives.

As far as GMP is concerned, the main concerns are with the microbial quality of the raw materials (particularly water) and with all aspects of the manufacturing process, the processing environment and equipment, the process itself and the personnel operating the process. These aspects are discussed in chapters 3 and 4.

As far as product formulation is concerned, any number of physicochemical factors can affect the fate of micro-organisms which enter a product. These factors include the conditions of water activity, pH and osmotic pressure within the product, the availability of nutrient material, the product storage temperature, and so on. In practice, it is found that some types of products are virtually 'self-preserving' such that residual contamination occurring during manufacture (or use) is reduced to

undetectably low levels during storage. In the pharmaceutical industry, there has been some tendency to overlook this aspect and to rely almost entirely on chemical preservatives. With increasing concern over possibilities of toxic side effects associated with chemical preservatives, it is important to consider carefully the possibilities of 'self-preservation or natural preservation' of products. This aspect is discussed in chapter 6.

The third aspect arising from the PSGB report relates to the inclusion of chemical preservatives. As stated in this report (but equally applicable to cosmetics and toiletries), although the microbial quality of products should be achieved by GMP, it may be necessary in certain types of product to include a preservative to protect the product against residual contamination introduced during manufacture and any further microbial contamination which might occur during use. It is clearly stated, however, that the function of the preservative must not be to cover bad manufacturing practice but to ensure that the product remains in a satisfactory condition during storage and use.

In solving problems related to chemical preservation, there are four main questions which have to be considered. Under what conditions is the inclusion of a preservative justified? What constitutes adequate preservation? What preservatives are available to meet our requirements? How do we establish that a product is effectively preserved?

In deciding under what conditions the inclusion of a preservative is justified, for some situations the indications are obvious whilst in others there may be room for disagreement. Thus, for example, in tablet film coating where working schedules demand advance preparation of film coating solutions, although the inclusion of preservatives in these solutions might be considered as an admission of bad manufacturing practice, in practice this is considered acceptable to prevent microbial growth which would otherwise occur during the unavoidable holding period prior to the film coating process. In addition to this, although tablets are assumed to be self-preserving, the question has been raised as to whether preservatives should be included to prevent incidents of mould growth which occur from time to time. Problems associated with control of microbial contamination in powders, tablets and capsules are discussed in chapter 8.

Secondly, it is necessary to consider what constitutes adequate preservation. As far as pharmaceuticals are concerned, it appears that the licensing authorities currently allow themselves to be guided by criteria defined in the *British pharmacopoeia* (*BP*) and *United States pharmacopoeia* (*USP*) tests for preservative efficacy. If one asks how such standards have arisen, it would be difficult to get a precise answer. It would be comforting to believe that they represent a definitive knowledge of the required activity to protect the product and user against the appropriate hazard, but in practice, as mentioned earlier, such information is not available, nor is it ever likely to be. In practice, the criteria appear to be based on a knowledge of what is achievable in relation to the range of preservatives available and a working knowledge of preservative efficacy in manufactured products. In recognition of the problems of deciding what constitutes adequate preservation, and the fact that this may vary from product to product according to its nature and intended use, the *BP* test states that, at present, compliance with the test is not rigidly demanded; deviation is acceptable where it is adequately justified by bacteriological and other

data (Anon. 1986a). For cosmetics and toiletries, at the present time there are no official standards, the industry preferring to work to 'in-house' standards which relate to levels of preservation demonstrated by established products known to be 'well preserved'. These aspects are further discussed in chapter 9.

Having considered what might constitute 'adequate preservation', the next problem is to find suitable preservatives which meet these requirements. Over the years, a very large number of antibacterial compounds have been examined and their suitability for preservation of pharmaceuticals, toiletries and cosmetics investigated. In practice, only a relatively limited range of about 13 preservatives, as illustrated in Table 1.1, is currently used in the majority of pharmaceutical products. For

Table 1.1 — Antimicrobial agents currently used in pharmaceutical products

(1)	*Single-dose and multiple-dose injections*	
	Chlorocresol (0.1%)	Benzylalcohol (1.0%)
	Cresol (0.3%)	Phenylmercuric nitrate, acetate or borate (0.002%)
	Phenol (0.5%)	
	Chlorbutol (0.5%)	
(2)	*Eye drops and contact lens solutions*	
	Chlorhexidine acetate or gluconate (0.1%)	Thiomersal (0.1%)
	Benzalkonium chloride (0.3%)	Chlorbutol (0.5%)
(3)	*Oral liquids*	
	Methyl, ethyl and propyl *p*-hydroxyhenzoate (parabenz) (0.3%)	
	Benzoic and sorbic acid (0.3–0.5%)	
	Chloroform (0.25%)	
	Bronopol (0.5%)	
(4)	*Creams*	
	Parabenz (0.3%)	Cetyltrimethylamonium bromide (1.0%)
	Chlorocresol (0.1%)	
	Bronopol (0.5%)	Phenylmercuric nitrate (0.01%)

cosmetics and toiletries the list is somewhat larger; the most recent EEC directive (Anon., 1986b) indicates some 39 compounds which are considered 'acceptable' and some further 25 compounds under consideration. Despite the much larger list of potential compounds, the most widely used compounds in cosmetics and toiletries are the *p*-hydroxybenzoates, Bronopol and the formaldehyde-releasing compounds;

a 1982 survey in the USA of some 20 000 formulations suggested that more than 90% contained only seven preservative types (Decker and Wenninger 1982). More than anything it is the problems of toxicity which limit the range of preservatives available for use in pharmaceuticals, cosmetics and toiletries. Problems of preservative toxicity and toxicity testing are discussed in chapter 11. The other main problem is that of cost. Although there is an urgent need to increase the range of preservatives suitable for products, the commercial advantages of providing new preservatives are generally outweighed by the substantial research and development investment required for their development. For this reason, the major research effort in this area is being directed towards better use of 'existing' preservatives including the use of potentiators and of synergistic preservative combinations. These aspects are covered in chapters 12 and 13.

In choosing a suitable preservative for a particular formulation, the compound is required to have certain characteristics.

(1) It should be active against the required spectrum of organisms.
(2) It must be effective at the pH of the formulation.
(3) It must be soluble in the aqueous phase of the formulation.
(4) It must be stable and non-volatile.
(5) It must be compatible with formulation ingredients.
(6) It should be colourless, odourless and tasteless.
(7) It must be of low toxicity.

In practice, as might be expected, the ideal preservative does not exist, and in many cases it is a matter of selecting the 'least unsuitable' rather than the 'optimum' preservative for a particular product.

In this text, no attempt has been made to discuss the properties of individual agents or groups of agents. For information related to selection of suitable preservatives for individual products, reviews by Bean (1972), Russell (1974), Block (1977), Cowen and Steiger (1977), Croshaw (1977), Bloomfield and Leak (1982) and Kabara (1984) should be consulted.

In addition to choosing a suitable preservative, or preservatives, for inclusion in a particular product, we also have the problem of maintaining an adequate preservative concentration within the product to ensure satisfactory preservation. Although simple aqueous formulations are relatively easy to preserve, many pharmaceutical and cosmetic formulations are complex systems consisting of a number of phases. Since micro-organisms will grow only in the aqueous phase of a product, it is vitally important to maintain an adequate concentration of free preservative in the aqueous phase of the product. Problems can also arise from partitioning of the preservative into packaging materials. These aspects will be considered in chapter 7.

Finally, there is the problem of establishing that a product is effectively preserved. Although a knowledge of the properties of a preservative may assist in the choice of a suitable system, it is well recognized that selection based on theoretical considerations can be regarded only as a guide, since in many cases the interactions are incompletely understood. For this reason, it is always necessary to undertake microbiological tests to determine the efficacy of the preservative system for each individual product. This usually involves the performance of simple challenge tests in

which a sample of the product is inoculated with the test organisms and the number of surviving organisms determined at intervals by viable counting procedures. The test system currently adopted for pharmaceuticals is usually based on the *BP* and *USP* tests. Although these tests may be suitable for establishing the 'bioavailability' of the preservative within the product over the designated period of storage, under defined conditions, concern has been expressed regarding the adequacy of such tests for predicting preservative failures in the practical situation. These aspects and the various alternative approaches to preservative testing of pharmaceuticals, cosmetics and toiletries are discussed in chapters 9 and 10.

In the following chapters of this book, the individual aspects of microbial quality assurance as outlined in this chapter are discussed in more detail. For each aspect, an attempt has been made to review theoretical as well as practical information available and to illustrate how this information may be developed and applied in achieving satisfactory microbial quality assurance for non-sterile pharmaceuticals, cosmetics and toiletries.

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2

Hazards associated with the microbiological contamination of non-sterile pharmaceuticals, cosmetics and toiletries

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SUMMARY

'Wise men learn by other men's mistakes; fools, by their own.'

Bacon

(1) Serious attention was not given to the hazards associated with contaminated non-sterile products until the 1960s. Patients, many with compromised defences, have been found to be at risk from organisms, particularly Gram-negative opportunistic bacteria, in non-sterile products administered by different routes.
(2) Non-sterile preparations have acted as intermediate reservoirs in common-source outbreaks of hospital infection.
(3) Infection from contaminated cosmetics and toiletries does not appear to occur readily, but isolated incidents in hospitalized patients, and some eye infections, have been reported.
(4) The spoilage of non-sterile products may be recognized organoleptically but changes indetectable to the senses could be hazardous.
(5) Continuous vigilance is necessary by those responsible for the production, distribution and administration of non-sterile products in order to protect patients and users from these microbiological hazards.

2.1 INTRODUCTION

The possibility that infection arises from microbial contamination of parenterally administered preparations has been appreciated from the close of the last century. Many incidents were reported (Groves 1973, Holmes and Allwood 1979) and steps

were taken to minimize their reoccurrence. Hazards arising from contaminated non-sterile products have been less readily appreciated. In the first half of the century, scattered reports of incidents of spoilage and infection appeared, particularly in connection with ophthalmic preparations which were then generally non-sterile, and disinfectants and antiseptics. However, serious attention was only given to non-sterile products after reports from Sweden indicated that they could constitute a real and direct hazard to the patient (Kallings *et al.* 1966). The thorough Swedish investigations initiated many surveys of non-sterile products for microbial contamination in a number of other countries over the next decade. Many of the results were summarized by Sykes (1971), who mentioned two important investigations in the UK organized by the Pharmaceutical Society of Great Britain (Anon. 1971a) and the Public Health Laboratory Service (Anon. 1971b). Many preparations were found to contain a surprisingly high number of viable organisms, including some harmful species. Later, interest spread to cosmetics and toiletries and these products were also found to constitute a potential hazard by virtue of their bioburden (Baird 1977, Malcolm 1976). More recently, an extensive independent survey of the microbiological quality of cosmetics and toiletries at the point of sale in Europe has been carried out. About 5% of nearly 5000 samples were found to have total viable counts greater than 1000 colony-forming units per gram (cfu g^{-1}) (some even exceeding 10^6 cfu g^{-1}), and a number of pathogens were isolated (Greenwood and Hooper 1982).

It has therefore been clearly established that significant microbial contamination of non-sterile products can occur. This constitutes a *potential* hazard, particularly to those people whose host defences are compromised, but it is the purpose of this review to indicate the circumstances in which such products have acted as a *real* hazard and have caused (or have been involved in) adverse reactions owing to the micro-organisms which they have harboured. Harm may result from the effect of spoilage, the initiation of infection directly or the facilitation of its spread. Most available information has originated from hospital practice where there is a greater chance of recognition of the connection between adverse reactions and contaminated products. Even in hospitals, such a relationship is not always obvious but chance has favoured the prepared mind and this has led to some fruitful investigations. In general practice the possibility of identifying the association must be much more difficult, and published reports may therefore represent a minority of unfortunate events that have occurred. Certainly, reports involving contaminated cosmetics and toiletries are very sparse and, while it is possible that such events still go unrecognized, it seems probable that the healthy user has proved relatively insusceptible to infections arising from this source.

Fortunately, man learns from his mistakes, and it is in this spirit that this summary of mishaps has been undertaken. A number of other reviews of different aspects of the topic have already appeared (Butler 1968, Parker 1972, Smart and Spooner 1972, Beveridge 1975, 1983, Somerville and Summers 1979, Baird 1981, Ringertz and Ringertz 1982). This chapter attempts to complement rather than to duplicate them.

2.2 INFECTION FROM NON-STERILE PRODUCTS

The most obvious and severe consequence of microbial contamination is the direct infection of the patient. Given that viable organisms exist in the preparation, the

chance that infection results depends upon a considerable number of variables, recently outlined by Ringertz and Ringertz (1982) and Parker (1984). The type of organism and the numbers present in the dose, or application, are probably paramount. The question of pathogenicity is a vexed one; probably any species of micro-organism may initiate an infection if conditions are suitable and, as virulence appears to be a fleeting characteristic, the factors involved are still poorly understood. Bruch (1972) pointed out the difficulty of deciding whether a micro-organism is objectionable in a non-sterile pharmaceutical. It is a complicated value judgement, which is particularly difficult for topical preparations. He contemplated the roles of many organisms in such preparations and concluded that most are objectionable for products applied to damaged epithelium, while others may be 'usually objectionable', depending on the species, the site and the health of the recipient. This concept of 'frank' and 'opportunistic' pathogens is fraught with difficulty because we still know so little about the factors affecting the properties by which a micro-organism causes death or reaction of the tissues invaded. Even absolute virulence, the ability of the organism to transcend all fluctuations of host resistance, is dependent on the number of organisms present. This has been established experimentally in normal volunteers and from chance observation of natural infection where, because the vector is known, the infective dose can be established. For example about 10^6 *Salmonella anatum* or *S. meleagridis* cells were found necessary to produce clinical symptoms of infection in healthy volunteers (McCullough and Eisele 1951) whereas, on average, only 50 or so cells of *S. napoli* constituted an infective dose for patients in an outbreak of salmonellosis following consumption of contaminated chocolate bars (Greenwood and Hooper 1983). Studies in volunteers have shown that the number of *Staphylococcus aureus* cells necessary to initiate a localized skin infection varies from less than 10^2 to more than 10^6, depending on local conditions (Marples 1976). In contrast, epidemiological evidence, coupled with extrapolation from clinical medicine and experimental pathology, has allowed tentative determination of the number of organisms necessary to initiate inhalation infections such as tuberculosis and anthrax (Williams 1967). Thus, infective doses vary considerably, even between similar species, but it is often difficult to decide whether this is a function of the organisms or of the host. Infection route is very important; for instance *S. aureus* and the anthrax bacillus are both far more virulent by the skin than by the alimentary tract. In contrast, many species, including *Salmonella*, infect via the mouth but cannot penetrate lightly abraded skin. Trauma obviously facilitates invasion of the skin by certain micro-organisms. This has been shown experimentally and hydration, superhydration, selective antimicrobial agents and a topical steroid have also been found to encourage experimental skin infections in volunteers (Marples 1976).

A number of natural conditions diminish host resistance and are particularly relevant to a consideration of infection arising from contaminated non-sterile pharmaceuticals. Age, genetic constitution, malignancies, diabetes and urinary tract abnormalities are all important while therapy with corticosteroids, chemotherapy, antimicrobial agents and radiation can facilitate infection.

2.2.1 Non-sterile pharmaceuticals

A number of reports of contaminated products, in some cases leading to infection, appeared in the 1960s. Before then publications were scant (Savin 1967). Sykes