STERILIZATION OF MEDICAL PRODUCTS

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STERILIZATION OF MEDICAL PRODUCTS VOLUME III

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PROCEEDINGS OF AN INTERNATIONAL SYMPOSIUM ON ADVANCES IN STERILIZATION OF MEDICAL PRODUCTS

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Contents

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SESSION IV

SESSION V – Part 1

SESSION V – Part 2

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SESSION V – Part 3

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PREFACE

This volume is the third in a series on 'Sterilization of Medical Products' that has been produced from international symposia. The earlier meetings were commemorative and known as the International Kilmer Memorial Conferences. They were held in the US in 1976 and 1980. Two other related symposia on 'Sterilization by Ionizing Radiation' held in 1974 and 1977, were, as this Symposium, sponsored by Johnson & Johnson and complement a published series on the subject of sterilization. All these conferences have enjoyed wide international acceptance and are amongst the most comprehensive meetings on the subject. The contents of this issue is an edited version of the proceedings of the meeting held on 4 – 5 November 1982 at The University of New South Wales, Kensington, NSW, Australia.

The Organizing Committee set out to achieve much the same objectives as those of the earlier meetings. We made every attempt to follow the very high standard and the pattern and tradition of our predecessors. The aims were comparable, namely to cover particularly significant emerging technologies involved in sterilization. We believe that the meeting is integral to the series in furthering the expansion of knowledge of experts involved in the discipline. We recruited experts in many new areas of sterilization practice. We hoped to impart some of their expertise to those who are involved in sterilization practice in Australia and to narrow possible information gaps. It has become obvious that there is an expanding need in health care to provide 'sterile' medical products, and that sterilization of medical products was receiving an increasing scrutiny by the Federal and State Departments of Health. We were also conscious of the increasing needs for 'aseptic care', and the protection of critical-care patients against infection. It is very disturbing that infection from contaminated products continues to remain an important cause of morbidity and mortality in hospital practice, especially in patients with impaired resistance to microbial invasion. In his summary of the meeting, Leigh Dodson, Director of the National Biological Standards Laboratory, reaffirmed that the subject matter of the meeting was of timely interest to people in sterilization practice in hospitals and industry in Australia. He also said that such symposia not only updated our knowledge, but provided many opportunities for revision. In particular, he said that the present high standard of health care was among other things due to clean water, sanitation, and the availability of sterile medical products, and that these were more important than antibiotics.

Acknowledgements

We would like to thank the authors for agreeing to participate at the meeting and for their excellent contributions.

On behalf of the Organizing Committee, we would like to thank the Director-General of Health, Dr. Gwyn Howells, for the participation of Leigh Dodson, and Mr. Fred Hooten, Chief, GMP Branch, Bureau of Medical Devices, Food & Administration, USA, for approving the visit of Richard DeRisio. We are very appreciative of the distinguished chairmen for their contributions and for providing a written appreciation of the subject matter of each session which has been published herein. Our gratitude is extended to those who made poster presentations during the meeting, to Joan Gardner, Jenny Sherrard, Barry Evers-Buckland, Anthony Herrmann, Robert Morrissey, William

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Murrell, James Lindsay, Alan Warth, Michael Izard, Ronald Glover, Marina Saunders, and Nazly Hilmy. Regretfully, we are unable to publish the poster presentations.

We are indebted to Colin Bull, Murray McNair, Alan Stahel, and particularly for the participation by Robert A. Fuller, Corporate Vice-President, Office of Science and Technology, Johnson & Johnson, USA, for their support and encouragement in organizing and conducting this Symposium. The secretariat, and in particular the administrative and secretarial help of Miss Lyne Peebles is gratefully acknowledged. Lyne not only co-ordinated our activities, but also typed all texts for this publication. We wish to thank Joseph Heyman for his assistance in editing and for undertaking the onerous task of proof-reading. Special thanks also go to the printer and his competent staff. Finally, we would like to express appreciation to our co-organizers from the US, to Robert Morrissey for his most able assistance in soliciting the overseas speakers, and to Eugene Gaughran who has played a major role in establishing the Kilmer Memorial Conferences, and who on the eve of his retirement stimulated our enthusiasm to hold this meeting outside the United States.

Leon E. Harris

Anna J. Skopek

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OPENING REMARKS

It is a pleasure for me to welcome you to Sydney on behalf of Johnson & Johnson and its family of companies. I hope, you will enjoy your stay in this beautiful host city. This Symposium is a continuation of a series of symposia that have been sponsored over the last several years by Johnson & Johnson. Being the broadest based company in the health-care business, sterilization has become extremely important to us. We have many companies manufacturing sterile products around the world, and we feel a real responsibility and an obligation to foster the study and improvement of the technology of sterilization.

Since our last meeting in Washinton, D.C., in October 1980, there has been further progress in the technology of sterilization. It has, perhaps, not been as obvious as advances in bio-technology and micro-electronics, which captures the attention of the press and have become popular issues. However, I am sure that those involved in this discipline are well aware that there has been great progress over the last few years, and we trust that the group of outstanding speakers assembled in Sydney will cover that progress.

It is a very pleasant occurrence that at this meeting there are many people participating from the South Pacific. Our initial conferences were held in USA and in Europe, and it is very satisfying that there is such good representation of the leading people in this part of the world, representing the pharmaceutical industry, hospital and health-care users, along with the medical devices industry and those from various government bodies. Your meeting has been structured to cover the progress in the science itself, standards, equipment, the regulatory trends, and, in fact, the whole picture of progress in sterilization over the last several years.

It is my pleasure to introduce Eugene Gaughran, the Director of Microbiology and Sterilization Quality Assurance at Ethicon, Inc., USA, a colleague of many, many years. Gene is a friend of many years; he has contributed greatly to the progress of sterilization procedures at Johnson & Johnson and was a prime mover in organizing the first international conference and has been active in all of them since.

Robert A. Fuller

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KEYNOTE ADDRESS

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Some 30 years ago, for those of us who were sterilizing medical materials, life was quite simple. We used steam and dry heat only and we sterilized most of our materials by a steam/pressure cycle. With varying frequency we subjected some of these materials to a standard pharmacopoeial sterility test. If we were sophisticated, we included in each sterilizer run some of the material contaminated with a resistant microorganism prior to the sterilization procedure. If no growth appeared in cultures of the samples subjected to the sterility test, the material was considered sterile and was labelled 100% sterile.

However, life became more complicated. Two new methods appeared on the horizon, gaseous ethylene oxide and ionizing radiation. Ethylene oxide permitted the sterilization of materials that could not withstand steam or dry heat and was rapidly accepted by both hospitals and industry. Its use gave rise to a multi-billion dollar, sterile, single-use device industry. Ionizing radiation was slower to be accepted. Although an electron accelerator was used in the late 50s in the United States to sterilize sutures, it was soon replaced by cobalt-60 irradiation and came into use in the 60s. The first cobalt-60 plant to sterilize medical products was the British installation at Wantage in 1960, although the first commercial *gamma* irradiation plant was the Australian plant at Dandenong, Victoria, built in 1959 for the sterilization of goat hair for the manufacture of rugs.

About this time there were certain medical microbiologists, like Jocelyn Kelsey of the Public Health Laboratory Service in England, who did not believe 100% sterility could be guaranteed. There was talk of 'virtual sterility' or 'partial sterility'. An awakening really came when the medical microbiologists and the aerospace microbiologists discovered the work of the food microbiologists, who 60 years ago were concerned about thermal death curves, and certainly 40 years ago were determining the probability of survivors in canned foods.

We recognize today that sterility cannot be tested into a product, and that assurance must be built into the manufacturing process, of which 'sterilization' is but one part. This has led to new approaches to process development, and process control, and the use of terms like 'bioburden' and 'validation' and the expression 'levels of sterility assurance'. It has lead to a better understanding of good manufacturing practices. All these are the subjects of this Symposium. Such things relating to sterilization are readily accomplished by a sterile medical products manufacturer, where a product is produced in large volume, and the composition of a sterilizer load can be standardized. The hospital, however, has a very difficult problem to follow in the path of industry, but has made great progress in

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what we now call ‘good hospital practice’ in sterilization.

With progress, there have come processes that provide in many cases an extremely high level of assurance of sterility. This has raised a number of questions that we will examine during this Symposium. Do these levels of sterility assurance exceed reasonable limits? Since absolute sterility is not a reality for medical or surgical materials, what degree or level of assurance of sterility do we need or do we want? Are those in the commercial world sterilizing products that really do not have to be sterile? Are there weak links, other than in sterilization, in the delivery of a sterile product to the patient in the hospital?

The groups charged with protecting the public health, the regulatory agencies, are involved with commercial sterile products. The regulatory control of such products is an extremely important subject of this Symposium. The medical and legal aspects, which are of great importance, will also be examined.

We have made considerable progress recently in the technology of sterilization. The radiation dose is an area of contention, and we have discovered a potential problem with ethylene oxide sterilization. There are other subjects still requiring a great deal of attention and research, e.g. packaging, and we propose to discuss and examine them.

We have been fortunate to have assembled an international array of experts from the medical profession, academia, government, hospitals, and the medical products industry to address the subject of Advances in Sterilization of Medical Products.

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SESSION I

Regulatory Aspects

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SESSION I

Regulatory Aspects

Introduction to Session
Leon E. Harris

There has been an increasing evolution of sterile medical products brought about by the needs of modern-day health care. This has invited compliance activities by regulatory bodies to define particular requirements for product use, to assess issues of performance qualification, and after sterilization processes, to make certain that there are adequate controls to ensure that the product is sterile. Thus, despite modern-day hygiene, there continues to be an increasing need for sterile medical products. The responsibility to provide such products rests with the health-care industry and the responsibility of the health authorities is to supervise generally the discipline.

The regulatory world revolves around pharmacopoeia, national standards, codes of good manufacturing practice, written guidelines and requirements. Most of what is promulgated is oriented towards industrial practice which will become evident during this Symposium.

Health-care users generally favour and manufacturers frequently want clear-cut acceptable rules. This is impractical where sterilization is concerned, so we are beginning to see a trend away from prescriptive regulations towards guidelines. It is believed that so-called prescriptive regulations tend to lack flexibility and limit innovation. We have approached the pinnacle of knowledge in microbiology and with the application of mathematical probability, as well as many new technologies, we are beginning to see many changes in approach. This phenomenon is coupled with refinements in the traditional methods.

By and large, the attitude of regulatory bodies has always been framed against a background of safety involving probability estimates. Inasmuch as sterilization is a probability phenomenon, namely the probability of eradicating microorganisms, such things as process validation and various operating controls have become important issues. Sterility assurance levels expressed in logarithmic powers, e.g. 10^{-6}, when translated is one chance in a million that the product is not sterile. This is the traditional figure which represents a safety factor that possibly could be varied to apply to different products depending on their intended use. Pragmatically, all safety factors should be based on estimates of ‘reasonable’ probability. Use of sterile medical products can never be free of negligible risk of infection because of the way of their application. Furthermore, it is probably unnecessary and impractical to sterilize all products in medical use. Thus, sterilization of medical products should be

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based on safety estimates which should be calculated against a background of community need and a subsequent risk-benefit ratio, risk factors being expressed as consequence times frequency. It is pragmatic that with standard sterilization practices there is a probability of one in a million that a product may not be sterile, but that the chance of infection associated with its use does not occur in the same ratio. Safety estimates make a lot of sense and these could become one of the challenges to regulatory bodies who have to define the extent of sterilization practice involving medical product use.

All other elements of sterilization practice arise out of general principles and practice, such as process validation, bioburden, biological indicators, dosimeters, and so forth, that are now well covered by codes of practice. These are the areas where regulatory bodies are best equipped to be the national custodians, so to speak. Regulatory bodies are also set up to examine and to test medical products on issues of safety and risk-benefit whether they are sterile or not. Regulatory bodies require proof of efficacy of a sterilization process, and do not dictate the means that must be used. New technologies and workable GMP regulations that are to incorporate new technologies, such as dose-setting strategies in radiation sterilization and assessing the adequacy of the manufacturing controls, are again some of the new challenges for the regulatory bodies. Manufacturers look to a close working relationship with regulatory bodies involving good sterilization practice based on scientific principles.

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Regulatory Review of Sterilization Control in Australia

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The requirements of the *British Pharmacopoeia* for individual products, which are the basic standard for Australia, are very simple. The goods must be sterile and comply with the tests for sterility. These two requirements are not synonymous. Compliance with a standard sterility test is not sufficient. The requirement that goods are sterile implies that, when necessary, nonstandard sterility tests may be used and that methods used to sterilize the goods must be efficient and reliable.

There are some considerations that over the years have guided my approach to this subject. The principles of sterilization are well-understood and the subject is of immense practical importance, but it is, with the exception of one university, taught very badly at the undergraduate level in Australia.

Sterilization is not infrequently supervised by individuals in industry whose primary training has been in organic chemistry rather than in microbiology. These considerations, borne out by our experience with new graduates, stress the need for each generation to be taught the subject in the work place. This consideration alone provides a continuing justification for conferences such as the present one.

My colleagues and I, in establishing the National Biological Standards Laboratory (NBSL) some twenty-five years ago, came to the subject of sterility and sterilization as ill-informed as most, but some of us were trained in microbiology and practical statistics and soon realized that the pharmacopoeial standards of that time were grossly inadequate.

The 1958 *BP* sterilization procedures and tests make interesting reading these days. In the sterility test, no sampling schedules were provided, incubation was at one temperature, 37°C, and for only five days. A product could be failed only if three successive cultures were positive or the same organism was detected twice. No mention was made of the testing environment, although the media were pretested for the capacity to grow some common but unspecified organisms. Clearly, only the heaviest contaminations or a failure to subject the batch to sterilization could be detected by such a test.

The literature showed that the food technologists had the firmest grasp of the subject and we tried to build from this basis. I don't think that we added any original ideas to the field, but at least we applied other people's good ideas logically to the regulation of these matters against some considerable resistance. I recall that Francis Bowman of the US Food and Drug Administration was responsible for a number of valuable innovations about this time.

Today it is widely recognized that sterilization is firmly based upon an excellent mathematical model, and requirements derived from this model are now being included in the pharmacopoeias. I suggest that it needs to be remembered that mathematical models are simplifications of complex situations, and that the large extrapolations of this model need to be checked against experience to

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make sure that some factor not represented in the model is operative, or that there has been a departure from an assumed linearity.

It is now universally accepted that, while any individual item of a batch either does or does not contain viable microorganisms, it is only possible to make probabilistic inferences about the sterility of the batch as a whole. The criteria for acceptance of a batch are to my mind social judgments including many factors, such as a margin of safety to provide for occasional human errors. Responsible people throughout the world eventually arrive at a consensus for acceptance criteria, based on long experience. It is for these reasons that regulatory authorities tend to be conservative in changing the acceptance criteria. Moreover, experience has shown that an error in sterilization technique may take a long time to be detected.

Another concept now universally accepted is that it is the sterilizing process that provides a guarantee of safety, not the tests for sterility. Indeed, pressures are developing to drop routine sterility testing for certain types of products and to substitute more intensive monitoring of the sterilizing process, as has been done in the case of radiation sterilization. Paradoxically, this will, I suggest, have the effect of increasing the importance of the sterility tests carried out to validate the sterilizing process.

A great deal more information about any particular batch of a product is or should be available to the manufacturer than is available to the regulatory agency. These data include the source of the raw materials, its bioburden and the variability of the bioburden, the opportunities for contamination to occur during processing, the thoroughness of the validation carried out for the sterilizing process, the records of the consistency of the overall process, and so on. Since the Regulatory Laboratory lacks this information, it should carry out more stringent sterility tests than is required of the manufacturer.

Our regulatory practices are also affected by the limited opportunities we have to influence the great pharmacopoeias of the world. I believe that the philosophy of the pharmacopoeias should be altered and no longer include descriptions of manufacturing or sterilizing processes. I suggest that the pharmacopoeia should be a collection of statutory specifications and not a sort of cookery book for manufacturers. It is, for both these reasons, that we include guidelines on sterility testing for manufacturers as an Appendix C to the Australian *Code of Good Manufacturing Practice for Therapeutic Goods (GMP)*. The guidelines are intended for manufacturers and are based upon, but differ somewhat from the official test for sterility used by the NBSL.

The *Code of GMP* is not a statutory document in Australia; it provides criteria used by inspectors to determine whether a manufacturer should have a licence. While no single provision of the Code is binding, our inspectors would only accept departures from the requirements of the appendix on sterility testing, or other requirements of the Code relating to sterilization, if the manufacturer could prove that his proposed practices are equivalent or better.

The official sterility test is only a requirement for industry when a manufacturer wishes to dispute the NBSL results in a particular case. In these circumstances, the manufacturer, as a minimum requirement, must use the official method.

The final generalization I wish to make is that in modern practice the tests for sterility usually have to detect small numbers of biochemically debilitated microorganisms, and the design of the test should reflect this.

The official Test for Sterility or Standard, as it is called legally in Australia, is very detailed. It provides:

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1. A schedule for the quantity and number of samples to be tested.
2. Precautions to be taken about the environment, equipment, dress, and a restriction that the area should be exclusively used for sterility testing.
3. A detailed description of the test methods, either by membrane filtration or by direct transfer, and instructions about sample treatment and for preparing solvents and diluents.
4. Additional tests that must be carried out are:

   – *Tests on media* – Media are preincubated to check their sterility and each batch, which must not be more than three months old, is inoculated with specified organisms to check their growth capacity for small inocula. The same test is carried out on the media after a 14-day incubation period at the end of the sterility test.

   – *Tests for inhibitory substances in the sample* – These are carried out by adding some 10 or 20 colony-forming units of specified organisms to the final wash passed through a membrane filter or by adding the inocula to the sample/media-combination when the method of direct inoculation is used.

   – *Tests on diluents and solvents* – These are preincubated and tested for the presence of inhibitory substances.

   – *Negative control tests* – Not less than ten items, usually doubly sterilized containers, are put through the whole testing procedure at intervals during each testing session. These are incubated with the samples. The purpose of this test is to check the manipulations and the environment and estimate the number of false positives that occur.

Two media are used; Fluid Thioglycollate Medium is incubated at 32°C ± 2°C, and of the items inoculated onto Soyabean Casein Digest Medium, one half are incubated at 23°C ± 2°C and the remainder at 36°C ± 2°C for a period of 14 days. Instruction on the methods for examination of containers and for subculture etc. are included.

Although the tests for sterility recommended for use by manufacturers are based on the official test, they differ significantly in various particulars.

Incubation needs to be carried out at only two temperatures, 23°C and 32°C, instead of the three required in official testing. The sampling schedules are different, being reduced by half if microbiological indicators are included with the sterilizer load. A distinction is made between goods that are terminally sterilized and those that are aseptically filled. About half as many again of the aseptically filled containers must be tested for sterility.

The NBSL places particular emphasis on the value of negative controls and record keeping. To use an electrical analogy, the negative controls provide a measure of the ‘noise’ of the test. Examination of our records shows that the frequency of positive tests, not confirmed in a retest, with some products such as penicillins is much higher than the ‘noise level’. It is suspected that at least 50% of these are examples of real, but very low level, contaminations. The use of records in this manner by quality control staff is to my mind a reasonably sensitive indication that problems exist. It also shows that the bias in sterility testing is towards passing contaminated products rather than failing uncontaminated ones.

I have dwelt on these matters, since our approach differs from some other countries and because some people think that we have taken a somewhat controversial attitude. This leaves little time to talk

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about other, probably more important, aspects of the regulatory process. These are inspections of factories using the criteria in the *Code of GMP* and evaluation of the sterilizing methods used for new products.

A GMP inspector has a heavy responsibility and in our experience performs a useful task that is in the long term advantageous both to industry and to the public. Such an officer has to be well trained and a previous background in industry is almost essential.

An inspector should check the environment for compliance with the specified requirements for particulate matter, surface textures, and the like. Double-barrier systems must be used for aseptically filled products and appropriate air pressure differentials maintained. Equipment must be regularly calibrated, including such objects as pressure gauges, temperature probes, filters in laminar flow cabinets, and UV lamps, etc. The personnel must be properly trained in the procedures used (the NBSL has assisted in the training of key personnel from time to time). The inspectors also check that the sterilizing process has been properly validated during the development stages for the product and that periodic checks are made on the bioburden. The heat distribution and penetration during steam sterilization must also be periodically checked. There are also requirements for written procedures for key processes. These matters are outlined in the Australian *Code of GMP*.

The evaluation of sterilizing processes for new products developed overseas is a somewhat unsatisfactory process, that may be compared to carrying out an inspection by correspondence. It is neither convenient, nor efficient, but this is the penalty we pay. Products that do not meet the accepted minimal standards will not receive approval for marketing. I believe, the criteria used in Australia are pretty much the same as those used in other countries such as the US and Canada.

The misgivings I have about the regulatory processes in Australia relate to three matters. The resources for enforcement are in my opinion inadequate. I have a suspicion that a number of manufacturers have items submitted to *gamma* radiation sterilization without an evaluation of the bioburden of the product and a validation of the method for the particular product, and do not carry out sterility tests. Finally, there is an uncritical acceptance of the quality of biological indicators by some companies. The products received at the end of a very long distribution chain in Australia have, in the few cases in which we have tested them, either failed to grow on incubation or could not be sterilized by standard sterilizing cycles. Some companies, I observe, feel compelled to manufacture their own biological indicators.

The regulatory controls briefly outlined are the product of many years' consideration and debate by government officers, quality control managers in industry, and microbiologists from the clinical and academic fields and the examination of practices in other countries. The controls have changed much during this time and no doubt will change again in the light of our experiences and the practices followed in other countries.

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Regulatory Review of Sterilization Control in the United States

Richard J. DeRisio

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The role of the sterilization technologist in a regulatory agency such as the Food and Drug Administration (FDA) is different from that of one in the industry. We do not have laboratories or manufacturing facilities in which to carry out experimental studies. Rather, we must be satisfied with the vicarious experience of recommending and monitoring experiments performed on our behalf by FDA's field laboratories. A symposium such as this one offers a regulator a valuable opportunity to learn about emerging sterilization technology from both formal presentations and informal conversations. On the other hand, regulatory and product safety concerns can be discussed with those having practical experience in areas including bioburden control and monitoring, residue dissipation and analysis, process uniformity and control among others.

Working in the FDA provides one with a broad perspective of the entire medical products industry. Although we have exposure to the level of science and medicine advocated by the participants in this Symposium, we see also in sharp contrast a segment of the industry that has much less interest in promoting public health, let alone complying with the Agency's *Good Manufacturing Practices (GMP)* regulations. We observe practices that would be considered unthinkable and perhaps unbelievable. Therefore, in developing compliance programmes and in establishing policies regarding medical product sterilization, we must take all segments of the industry into consideration. Accordingly, some FDA policies may appear too restrictive, perhaps even naive, to a large sophisticated manufacturer.

The aim of a regulatory professional should be to establish policies that provide adequate control to protect the public health, and yet ones that are not so rigid as to stifle new developments in science, medicine, and technology. Companies that are willing to invest in research in the health sciences should be able to make practical applications of their findings without undue regulatory burden. This approach is consistent with the current Presidential Administration's Mandate for Regulatory Reform.

In the area of sterilization, the Office of Medical Devices had traditionally sought to encourage the adoption of new technology in device manufacturing. For example, alternatives to finished device

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sterility testing are permitted as a means of product release. Companies may replace all or part of their device sterility testing with biological indicator monitoring, use of inoculated products or a system of dosimetric or process-control release. These provisions pertain even to those devices, such as sutures and gauze bandages, for which there is a *United States Pharmacopeia (USP)* monograph requiring that sterilized items must meet the requirements of the *USP* Sterility Test.

The Office of Medical Devices notes that the sterility test section of the *USP* states that 'alternative procedures or procedural details may be employed to demonstrate that an article is sterile, provided the results obtained are at least of equivalent reliability'. Furthermore, the *USP* text states that 'no sampling plan for applying sterility tests to a specified proportion of discrete units selected from a sterilization load is capable of demonstrating with complete assurance that all of the untested units are in fact sterile'.

It is recognized that quality, in this particular case sterility, must be designed into the product through a comprehensive validation and quality assurance programme, and not be tested into the product by means of one finished product test involving a small number of units. In fact, the *USP* states that process validation studies and the use of in-process controls may provide a better assurance that an item meets a particular compendial requirement than the test itself, where that test is performed on a sample of a few discrete units.

The *GMP for Medical Devices* requirement for finished device inspection, 21 *CFR* 820.160, states that 'prior to release for distribution, each production run, lot, or batch shall be checked and where necessary tested for conformance with device specifications'.

We believe that a firm choosing to use an alternative 'check' such as biological indicators or dosimeters in lieu of an actual finished product test for sterility does meet the intent of this *GMP* requirement.

The current position of the Office of Medical Devices in this matter is based in large part upon the findings and recommendations in the evaluation report of the fiscal year 1976 (FY76) Device Sterility Compliance Program. It was found that nonsterile devices could not be reliably detected unless the percentage of nonsterile units was relatively high. Among lots tested in FY 76, the lowest percentage of nonsterile lots detected by a *USP* test using 40 samples was 21.7%, far above the one in one million probability that represents the minimum sterility assurance level desirable for many sterilized medical products. Moreover, the chance for an accidental laboratory contamination, if assumed to be once in every 2000 tests, was much higher than the 10^{-6} level sought as a minimum for parenteral and other invasive devices.

Of a total of 82 samples (40 per sample) of devices tested in FY 76, 87% were found to be sterile as tested. In 10% of the cases, the conclusion was equivocal, that is, there were positive samples on the initial test, but none on the retest. In these cases, use of the most probable number (MPN) equation of Halvorsin & Zigler estimated the levels of nonsterility to be in a range of 2.5 to 4.0% nonsterile. In the cases where a check analysis clearly demonstrated nonsterility not occurring as a result of adventitious laboratory contamination, the levels of nonsterility in the lots were estimated to be 21.7%, 53.3%, and 72.2%.

As part of the FY 76 evaluation, the probabilities of detecting nonsterility in a lot using the *USP* test were calculated and tabulated. Table 1 demonstrates that a forty-unit sample using twenty samples in each of the two prescribed media can reliably detect nonsterility only if the proportion of nonsterile units in the lot is very high. For example, if the particular contaminating organisms were

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fastidious and could grow on only one of the two media, then in order to be sure 95 times out of 100 that nonsterility would be detected, the lot would have to contain 13.9% nonsterile units, or almost 1400 nonsterile units in a sterilizer load of 10 000 devices. Clearly, this level would not be expected unless there had been a major cycle malfunction. Moreover, the table indicates that 5 times out of 100, the 13.9%would go undetected in a forty-unit sample, assuming the organism would grow on only one of the two media.

Table 1

Nonsterile units that may be present in a lot and not be detected in a given sample size

Sample size		Growth characteristics of contaminating microorganisms			
Total units tested	Units in each test medium	Both media		Only one medium	
		0.5*	0.05*	0.5*	0.05*
10	5	6.7%**	25.9%	12.9%	45.1%
20	10	3.4%	13.9%	6.7%	25.9%
30	15	2.3%	9.5%	4.5%	18.1%
40	20	1.7%	7.2%	3.4%	13.9%
50	25	1.4%	5.8%	2.7%	11.3%
60	30	1.1%	4.9%	2.3%	9.5%

* Probability of sample containing no nonsterile units.

** Percentage of nonsterile units.

Consequently, FDA investigators are instructed that collection of samples of finished devices for sterility testing is nonproductive unless it is apparent that the sterilization process cycle, equipment, or controls are seriously deficient. Collection of finished devices would be indicated in a situation where the sterilization process was based upon product bioburden and inspection evidence indicated that actual bioburden levels were likely to be considerably higher than those upon which the process was based originally.

It became clear to the Office (then Bureau) of Medical Devices that it would be worthwhile to promote process validation including equipment and cycle qualification and overall production quality assurance, rather than sterility testing, as a better means to ensure that an acceptable sterility assurance level is reliably maintained. As a result, one of the recommendations of that compliance programme evaluation was to develop a GMP regulation for sterile devices. The Office of Medical Devices has determined, however, that it is more effective working with other health professionals through organizations such as the Association for the Advancement of Medical Instrumentation (AAMI) to develop voluntary guidelines or standards of practice that deal with sterilization operations in detail. Of course, the umbrella *GMP* regulation is applied to a sterile manufacturing operation in a manner appropriate for the particular device inspected.

I would like to present some background regarding FDA activities. FDA performs inspections of foreign medical product manufacturers and clinical laboratories. The *Federal Food, Drug, and Cosmetic (FDC) Act*, as amended, stipulates that we have jurisdiction over interstate commerce. Trade between the US and a foreign country meets that definition, as does that between states, or

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between a state and the District of Columbia or a US territory. Moreover, this Act states a requirement that manufacturers of drugs and Class II and Class III medical devices must be inspected every two years. Class II and Class III devices are those judged to require a performance standard and a premarket approval (PMA) application, respectively. For Class III devices, the PMA process is analogous to the New Drug Application (NDA) procedures for certain drugs. Class I devices are those subject only to general controls such as registration, listing, notice of intent to market the device, conformance with the *GMP* regulation site inspections, labelling, record keeping, and certain other FDA regulations.

Table 2 summarizes the FDA's foreign inspection activity by product types for the last three fiscal years ending 30 September 1982.

Table 2

	FY 80	FY 81	FY 82
Drugs subject to an NDA	121	82	84
Antibiotics (Form 5/6)	82	50	39
Medical devices	70	79	85
Clinical laboratories	17	7	2
Totals	290	218	210

It is noteworthy that we hope to accomplish 150 inspections of foreign medical device firms in FY 83. In contrast, the total number of domestic and foreign inspections of medical device establishments was 1403, including 206 manufacturers of sterile devices. Foreign firms are evaluated under the same inspection programmes as are domestic firms. The investigators are part of an experienced cadre of personnel who are periodically detailed from their US inspectional work to perform four- or five-week foreign inspection assignments. Inspections of US firms are unannounced; in contrast, foreign firms are contacted several weeks in advance to request permission to inspect their facility. The *FDC Act* does not grant authority to perform unannounced inspections in foreign countries. These preliminary contacts are necessary also in order to establish a very tight inspection and travel schedule for the investigator on foreign assignment. During my two- year tenure as Assistant Chief of the Foreign Inspection Staff, I know of only one instance wherein FDA was not granted clear approval to inspect. In fact, no reply was received from the firm. Shortly thereafter, an investigator on another assignment visited the same city in which the nonresponding firm was located and determined that the site was only a distribution centre. In this case, products imported by that company were detained by the US Customs officials until the actual device manufacturing site could be identified and inspected. Following a domestic or overseas inspection, a firm with minor *GMP* deficiencies may be sent a Notice of Adverse Findings (NAF) Letter. For more serious violations, however, the regulatory follow-up for US firms, as prescribed by the Act, is different from that permitted against manufacturers of products imported into the US. In the US, the FDA may seek, through the courts, to seize unsafe or ineffective products, or prevent (enjoin) a manufacturer from producing potentially unsafe or ineffective products. On the other hand, our recourse against a foreign firm found to be out of control with respect to *GMP* compliance and product performance is to detain the product at the point of entry into the US (in co-operation with US Customs or Postal Service) and thus prevent its

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interstate distribution into the US.

In its regulatory control of sterilization in the US, FDA has always required evidence of process efficacy and equipment reliability for regulated products that are purported to be sterile. The *GMP* regulation for low-acid canned foods (LACF), *Code of Federal Regulations*, Title 21, Part 113 (21 *CFR* 113), published in 1973, specifically requires proof that the scheduled process cycle is effective in assuring that no container is contaminated with viable cells of *Clostridium botulinum*, and also that there are no surviving organisms that could lead to economic spoilage under the normal conditions of storage.

This regulation also requires that the persons who establish such cycles and also those who evaluate process cycle deviations have suitable training and experience. Requirements for qualification of the process equipment and calibration of the measurement and control systems are clearly described. The regulation prescribes specific equipment designs and operating procedures for the chambers (retorts) used to process low-acid canned foods. For installations different from those in the regulation, heat distribution data must be kept on file demonstrating uniformity of temperature distribution and adequacy of venting. It was the National Canners Association, now National Food Processors Association (NFPA), that originally petitioned the Agency to adopt a regulation that was identical not only with the code of practice used by their members, but also with the canning regulation enforced by the State of California. Although highly technical, the LACF regulation was implemented quite easily because it was practicable and represented the state of good industry practice at that time. Within a few years, the regulation effected a substantial increase in the industry level of compliance, particularly among those firms that previously had not followed any code of practice. FDA continues to work closely with NFPA, particularly in co-ordinating the follow up to health-threatening food hazards involving their member firms.

In 1976, FDA published a proposed *GMP* regulation for large volume parenteral (LVP) drug products for human use (*Federal Register*, Vol. 41, p22202, i.e. 41 *FR* 22202). In the preamble to that proposal, it was made clear that the proposed LVP regulation would *supplement* the more general *GMP* provisions of 21 *CFR* 210 and 211. The comments received by FDA concerning this proposal stated that it was over restrictive and, in some respects, impossible to implement reasonably. This proposed regulation is still officially under review with the likelihood that some portions will eventually be published as regulations or guidelines. Several provisions of the proposal, however, do reflect current industry practice for LVP manufacturing and define a baseline for FDA expectations regarding the manufacture of parenteral drugs. It is difficult for a regulatory authority to promulgate a detailed technical regulation without input from those with current practical experience.

In 1978, FDA promulgated a *GMP* regulation for the manufacture, packing, storage, and installation of medical devices (43 *FR* 31508). The device *GMP* regulation is an ‘umbrella’ regulation applicable to all medical device manufacturers. In view of the diversity of manufacturing processes and finished product types, it was recognized that the regulation should not be so specific as to prescribe the precise details of how each firm must manufacture its devices. Rather, the *GMP* regulation contains general requirements in specific areas of concern applicable to all manufacturers who then develop procedures that not only fulfil *GMP* requirements, but also are appropriate for their particular device.

I believe, it would be worthwhile to discuss the applicability of this *GMP* regulation to sterile devices. The preamble to the final order (43 *FR* 31508) contained this statement: ‘The Food and Drug

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Administration (FDA) expects to publish additional GMP regulations applicable to specific types of devices. These future regulations will supplement the "umbrella" GMP regulation and will be of two types: One will contain requirements that will apply only to generic types of devices or classes of devices, e.g., pacemakers, eyeglasses, etc.; the other will contain requirements that will apply to certain devices or cross-class characteristics or processes, e.g., sterile devices, plastics, electrical properties, etc.'

It has been suggested by some individuals that the above wording implies that sterile devices should not be regulated under the umbrella *GMP* regulation. Yet, using the same rationale, this line of reasoning could be extended also to all plastic devices and all devices with electrical properties. The above wording clearly states that these future regulations would *supplement* the umbrella *GMP* regulation.

It was never the intent of Congress or FDA to exclude sterile devices (or those made from plastic or those having electrical properties) from *GMP* controls. Moreover, the general provisions subpart of the *GMP* regulation in the selection on Scope (21 *CFR* 820.1) states: 'The regulation set forth in this part describes current good manufacturing practices for methods used in, and the facilities and controls used for, the manufacture, packing, storage, and installation of all finished devices intended for human use.'

The wording 'of all finished devices' does not exclude devices that are sterilized. In fact, this section (Scope) goes on to exclude specifically those devices that were not intended for coverage, and sterile devices were not among those excluded. Section 820.3(j) defines a 'finished device' as 'a device, or any accessory to a device, which is suitable for use, whether or not packaged or labeled for commercial distribution'. Sterile devices are not excluded from this definition.

An example of the Bureau's commitment to the application of the device *GMP* regulation to sterile devices is the fact that no manufacturer of a sterile device (even one that is Class I) has been exempted from the device *GMP* regulation. The final classification order for patient examination gloves (21 *CFR* 880.6250, 45 *FR* 69723) contains the statement that 'if the device is not labeled or otherwise represented as sterile, it is also exempt from the good manufacturing practice regulation in Part 820, with the exception of Section 820.180, with respect to general requirements concerning records, and Section 820.198 with respect to complaint files'.

Other examples of this reasoning can be found in classification orders for haematology and pathology devices, among others. Moreover, the Division of Compliance Programs has developed a guideline for the application of the umbrella *GMP* regulation to a sterile device manufacturer. This document is included as an attachment to the Compliance Program Circular that is used to guide the investigator during the inspection of a sterile device manufacturer.

In summary, the Bureau has always intended that sterile devices be covered by the *GMP* regulation. The concept of developing a separate supplemental regulation for sterile devices (and other 'cross-class characteristics') evolved during a period when the Agency was moving toward promulgation of product-class specific regulations that were intended to *supplement* the umbrella *GMP* regulations. Examples include the low-acid canned food regulation, the acidified foods regulation, and the proposed large volume parenteral regulations. However, the Agency is now adopting alternatives to such regulations that may have less impact on the regulated industry but still attain the goal of protecting the public health. An example of one such alternative is the *Guideline for Industrial Ethylene Oxide Sterilization of Medical Devices*, a recommended practice of the

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Association for the Advancement of Medical Instrumentation (AAMI). A subcommittee that included representatives of industry, the academic community, consulting firms, equipment manufacturers, several professional associations, and FDA collaborated on the final drafts of the AAMI guideline. As a result, this document represents a consensus guideline of good practices for the design, validation, and control of ethylene oxide (EO) process cycles.

The FDA cannot recognize the AAMI document as an official guideline or regulation, because it was not developed in accordance with the Administrative Practices and Procedures Regulations of the FDA, in particular with respect to publishing such documents in the *Federal Register* for public comment. Nonetheless, the extent of FDA participation is well known and FDA's Office of Medical Devices (OMD), formerly the Bureau of Medical Devices (BMD), considers firms following the AAMI guidelines to be meeting the *GMP* regulation requirements for validation and process control of their EO sterilization operations. AAMI has invited FDA input on other guidelines and standards including, for example, those for steam sterilizers and biological indicators.

I would now like to detail a brief overview of the strategy used to evaluate a manufacturer's level of compliance with the *GMP* regulations.

The Agency is preparing to publish a guideline intended to outline general concepts and key elements that FDA considers to be acceptable parts of process validation associated with the manufacture of drug products and medical devices. The introduction to a recent draft of the guidelines states: 'This guideline discusses process validation elements and concepts that are considered by the FDA as acceptable parts of a validation program. The constituents of validation presented in this document are not intended to be all inclusive. The Agency recognizes that because of the great variety of medical products, processes, and manufacturing facilities, it is not possible to state in one document all of the specific validation elements which are applicable. Several broad concepts, however, have general applicability and provide an acceptable framework on which firms may build a comprehensive approach to process validation based upon the needs of each situation.'

An FDA guideline states principles and practices of general applicability that are not legal requirements but are acceptable to the Agency. A person may rely upon the guideline with the assurance of its acceptability to FDA, or may follow different procedures. When different procedures are chosen, a person may, but is not required to, discuss the matter in advance with FDA.

It is through careful design and validation of both the process cycle and process controls that a manufacturer can assure that there is a very high probability that all manufactured units from successive lots will be acceptable. This objective reduces the dependence upon intensive in-process and finished product testing. Reliable process control also lowers costs by reducing the proportion of defective finished products that must be scrapped or reworked.

The AAMI EO guideline contains a definition of validation as a documented programme to demonstrate that a specified product can be reliably sterilized by the designed process.

The definition implies the need to maintain records demonstrating that from run to run, a particular product will be sterilized by a cycle originating from a suitable data-based design.

A validation protocol should be developed which calls for a sufficient number of replicate process trials in order to demonstrate reproducibility. The number of trials should be selected so as to provide a statistically valid measure or variability among successive runs and include worst-case challenges to the process.

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The basic concept of validation is inherent throughout the current *GMP Regulations for Finished Pharmaceuticals*, 21 *CFR* 210 and 211. A general requirement for process validation is contained in 21 *CFR* 211.100 – Written procedures; deviations – which states in part that 'there shall be written procedures for production and process control designed to assure that the drug products have the identity, strength, quality, and purity they purport or are represented to possess'.

Several sections of the *Code of GMP* regulations state validation requirements in more specific terms. Excerpts from some of these sections are:

Section 211.110, Sampling and testing of in-process materials and drug products.

(a) '… control procedures shall be established to monitor the output and VALIDATE the performance of those manufacturing processes that may be responsible for causing variability in the characteristics of in-process material and the drug product.' (emphasis added)

Section 211.113, Control of microbiological contamination.

(b) '… Appropriate written procedures designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include VALIDATION of any sterilization process.' (emphasis added)

Section 211.165, Testing and release for distribution.

(e) '… The accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented. Such VALIDATION and documentation may be accomplished in accordance with Section 211.194(a)(2).' (emphasis added)

A requirement for process validation is inherent in the medical device *GMP* regulation, 21 *CFR* 820. Section 820.5 requires every finished device manufacturer to 'prepare and implement a quality assurance program that is appropriate to the specific device manufactured'. Section 820.3(n) defines quality assurance as 'all activities necessary to verify confidence in the quality of the process used to manufacture a finished device'.

Process validation is the major activity used to provide confidence that a process will consistently produce a product meeting the designed quality attributes.

A general stated requirement for process validation is contained in Section 820.100: 'Written manufacturing specifications and processing procedures shall be established, implemented, and controlled to assure that the device conforms to its original design or any approved changes in that design.'

Validation is an essential element in the establishment and implementation of a processing procedure, as well as in determining what process controls are required in order to assure conformance to specifications.

Section 820.100(a)(1) requires that 'procedures for specification control measures shall be established to assure that the design basis for the device … is correctly translated into approved specifications'.

By establishing procedures for sterilization process validation, a manufacturer assures that the design criteria defining a particular sterility assurance level for the device will be reliably achieved in production. In addition, Section 820.100(b)(1) states that 'where deviations from device specifications could occur as a result of the manufacturing process itself, there shall be written procedures describing any processing controls necessary to assure conformance to specifications'.

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In addition to requiring that there be effective controls for process variables, this section also implies a requirement for periodic revalidation for such operations as sterilization, particularly upon the initiation of significant changes in the process, equipment, product, or package.

In response to several inquiries from small manufacturers, OMD has prepared a draft document entitled 'Adopting a Medical Device Into a Validated Sterilization Cycle'. This practice has been used by firms that market sterile hospital trays whose components change for custom orders. These items may be sterilized without revalidating the process cycle. Such decisions are based on scientific judgment and can be simplified if the original validation studies are performed on worst-case product types and load configurations. The adoption document permits very small manufacturers whose monthly output might not comprise one pallet load to select a process cycle that is adequate for the device without incurring a validation cost that would be excessive. Adoption of a device into a previously validated cycle relies on assumptions that the physical and microbiological characteristics of the adopted device and packaging are very similar to the parent load. For EO processing, it might be necessary to adopt the device into a cycle that included a rigorous external preconditioning to ensure that the lethality delivered to the cartons of adopted devices was achieved at a rate comparable with that of the parent load.

The following is a brief overview of the strategy used to measure a device manufacturer's level of compliance with the *GMP* regulations and to evaluate a company's state-of-control for sterilization and related activities.

The FDA Compliance Program 7378.008 entitled *Inspection of Medical Device Manufacturers* provides guidance to the FDA field staff for the enforcement of the requirements of the *GMP* regulation and applies to all devices including those labelled 'sterile'. The objectives of the current programme are:

1. To identify domestic and foreign manufacturers who are not operating in a state-of-control.
2. To bring such manufacturers into a state-of-control through voluntary or regulatory means as appropriate.

The programme defines state-of-control by noting that when manufacturing is organized in a way which enables a device manufacturer to have full mastery over the attainment of *quality of conformance*, the device manufacturer is said to be in a state-of-control.

It is the manufacturer who is responsible for developing a suitable QA system that is appropriate for the device and meets *GMP* requirements. The issue of flexibility is reiterated in the following portion of the programme text:

> 'The FDA intends to measure GMP compliance in light of this discretion and flexibility accorded the manufacturer. Therefore, the investigator should not approach the inspection with the expectation that a manufacturer is rigidly and absolutely in compliance with each GMP requirement since each requirement is both general and objective in nature. The investigator should expect to find situations where a manufacturer has tailored the quality assurance program to his particular needs after taking into account such factors as necessity of a procedure, or the uniqueness of a device or component … Each variation from the GMPs needs to be carefully examined in terms of its impact on the fitness for use of the finished device.'

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The recognition of flexibility is clear and supports the idea that FDA discourages formal variance

petitions. Instead, manufacturers choosing to meet a *GMP* requirement through a means other than that explicitly stated in the regulation must satisfy themselves that safety and efficacy will be attained, and then be prepared to support their variation if questioned by the investigator.

Class II and Class III device manufacturers are inspected every two years, as a statutory requirement imposed upon FDA by Congress in the *FDC Act*. Companies are scheduled for coverage using a computer program that alerts FDA district offices that particular companies are due for their biennial reinspection. There may be follow-up inspections within the two-year period as dictated by a company's state of compliance. Manufacturers of Class I devices are not inspected on a biennial schedule unless the device is sterilized or marketed for sterilization by the user (in which case the device would not be exempt from the *GMP regulation*). Also Class I devices may be inspected as a result of a specific BMD assignment, or if there is a problem with the device.

The investigator's inspection is called a 'quality audit' and is guided by a Quality Audit Worksheet, a portion of which is reproduced below:

820.61 MEASUREMENT EQUIPMENT

23A.	All QA and production measurement equipment is routinely checked, calibrated, and inspected according to written procedures.	NDN D A N/A
23B.	Records documenting these activities are maintained.	NDN D A N/A
23C.	When automated production or quality assurance systems are used, programs are validated by adequate and documented testing.	NDN D A N/A
23D.	All program changes are made by a designated individual(s) through a formal approval procedure.	NDN D A N/A
	(a) Calibration procedure	
24A.	Calibration procedures include specific directions and limits for accuracy and precision.	NDN D A N/A
24B.	There are provisions for remedial action when accuracy and precision limits are not met.	NDN D A N/A
	(b) Calibration standards	
25.	All production and quality assurance measurement equipment are calibrated using traceable standards, i.e. traceable to: (1) an NBS standard, (2) an independent (not in-house) standard, or (3) an in-house standard.	NDN D A N/A
	(c) Calibration records	
26A.	The calibration date, the calibrator, and the next calibration date are recorded and displayed, or records containing such information are readily available for each piece of equipment requiring calibration.	NDN D A N/A
26B.	A designated individual(s) maintains a record of calibration dates and of the individual performing the calibration.	NDN D A N/A

This section pertains to calibration, an important aspect of sterilization process development, validation, and control. In responding to questions on the Quality Audit Worksheet, the investigator marks the question: 'NDN' if there was No Deficiency Noted; 'D' if that area was Deficient; 'A' if there was a total Absence of compliance with the pertinent section; and 'N/A' if the question was Not Applicable for the particular device and manufacturer being inspected. Unlike the original Worksheet, the new version has a provision for the 'N/A' response after every question, thereby permitting and fostering an attitude of flexibility in applying the *GMP* regulation.

At the conclusion of the inspection, the investigator is required to issue a list of inspectional observations, the Form FD 483, if he or she has marked any responses 'Absent' or 'Deficient'. The investigator, during the exit discussion with management, will ask the company to describe the corrective action management intends to take to correct the observed deficiencies, discuss the timetable for the promised correction, and clarify any questions regarding the *GMP* regulation deficiencies.

The purpose of the Worksheet is to:

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1. identify the specific requirements of the *GMP* to assure that the audit covers all applicable

sections of the regulation;

2. guide the flow of the audit, since the inspection should be conducted not in the order of *GMP* sections, but along sensible lines which expose the investigator first to the device master record, then to complaint files, and so on;
3. assist the investigator in identifying deviations from the *GMP* regulation and communicating them to management;
4. provide a framework for the orderly receipt and retrieval of data essential to programme evaluation; and
5. foster uniformity and consistency by providing a text for the reporting of deviations.

Table 3

Compliance indices by firm size and type of device manufacturer firms receiving initial GMP inspection in Fiscal Year 1981

Type of firm	Firm size			
	All	1-10	11-50	>50
ALL				
N*	762	336	225	201
$\bar{X}$**	87	82	88	93
SD***	16	19	15	10
CRITICAL				
N	71	24	20	27
$\bar{X}$	88	82	87	95
SD	17	20	18	9
NONCRITICAL				
N	559	254	166	139
$\bar{X}$	86	82	87	93
SD	17	19	16	11
IN VITRO DIAGNOSTIC				
N	132	58	39	35
$\bar{X}$	90	84	94	97
SD	15	20	7	5

N* = Number of firms

$\bar{X}$** = Mean

SD*** = Standard deviation

In order to compare levels of compliance between firms, for different inspections of the same firm, among different products, and so on, a Compliance Index was developed. The Compliance Index is expressed as a percentage and is calculated by assigning different point scores for each response received on the Worksheet. This Index, while useful for tabulating and expressing the data,

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has shortcomings that are clearly recognized by the Bureau. In particular, it does not give more weight to those *GMP* requirements that could have a greater impact upon safety and efficacy. Also, the number of questions used on the Worksheet to evaluate compliance with a particular *GMP* requirement affects the weighting of the Index. The evaluation report notes that 'differences between various Compliance Indices does not necessarily mean that the quality of one firm's product is any better or worse than another's product'.

In fiscal year 1981, 1403 medical device manufacturers were inspected under the *GMP* compliance programme. Table 3 summarizes the findings for 762 of the companies that underwent their first *GMP* inspection. Accordingly, there was a higher proportion of smaller companies than is actually represented in FDA's entire inventory of medical device manufacturers.

Table 4

Compliance indices by firm size and type of device manufacturer Second biennial GMP inspection

Type of firm	Firm Size			
	All	1-10	11-50	>50
ALL				
N	641	182	195	264
$\bar{X}$	94	89	94	96
SD	10	13	8	6
CRITICAL				
N	76	13	16	47
$\bar{X}$	94	86	96	96
SD	8	15	6	4
NONCRITICAL				
N	433	125	130	178
$\bar{X}$	94	89	94	96
SD	10	13	8	6
IN VITRO DIAGNOSTIC				
N	132	44	49	39
$\bar{X}$	93	89	94	96
SD	10	13	7	5

The remaining 641 companies had been inspected for the first time under this programme in FY 79 and thus the FY 81 *GMP* inspection was their second. The inspection findings for these companies are shown in Table 4. There were more large firms (750 employees) in this latter group, because the initial inspection model called for visits to large manufacturers before smaller ones with some adjustments for type of device manufactured (critical vs. noncritical), or the history of device problems.

The salient inspection findings from Tables 3 and 4 can be summarized as follows:

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1. The average ($\bar{X}$) compliance index (CI) for firms that received their second *GMP* inspection in 1981 (biennial follow-up) was higher than that for firms receiving their first *GMP* inspection (94 vs. 87).
2. There was no significant difference in CI among manufacturers of critical, noncritical, and *in vitro* diagnostic products (IVDP) within each table: first *GMP* inspection – 88, 86, 90; second – 94, 94, 93.
3. The CI for large firms (750 employees) is higher than that for the smallest firms (1-10): Table 3 – 93 vs. 82; Table 4 – 96 vs. 89.
4. The variability in CI (SD) among manufacturers is (a) lower for larger firms, e.g. in Table 3, 10 vs. 19; and (b) is lower after a second *GMP* inspection, e.g. 16 in Table 3 vs. 10 in Table 4.

Of the 641 companies that received their second *GMP* inspection in FY 81, 90 had received yet another follow-up visit between those two inspections, most likely to review corrections made to objectionable conditions observed during the original FY 79 inspection. An analysis of these firms is shown in Table 5 and demonstrates that the 90 firms receiving an intermediate inspection had a significantly lower CI (83) than that (88) for the firms that were not reinspected since FY 79. A comparison of the CIs for the two groups of companies at the second *GMP* inspection (the biennial reinspection) indicates that there is no significant difference between the mean CIs (94 vs. 93). These data suggest that an intermediate follow-up inspection of firms having considerable *GMP* deficiencies may be an effective means of raising their level of compliance. Note also that variability, as measured by the standard deviation (SD), was also reduced significantly following an intermediate follow-up inspection.

Table 5

Extent of improvement in compliance indices

	Firms not receiving an intermediate inspection	Firms receiving an intermediate inspection
Number of Firms	551	90
Initial *GMP* inspection		
$\bar{X}$	88	83
SD	14	17
Second biennial *GMP* inspection		
$\bar{X}$	94	93
SD	10	9
Delta (CI second biennial *GMP* inspection minus CI initial *GMP* inspection)		
$\bar{X}$	6	10
SD	13	16

One can suggest possible explanations for the observation that smaller firms have a lower mean Compliance Index:

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1. small firms, if newer, have not been inspected as many times and thus have less opportunity to evaluate compliance;
2. the small staff size precludes having an individual responsible full-time for *GMP* conformance;
3. resource limitations prevent participation in *GMP* training programmes;
4. small firms may be reluctant to confront FDA regarding a difference of opinion or to ask for guidance.

The data have not resulted in FDA having a predisposition against smaller firms because their mean Compliance Index is lower. Nonetheless, the Agency maintains that it shall not have a double standard that permits a lower level of conformance to *GMP* requirements for small firms than for large firms. To assist small firms in complying with the *GMP* regulation, the Office of Small Manufacturers Assistance provides guidance through workshops, telephone and memo correspondence, a newsletter, and on-site visits.

The most frequently cited problem areas among inspected companies include:

1. failure to audit properly the QA system for conformance with *GMP* regulations;
2. deficiencies in the device master record,
3. deficiencies in calibration procedures.

It was found through an analysis of the data that firms not performing audits of their quality assurance systems are much more likely to be deficient in many other areas of the *GMP* regulations than firms routinely performing audits. A similar correlation is found between low overall *GMP* compliance and deficiencies in the device master record and/or device history record.

Manufacturers of sterile devices are inspected under the overall *GMP* compliance programme and also under an addendum designed to direct inspection of sterile device manufacturing systems. This programme supplement provides a data collection form that is used to ensure complete and consistent coverage of sterilization processes worldwide. Two pages of that form are shown in the Appendix as examples. One, entitled ‘Cycle Parameters’ is a page from the section on EO sterilization; the other is one of the pages pertaining to process validation. Copies of the entire 21-page worksheet as well as other GMP and sterility materials may be obtained from the FDA Office of Small Manufacturers Assistance in Silver Spring, Maryland.

The following is a summary of a portion of the findings for the 206 sterilization processes reviewed within the 1403 *GMP* inspections that were accomplished:

— Types of sterilization processes used:		
Ethylene oxide (EO)		129 (63%)
12% EO/88% Freon* carrier	75 (36%)	
100% EO	32 (16%)	
Miscellaneous EO processes	9 (4%)	
EO processes, type unknown	13 (6%)	
Steam		46 (22%)
Radiation (cobalt 60)		17 (8%)

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Miscellaneous	14 (7%)

* Trade Mark

— Location of the sterilization process:

At the manufacturing site	138 (67%)
At a contract sterilizer	56 (27%)
At another company site	4 (2%)
Unknown	7 (4%)

— By sterilization type:

	EO	Steam	Radiation	Other
At the manufacturing site	61%	87%	35%	100%
At a contract sterilizer	34%	2%	65%	
At another company site	2%	2%		
Unknown	2%	9%		

— Method of product release used by firms reviewed for their evaluation:

Biological indicator testing only	62 (32%)
Finished product testing only	34 (17%)
Dosimetric monitoring only	10 (5%)
Process control only	1 (1%)
Finished product and biological indicator testing	88 (45%)

The small proportion (17%) of firms relying solely upon finished device sterility testing as a means of product release reflects the OMD position that it is more effective to build in process control through validation, calibrated instruments, and certified biological monitors rather than to rely upon sterility testing a few discrete units.

At the conclusion of all FDA inspections, the investigator is obligated to present the company's top management with a list of objectional conditions. This form, FD 483, is entitled 'Inspectional Observations'. Of the 206 sterile device manufacturers inspected, 118 received an FD 483 with items pertaining to sterility. The five most frequently cited problems were as follows:

1. The process was in some way inadequate. This broad category included generally poor manufacturing practices including controls insufficient to ensure that the finished device met its claim for sterility.
2. There were deficiencies in the Device Master Record (DMR). In some cases, the current sterilization procedures and specifications were not included in the DMR. In others, the DMR was not signed or dated as required, or there was inadequate control of changes in the DMR.
3. There were problems regarding the use and testing of biological indicators (BI). In some cases the number used or placement in the load did not meet the DMR requirements or the specifications established during validation. Some citations noted that positive unexposed controls were not always tested along with exposed BIs from the same lot. Some companies had apparent surviving BIs, but released the load anyway without an adequate investigation into

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the reason for BI survival.

4. Process validation was lacking or incomplete. There were deficiencies in equipment qualification and process performance testing using suitable monitors and microbial challenges.
5. There were problems with finished product testing for sterility or EO residues. In some cases, companies failed to follow their own procedures for testing, in others, the test method was not a compendial (e.g. *USP)* method, and its use as an alternative was not validated. There were instances of improper follow-up, including retesting in cases where positive product samples were found.

Following its review of the objectionable conditions reported, the FDA may send a letter to the company's top management requesting an action plan for correction of the deficiencies. The requested response time may be ten or thirty days, depending upon the severity of the findings. Firms in the US and other countries are always encouraged to reply in writing to FDA concerning an FD 483 or written letter.

The use of a special compliance programme attachment for sterile devices reflects the FDA's concern that all devices which must be sterile at the time of use be safe and effective. Thus the inspectional strategy includes thorough coverage of validation, process controls, biological indicators, and package integrity. It is obvious that industry shares this concern. There has never been adverse industry reaction to Office of Medical Devices coverage of sterile device manufacturing. Moreover, the industry itself is very active in research and in sponsoring conferences and symposia such as this one. Industry and the regulators working together in advancing sterilization science can achieve improved levels of patient safety.

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Appendix

CYCLE PARAMETERS

Determine the device master record specifications and determine the conformance to these specifications by reviewing sterilization process lot history records for the following parameters: (attach one representative sterilization history record including charts)

		FIRM'S SPECIFICATIONS	OBSERVED PARAMETER
VACUUM (Specify mmHg, in. H_20)	:	_____	_____
AIR VENTING OTHER THAN BY VACUUM (prior to or during gas charging)	:	YES/NO	YES/NO
TEMPERATURE	:	_____	_____
OPERATING PRESSURE	:	_____	_____
RELATIVE HUMIDITY (%)	:	_____	_____
PREHEATING (heat exchanger) OR HOLDING TEMPERATURE OF GAS WHEN INJECTED INTO CHAMBER	:	_____	_____
GAS CONCENTRATION IN CHAMBER (mg/liter)	:	_____	_____
IS CIRCULATION FAN USED	:	YES/NO	YES/NO
EXPOSURE TO STERILANT (hrs.)	:	_____	_____
ARE MULTIPLE EVACUATION CYCLES USED (number of cycles)	:	_____	_____
COME-DOWN OR EVACUATION RATE	:	_____	_____

HOW IS EACH OF THE ABOVE PARAMETERS MONITORED?
(specify when not monitored):

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SECTION II –

VALIDATION OF STERILIZATION PROCESS

HAS THIS CYCLE BEEN VALIDATED:

YES_____ NO_____ PARTIALLY_____

LIST QUALIFICATIONS, BACKGROUND AND EXPERIENCE OF INDIVIDUALS RESPONSIBLE FOR PERFORMING, REVIEWING AND APPROVING VALIDATION STUDIES:

_____

_____

_____

METHOD TO DEVELOP STERILIZATION CYCLE
(specify overkill or bioburden):

A. Summarize cycle development test results (e.g. laboratory or pilot plant studies):

_____

_____

_____

B. Describe microbial challenge system used in above studies:

_____

_____

_____

STUDIES CONDUCTED TO VALIDATE THE STERILIZATION PROCESS

A. INSTALLATION QUALIFICATION OF EQUIPMENT (describe when applicable)

1. Empty chamber temperature distribution studies:

_____

_____

_____

2. Is the measurement equipment calibrated before validation studies (including thermocouples):

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A Regulatory Review of Sterilization Control in Europe

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The developments in the regulation of sterile single-use medical devices which are taking place in Western Europe are reviewed. Significant differences in approach to the regulation of sterilization of medical devices may be observed when one country's requirements are compared with those of another.

Table 1

Europe – Its main politico-economic groupings

	No. of Member States	Population (millions)
1. The European Community (EEC) 1957 [Spain (37 millions) now negotiating entry]	10	279
2. The European Free Trade Association 1960	7	41
3. The Council for Mutual Economic Assistance (COMECON) (Excludes USSR)	7	99
Others: Spain, Yugoslavia, Albania	3	62
	27	481

There are some 18 countries in Western Europe (Table 1). Few share a common language and the peoples in those countries inherit distinctly different cultures and traditions. While it is not surprising then that the approaches to regulation vary widely, it does make things complicated. The same medical device may be submitted to quite different requirements in each of several countries. This can make it difficult to market a particular device, even with multilanguage labelling, in the same configuration in several European countries.

Requirements in a given country for sterile single-use medical devices generally fall in one or more of these categories:

Good Manufacturing Practice (GMP) codes
Registration – of the products
– of the manufacturing facility
Notification – of products
Labelling
Expiration dating

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National languages
Packaging material specifications
Product standards or specifications
Presterilization microbial load ('bioburden') determinations
Sterilization cycle requirements

Good Manufacturing Practice Requirements

Codes of GMP have been written in Europe for medical devices and have been published in Denmark (MEDU 1967), in France (1978), and in the UK (1981). Draft documents have been prepared in Sweden and in Italy.

The Danish GMP code for medical devices is contained in the recommendations of the influential MEDU (Medical Utensils) Committee and was published in 1967. It focuses on the need to maintain hygienic conditions and control over the manufacture of sterile medical devices. The French GMP document, *Pratiques de Bonne Fabrication* (Maisonneuve S.A., 1978) is a more general guide and is not specific to medical devices.

The current UK GMP document, *Guide to Good Manufacturing Practices for Sterile Medical Devices and Surgical Products* (H.M.S.O., 1981), which is a revision of the Guide published in 1979, represents the fruits of a co-operative effort between the Department of Health and Social Security and the major trade associations whose members manufacture these types of products. In the revised edition, a complete chapter on sterilization was added.

Registration Requirements

Registration schemes for groups of products have been introduced in Belgium (1966), Italy (1975), and Spain (1976). Each of these schemes varies in scope, but in Belgium and Spain they cover sterile single-use medical devices. In general, devices for *in vitro* diagnostic use are not included in their scope.

In Italy, the scope of the scheme is limited to a list of products ('Presidi Medico-Chirurgici') as defined in the Ministry of Health memorandum ('Circolare') No. 74 of 1 September 1975 (Appendix 1). Although a revised set of regulations for medical devices has been drafted, it has not yet been finalised.

In Spain, the registration of sterile medical devices is regulated by the order of 21 October 1976, published in the Official Gazette on 19 November 1976 (see Appendix 2 for an unofficial translation). This order deals with the technical management by a qualified person (pharmacist) of manufacturing, control of materials, process and product quality, facility licensing, registration of sterile devices, packaging, labelling, distribution and sale. The registration requirements demand that information be provided on the product, the manufacturing, packaging and sterilization processes used on the quality control of raw materials, finished goods and packaging, and on the estimate of the product's shelf life. Samples of the final packaging materials must also be supplied. Authorization to manufacture and/or distribute the product will include the requirement to show the registration number and the name of the registered qualified technical manager (pharmacist) on the product packaging.

Product registration schemes have been proposed in Norway, Sweden, and th Netherlands. Sweden has a product notification scheme for industrially sterilized medical products in place

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already.

In the UK, the Department of Health and Social Security (DHSS) decided against product registration for medical devices although the DHSS Supply Division, Scientific and Technical Branch (STB) have in place a number of product approval schemes based on technical specifications. The 25 or so products are subject to testing against the relevant technical specification and the manufacturers' facilities are also subject to inspection by the DHSS STB staff. Extension of this scheme to all medical devices was seen to be costly, and so the DHSS has introduced in 1982 a voluntary registration scheme for manufaturers of sterile products which qualifies manufacturing sites as being in compliance with the DHSS *Guide to GMP for Sterile Medical Devices and Surgical Products*, referred to above.

The scheme is designed to provide the National Health Service (NHS) with the names and addresses of manufacturers whose product or processes comply with the DHSS Guide to GMP. Details of the scheme appear in Appendix 3. A Declaration (of compliance) Form is part of the application for each registration, to be signed by the Chief Executive, the Production Manager, and the Quality Controller at each manufacturing site. (Provision is made in the application for listing point(s) that are not yet in accordance with the Guide, with anticipated date(s) of completion of necessary changes(s).) Teams of three STB inspectors tour the manufacturing and sterilization areas; check the air handling and environmental controls; review the cleaning equipment, practices, and schedules; investigate physical and microbiological quality control procedures; evaluate materials control, identification, and segregation; ask questions about the organization structure and quality systems, and so on, using the Guide as their reference.

Satisfactory outcome of the inspection by STB staff will be required to achieve or maintain the inclusion of the manufacturer's name on the register of manufacturers. The list of registered manufacturers will be promulgated to the NHS with the recommendation that sterile products should be purchased only from registered manufacturers.

Labelling and Expiration Dating Requirements

For manufacturers aiming to produce in one location for several European countries – even using a multilingual approach – these requirements pose a problem. Several countries impose mandatory expiration dating of most sterile products regardless of the real shelf life of the product; these are France, Italy and Spain. France and Italy have changed the maximum shelf life permitted over the years – Italy started with a maximum of five years and reduced it to three years in 1975; France started with one or two years maximum shelf life (depending on the sterilization process or on the type of packaging) and increased it to a maximum of five years earlier this year. Spain maintains that the maximum term allowed for expiration dating of drug products (five years) shall apply to sterile devices also.

The many items of information which must appear at each level of packaging can and do crowd the surface of the individual (unit) packaging of smaller devices. A number of countries have required that their registration number for the product, or the company, appear on every item (Italy, Belgium, Spain). As we have seen above, the name of the (registered) responsible pharmacist must appear on the product labelling to satisfy Spanish requirements.

A Labelling Working Group was set up in 1979 y EUCOMED (the European Confederation of Medical Suppliers Associations) to survey the diversity of labelling requirements in Europe and to

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prepare a recommendation to harmonize these requirements. Its draft recommendations and the underlying rationale may be found in Appendix 4. As a result of the work, those countries about to introduce labelling requirements were in a position to consider these carefully prepared recommendations.

National Languages

While only France has a law requiring everything printed on and with the product which is not in French to appear in French, in practical terms the national language is necessary in countries where product registration schemes are in place for sterile products. It is also desirable, if not essential, because of product liability considerations, to translate warnings, safety precautions, and related aspects of the instructions for use.

Packaging Material Specifications

To date, packaging material specifications have only appeared in the UK and for a limited range of papers. France, through the Pharmacopoeia Commission, has prepared draft monographs for a number of grades of paper for packaging sterile products. The measurement of microbial barrier properties have not, thus far, been included in any of these specifications.

Product Standards

There are a growing number of national and international product standards for sterile medical devices. These are generally prepared by committees set up by a national standards body such as DIN (Germany), AFNOR (France), BSI (UK), etc. Standards have been published for products such as hypodermic needles, syringes, catheters (urinary), transfusion equipment, anaesthetic and respiratory equipment, mechanical contraceptives, surgical implants, etc. Occasionally, one finds a second standard written for the same product by a national Pharmacopoeia Commission, but written, of course, from a different point of departure. In certain countries conformance to product standards is mandatory for that part of the market that is partly or fully reimbursible by public funds.

Sterilization-related Requirements

Regulatory controls of sterilization processes are defined in different ways in the European countries. Sweden has ‘guidelines for the control of sterility of industrially sterilized single-use medical devices’. France and Italy have issued pharmacopoeial monographs. In the UK, the DHSS *Guide to GMP for Sterile Medical Devices and Surgical Products* contains requirements in Chapter 8 and the ‘Orange’ *Guide to Good Pharmaceutical Manufacturing Practice* covers the same sterilization processes, but with some differences (mainly minor in nature). The MEDU Committee recommendations of 1972 set out the Danish requirements. It is proposed to review some of these requirements.

Presterilization Microbial Load (Bioburden) Determination

A number of countries require bioburden numbers, as opposed to bioburden resistance, determinations to be carried out. In the Annex to the 9th Edition of the *French Pharmacopoeia*, in the monographs on ethylene oxide and radiation sterilization (see Appendix 5), reference is made to the need to establish the bioburden number, and in the case of radiation, a qualitative analysis of the bioburden is also required. This is aimed at detecting microorganisms of lower sensitivity to radiation treatment. Apart from the above cited French requirements, no regulations demand the

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determination of the bioburden sensitivity to the chosen sterilizing agent.

In general, no country has set criteria for acceptable bioburden numbers with the exception of Denmark and Sweden. The MEDU radiation-sterilization recommendations for sterilization dose levels are as shown in Table 2.

Table 2

Denmark – MEDU Sterilization dose requirements as determined by bioburden levels

Average Initial Count per product Item	Minimum Absorbed Dose to be Used
> 50	35 kGy (3.5 Mrd)
> 500	45 kGy (4.5 Mrd)
>5000	50 kGy (5.0 Mrd)

In Sweden (Appendix 6), a sterilizing dose of 32 kGy is required for products having less than 50 microorganisms/product item. A dose of 25 kGy is considered acceptable for sterilization where the bioburden is less than one microorganism/item and where long experience, properly documented, demonstrates the consistently low bioburden level.

Sterilization Dose Requirements for Radiation Sterilization

With the above exceptions, where a dose requirement has been specified in Europe, it has been 25 kGy (2.5 Mrd). In the Netherlands, no dose has been specified for medical device sterilization. The UK DHSS Supply Division (STB) states: 'A minimum dose requirement of 25 kGy (2.5 Mrad) is generally accepted as adequate for this purpose'; and the Medicines Division states: 'A radiation dose requirement of 25 kGy (2.5 Mrad) is regarded as adequate from the microbiological viewpoint … Other doses may be used subject to adequate biological validation being performed'.

Biological indicators are required routinely in radiation sterilization only in France.

Cycle Requirements for Ethylene Oxide (EO) Sterilization

Generally speaking, no specific requirements have been laid down for gas concentration, relative humidity, temperature, or time of exposure to the EO gas. Most countries requirements spell out that biological indicators (BI) must be used. The number of BIs required varies from 'at least 10^6 *B. stearothermophilus* spores' (France) to 3 to 6 spore preparations (BIs) per cubic metre of sterilizer volume, but not less than 6 (Sweden). In the UK, a minimum of 10 BIs per cycle must be used.

Product Sterility Testing

Product sterility testing remains a requirement in a number of countries although awareness of its low value is spreading. In the UK, product sterility testing is not required for EO or radiation sterilization. Some European countries accept dosimetric release of radiation-sterilized products.

Ethylene Oxide Residuals

Established limits are the exception rather than the rule. France has a limit of 2 ppm for all EO-sterilized products and Italy has a similar limit for certain medical devices.

Trends

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It may be said that the trends in regulatory controls are moving slowly towards abandoning the

product sterility test as a release criterion, towards dosimetric release of radiation-sterilized products, towards validation of sterilization cycles, and, very slowly indeed, towards permitting the matching of sterilization cycles/doses to bioburden to give calculated levels of sterility assurance.

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Appendix 1

Italy – Registration Scheme
List of Affected Products
(Unofficial Translation)

Circular No. 74 Roma 1.9.1975

Object: **Medical-Surgical Devices**

From the part of the Trade Associations and Pharmaceutical Companies are often requested information concerning which products are today subject to registrations as medical-surgical devices, according to the article 189 of T.U. (Text Unique) of the medical laws of 1934.

With regard to these requests, we make it clear that at the present are subject to registration, as medical devices, the following products:

1. Pessaries
2. Irrigators, douches, syringes, vaginal insufflators, vaginal cannulas.
3. Disinfectants and substances commercialized as bactericides or germicides.
4. Instruments to contain intestinal hernias or abdominal organs.

The numbers 1-2-3-4 are included in the list enclosed to the R.D. (Royal Decree) 6.12.1928 no. 3112.

5. Instruments and auricular prosthesis, ear-trumpets and similar (Ministerial Decree 5.2.1929)
6. Insecticides (D. ACIS 21.9.1954)
7. Insect repellants (Ministerial Decree 31.7.1971)
8. Plastic syringes single-use (Ministerial Decree 27.2.73)
9. Pipes, masks and applications for reanimation (Ministerial Decree 22.4.1963)
10. Non pharmaceutical products containing esachlorophene (Ministerial Decree 8.1.73 – circular No. 100 of 2.9.74).
11. Plastic defluxion instruments for blood and hemoderivatives.
12. Plastic containers for blood and hemoderivatives.
13. Plastic defluxion instruments for infusional solutions.
14. Plastic containers for infusional solutions.
15. Pipes, containers and instrument parts for dialysis – including membranes (circular No. 28 of 2.4.75).
16. Pipes, or instrument parts for extra-bodily circulation.
17. Catheters for cardiology and vascular prosthesis.
18. Electrodes for pacemakers.

The medical devices from No. 11 to No. 18 are all subject to registration according to Ministerial Decree 27/2/73 – circular No. 28 of 2.4.75 – circular No. 49 of 30.5.75 – circular No. 50 of 30.5.75.

19. Orthopedic shoes for children (Ministerial Decree 22.3.75 G.U. No. 129 of 17.5.75).

We would also like to remind you that the use of the following products has been forbidden:

1. DDT in aerosol (Circular No. 3 of 11.1.71)
2. DDVP in aerosol (Circular No. 196 of 12.11.71)

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3. Chlorocyclodienics (Ministerial Decree 1.2.74)
4. Aminotriazol as herbicide for civic and domestic use (Ministerial Decree 9.10.74).
5. Lindane and BHC (Ministerial Decree 9.11.74).

We believe it is also important to remind some directions which have been issued for the medical-surgical devices.

– Taxes to pay for the medical-surgical devices (G.U. No. 292 of 11.11.72)
– Solvents and propellants authorized (Circular No. 32 of 14.3.72 – circular No. 119 of 14.9.72 – circular No. 4 of 13.1.72)
– Modifications to the chemical tests for the plastic (Ministerial Decree 5.7.74 – G.U. No. 189 of 19.7.74)
– Blood preservation and hemoderivatives in PVC bags – max 72 hours (Ministerial Decree 28.3.75)
– Medical devices containing pyrethrum and pyrethrins – modification of instruction sheets (circular No. 135 of 20.11.74).

All analysis certificates made by University Institutes must be on stamped paper and countersigned by the University President.

All certificates released by provincial Laboratories of Public Health and Prophylaxis must be on stamped paper and signed by the managing director of the lab.

We invite the addressees to give the maximum urgent promulgation to the present circular in order to avoid any possible doubt by the interested parties.

The police HQ of NAS (Nuclei Anti Sofisticazioni) is kindly requested to take urgently all provisions in the case medical devices could be found in the market which are not regularly registered as such.

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Appendix 2

Spain – Registration of Sterile Medical Devices
(Unofficial Translation)

Official Gazette No. 278, 19th November, 1976.

MINISTERIO DE LA GOBERNACION
(Ministry of the Government)

ORDER OF OCTOBER 21, 1976 CONCERNING THE MANUFACTURE, REGISTRATION AND CONTROL OF STERILE MATERIAL.

The medical-pharmaceutical practice, for greater effectiveness, guarantee of use and health protection makes it necessary for certain materials, utensils and products be manufactured, sold and used after previously being submitted to sterilisation processing and under the condition that they are used only once.

Their growing use, particularly of those for "single-use", can involve certain health risks which must be prevented by subjecting their production, preservation and sale to standards and control.

Therefore, as proposed by the Director General of Health, I have found it advisable to decree:

1. **Field of Application**

1.1 This order refers to those articles currently used in the medical-pharmaceutical practice which must be or are assigned as being sterile, whether or not for single use only, such as syringes, hypodermic needles, sutures, spatulas, lancets, depressors, probes and catheters, sets for the administration of solutions and blood extraction, and any other similar devices, excluding sterile dressings and sutures, which will continue to be regulated by Decree 2464/1963 of 10th August, and Ministerial Order of 5th May, 1964, as well as to organisations and Companies which manufacture, import, distribute and sell them.

2. **Manufacturers**

2.1 All firms which are engaged in the manufacture of the aforementioned articles will be subject to health authorisation and registration with the appropriate department of the Directorate-General of Health, and their installations will be subject to the Departments inspection and control.

2.2 The processing of authorisations and registrations to which this Order refers shall be addressed to the Deputy Director of Pharmacies, Directorate-General of Health.

3. **Technical Management**

3.1 The manufacture of products detailed in this Order will be carried out under the supervision and responsibility of a qualified technical person who will guarantee the cleanliness of manufacture and the control of sterilisation.

3.2 In any case, the appointment of a qualified technical person shall be notified within one month to the Provincial Health Authorities who, respectively will notify the Directorate-General of Health within 15 days.

If the qualified technical person resigns from the position, the organisation will name a substitute and confirm the appointment within one month, following the indicated procedures.

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3.3 Companies which import sterile articles for distribution, must justify to the Directorate-General

of Health that sufficient health guarantees exist for the imported product.

4. **Requirements for Materials and Controls**

4.1 All organisations engaged in the production of articles detailed in this Order shall as a minimum have the following sections:

Warehouse: It shall consist of three distinct parts: one, for raw materials used in production; two, for manufactured products not yet sterilised; three, for finished products ready for distribution.

Manufacturing: In this section besides the packaging and sterilisation of products, the operations of material technology shall be carried out.

If the packaging process is carried out after sterilisation of the product, it (the packaging) will have to be undertaken in a sterile area unless the method does not require it; as a minimum this would consist of an ante-room fitted with germicidal lamps where personnel change clothing and a sterile area for aseptic packaging. Communications with this sterile area from the outside and the supply of materials shall be by adequate means to avoid contamination; all technical and auxiliary personnel employed on product preparation shall be subject to periodic health checks to guarantee that they are not suffering from any contagious illness or skin diseases.

Packaging and sterilisation shall in any case be independent and suitable to the processing methods employed, or to the nature of the material to be sterilised and, when necessary, an intermediate quarantine department shall be established.

Control Laboratory: It shall be equipped with apparatus to carry out tests which guarantee quality, non-toxicity and sterility of the products.

4.2 The controls will be appropriate to the nature of the substances and products and will be undertaken on raw materials, during production and on finished and packed products.

These will include the physical, chemical and biological controls which guarantee the quality and preservation of the finished product e.g., corrosion, polishing, presence of particles, oxidizable substances, leachable substances, haemolitic action, treatment residues, toxicity, irritation, pyrogens, dimensions, calibration and sterility.

The manufacturer will be under obligation to keep the protocols of the controls carried out, for the raw materials the manufacturing process and the finished product, holding itself responsible for the said analyses and controls.

4.3 The minimum controls to be performéd in finished products shall be:

Sterility tests.

Pyrogens.

Functionability (dimensional and calibration).

Residues of sterilising agents and of residual radioactivity.

Permeability and strength of packaging.

4.4 The Directorate-General of Health, in duly justified cases, and subject to petition by the firm, may authorise the analytical controls of raw materials and finished products to be carried out in Specialised Centres.

5. **Company Authorisation**

5.1 The authorisation shall be initiated by a petition to the Directorate-General of Health, in which the following information and documents will be included:

Full name of the owner of the firm or name of the Company.

Full address of the establishment.

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Name of responsible Director or Guarantor.
Map of the establishment.
A technical specification of the manufacturing and sterilisation processes and of the controls to be carried out pursuant to those provided for in Clauses 4.2 and 4.3.

5.2 In the light of the documentation submitted and other appropriate information obtained, and an inspection visit of the premises, the Directorate-General of Health will be able to declare the Company suitable for operation and assigning to it an identification number.
In the case of dealing with previously authorised Pharmaceutical Laboratories the identification number shall be the number already assigned.
If, as a result of the visit to the premises or other causes, the established conditions are not met, authorisation shall not be granted. However, the Company may re-apply for authorisation after the deficiencies which resulted in the original refusal have been corrected.

5.3 All substantial modification to the installations, processing or control systems, or changes in address must be authorised by the Directorate-General of Health, subject to the inspections and reports deemed appropriate, as long as the requirements of other Ministerial Departments are not affected.

6. Registration of Sterile Material

6.1 The registration procedure for the products subject to the present regulation will be initiated by a petition to the Director-General of Health, by the manufacturing or import Company, signed and authorised by a responsible Director or Guarantor, in which the following information and documents shall be enclosed:
Name and address of the manufacturing or importing organisation.
Name of responsible Director or Guarantor.
Type of product and description of its basic nature.
Manufacturing and packaging processes.
Sterilisation systems used.
Controls made on raw materials, finished products and packaging materials.
Estimate of expiration date of sterility.
Name and authorisation number of the Control Laboratory, approved by the Directorate-General of Health, carrying out analytical controls in accordance with Clause 4.4.
Samples of the packaging or conditioning (finishing) material.

6.2 In the light of the information contained in the registration proceedings and the statement from the National Pharmacobiology Centre, the Director-General of Health will:-
Pass a resolution denying authorisation of registration for manufacture.
Request the firm to provide further studies, explanations and modifications.
Authorise the manufacture and distribution of the product.
The authorisation shall be assigned a Directorate-General of Health registration number …. M.E. (Numero Registro D.G.S. Material Esteril), which, compulsorily, shall be printed on the outer and/or inner packaging material, and, if possible, on the product in very clear printing.

6.3 For these sterile products and materials, a lot shall be defined as each sub-batch (fraction) of production which has been subjected to the same manufacturing and sterilisation process.
Lots shall be identified by a code consisting of a single capital alphabetical letter and a number correlating with each lot of products manufactured with the same material, in accordance with the

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Decree No. 2828/1965, 14th August concerning numbering of lots.

6.4 On each occasion when a manufactured lot is finished or imported, the Technical Director or Guarantor shall notify the Directorate-General of Health, quoting the lot number and number of items contained in the lot.

7. **Conditioning (Packaging) Material**

7.1 The conditioning (packaging) material to be used must be adequate to guarantee the quality and sterility of the product when it reaches the consumer.

7.2 In addition to the words 'Sterile' and 'Valid for Single Use Only' the packaging material shall be clearly printed with the following data:

Name of product.

Registration Number.

Lot Number.

Name of the responsible Director or Guarantor.

Name of manufacturing or importing firm.

Use before date.

8. **Distribution and Sale**

8.1 Although the distribution is considered to be free, the Companies must comply with the conditions which guarantee storage and control.

If at any time, in the judgement of the Health Authorities, the distributing organisation does not meet the minimum conditions guaranteeing proper warehousing of the products subject to these regulations, the Directorate-General of Health, after receiving the necessary reports, may order the immobilisation, confiscation and prohibition of the distribution of the materials.

8.2 Sales or dispensing shall be effectuated through Pharmacies and establishments specialising in medical and sanitary materials and products; these establishments shall similarly be subject to inspections by the Directorate-General of Health.

Such specialist establishments shall report to Provincial Health Departments their existence and activities in accordance with this resolution.

9. **Violations and Penalties**

Infringement of these regulations will be penalised according to the type of offence and the scale of penalties contained in Decree 2464/1963, 10th August, following the sanctioning procedures provided for in the Law of Administrative Procedures.

10. **Final Provisions**

10.1 The Directorate-General of Health is empowered by Resolution to expand and complement the provisions established in this Order, both in general aspects of the present standards and in those cases where additional standards are necessary due to the special health characteristics of some products and materials.

10.2 Manufacturing organisations, authorised and registered specialist pharmaceutical laboratories, importers, distributors and specialised sales establishments referred to in Section 8.2, shall have one year from the date this Order comes into force, to adapt themselves and comply with the established standards.

Within the same period of time, inscription and registration shall be solicited for all manufactured and imported sterile products.

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Madrid, 21st October, 1976.

Directorate-General of Health.

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Appendix 3

United Kingdom – Registration Scheme

Scheme for the Registration of Manufacturers of Sterile Medical Devices and Surgical Products

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Introduction

1. Following discussions with Trade Associations, the Department has decided to establish a register of manufacturers of sterile medical devices and surgical products.* The Department's main intention is to aid the National Health Service to obtain products of uniform quality, safety and performance by making available the names of firms which manufacture sterile products according to recognised principles of good manufacturing practice. The scheme does not involve checking the design and performance of individual products and should not be confused with existing or planned product approval schemes which may need to be continued as specific extensions of the manufacturers' registration scheme.

2. The Guide to Good Manufacturing Practice for Sterile Medical Devices and Surgical Products which has been prepared in collaboration with Trade Associations will form the basis of the Registration Scheme. Manufacturers, whether based in the U.K. or abroad, who wish to be registered will need to declare in writing their compliance with the principles and requirements set out in the Guide.** They will need to confirm, in respect of themselves and their subcontractors, that premises and records (as specified in the Guide) will be made available for inspection by officers of the Scientific and Technical Branch (Supply Division) of the Department of Health and Social Security. Complete compliance with all the requirements of the Guide is the normal condition for registration but attention is drawn to Sub-Clause 1.1.3 of the Guide which states that "Systems other than those described but which achieve the same ends may be equally acceptable". Acceptance of alternative methods or any other departures from the full provisions of the Guide will be subject to terms and conditions laid down by the DHSS. (Manufacturers of implantable cardiac pulse generators should also refer to the document "Specific Requirements for Quality Systems for Manufacturers of Implantable Cardiac Pacemakers", HMSO 1981).

3. The list of registered manufacturers will be promulgated to the National Health Service with the recommendation of the Health Service Supply Council that sterile products should be purchased only from registered manufacturers.

4. The National Health Service purchases many sterile products through wholesale firms and other agents. Since these are not manufacturers the registration scheme will not apply to them, but they should note that the National Health Service will be advised to ensure that the items purchased are produced by registered manufacturers.

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Eligibility and Application for Registration

5. Applications should be made by completing and submitting the enclosed application form and declaration to:-

The Registration Scheme Officer
DHSS
Supply Division, HSSB,
Room 316
14 Russell Square
London WC1B 5EP

The initial application should NOT include a payment for registration. The Department will notify applicants when payment is due.

6. Manufacturers* whose sterile products have been supplied to the National Health Service on or before 1 January 1981 will be registered when examination of the information provided and any subsequent enquiries have been satisfactorily completed.

7. For other manufacturers, satisfactory inspection by Scientific and Technical Branch (Supply Division) DHSS will be necessary before registration can be completed. Exceptionally the Department may exercise its discretion to dispense with this pre-registration inspection where a manufacturer has been registered or approved under a similar scheme which offers equivalent assurance of good manufacturing practice.

8. Unsatisfactory or incomplete information may delay inspection and/or registration.

9. After registration all manufacturers and their subcontractors will be liable to inspection by officers of Scientific and Technical Branch at any time.

10. A decision not to include a company on the register will be taken at a senior level in the Department as part of the administrative arrangements for operating the scheme. A company that wishes to object to non-inclusion in or to removal from the register should write to the Controller of Supply at DHSS, Room 211, 14 Russell Square, London, WC1B 5EP.

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Declaration

11. It will be seen from the Declaration Form that the undertaking regarding manufacture in accordance with the Guide must be signed by the Chief Executive of the manufacturing Company and the key personnel – the Production Manager and the Quality Controller, and that qualifications and/or experience must be given for the last two signatories. A declaration stating compliance of each manufacturing site with the Guide must be provided. THE DEPARTMENT MUST BE INFORMED OF ANY SUBSEQUENT CHANGE.

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Charges

12. A non-returnable charge of £500 will be made for registration. This charge will cover an initial registration period of three years and further payment will be required each time an application is made for continued registration. In addition applicants may be liable to a further charge if, in undertaking inspections, the Department incurs expenditure substantially in excess of £500.
All cheques should be made payable to the Department of Health and Social Security.

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Removal from the Register

13. A manufacturer may be removed from the register at any time if the Department is satisfied that:-

(i) false particulars have been submitted to the Department or to its inspectors; or

(ii) the results of inspection show standards significantly below those required and the manufacturer is unwilling or unable to take corrective measures; or

(iii) the appropriate charges for registration or inspection have not been paid.

14. Manufacturers wishing to withdraw from the register should advise the Department in writing.

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Publication of Register

15. The register will be published and circulated to the National Health Service and to registered manufacturers annually. Changes will be circulated regularly.

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Enquiries

16. All enquiries about the scheme should be addressed to:-

The Registration Scheme Officer
DHSS
Supply Division, HSSB,
Room 316
14 Russell Square
London WC1B 5EP
Telephone: 01-636 6811 Ext 3600

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DHSS

Supply Division, 1982

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Appendix 4

EUCOMED – Labelling Recommendations for Sterile and Non-Sterile Medical Devices

Final Draft

	Sterile	Non-Sterile
Unit Container		
Description of Contents	X	X
The word "STERILE"	X	–
The words "SINGLE USE"	X	–
Identity of Manufacturer or Supplier	X	X
Batch Number	X	X
Instruction for Use (if required)	X	X
Special Precautions (if any)	X	X
Date of Expiration (if applicable)	X	X
Shelf Container		
Description of Contents	X	X
The word "STERILE"	X	–
Name and Address of Manufacturer or Supplier	X	X
Batch Number	X*	X
Date of Sterilization (month, year)	X*	–
Date of Expiration (if applicable)	X	X
Instruction for Storage (if applicable)	X	X

* If the Batch number clearly identifies the date of sterilization (month, year), date of sterilization is not required in addition to a separate batch number, and vice versa.

RATIONALE FOR RECOMMENDATIONS BY EUCOMED WORKING GROUP

1. *Country of Origin*
   Requirements for identification of Country of Origin are usually governed by Trade Description Legislation and/or Customs and Excise requirements and are therefore not included in these recommendations.
2. *Interpretation of Government and Regulatory Body Legislation*
   The Group found it difficult to interpret some of the labelling requirements for committed countries. In some cases dual standards are being applied by governments and regulatory bodies. This matter can only be resolved by the appropriate local Association representing EUCOMED's interests.

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3. *Expiry Date*

The philosophy adopted by the Group was that if the product is subject to material degradation within five years the expiry date (month and year) should be printed on the unit and shelf container. For all other practical purposes, products will remain sterile indefinitely if handled correctly. Maintenance of sterility is event related rather than time related.

4. *Language Requirements*

The Group recommended that the following four languages should be adopted and used singly or in any combination.

English; French; German; Spanish

Where products are manufactured in countries other than those mentioned above, the local language may be printed if required by legislation.

5. *Non-Committed Countries*

In preparing the charts it is assumed that the following are non-committed countries and will accept EUCOMED recommendations.

5.1 Medical Devices

Austria, Cyprus, Finland, Greece, Iceland, Luxembourg, Malta, Portugal, Switzerland, Turkey.

5.2 Surgical Products

Austria, Cyprus, Finland, Greece, Iceland, Ireland, Luxembourg, Malta, Portugal, Switzerland, Turkey, Spain, Norway and Italy.

6. *Rationale for Omitting Some of The Committed Country Requirements for Sterile Surgical Products and Single-Use Medical Devices*

The Group considered a rationale for omitting from the EUCOMED recommendation charts some of the requirements of committed countries, e.g.

6.1 Method of Sterilization

The indication is of no help or importance for the end user. The indication “STERILE” implies that the products have been sterilized by an accepted method under the responsibility of the manufacturing company. The indication “STERILE” gives an assurance of the microbiological safety of the product.

6.2 Registration Number and Authorisation Number

These indications are of no help to the end user. They are code numbers for the identification of the product (registration number) and for the manufacturer (authorisation number) which are already clearly identified.

6.3 Name of Responsible Person/Pharmacist

The identity of the manufacturer or supplier and the lot or batch numbers will automatically lead to the responsible pharmacist.

6.4 Dose of Radiation required

The assurance of microbiological safety, given by the indication “STERILE” implies, for gamma sterilization, that the required dose has been given in compliance with the relevant GMP.

6.5 Identity of Sterilization Facility and Address

The identity and address of the sterilization facility is traceable through the manufacturer and therefore need not be specified separately.

6.6 Pyrogen-Free Claim

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National and international standards require that some single-use medical devices are free from

pyrogens. It is therefore not considered necessary to make this a mandatory requirement on the package.

6.7 Sterile Provided Pack Unopened or Undamaged

This statement is considered not to be necessary on the unit or shelf container. For all practical purposes products will remain sterile indefinitely if stored and handled correctly. Maintenance of sterility is event related rather than time related.

GLOSSARY OF TERMS

Terms	
Unit Container	A package containing an individual device or dressing or a number of devices comprising a procedural kit to be used on one patient.
Shelf Container	A package containing a number of unit containers.
Committed Countries	Those countries where there are existing requirements supported by legislation and/or requirements enforced by local inspectors.
Non-Committed Countries	Those countries where there are no existing requirements supported by legislation or if legislation exists the country concerned is not enforcing stated requirements.
Batch Number (or Lot Number)	The designation of a batch by means of distinctive combination of numbers and/or letters which identifies it and permits its history to be traced. A batch may be a single product in which case this may be referred to as a serial number.
Address of Manufacturer/Supplier	The Town and/or Postal Code and Country sufficient to locate or contact the Mnufacturer.
Medical Device	An instrument, apparatus, implement, appliance, implant, or other similar or related article, which is intended for use in the treatment of humans, contraception, or in diagnosis. A device achieving its principal intended purpose through chemical or biological action is excluded from this guide.
Surgical Products	Adhesive and non-adhesive non-medicated surgical products. Surgical devices are designed to cover surface damaged body parts and/or absorbed body fluids.

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Appendix 5

France – Sterilization

(Unofficial Translation)

Published
October 1976

FRENCH PHARMACOPOEIA

Annex to 9th Edition
Part II, pp. 212-6

STERILIZATION

Four sterilization processes are in general use in the pharmaceutical industry:

a) sterilization by heat,
b) sterilization by filtration,
c) sterilization by contact with gaseous substances,
d) sterilization by ionizing radiation.

The National Pharmacopoeia Commission is at present revising the definitions, methods and operating conditions to be respected when using these various processes.
In this connection, the following monographs have already been prepared and are published below:

– a monograph concerning the sterilization of medical-surgical instruments by ethylene oxide,
– a monograph concerning the sterilization of single-use medical-surgical instruments, dressings, and sutures by ionizing radiation.

Other complementary monographs will be published at a later date.

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STERILIZATION OF MEDICAL-SURGICAL INSTRUMENTS BY ETHYLENE OXIDE

Ethylene oxide is used to sterilize medical and surgical instruments such as syringes and the necessary articles for perfusions, probes and catheters etc. (1)

Sterilization by ethylene oxide (substance listed in table A of poisonous substances, Section II, decree of 21 January, 1975, Official Journal of 5 February, 1975) must be carried out by trained and experienced personnel.

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Initial Contamination

As with other sterilization methods, the effectiveness of this method depends on the initial quantity of contaminating germs on the product before sterilization.

All stages of manufacture or preparation of the equipment to be sterilized are carried out in such a manner as to reduce contamination to the absolute minimum; rules on personal hygiene and working procedures must be strictly adhered to in order to reduce the risk of contamination, with handling of products being restricted to the absolute minimum.

The articles are packed in materials which are permeable to ethylene oxide and wrapped so as to maintain sterility after sterilization. The articles will be packed in such a manner that they can be removed from their package aseptically (2).

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Sterilization Method

Sterilization is carried out using ethylene oxide, generally mixed with air, fluoroalcanes, carbon dioxide or any other suitable gaseous diluent. The sterilization lot consists of a set of articles which are identical in composition, shape and intended purpose and which are sterilized at the same time; they must not undergo any physical or chemical change which would be incompatible with their subsequent use.

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Test Requirements

1 – Determination of residual ethylene oxide

The apparatus (see figure) consists of a 1,000mL capacity round-bottomed glass flask, having a diameter of approximately 140mm, which is equipped with three openings, a, b and c, having standard No. 2 ground glass joints, for connection to a 330mm high condenser, A, for an air inlet through a capillary tube connected to a 200mL wash bottle (1) and for the introduction of the sample. The flask is heated by a heating mantle. The condenser is connected to two bubblers (3) and (4), 220 to 230mm high and 25mm diameter, arranged in series having drawn ends and fitted inside two double-walled vessels containing an ice-water mixture. A bent tube connects the bubbler (4) to a 200mL wash bottle (5) which itself is connected to a water driven vacuum pump.

Sampling – For instruments of homogeneous composition, one sample is taken from each sterilization lot.

For instruments made of several materials, carry out the test on standard samples of each of the constituent materials of the instrument, taken from the same package and coming from the same manufactured batch.

Take an accurately weighed sample, p, between 5 and 20g, and cut it into pieces of about 0.10g.

Method – Introduce a freshly prepared solution of 1.7g of hydroxylamine hydrochloride (R) and 3.3mL of triethanoloamine(R) in 100mL of water into the bubbler (wash bottle) (1), 100 to 150mL of water into the flask (2), 50mL of water cooled to 0°C into each of the bubblers (3) and (4) and 50mL of water into the wash bottle (5).

Heat the contents of the flask (2) to boiling point and connect up the vacuum pump (to give a rate of four bubbles of air per second). When the delivery level in the wash bottle (1) and (5) becomes stable, introduce the sample, p, into the flask (2). Distill for forty-five to sixty minutes.

Remove the bubbler (3) and (4) and transfer their contents to a 150mL conical flask fitted with a ground glass stopper. Rinse each bubbler with water and add the washings to the conical flask. Prepare a standard solution by placing 80mL of water in an identical flask. Carry out the following procedure for each of the two flasks.

Introduce 1mL of 0.5 N sulphuric acid; close off the flasks hermetically and immerse them in a boiling water-bath for one hour. Cool to room temperature. Neutralise the solution with 1mL of 0.5 N sodium hydroxide and transfer the solution to a 100mL volumetric flask. Rinse the conical flask with water and add the washings to the volumetric flask. Add 2mL of 0.1 M sodium periodate (R). Allow to stand for 15 minutes shaking frequently. Add 2mL of an 11 per cent W/V solution of sodium sulphite and make up to 100mL with water. Transfer 5mL of the solution to a 10mL graduated test tube immersed in iced water. Using a graduated burette, add 5mL in drops of a freshly prepared solution consisting of 0.10g of chromatropic acid sodium salt, (R) dissolved in 2mL of water and then mixed with 50mL of sulphuric acid (R). Heat the test tube in a water bath for 10 minutes; cool to room temperature and make up to 10mL with 18N sulphuric acid (R). Measure the extinction (page II-345) at the maximum absorption of about 570nm in a 1cm cell using the standard solution as control.

Calibration curve – Dissolve an accurately weighed sample, about 1.4g, of pure ethylene glycol in one litre of water. Dilute to 100 times its volume. From the diluted solution, transfer 1, 2, 3, 4 and 5mL samples to 100mL volumetric flasks and carry out in each flask the periodic oxidation and the assay of the formaldehyde formed as described above.

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Draw a curve on the basis of 1.409g of ethylene glycol being equivalent to 1g of ethylene oxide.
The ethylene oxide content of each sample shall not be greater than 2ppm for each of the materials examined. This level of 2ppm of residual ethylene oxide is usually achieved only when a suitable aeration time has elapsed, which depends on the equipment sterilized.

2 – Microbiological Quality Control

This verification is made for each sterilization lot using biological indicators placed inside or on the surface of the articles to be sterilized. These indicators are either articles deliberately contaminated by at least 10^6 Bacillus stearothermophilus spores, or substrates deliberately contaminated and made of a material as similar as possible to that of the article to be sterilized placed in an identical package.
Twenty-four hours after sterilization immerse the spores in a suitable medium and incubate this liquid for forty-eight hours at 56°C. No living spore shall survive.

3 – Sterility Controls

Sterility controls shall meet the requirements of regular sterility tests, page II-238.

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Storage

The integrity of the package must be checked before use.

Expiration date – One year after the date of sterilization; in cases where special packaging is used, especially double packaging or rigid packaging, the expiration date may be extended to two years.

(Note: a more recent monograph has modified this expiration dating requirement).

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Marking

Marking shall comply with the appropriate general national and international regulations.

Each sterilization lot and each article shall also be marked with the following information:

– the sterilization method;
– the name and address of the sterilization company;
– the sterilization lot number;
– the date of sterilization;
– the expiration date.

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STERILIZATION OF SINGLE USE MEDICAL AND SURGICAL INSTRUMENTS, DRESSINGS AND SUTURES BY IONIZING RADIATION

Gamma rays and accelerated electrons are two of the kinds of ionizing radiation used to sterilize single-use medical and surgical devices such as syringes, needles, perfusion sets, probes, blood dialysis equipment, as well as dressings and sutures in their single or multiple-unit airtight packaging.

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Initial Contamination

As with other sterilization methods, the effectiveness of this method depends on the initial contamination load on the product before sterilization. All stages of manufacture must, therefore, be carried out in such a manner as to reduce contamination as much as possible; the level of microbial contamination of the environmental air must be checked at regular intervals, regulations on personal hygiene and working procedures must be strictly adhered to in order to reduce the risk of contamination; handling during production must be limited to the absolute minimum.

Contamination will be qualitatively and quantitatively measured after manufacture and before sterilization of the product. The aim of these measurements will be to check that the number of germs found does not exceed the maximum permissible limit per package. This limit is prescribed in special monographs, or failing this, determined by preliminary studies; unless specified to the contrary, this number represents the total number of microorganisms found on the article and inside its immediate packaging.

The purpose of identifying these contaminating agents is to ascertain that they belong to species known to be sensitive to the method of sterilization involved or to detect the species of lower sensitivity.

The frequency with which such determinations are made depends on the nature of the article and on the experience acquired from previous measurements; it is defined at the time of use.

The articles are packaged in such a manner as to maintain their sterility, and the package is designed so that the contents can be removed aseptically.

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Irradiation Method

Before proceeding with this sterilization process, a preliminary study is carried out. This involves irradiating several packages (1) suited to this process, each containing several dosimeters (2), devices used for physico-chemical measurement of the dose absorbed and which are distributed over the surface of and inside the package in order to define the conditions under which the minimum dose to be administered is achieved at any point of the package.

The physical operating characteristics of the selected irradiation facility and the distribution of the corresponding dose in the centre of the article are noted in a preliminary test report so that adequate irradiation conditions may be defined.

During industrial treatment, checks are made on each sterilization lot by means of dosimeters in order to ensure that the doses absorbed at well-defined points of the package units are in accordance with the doses obtained during the preliminary tests. The sterilization lot consists of all the packages having similar contents and irradiated successively without interruption, in the same facility, over a given period of time which must not exceed twenty four hours.

In the case of irradiation facilities using accelerated electrons, checks will be made by means of a dosimeter suitably placed on each package unit and by continuous monitoring of the main physical operating characteristics of the sterilization facility, in order to ensure that the physical irradiation characteristics are always maintained at the values defined in the preliminary tests. The electron energy level must be such that no induced radioactive effects are produced.

In facilities containing radioactive sources, a dosimeter is fitted to the first five and last five packages of the sterilization lot and on every tenth package. The irradiation time is accurately measured.

In all cases, a direct reading “irradiation indicator” is fitted to each package in order to avoid any possible confusion between irradiated and non-irradiated articles.

The choice of irradiation dose will depend on the initial contamination, the radiosensitivity of the germs and the safety margin required. For articles manufactured in accordance with the abovementioned regulations on working procedures and hygiene and on which the number of contaminants is less than the maximum permissible value, taking the radiosensitivity of the contaminating microorganisms into account, a minimum dose of 2.5 Mrads, uniformly distributed, generally affords a satisfactory safety margin. At this dose, the articles must not undergo any physical or chemical change inconsistent with their subsequent use.

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Test Requirements

Test of microbiological efficacy

This test is made for each sterilization lot using biological indicators placed inside or on the surface of the articles to be sterilized. These indicators are either articles deliberately contaminated or test pieces consisting of a material as similar as possible to that of the article to be sterilized or its package, contaminated by a known number of dried bacterial spores of known radiosensitivity (Bacillus pumilus E 601 spore or Bacillus sphaericus C_1A spores) (1).

In the most general case of a minimum irradiation dose of 2.5 Mrads, use the "growth or no growth" sterility test with Bacillus Pumilus E 601 or determine the inactivation factor of Bacillus sphaericus C_1A, which must be around 10^8.

In the case of a higher minimum absorbed dose of irradiation, determine the inactivation factor of Bacillus sphaericus and check, using an inactivation curve, that is to the required dose.

a) (growth or no growth) Sterility Test with Bacillus pumilus E 601 – Place the indicator in a known volume of isotonic solution of 0.9% sodium chloride containing polysorbate 80 (0.1%) and shake so as to bring the spores into suspension. Transfer the suspension into glass petri dishes containing a suitable gelose culture medium (for example, gelose-trypticase-soy broth); incubate for 48 hours at 37°C or for 5 days at 32°C. No living spores should remain.

b) Determination of the inactivation factor with Bacillus sphaericus C_1A – Put the spores into suspension as described above; prepare successive 1:10 dilutions of the suspension; transfer them into petri dishes containing gelose culture medium; incubate as described above, count the colonies, calculate the number of living spores on the indicator and then the inactivation factor as follows:

$$\frac{\text{Number of living spores before irradiation}}{\text{Number of living spores after irradiation}}$$

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Appendix 6

Sweden – Sterilization Guidelines

National Board of Health and Welfare (of SWEDEN)
Department of Drugs

Guidelines for control of the sterility of industrially sterilized single-use medical devices:
26th May 1976

Introduction: The Act of the Riksdag of 7th May 1975 (SFS no. 187; MF no. 51) concerning the control of industrially sterilized single-use medical devices requires persons manufacturing or otherwise handling industrially sterilized single-use medical devices to take such precautionary and other measures as may be necessary to ensure that the products are sterile when used. In its instructions, the National Board of Health and Welfare has been authorized to issue supplementary directives relating to the Act. Part of the supervision under the Act – particularly with respect to sterility – is carried out by the Sterility Section of the Bacteriological Department of the National Bacteriological Laboratory. The Section has drafted guidelines for sterilization and sterility testing. The draft has been discussed and, to some extent, revised by the Board's advisory committee for control of sterile single-use medical devices and the Board has decided to apply the amended guidelines, until further notice, in connection with inspections and when scrutinizing applications submitted to it. When sufficient experience has been gained from inspections and scrutiny, the guidelines will be revised and given their final form.

1. *Sterilization*

Sterility

Industrially sterilized single-use medical devices shall be manufactured and sterilized under such conditions that not more than one living microorganism is present per million units produced. The pack shall be so designed that the microbiological quality of the products is maintained. Transport and storage shall also take place under such conditions as not to jeopardize this quality.

The quality of the raw materials and hygiene precautions during production shall be such that contamination with particles and foreign matter is prevented and the number of microorganisms in the product before sterilization is kept low. The ability of the production process and hygiene conditions to fulfil these requirements shall be controlled at regular intervals. The National Board of Health and Welfare will issue advice and directives for production hygiene during the manufacture of industrially sterilized single-use medical devices in due course.

Industrially sterilized single-use medical devices are to be sterilized in the sealed product pack. Depending on the material contained in them, different products may need to be sterilized in different ways. The following are examples of suitable methods for industrial sterilization of single-use medical devices.

Autoclaving

Heating in saturated steam under suitable conditions of temperature and time e.g. 121 °C for 15

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minutes. The sterilization time is to be taken starting from the time at which the least accessible parts of the item being sterilized reach the specified sterilization temperature.

The autoclave is to be provided with automatic recorders for time, pressure and temperature. The physical parameters are to be registered for each batch sterilized.

The reliability of the autoclaving procedure is to be controlled at regular intervals using Bacillus stearothermophilus spore preparations intended for control of autoclaving procedures and of identical resistance to the preparations that can be requisitioned from the National Bacteriological Laboratory (1). The spore preparations are to be placed in those places where the sterilization conditions have been found by experience to be least favourable.

Dry sterilization

Heating in dry air under suitable conditions of temperature and time e.g. 160°C for 2 hours or 180°C for 30 minutes.

Dry-sterilizers shall be of such design that an even temperature is reached in all parts of the oven. The apparatus shall be provided with instruments for measuring time and temperature. The physical parameters are to be recorded for each sterilization batch, preferably with an automatic recorder.

Control of the physical parameters is normally sufficient. The reliability of the dry-sterilization procedure is to be controlled at regular intervals, however, using Bacillus subtilis spore preparations intended for control of dry-sterilization procedures and of identical resistance to the preparations that can be requisitioned from the National Bacteriological Laboratory (1).

Ethylene oxide sterilization

Sterilization by means of ethylene oxide gas at suitable concentrations and temperatures and for suitable times, and under conditions ensuring a homogenous gas mixture and suitable humidity in the products sterilized. It is particularly important to check the number of microorganisms on the products before sterilization at regular intervals for products which are to be sterilized by ethylene oxide.

The physical parameters are to be controlled and recorded for each batch sterilized, whenever possible with automatic recorders.

The reliability of the sterilization procedure is to be controlled for each batch sterilized using spore preparations intended for control of ethylene oxide sterilization procedures. The properties of the spore preparations shall be such that they will reveal the presence of insufficient humidity in the products sterilized. Suitable spore preparations can be requisitioned from the National Bacteriological Laboratory (1). The number of spore preparations is to be varied according to the number of units in the sterilization batch, the size and design of the sterilizer, and experience of the reliability of the sterilization procedure with the apparatus used. At least 6 spore preparations per sterilization batch are normally to be used. When using larger sterilizers, 6 spore samples per cubic metre of goods sterilized should be used. If the efficiency and reproducibility of the sterilization procedure can be documented by long experience of such tests, the number of spore preparations may be reduced to 3 per cubic metre of goods sterilized, but with a minimum of 6 per sterilization batch. The spore preparations are to be placed in those places where experience has shown the sterilization conditions to be least favourable e.g. inside packed disposable syringes, tubing etc.

Sin[illegible] distribution prohibited.

Ionizing radiation

Gamma- or electron-irradiation in accordance with the recommendations issued by the International Atomic Energy Agency, 1967, in the Code of Practice for Radiation Sterilization of Medical Products (2).

The reliability of the radiation sterilization procedure is to be controlled in accordance with the requirements of the Code of Practice. The microbiological efficiency of the apparatus shall be established before it is taken into operation and after any essential alterations to the radiation sterilizing equipment (3.1 note 1), but not less often than once a year. Information on suitable spore preparations may be obtained from the National Bacteriological Laboratory (1). The number of microorganisms on the product before sterilization shall not exceed the level at which the radiation dose used will give sterility as defined in the first paragraph of these guidelines under the heading "Sterility". For products with less than 50 microorganisms per unit, for example, a radiation dose of 3.2 Mrad from a cobalt apparatus for sterilization of dry products is to be used. If the manufacturer, on the basis of data collected during long experience with the production concerned, is able to document that the magnitude and type of microbiological contamination (e.g. an average of < 1 microorganism per unit) before sterilization and the humidity, temperature, dose-rate etc. during sterilization are such as to satisfy the sterility requirements as defined in the first paragraph under the heading "Sterility", a minimum dose of 2.5 Mrad may be considered.

Documentation of the sterilization procedures

Before being taken into operation, the sterilization procedures used are to be thoroughly tested so that efficient sterilization of the product concerned can be documented. Instructions for carrying out the tests and unambiguous criteria for assessment of the results of routine controls and measurements are to be drawn up. The results are to be recorded for each batch produced and filed for as long as the batch may be expected to be available on the market, but not less than 3 years. The records are to indicate where and how the individual spore preparations were placed in the sterilizer.

2. *Sterility Testing*

Industrially sterilized disposable articles shall comply with the test for general microbial contamination as stipulated in the Nordic Pharmacopeia.

For product sterilized by autoclaving, dry-sterilization or radiation sterilization as stipulated above, sterility testing of each batch is not normally necessary. For production batches sterilized by other methods of sterilization, each batch is to be submitted to sterility tests according to the methods specified in the Nordic Pharmacopoeia or alternative methods of equivalent sensitivity.

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DISCUSSION SESSION I

Q. by J.E.W. Nygard – USA

Dr Dodson, you mentioned that manufacturers are releasing radiation-sterilized goods without completing a validation of the process or doing a sterility test. I was wondering what the basis of that release is and how it is related to Australian regulations.

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A. by L.F. Dodson – Australia

There are no regulations in Australia prescribing just how sterilization must be performed. The requirement is for the product; it must be sterile and it must comply with the sterilization test. There is, of course, considerable influence of government over this process by way of inspections under the Code of Good Manufacturing Practice, but we have no registration system in this country that requires people to submit for each product what they actually do about it. This was a proposal that was, in fact, rejected by government. The resources we have available do not, in fact, permit us to chase up the devices as closely as we could. The resources are shared between the State and Commonwealth governments. In fact, there has been very little regulation of the device field in Australia.

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Q. by D. McKay – Australia

Mr DeRisio, we have heard mention this morning of the concept of tailoring the level of sterility assurance to the nature of the product or its intended use. This strikes me as being an exceedingly difficult quantity to measure in any logical fashion. Arbitrary levels have already been set up, like this level of 10^{-6}, that is generally accepted. It is just a convenient number, I suspect, rather than being related to anything more logical than that. Is there any sound way of developing these required sterility assurance levels or are they just going to be entirely arbitrary?

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A. by R.J. DeRisio – USA

The Bureau of Medical Devices has not listed specific levels of sterility assurance for particular devices, and maybe if we ever do promulgate standards or work in the development of a voluntary standard development, such a document could define a level of sterility assurance and could define whether or not a particular product should be nonpyrogenic.

In the Agency, we have always regarded that any invasive device, and that is one that is defined as crossing a natural body barrier, must have a sterility assurance of 10^{-6} (probability of one in a million). For certain devices that are noninvasive, such as drapes which are used in a hospital theatre but which do not come into contact with the patient, the level of sterility assurance of 10^{-3} has been accepted.

In the medical pharmaceutical products, of course, we recognize that aseptically filled sterile filtered products are probably processed at a level of sterility assurance of perhaps as good as 10^{-4} and so the argument has been made that perhaps some devices that cross body barriers, and are used to administer these drugs, such as small volume parenterals, might themselves not have to have a sterility assurance level of 10^{-6}. Personally, I somewhat resist that, and because we recognize that probability of a contamination and of an infection is an additive concept, where practical the manufacturer should do what he can to maintain an acceptable level of sterility assurance.

We see also that the new dose-setting technologies are an attempt to provide a lower sterility assurance level for devices than the traditional 10^{-6} level. One other thought currently with medical devices is that many processes are validated to 10^{-6} on the basis of a biological indicator in an overkill situation. We recognize that even if there is variability in the cycle or the bioburden, the degree of sterility assurance is far better than one in a million. I believe, the crucial area is the devices that are *in vitro* products and are handled aseptically. We do not expect a sterility assurance level better than 10^{-3}.

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Comment by L.F. Dodson – Australia

I am not so impressed by the concept of the sterility as one in 10^6 because it is a mathematical extrapolation of pretty inadequate data, and I think it is inadequate because I do not believe that it is feasible to determine accurately the bioburden on particular products. I just do not think, we get a very good estimate by bioburden and, for example, David McKay will be referring to something that happened many years ago. Someone complained that a 'giving-set' was not sterile, and it was sent to us by a microbiologist in Tasmania who said: 'this is not sterile; you had better check it out'. So, we checked it out by using what is still the standard US sterility test for these things, running a solution through it, and we could not grow any organisms; and then he pointed out that we should incubate it with the medium inside the material instead of inside the tube; and as soon as we filled the tube up and clipped the ends and threw it in an incubator for a few days, we grew large numbers of organisms. Now, if the tests for determining bioburden are susceptible to errors like that, and I suspect often that microorganisms are very firmly attached to devices, I wonder, are we perhaps rather bemused by these figures. They are only as reliable in my mind as the estimate of bioburden, and if that estimate is not accurate, can we be sure that this concept is as valid as all that.

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Q. by S. Riley – Australia

Mr DeRisio, you stated that the FDA has no data available stating any safe use of resterilization of single-use items. Is it your opinion that the FDA will look into this and state any safe use of resterilization of single-use items?

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A. by R.J. DeRisio – USA

This compliance policy guide was originally prepared in response to some criticism from health-care providers that we were over restrictive and that our position could result in very high health-care costs, and I think that this is why the statement was made that there were no data available. In fact, we have not done any work within the Agency because of our limited laboratory work, and if work had been done, it was not readily published or available to us.

Now, I want to make sure that I did not miss the last part of your question; could you repeat the last part of your question and comment, please.

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Q. by S. Riley – Australia

Is it likely that the FDA will set any regulations or specifications for safe reuse of single-use items?

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A. by R.J. DeRisio – USA

I do not believe that we would attempt to do that. We tend to be very industrially and commercially oriented, even in our staff. Although we do have health professionals (nurses and doctors), I do not think that we have the resources in terms of laboratory or even in terms of availability of samples to conduct studies on resterilization, so we have looked to the health professional associations to do that kind of work and to the device manufacturers themselves.

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Q. by B. Rawal – Australia

I am interested to know if there is any development with regard to the role of microbial products in terms of toxins. Mention was made this morning about the presterilization microbial load. I assume, it refers to the number of viable organisms, but in such a situation we could also have preformed microbial products, like toxins, and these could be very important in terms of products that are sterilized for human use and sterilized by gas sterilization, which may not have any effect on toxins.

My question to the panel, therefore, is whether or not preformed microbiological toxins are required, or would be required in the future, to be examined before sterilization by gas or irradiation.

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A. by P.T. Doolan – France

I know of no European regulation in draft form or in place right now that envisages that subject. When you say toxins, are you referring to dead microorganisms.

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Q. by B. Rawal – Australia

I am referring to staphylococcal toxins often found in products used for implants and in things of that nature. I am aware that there is no statutory regulation at present, but I am talking about the possible development in the future.

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A. by P.T. Doolan – France

I think, the answer is ‘No’. I am sorry not to be able to answer it more fully, but I am having difficulty in getting all of the question.

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A. by L.F. Dodson – Australia

I know of no evidence for the need to do that except that there is a need to have a very low bioburden to reduce the possibility of endotoxins occurring, which is of course checked by pyrogen testing.

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A. by R.J. DeRisio – USA

We have always advocated that manufacturers control the bioburden to prevent the possible increase in endotoxins because of a large bioburden on a device. Fortunately, most of the products that we regulate tend to have low bioburden to begin with and also do not support growth. Now, with the LAL Method there is a provision for approval of a process control release procedure where the manufacturer would look at possible sources of contamination with particular bacterial endotoxins from gram-negative species, but it could also include other types of toxins, presumably from a microbiological control standpoint. However, from the endotoxin standpoint, we would encourage the firm to look at components and personnel, water that might be used for rinsing the device, or at a dipping process, or to look at possible sources of contamination in the product that might be masked by the time the finished product was tested. Firms that elect to validate a process control procedure for pyrogen control or endotoxic control can then reduce other finished product testing requirements to as few as just one device per lot, instead of the current *USP* provision for ten subassemblies, for example, per production day.

I know of no incident in my country of staphylococcal toxin contamination. It has been reported in other products that we regulate, such as foods, when a product after formulation was held in warm conditions and not sterilized for some reason for an unduly long period.

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SESSION II

Hospital Sterilization

Chairman
John K. Clarebrough

Royal Australasian College of Surgeons
Melbourne, Victoria, Australia

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SESSION II

Hospital Sterilization

Introduction to Session
John K. Clarebrough

This Symposium is timely as I believe that the subject of infection will be of continuing concern over the next ten years. It is wise to adopt preventive measures rather than be forced into the defensive position of having to react to an unforeseeable situation. We have been put in this situation by the current epidemic in hospitals of multiple resistant strains of *Staphylococcus aureus*.

Thus, infections are, and will continue to be, a major problem. There are clearly many factors to be considered and in looking at the hospital scene many changes have occurred over recent years. One of the most significant changes is the escalating use of non-natural materials, the use of artificial aids towards healing, and the replacement of human parts. These replacements may be by human organs or more frequently by synthetic products. In the case of transplantation procedures, success often depends on the interference with a patient's resistance to infection by chemical or other means.

The implantation of foreign materials, such as hips, knees, heart valves, arterial prostheses, is now being performed on an escalating scale. Just over one thousand patients had artificial valves inserted in this country in 1980-81.

In addition to routine surgical procedures, renal dialysis, cardiopulmonary bypass, and total parenteral nutrition all demand the insertion or infusion into the patient of non-natural products in situations where infection can be the real problem and the outcome may be devastating.

The safe passage for a patient through today's modern hospital and the freedom from major infection depend on many factors, such as the environment of the hospital, freedom of the staff from a carrier role, meticulous surgical and medical techniques, and particularly the sterility of those substances that will be inserted or infused into the patient.

There is an assumption that products used in hospitals are 'sterile' but in fact may not be. Jocelyn Kelsey in his classic paper on 'The Myth of Surgical Sterility', pointed out that sterile meant by definition 'freedom from microorganisms'. This, he went on to show, was not always possible, and that in practical terms he suggested a new term, 'the state of having been sufficiently freed from micro-organisms to be deemed safe for some special purpose by some competent body'.

It is implicit that the setting of standards by an appropriate authority and a review of these standards are maintained. These concepts are termed 'quality assurance' and should be applied to

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health care, and should be particularly operative in health-care delivery systems.

In the ten years since Jocelyn Kelsey made his statement, we have come a long way. Hopefully, we may in the field of hospital sterilization attain practical use sterility.

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Good Hospital Practice – Australia

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Sterile manufacture in hospital pharmacies in Australia is covered by the *Code of Good Manufacturing Practice for Therapeutic Goods* of the National Biological Standards Laboratory. Compliance is expected to be much more rigidly enforced in the future. There is no similar nationwide code for the operation of hospital Central Sterile Supply Departments (CSSD) although standards for sterilizing equipment and packaging materials are laid down by the Standards Association of Australia. There are substantial variations in CSSD operation between the States, and a number of problems are faced by personnel. It is anticipated that the Australian Council on Hospital Standards may be influential in the future in promoting a more uniform approach to hospital sterilization.

Compliance with codes of good practice is complicated by the lack of satisfactory evidence for a beneficial effect on patient safety of many of the procedures involved, and by the lack of suitable guidelines for quality control of very small scale operations. Both regulators and manufacturers would be unwise to underestimate the confusion about many aspects of decontamination, sterilization, and the usage of sterile materials that may occur in Australian hospitals.

In a symposium such as this, 'good practice' in sterilization inevitably has to be defined as a code of regulations relating to the manufacture or subsequent processing of goods designated as 'sterile'.

The major stimulus for the formulation of such codes has been the occurrence of episodes of serious infection, usually involving patient deaths, that can clearly be attributed to materials used in patient care. When such a disaster occurs, then obviously steps must be taken to prevent a recurrence.

We can therefore say that the code of regulations which constitute good practice in sterilization is aimed at preventing the recurrence of episodes of infection caused by equipment or materials used in patient care.

This intention of good practice is one that cannot be argued with. It can, however, be argued that this essentially passive approach – waiting until a disaster occurs (and remember that usually deaths will occur before the true nature of this sort of problem is recognized) – is inadequate. The aim of such a code of regulations should be to prevent any possibility of infection occurring due to microorganisms derived from items used in patient care. Unfortunately, the absolutes that are implied in this statement are totally impracticable, so that in the end we are left with a statement that is filled with some very uneasy compromises – such as this one.

'Good practice in sterilization is a code of regulations aimed at preventing the failure of procedures which were designed to ensure that designated equipment and materials achieve a state corresponding to the current accepted definition of sterility.'

The current definition of sterility is, of course, very much the overall concern of this Symposium. It is not the absolute state that the dictionary would have us believe, and this fact is not sufficiently appreciated in medical practice. Jocelyn Kelsey gave a practical definition of sterility as 'the state of

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having been sufficiently freed from microorganisms to be deemed safe for some special purpose by some competent body'. Again some very uneasy compromises are implied, so perhaps we should look at some of the questions that are raised by the concept of good practice as a code of regulations.

The first question is who should define the elements constituting good practice in any given process. In theory, of course, the elements would be produced by agreement between the practitioners of the process and the regulatory authorities. In practice, this sometimes does happen, at least in part.

The second question concerns some of those uneasy compromises that we skated over earlier. What sort of evidence do we have on which to base our working definition of that apparently simple word 'safe'? What sort of things need to be 'safe'?

In terms of preventing the recurrence of established disasters, we do have at least some information on which to base decisions, but when we go beyond this to consider sources of potential infection, then the quality of evidence becomes very important. Let us take a simple example. Let us postulate that standard bed linen is a potential source of infection to hospital patients. It would be easy to devise experiments supporting this hypothesis. Why therefore do we not sterilize all bed linen?

The cost would be formidable, and cost is an increasingly important consideration. There are practical problems of processing and storage and perhaps the most formidable problem of all is the difficulty of overcoming the inertia inherent in all established hospital routines.

We would hastily re-examine our evidence and decide that the simple decontamination of standard laundering is quite adequate.

Achieving a practical compromise by setting reasonable minimal standards is quite easy in this case. For many processes and many items, it is much more difficult. Who should determine the minimal standards – the regulators who have their eyes firmly fixed on the potential infection, or the regulated who have their eyes equally firmly fixed on the practical problems.

I must say at this stage that I have considerable sympathy with the regulators. Regulation of Australian hospitals is a particularly complex subject. I shall not attempt to explain the system of health-care delivery in this country, partly because I am not sure that I understand it myself, but something does need to be said about the hospital system. The great majority of acute care hospital beds in this country are in public hospitals for which the various State Departments of Health are responsible for funding, private hospitals accounting for only a small proportion of acute care beds. Public hospitals are controlled by local hospital boards responsible to the State Governments. The State Departments of Health can exercise only a very limited direct control over the hospitals for which they are responsible, mainly in such areas as staff establishment and the purchase of major items of equipment.

Thus, there is a curious anomaly. Private hospitals (and manufacturers) are regulated by means of a licensing system. There is no licensing system for public hospitals and these hospitals until very recent times have been virtually unregulated in the terms that we are talking about today. If a State Department of Health was dissatisfied with the activities of one of its own hospitals and that hospital refused to mend its ways, then the only recourse open to the Government was to dismiss the hospital board.

For a number of reasons, this system has been found to be unsatisfactory in recent times and changes are in progress that will provide a more direct control over the day to day running of public hospitals.

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One of the first areas where these new policies will be manifested will be in the area of sterile manufacture.

I have already stressed the importance of the occurrence of disasters as a stimulus to the formulation of codes of good practice. Such an episode occurred last year in a hospital in New South Wales involving a parenteral solution manufactured in the hospital pharmacy.

Of all hospital departments, the pharmacy is the area where the greatest risk is likely to arise from faulty sterile manufacture.

Certainly, it is the area where there has been most activity in defining good practice in recent years. In 1975, an epidemic of hospital-acquired urinary tract infections due to *Pseudomonas cepacia* was tracked back to a contaminated pharmacy water still. Coincidentally a code of disinfection practice was being prepared for the New South Wales Health Commission. Following the introduction of this code, amendments were made to the *Therapeutic Goods and Cosmetics Act, 1972*, dealing with antiseptics and disinfectants. These regulations included a new set of performance standards, a labelling standard, and prohibition of certain representations. One such prohibition concerned the use of the word 'sterilization' in connection with disinfectants, except in specific cases where use of a product as directed can be shown to achieve sterility with accepted probabilities of success. Written authority for this use is required.

More recently, a recommendation has been made that all antiseptics (defined as agents used for preventing, arresting or treating infections, or destroying or inhibiting pathogenic microorganisms on the human body or its mucous membranes) used in hospitals should preferably be sterile. A further guideline states that antiseptics for use in body cavities must be sterile.

The problems involved in sterile manufacture in hospital pharmacies have, of course, been recognized for some time. The *Code of Good Manufacturing Practice for Therapeutic Goods* formulated by the National Biological Standards Laboratory has an appendix entitled 'Supplementary Notes for Hospital Pharmacists'. The Australian Council on Hospital Standards in its 'Accreditation Guide' specifically refers to adherence to this Code and its Supplementary Notes as one of the essential components of Pharmacy Service policies and procedures. Accreditation in this country is voluntary and no penalties result from failure to achieve it. Nevertheless, the Council has been extremely influential.

While the appendix is designed to make the Code applicable to bulk compounding and packaging of sterile therapeutic goods, there is also a section devoted to the addition of drugs to intravenous fluids, also regarded as a manufacturing process. Intravenous administration of drugs is nowadays the general rule in hospitals in this country. Intramuscular injections have become relatively uncommon. This has been a worrying trend since there are a number of risks involved in intravenous administration, quite apart from that of bacterial contamination.

The most hazardous procedure in the hospital pharmacy, however, is the bulk compounding of sterile fluids for parenteral administration. In recent years, this has most commonly been undertaken in the preparation of intravenous feeding solutions for total parenteral nutrition.

It should be made clear that only a relatively small proportion of hospitals in Australia are engaged in bulk sterile manufacture of this sort. At the Royal North Shore Hospital, it has been the policy for some years that sterile parenteral fluids should be obtained from commercial sources. We do, however, carry out aseptic admixture procedures to prepare specific formulations for individual patients.

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Following last year's incident, referred to earlier, the Health Commission of New South Wales issued a circular requesting hospitals to cease all bulk manufacture of large volumes of intravenous fluids. At the same time, the Health Commission convened a Working Party to investigate the manufacture of intravenous fluids in hospitals.

When the Working Party had completed its review of the bulk manufacture of large volume intravenous fluids in public hospitals, the Health Commission issued a further circular. The phrase 'are requested' was no longer used. The word 'required' was used instead. Specifically, the Health Commission required 'public hospitals to cease all bulk manufacture of sterile therapeutic goods until an approval has been given in each specific case. Any approval will be subject to:

1. a general policy on the bulk manufacture of sterile and other therapeutic goods to be determined by the Commission;
2. compliance with the Australian Code of Good Manufacturing Practice for Therapeutic Goods.'

Hospitals were further advised not to commit resources to bulk manufacture pending finalization of policy guidelines.

In the meantime, the Working Party, now enlarged, was investigating the whole area of pharmaceutical manufacture in public hospitals in New South Wales. The first guidelines were issued on 10 May 1982 and outlined good manufacturing practices for the preparation of bulk nonsterile and nonbulk sterile pharmaceuticals.

A draft report covering bulk sterile manufacture has been issued, causing some consternation.

The first surprise lay in the definition of bulk sterile manufacture.

Bulk manufacture in relation to sterile parenteral products was defined as including preparations made in advance for potential patients and total parenteral nutrition preparations made more than 24 hours in advance. So defined, the practice followed that aseptic admixture of commercial sterile fluids was regarded as bulk manufacture, unless the solutions were for immediate use. The working party defined immediate use as 'use within 24 hours'.

The 24-hour rule is an interesting one. It seems to stem from the experience in the United States during the epidemic of septicaemia attributed to microbial contamination of elastomer linings in the screw caps of commercially prepared intravenous solutions in 1970 and 1971. In investigating this epidemic, the US Center for Disease Control (CDC) found that patients who had their intravenous fluid administration sets changed within 24 hours had a substantially lessened risk of septicaemia. The CDC consequently recommended that intravenous administration sets should be changed routinely every 24 hours on the basis that between 4 and 10% of intravenous fluid in use contained microbial contamination. The publicity consequent upon this episode and similar incidents in Great Britain did a lot of damage to the image of commercial sterile manufacture. This apparently poor record of quality control, along with an increasing requirement for specialized fluids, tended to encourage hospitals to undertake manufacture themselves.

In fact, regrettably, although rare, infection does occur in hospital patients associated with intravenous therapy. Clinically significant sepsis occurs as a consequence of intrinsic contamination of intravenous fluids. The great majority of significant episodes appear to be due to extrinsic contamination resulting from manipulations of the administration set or from infection of the cannula puncture wound. A recent study of the rate of contamination of intravenous infusion fluid associated with a change of administration set every 48 hours as opposed to a change every 24 hours showed no

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significant difference. This study showed a 2% contamination rate of fluids in 600 patients, but in no case did clinical bacteraemia occur. Some idea of the difficulty of obtaining useful evidence in this type of situation can be gauged from the estimated requirement for a population of 50 000 patients to obtain information on clinically significant sepsis.

Instituting a policy of changing administration sets every 48 hours offers substantial savings in the cost of equipment and in personnel time. This interval of change has now been recommended by the CDC in its most recent guidelines for prevention of intravascular infection.

Twenty-four hours is thus no longer quite the magic interval that it once was. It is even less so when one considers that the high dextrose concentrations in total parenteral nutrition solutions are actually toxic to bacteria. (This is not true of lipid solutions, of course.) I believe that there is no evidence to support the implication that 'immediate use' of total parenteral nutrition solutions circumvents the problems of microbial contamination or confers additional patient safety.

This broadened definition of bulk sterile manufacturing presents some very definite problems for hospitals faced with the need to provide a seven-day-a-week service. The Australian working weekend is peculiarly expensive. The industrial awards under which most hospital employees work provide for an additional 'penalty' payment of 50% for hours worked on Saturday and 75% for hours worked on Sunday. Furthermore, in any hospital pharmacy there will only be a very limited pool of personnel sufficiently experienced in aseptic procedures to be entrusted with the task.

The thrust of the Working Party's draft recommendations has thus been to divide the items likely to be manufactured in hospital pharmacies into four lists (see Appendix). List A are products under the heading of bulk nonsterile manufacture. List B includes the nonbulk sterile products defined as either volumes of 100 mL or less or larger volumes for 'immediate' use. Both of these categories are covered by the previously published guidelines and endorsed by the Working Party. It added an additional comment for the List B nonbulk sterile pharmaceuticals. The Working Party said that: 'random routine laboratory testing should be carried out on products within this group'.

This is all that was said on the subject. I presume that what was said was intended to be a vague gesture in the direction of quality control and I find this very worrying. Quality control and quality assurance are subjects that are too frequently misunderstood in hospitals. Many hospital personnel find the concepts and their implications very threatening, and there is a great temptation to reassure oneself with easy but essentially meaningless procedures. I believe that either proper quality assurance regimes should have been specified here or the subject omitted completely.

List C includes unusual or limited-batch sterile pharmaceuticals and also, by default, aseptic admixtures that will be kept for more than 24 hours before administration.

The Working Party stated that these products should be manufactured in as close a conformity as practicable to the Australian Code of GMP. Further, hospitals must receive prior approval from the Health Commission before embarking on such manufacturing. Finally, these hospital pharmacies will be subjected to regular inspection under the Code of GMP.

All this seems very reasonable. However, on several readings of the Supplementary Notes for Hospital Pharmacists and the Guidelines on Tests for Sterility, I cannot find the sort of guidance for process validation that seems to be called for.

The final group of products, in List D, includes standard bulk sterile solutions. It is recommended that these products should only be prepared in full compliance with the Code of GMP. The Working Party considered that at present no NSW hospital facilities meet full Code requirements.

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All in all, the working party recommendations add up to very stringent controls on manufacturing in hospital pharmacies that are far more stringent than the recently published CDC guidelines on infection control relating to the pharmacy (containing a recommendation that fluid should not be routinely cultured, either before or after admixing). It seems certain that part or all of these recommendations will be adopted. Needless to say, at present hospital pharmacists in New South Wales and in the other states are anxious and confused, and the sooner firm decisions are made and guidelines issued on process validation, the better.

There is no doubt that considerable cost will be involved. Will the cost be justified in terms of additional patient safety? Frankly, I do not know.

Over the last six years there has been increasing pressure in the area of infection control to produce evidence that measures advocated to prevent infection do in fact achieve this end. It has become clear that hard evidence of efficacy exists for only a handful of measures. Further, the task of collecting convincing proof in many instances appears to be beyond our resources, as exemplified by the simple problem of the interval for changing administration sets.

I have concentrated on sterile manufacture in the hospital pharmacy because that is where most of the activity in relation to good practice in Australia has been. There is really no equivalent to the Australian Code of GMP regulating the manufacturing processes of hospital sterile supply departments, although there are some elements in the Code which are clearly relevant.

When we look at the elements of good practice in hospital sterilization there is really only one area that could be considered to be codified on a nationwide base and that is equipment and materials. The Standards Association of Australia has been laying down for some years now requirements for sterilizers, packaging materials for sterile goods, and other equipment. The purchase of major items of equipment by public hospitals is one area where State Departments of Health usually exercise direct control. Hence the recommendations of the Standards Association are of some importance in determining the practice of sterilization.

The Accreditation Guide of the Australian Council on Hospital Standards has virtually nothing to say specifically about Central Sterile Supply Departments. The CSSD is lumped together with housekeeping, laundry, maintenance, infection control, sanitation, and fire safety under the heading of 'Environmental Services'. In all fairness, it should be made clear that hospital accreditation has mainly concentrated on organizational and safety aspects in most departments.

Failure of sterilization procedures in a hospital CSSD is far less likely to engender recognizable clinical consequences than failure of sterilization procedures in a hospital pharmacy. The most critical part of the sterilization of reusable equipment is the initial cleaning rather than the sterilization process itself. Hospital staff can generally be relied on to refuse to accept obviously dirty instruments or wet 'sterile' linen. There are, however, some areas where a code of good practice might be welcomed, apart from the areas of safety and basic efficacy of the sterilization process that are already regulated.

The first of these which might be termed 'professional standards' is the recognition of hospital sterilization work as a skilled activity, requiring appropriately qualified personnel who have undertaken recognized programmes of training. The Sterilization Research and Advisory Council of Australia has been active in promoting this concept but has not yet been totally successful. There are still some parts of the country where the sterile supply department is regarded as a simple adjunct of the operating theatres, requiring no particular expertise.

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Some of the other problems of hospital sterilization departments in Australia are universal. It is equally difficult to try to determine standards in relationship to storage facilities, and consequently shelf life, here, as it is anywhere else in the world. One of the greatest failures of the sterilization industry as a whole has been its inability to present a clear message to consumers on this matter.

Similarly, the problem of decontamination of delicate and sensitive instruments has been handled no better in Australia than anywhere else. In most hospitals, the CSSD is neither offered, nor accepts, any responsibility for cleaning and decontamination of flexible fibreoptic endoscopes. The problem in Australia is that the demands of the physicians for a very rapid turn-around time for these expensive instruments are not compatible with the requirements for effective decontamination. The manufacturers of these instruments have been somewhat unhelpful.

New challenges are emerging all the time as invasive monitoring procedures are increasingly carried out on susceptible patients using pressure monitors, television monitors, etc., clearly not designed with the problem of sterilization in mind.

As these procedures become more common, the cost rises. Frequently, the procedures involve quite expensive items of equipment that are intended to be disposable, for example a Swann-Ganz catheter may cost the hospital $97. Sterilization of these items for reuse appears to be a very attractive proposition and the manager of the CSSD is often put under intense pressure to co-operate. This problem was recently tackled by the Health Commission of New South Wales in a circular dated 19 May 1982. This circular advised that' single use' items should not be reused unless all criteria for safety and efficacy of the processed product could be met. An additional criterion was added on cost effectiveness, namely that reuse and/or sterilization had to offer significant savings. Unfortunately, this circular did not receive the publicity it deserved.

The extent to which hospitals prepare their own sterile disposable items varies widely. In October 1981, the National Biological Standards Laboratory, in carrying out tests on wound dressings labelled or required to be sterile, found many in a state of contamination which was described as 'heavy and serious'. Interim controls were instituted, requiring sterility testing of sterile goods and testing of nonsterile goods for the presence of pathogenic bacteria.

Revised controls are now proposed. Nonsterile goods will be required to have a total count of less than 10 000 aerobic microorganisms per gram. The draft proposals make no attempts to distinguish between pathogenic and nonpathogenic organisms. Absolute bioburden will be the criterion. As a clinical microbiologist, I must agree whole-heartedly with this concept.

Of three categories of goods, there are goods that are required to be 'sterile-in-use'. These involve dressings intended to be applied to broken skin or burns and goods used in surgical procedures.

Another category will be goods required to be sterile-in-use, which are supplied to hospitals in a nonsterile state. Such goods will be required to be clean and have a total count of aerobic organisms of less than 10 000 microorganisms per gram. It is proposed that such goods shall be labelled 'Non-sterile – For Hospital Use Only – Sterilise Before Use'. The intention of this labelling seems quite straight forward. However, at least two companies which introduced this type of labelling as an interim measure have discovered that the sudden appearance of such labels in hospital stores departments leads to confusion and outrage.

This category is a valid one, but I believe that any manufacturer who proposes to supply goods with such a label would be well-advised to make absolutely sure that the recipients understand what

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it means. The events of the last 18 months in the area of sterilization have, unfortunately, made it clear that the present lines of communication within the health-care industry cannot necessarily be relied upon to ensure effective dissemination of information.

The application of microbiological quality controls to this class of goods will be of help to hospitals preparing their own sterile disposable goods. However, the onus will be on the hospitals to examine the uses of items in this category to ensure that sterilization is performed where the subsequent use requires it.

In summary, sterile manufacturing has been performed in Australian hospitals in the past with surprisingly little, if any, regulation. In response to recent events, however, one expects that considerably more attention will be paid to good practice in this area.

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Appendix

List A

The products that it is considered could still be prepared properly and safely under present facilities in hospital pharmacies are:-

(i) ENT preparations

(ii) oral preparations

(iii) rectal and vaginal preparations

(iv) topical preparations

(v) disinfectants

(vi) repacking of prepared pharmaceuticals from bulk

List B

There is a second group of products that also may need to be prepared regularly in hospital pharmacies but to which certain conditions for their preparation should apply. These products with their conditions are:-

(i) single units of TPN for "immediate use" as defined in Section 3 – need to be prepared aseptically, by suitably trained staff, under laminar flow conditions

(ii) cytotoxic drugs
– need to be prepared aseptically under suitable conditions as specified in a separate report on cytotoxic drugs

(iii) I.V. additives (when prepared in the Pharmacy) for "immediate use" (not bulk)
– need to be prepared aseptically as single bottles, by suitably trained staff, under laminar flow conditions

(iv) eye drops
– only single units aseptically filled, or bulk quantities which are terminally sterilised.

(v) ampoules and vials
– small batches (approximately 10 units) which are terminally sterilised

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– prepared aseptically for "immediate use" only

(vi) radiopharmaceuticals
– made up by specially trained personnel under appropriate conditions

(vii) antiseptics
– should preferably be sterilised

(viii) bladder irrigations
– prepared aseptically in small batches which have an expiry date of less than 5 days.

These operations should be conducted, where applicable, in conformity to the Guidelines in Commission Circular 82/133. The Commission should extend the present Guidelines by including, in due course, a guideline on random monitoring of chemical and microbiological quality. In the meantime, protocols may be developed by hospitals to provide an index of quality of the products made.

List C

Under some circumstances, hospital pharmacy departments may be required to manufacture unusual or limited batch sterile pharmaceuticals not included in the previous list of products.

These may include:-

(i) specialised bulk TPN and specialised Large Volume Parenteral fluids
(ii) specialised bladder irrigations
(iii) ampoules and vials in batches of greater than 10 units
(iv) bulk eye drops, aseptically filled
(v) specialised peritoneal dialysis solutions

When such hospitals must meet the need of their patients for those products they should be manufactured in as close a conformity as practicable to the Australian Code of Good Manufacturing Practice, and the hospitals must have received prior approval from the Commission. These hospitals should be regularly inspected under the Code.

List D

The following products should only be made in approved commercial or hospital manufacturing facilities and in full compliance with the Australian Code of Good Manufacturing Practice. At present, no NSW hospital facilities meet full Code requirements. Therefore, none of the products on this list should be made in hospitals:-

(i) bulk standard-formula TPN solutions
(ii) bulk bladder irrigations
(iii) bulk cardioplegic solutions
(iv) peritoneal dialysis solutions

The Working Party agreed that these products would usually have standard or standardisable formulae and hence could be made available commercially.

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Good Hospital Practice – Worldwide

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Introduction

The phrase ‘good hospital practice’ encompasses a broad range of hospital activities, but this presentation will be selective in addressing specific issues of hospital operations that are involved in sterility attainment, measurement, and retention. Examples in the areas of hospital processing procedures, infection control situations, aseptic medical practices, sterility testing, and parenteral solution manufacture will be specifically discussed. I will point out scientific issues that are controversial and questionable, invite the audience into a dialogue with regard to methods, procedures, and standards that should be developed to resolve these issues and develop acceptable worldwide standards for hospital operations. Through co-operative dialogue and agreement of medical professionals, the problems of infection control and medical practice will lead to better controls of hospital operations and reduced potentials of treatment-induced and nosocomially-acquired infections.

The title of this presentation has an awesome note, because it suggests that the author will take it upon himself to be judge and juror for hospital practice procedures that are in use worldwide. To dispel this notion early, the subject matter will encompass some common controversial issues that hospitals face in various countries of the world. Due to the knowledge and personal experience of the writer, there will obviously be some bias, but I trust that it is based upon scientific and technical judgment rather than the narrow-mindedness based upon unproven academic or technical trivia.

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Good Manufacturing Procedures Issues

As the title suggests, the phrase 'Good Hospital Practices' encompasses a broad range of hospital activities from administrative procedures to clinical practices. Organizational charts, building designs and maintenance, and other aspects of hospital operations will not be discussed since they are not directly involved in the issues of clinical practice or to procedures to reduce or control nosocomial and other types of infections. Hospitals of all countries should develop their own locally necessitated operational procedures that best suit their administrative control and these procedures should preferably be documented in written form that is available to hospital personnel involved in specific activities. The document should set the stage and the basic rules of what is expected as a standard within the hospital and also the satisfactory expectations of routine operational activities within the hospital or specific departments. In the US, each hospital sets up its own operational control manual based upon guideline recommendations by a number of publications that include the *Accreditation Manual for Hospitals*, published by the Joint Commission on Accreditation of Hospitals (1), the numerous guidelines published by the Communicable Disease Centers (2-6), and the American Association for the Advancement of Medical Instrumentation (AAMI) documents entitled Good Hospital Practice: Steam Sterilization and Sterility Assurance (7) and others (8-10). In other countries, somewhat similar documentational approaches are performed and many are patterned similarly to those published in the US. Most operational control manuals are highly idealized approaches to the specific issues of infection control and hospital operations. More importantly, these guideline manuals serve as the basis of hospital internal standards by which administrative judgments can be made on departments and individuals. Whenever governmental or pseudo-governmental guidelines are published, enforcement and control of these proposed regulations are problematic because of internal hospital administrative practices, governmental restraints, and legal aspects. However, it is good business sense to set up a form of Good Hospital Practices Guidelines to set minimal or basic standards for hospital operations. In the US, private, city, state, and federal hospitals act independently without heavy government controls, but minimal general standards must be maintained to protect the patients. However, in other countries where hospitals are federally controlled and supported, patient care standards are governed by bureaucratic agencies rather than local hospital-developed guidelines, and all too often these bureaucratic regulations or guidelines are impractical or redundant for a particular hospital's operation. Another issue of difference in world hospital operations is whether or not health care is nationalized. Under those conditions, bureaucratic regimentation prevails over flexible local needs and, hence, brings up questions of effectiveness and accomplishment. In the US, where medicine is not nationalized, the patient has several recourses in the event of suspect medical services, including the ever ominous presentation of a malpractice suit. All hospitals do require some types of standards and guidelines under which they operate, to protect the patients and continually improve patient care.

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Infection Control Issues

One of the most overworked and abused medical phrases, 'Infection Control', is the catchcry of most hospital operations relating to patient care. All medical and administrative issues generally are focused on techniques, procedures, general operating procedures, and treatments to control infections in hospitalized patients. Both medical and surgical patients have equivalent propensity to exacerbate the conditions of the illness under which they came to the hospital, and have also the potential to acquire additional health problems as a result of their hospital stay. All hospitals of the world are similar in that it is necessary to have good hospital practices to control naturally and hospital-acquired infections in the health treatment centres. Clinically recognized infections of incoming patients can be easily managed and controlled by applying precautions and treatments generally recognized internationally and standardized. However, two spectres hover over hospitals and have no geographic bounds: indigenous or endemic infections that are becoming epidemic through the jet age and the ever-present nosocomial infections with their changing panorama of causative or implicative infective agents. A rather naive solution to control these infections is to have all patients under strict isolation with tight infection-control practices, namely to treat by decontamination procedures or sterilization all recognized and unrecognized items having patient contact as being potentially infectious. Such a practice would not only be futile but also highly costly. Since this conference focuses on sterilization procedures, it is obviously advantageous to use sterilization processes to reduce or break the chains of spread of hospital infections. Using a sterilization process is a guarantee of the destruction of known and unknown infectious agents on articles having patient contact and is the prime method of finite decontamination. Unfortunately, not all objects are stable to standard hospital sterilization processes and alternatives of decontamination, such as disinfection, are employed that do not produce absolute microbial destruction. The decision of whether to use sterilization or disinfection for rendering articles safe to handle by reprocessing personnel, or safe for subsequent contact with patients, is a foreboding one that all hospitals in the world must make at one time or another. The absolute and not debatable approach is sterilization, because the processes are well developed and easily controlled and monitored. Disinfection, on the other hand, with well-proven chemical or physical processes, can fail in the destruction of all microbial forms, and routine monitoring of the process is not feasible or practical at present within the time limits of hospital operations. The efficacy of any chemical and physical disinfection process is not only dependent upon the disinfectant and its ultimate total contact with the object, but also upon the degree of cleanliness of the article before the disinfection process is applied. Soil residues left on the article will severely inhibit or prevent attainment of microbial disinfection satisfactorily to provide adequate patient safety. Some articles requiring disinfection as the only microbial control measure may not be amenable to total disinfectant exposure by immersion or contact due to component incompatibilities. It is therefore very questionable whether disinfection can be adopted in world hospitals as a safe method for decontamination and reuse of articles subjected to the process. A good example of worldwide dilemma of the disinfection/sterilization issue is the reuse of all types of endoscopes. Numerous recognized health agencies worldwide have taken different positions on this particular issue but there are very few authorities, health care and other, who will totally support the absolute standard that all endoscopes must be delivered and employed sterile for any patient procedure. Arguments are continually made by physicians, hospital administrators, nurses, and paramedics that

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sterilization cannot be employed, except for the first patient of the day, because the endoscope turnover time is short, or that the costs of additional endoscopes are too high to have a satisfactory inventory of them for each day's operations. This argument has always been weak even though publications supporting sterilization have appeared (11). Recent articles by Hawkey et al. (12) of the UK and M.T. Sammartino et al. (13) describing specific patient cross-infection by endoscopes with *Salmonella* and *Pseudomonas* spp. are documented evidence to justify the need for sterilization of endoscopes. Data and publications from the US and other countries may not have appeared because of the high potential of litigation by patient malpractice suits. Nationalized health care countries may provide additional documented clinical evidence to support the position that sterility is a requirement for all endoscopic instruments.

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Aseptic Medical Practices

In concert with any hospital infection control programme and controlled hospital sterilization procedures, reinforced programmes should be instituted to have total agreement and acceptance on aseptic practices of many hospital operations and procedures. Breaches of aseptic practice occur daily in all world hospitals to the jeopardy of the hospitalized patients. All too often the principles of aseptic practice are given in theoretical terms during the early training and education of key hospital professionals, i.e. physicians, surgeons, paramedics, nurses, and clinical technicians, but by the time they have reached clinical practice or graduation, these concepts are totally forgotten or abandoned. Take for example the routine practice of drawing blood samples. In hospitals, sterile lancets and pipettes are provided in commercial and hospital packaging. Sterility of these articles becomes academic, as the hospital professional rips the articles from their packaging with unwashed hands. The skin site is rinsed with an alcohol or iodophor swab or pledget in a time inadequate even to disturb the skin microbial colonies. This is then followed by lancet puncturing, that may have been mishandled and contaminated by the hands. Blood is sampled by the pipette smeared by skin bacteria and finally followed by the magic alcohol or iodophor pledget. The picture is quite clear that in simple procedures such as these, all the elements of aseptic procedures have been provided with sterile supplies, but sterile practice is left behind. Many will argue that the safety issue of a blood-letting procedure is inconsequential, but no one takes time to think about the fact that the same procedure is used on critically ill patients, such as in renal transplantation, cardiac problems, and patients treated with immunosuppressive drugs, where there is a high risk of contamination.

Another area of worldwide breaching of aseptic practice is in the use of isolation techniques for patients of different types of medical problems. The principle of isolation is an extremely good and technically accepted one, and was derived from health care practice even before the microbes were recognized as the causative agents of some diseases. Today there are many sophisticated isolation procedures ranging from total room control to bed plastic isolates. Procedures for patient contact within the isolation systems vary but, generally, the litany consists of donning sterile cover gowns, sterile gloves, and sterile masks. However, all these actions are exercises in futility, because the medical professional does not treat these basic items as sterile, nor makes any attempt to preserve their sterile integrity before any patient contact is made. Last but not least, many professionals forget one of the first basic principles of aseptic practice, that is hand washing before and after all patient contacts. The irony of this example is that the hospital central service department, or the commercial medical device manufacturer, have gone through a systematic process of packaging and sterilization to assure delivery of each item in the sterile state up to the time of use.

Another common breach of aseptic practice which occurs routinely in all medical hospital operating rooms is the handling of sterile surgical instruments during any operative procedure. The instrument processing or central service department are diligent and scrupulously careful to assure that all surgical instrument packs contain the correct number of scrupulously clean instruments, packaged in accordance with internationally accepted standards, sterilized by well-controlled sterilizers of steam or ethylene oxide gas, and delivered to the operating room in the accepted double wrap to allow for retention of instrument sterility until the time of need and use in the operating room or emergency room. All this is in vain because the instrument packs are opened far in excess of their time of need and are open to the environment in an organized display during the entire operative

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procedure that may be 30 minutes to hours. No operating room, no matter how well environmentally controlled, is sterile, and the articles within the room and, most importantly, all the operating room personnel are shedding microbes that can, and do, deposit on the surgical instruments by the normal eddying currents of the environmental air. How many nosocomial infections of surgical patients occur because of this breach of aseptic practice is unknown because the finger can be pointed toward many other breaches in health care that can occur during a patient's hospital stay.

There is also 'overkill' of good hospital practices worldwide without full comprehension of the scientific or technical basis for incorporating these practices into routine hospital operations. The continued use of ultraviolet light in specific departments of the hospital, such as central service and the operating room, is still believed to be an important infection control device. Although the scientific literature has shown years ago that ultraviolet on ceilings etc. has little effectiveness except for a few centimetres away from the light source, ultraviolet lights are still seen in many hospitals of the world. Even as psychological crutches of passive infection control, ultraviolet light can be forgiven, but these lights may, and do, cause serious cumulative retinal damage to patient and hospital personnel who have continuous contact in these ultraviolet light rooms. Eye safety is far more important than the miniscule reduction of airborne microbes.

Laminar-flow hoods are another example of products that have been professionally touted to be an effective part of some infection-control programmes and have been incorporated into operating rooms, intensive care wards, isolation areas and rooms, and clinical and pharmaceutical laboratories. Generally, the worldwide hospital use of laminar-flow hoods, whether they be horizontally, vertically, or diagonally sterile, delivering laminar-flow air is fraught with more technique breaches than their value in any hospital applications. Sterile laminar-flow air can be used in its proper environment and for specialized applications, but it is not recommended for the many hospital applications in which it is currently employed, because the integrity of the laminar-flow sterile air is never maintained intact whenever any movements take place within the path by patients or hospital personnel. There is also no documentation to support the premise that the infection rate of patients under sterile laminar-flow air is smaller than under standard hospital procedures of good patient care. The strongest argument, in the past, for sterile laminar-flow air came from orthopaedic surgeons performing hip or joint prosthesis surgery. However, today, the data collected on infection rates of total hip replacements support the premise that surgical procedures are better and shorter and the contribution of sterile laminar-flow air is nominal or nonexistent.

Another area of hospital operations where there is questionable value of its continued practice are the hospital laboratory analyses of attainment of sterility using product sterility test as the index of sterility measurement. Many hospitals worldwide still live under the fallacy that sporadic or routine performance of product sterility tests is an integral portion of their infection-control programme. Simple to elaborate benches and rooms have been set up to perform these tests. These may include laminar-flow benches. The sterility test laboratory is one of the few places where laminar-flow hoods or benches can be fully justified technically. Hospitals generally do not have personnel knowledgeable and/or adequately trained to perform product sterility tests even if the sterility test could have any technical significance. The debate between the value of product sterility tests and validated sterilization processes with biological indicators for sterility measurement is basically worldwide and, in general, acceptance of the validated sterilization process technique is prevalent. For aseptically manufactured items, the product sterility test may still be required but it is a weak

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measurement of sterility attainment. Routine product sterility test in hospitals is unnecessary and should not be performed except in very special circumstances.

Manufacture of sterile parenteral solutions is carried out in all world hospitals except the US. The procedures for manufacturing these solutions within the hospitals, from simple saline solutions to complex mixtures of nutritive solutions, are highly variable. Control of these products within the hospitals is by personnel in departments of diverse scientific and technical training such as pharmacists, central service technicians, and clinical laboratory professionals. There are invariably many instances of breaches of scientific and medical principles occurring in this area. Hospital manufacturing processes of parenterals should be rigidly controlled by precise written procedures and under the control and supervision of knowledgeable trained professionals who appreciate the safety and efficacy of the sterile solution products and do not compromise scientific principles in times of manufacturing urgency. Two areas of problems in solution manufacture that deserve special mention are the validation and monitoring of the sterilization process for the liquid-filled containers and the tests for pyrogens (14, 15). Special procedures and products have been developed to enable the hospitals to carry out routinely these quality control functions with a minimum of error. Sterilization validation systems for solutions have been published by many agencies and reference is made to those publications on the subject. Similarly, the *Limulus* Amebocyte Lysate (LAL) test for pyrogen reduces or eliminates the need for the rabbit pyrogen test. The LAL test is simpler, more rapid, accurate, and more precise than any rabbit test. Corrections of other breaches of solution manufacture are easily made following the techniques and procedures developed and published worldwide.

Last but not least of worldwide hospital practices that are controversial, is the continuous professional debate over reprocessable hospital supplies and disposable or one-time-use items. This issue will continue on for many decades because the justification of one position over the other encompasses not only professional and ethical implications but also economic considerations. In the US, the use of disposable products far outweighs the reuse of reprocessable hospital supplies. The cost justification for the shift has been touted and published by medical device manufacturers and has lead to the high acceptance of sterile disposable items. However, the continued price increases of one-time-use items has far escalated the previous hospital cost justification. Questions are being made on the value and cost economics of these items by the now cost-conscious US hospitals as well as the administration of the United States Veterans Administration Hospitals group. Although all the answers are not in, it is fair to say that the cost factor of health or hospital care is forcing the pendulum the other way and many items deemed ‘necessary and safer’ as one-time-use items will again be cost-justified by hospital reprocessing. Examples of products which should never be reprocessed are IV needles, sutures, and many types of cardiac catheters and diagnostic devices. The risk/benefit involved cannot be justified. Other products of less cost will return to standard hospital cleaning, reprocessing, sterilization and packaging operations for recycling, either to reduce or to minimize rising hospital costs.

In summary, this paper has pointed out several controversial issues that all hospitals are facing today. Many of the technical issues and problems do have resolutions and compromise, but it is the questioning and debating that all of us play in our professional roles that will continue to raise the standards of world hospital patient care and lower infection.

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The Expectations and Demands on the Hospital System for Sterile Products

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Twenty five years ago, I can remember, as a student, being introduced to the mysteries of sterile technique and surgical sterility. Sterility was explained to us as an absolute situation rather akin to pregnancy! Indeed, I am still convinced that many of my surgical colleagues and scrub nurses retain similar views today.

Over the years in between, I became aware of great changes in the management of patients in our modern hospitals. Sterilization has graduated from being a side-room activity to become a new area of specialization in itself.

Hospitals today are confronted by a bewildering array of sterile products, both disposable and reusable. Demands for improved products and techniques are insatiable and expectations of quality, performance, and availability are infinite.

To understand the present situation, it is helpful to review the history of modern hospital practices and the way in which sterilizing services have had to respond to the demands of the users. These demands have been governed by the development of technology and the economic constraints affecting modern hospitals.

To review this situation, I would like to illustrate some of the present problems in a large Australian hospital and give some idea of the workload that produces the demand, and how the sterilizing services are organized to respond.

It is difficult to accept that modern concepts of surgical technique and sterility in hospital practice are relatively recent historical developments; in fact, little more than one hundred years old. To those of you from microbiological backgrounds, the surgeons' concepts of absolute sterility and the sterile surgical field must appear a little dubious. However, the strange rituals of the operating room, although perhaps lacking in scientific basis, have an enviable track record in the growth of safe modern surgery.

Developments during this century have been progressive, but the pace of progress has accelerated over the last thirty-five to forty years. These latest advances owed their beginnings to the Second World War, and speeded up with the widespread use of antibiotics and other modern technology. Hand in hand with the surgical advances have come better anaesthesia, better understanding of physiology, and the easy means of measuring physiological data in clinical situations.

Sterilization has kept pace with the times. Great strides have been made over the last thirty years, when hospitals began to make their first steps into central sterilizing to improve standards and quality

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control. The range of goods processed at first was not vast, then again neither was the demand. Most departments dealt with surgical instruments, gowns, gloves, sterile dressings, linen, and miscellaneous sterile hardware. Needles were sharpened, gloves processed, and intravenous giving sets were Heath Robinson contrivances of glass, joined by rubber tubes with screw clamps to control flow rates. All these products were sterilized in simple autoclaves.

In the early days of central sterilizing, there were clearly double standards. In the wards of the typical hospital, there was a much more permissive set of rules. Here, syringes and needles used for intramuscular injections were simply boiled in water, and dressings for ward use were bulk-sterilized and placed on a rather majestic vehicle called the dressing trolley. This conveyance moved from bed to bed and these bulk dressings were applied using the boiled instruments when dressings were changed or sutures removed. These practices seemed satisfactory, even advanced, for times when older physicians and nurses could easily remember spirit containers for syringes, and spirit lamps and teaspoons to make up injections from tablets.

The beginning of the technological explosion cannot be pinpointed exactly, but there were a number of significant advances at about the same time that played a major role. The availability of intermittent positive-pressure ventilators, automated biochemical analysers, and physiological monitors placed powerful weapons in the right hands at the right time. Added to this, pharmacology had provided a whole new range of powerful drugs, active on the vasculature and central nervous systems. Surgery, making use of these new tools, obliged by attacking the vascular system, the heart, the great vessels, and the brain. By using metallurgical advances, fractures and joint disease were treated with better means of internal fixation and joint prostheses. Extra-corporeal circulations were developed to allow more time to repair cardiac defects, and immunology provided the ability to suppress the immune mechanisms to permit organ transplantation.

The whole process has been relentless and the performance, as measured by mortality, morbidity, and improved quality of life, has been one of steady improvement. Cardiac surgery which carried mortality rates of 20% in early days is now being performed with rates of 1% or less.

The improvement in performance is a result of experience, better techniques, better technology, and better equipment in the right hands. Many of the problems of the early days have been solved. Many of the solutions have been the result of sheer hard work and many have resulted from the ability to innovate. As succeeding generations of students will testify, the explosion of knowledge has provided a lot more to be learnt in the same amount of time.

Along the way, the science of medicine has benefited from advances in technology in plastics, metallurgy, and above all, in electronics. Some of these developments brought their own problems with them. Foremost in these were the problems of how some devices could or should be sterilized, and many previously well-accepted procedures and products had their shortcomings highlighted.

To illustrate the point, intravenous fluid had changed from being locally prepared in bulk to being commercially prepared in litre flasks. After particulate matter had been demonstrated in fluids, plastic packs which were easier to store and use began to appear. The same problems are now being reported from this presentation. Similarly, giving sets had developed from the glass and rubber contraptions to disposable plastic sets with silicone-coated filters. Various measuring devices had also become available to regulate drug dosage and drip rates. The height of these developments is seen in the drip pumps and Injectomat* types of apparatus. These changes had taken intravenous therapy from a stage at which pyrogenic reactions were commonplace to a situation of greater safety,

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in which graduated quantities of fluid with measured doses of additives could be accurately administered. Today's quality control and product assurance programmes tend to be taken for granted until tragic events re-emphasize the need for their constant reinforcement.

The Central Sterilizing Department which had been dealing with a limited range of products was soon presented with a greater variety of strange materials and instruments in need of processing. Plastics and other synthetic goods lead to the use of ethylene oxide sterilizers. Ultrasonic cleaners were developed to deal with those instruments and devices that could not be cleaned satisfactorily manually. The success of this sort of machine made hospitals recognize, in retrospect, that they had cleaner surgical instruments than they had ever had, rather than 'sterile' instruments that contained 'sterile' organic matter on their inaccessible parts.

The expansion of the horizons of surgery brought other problems, as the scourge of antibiotic resistance of the late 50s. This was the late legacy of the introduction and widespread use of the wonder drugs of the same decade. The problems showed up as wound infections, respiratory infections, and septicaemia, and their appearance caused widespread revision of the procedures to control infection and cross-infection in hospitals. Valuable lessons were learnt which combined the best of the old and new methods. Generally, these solutions proposed better techniques and procedures. Better quality products became mandatory as normal everyday requirements. Old routines disappeared. Single-use syringes and needles appeared, catheters, dressings, and endotracheal tubes were individually packed and sterilized. Local sterilizing was done away with completely.

Nevertheless, these extensive procedures had considerable associated morbidity and were not achieved without clinical risk. Heroic surgery required prolonged intravenous fluid therapy, drainage tubes in body cavities, indwelling bladder catheters, and often tracheostomy and intermittent positive-pressure ventilation. Monitoring of the patient's condition required arterial and venous pressure monitoring and needle electrodes under the skin. All these techniques, apart from their inherent complexity, breached the body's first line of defence against infection.

Intensive and Coronary Care Units had become necessary to deal with all the elements of the complex treatment and their complications.

However, another interesting observation had been made about costs on the way. Not only had this new technology permitted more complex procedures to be undertaken, but the ordinary processes of metabolic recovery after less arduous procedures could also be accelerated. Lengths of hospital stay could be significantly shortened and some clinical complications thereby avoided. Economically, these were discoveries of profound significance. More patients could be treated in the same number of beds. However, it was also apparent that this increased level of activity made increased demands on staff. Staff levels began to rise both at direct care levels and in the staff necessary to maintain the logistic support.

Instead of the previously recognized 10-12-day stay of the general teaching hospital, stays have tended to fall to 5-6 days. Wards contain much sicker patients and the workload does not allow much relief for tired staff.

Hospital workloads have increased in terms of the total numbers of patients treated. Demands for services have risen as the users have come to realize that medicine has something to offer. Many other factors have contributed to the rise in demand like the ageing of the population generally and the increased incidence of road traffic accidents.

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Despite the technological miracles of the age, governments all over the world have shown increasing concern as the overall costs of health care have continued to rise. Clear messages have been delivered in this country that the hospital system has to tighten its belt and consume less resources. Emphasis has been correctly placed on efficiency, the elimination of waste, and the improvement of budgeting. Blame for the increased costs has been laid at the door of rapacious doctors, profligate administrators, unnecessary operations, and the unjustified use of technology without due regard to cost. Whilst all of these charges have been supported by examples, two facts are quite clear: first that staff costs have risen enormously due to increases in numbers and in individual earnings, and secondly, that numbers of patients treated have increased disproportionately to the growth in hospital bed numbers.

Whilst great economies can be achieved by the elimination of unnecessary and wasteful practices, major savings cannot be achieved without some reduction in the levels of services provided, either by limiting numbers or allowing lengths of stay to blow out. The result of the economies, however effected, will be waiting lists for hospital admission, reduced levels of service, and dissatisfied staff and customers.

These historical and economic digressions have been necessary in order to set the scene of the present hospital situation. Let me take you through one modern teaching hospital and try to give you some idea of the services provided and the workloads generated.

The Westmead Centre of The Parramatta Hospitals is the first teaching hospital completed in NSW this century built with a purpose. It was built to supply referral and general hospital services to the large population of about 1.2 million people living in Sydney's Western Metropolitan Health Region. This area has undergone dramatic growth and development over the last 25 years. The provision of the Westmead Centre sought a reduction in long travel distances for the local population to the traditional inner city referral hospitals for some of the basic and all of the more esoteric forms of hospital treatment and diagnosis.

The area served by the hospital is vast – about 5300 km^2. Transport is poor and the population is relatively young by Australian standards – mostly young families from middle and working class backgrounds, attracted by the cheap land and houses available in the area. Many of the residents commute daily to the city although there is considerable employment in light and medium industry and clerical work in the area. The Region shows a strong community identity and spirit, although natural, community, and social amenities are relatively deficient. In the early 1970s, it was recognized as a 'Health Services Scarcity Area' – undersupplied with hospital beds, doctors, and paramedical staff. Leakage from the area to inner city hospitals was high and the local hospitals were among the most heavily utilized in the nation.

As an attempt to redress some of this health service imbalance, it was proposed to build this new teaching hospital of 925 beds and, through Federal and State Government co-operation, the project was begun in 1975 and completed in 1980 at a cost of $A181 million. The politics of both Federal and State Governments changed during the course of construction, yet the project continued without interruption, such was the level of commitment to the project.

The first patients were admitted to the hospital in November 1978, three and a half years after building work had begun. In the four years since opening, the Centre has expanded rapidly and the services provided have been enthusiastically received by the population.

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Annual growth of services

	Available beds	Inpatients	Outpatients
1978-79	360	4 516	33 136
1979-80	710	25 349	190 958
1980-81	800	37 044	322 207
1981-82	844	38 641	437 768

The services supplied in 1981-82 included:

Output		Input	
Inpatients treated:	38 641	Staff employed:	3 500
Babies born:	3 510	Salaries and wages:	$54 508 000
Outpatients:	437 768	Goods and services:	$19 154 000
Operations:	15 000	Other costs:	$ 4 046 000
Accident & Emergency attendances:	75 000	Measured as cost/bed/day:	$261
		Income – all sources:	$16 058 000
		Government subsidy:	$61 750 000

Against this strictly statistical background, you must also try to gain an impression of the practical operation of the hospital. Every day, there are approximately 120 new admissions comprising:

10 Obstetric
55 Emergencies
55 Booked and referred admissions

Procedurally there are daily:

10-15 Confinements
80 Operations (major)
1500 Outpatient visits
220 Casualty attendances
430 Dental attendances
350 Litres of intravenous fluid used
3000 Injections
120 Anaesthetics

These daily workload figures will give you more of an idea of the demands existing for sterile products. The expectation is that every case in need will have all foreseeable requirements instantly available, with reserve stock available to allow multiple cases to be carried out successively.

The only work that can be booked involves those patients who belong to the booked and referred admission group. These include certain obligatory admissions such as haemodialysis patients, patients for cardiac catheterization, and for semi-urgent surgery for cancer. It is obvious that this group does not include many patients in the discretionary surgery group.

In the event of excess demand, they are the same booked and referred (so-called nonurgent or elective) admissions who suffer first, so that more acute admissions can be taken. There is a certain urgency about obstetric admissions which will not be denied.

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To operate effectively, the hospital has been built with three major sterilizing units, each

concerned with the receipt, sterilization and delivery of sterile products for specified areas.

The principal unit (called Central Sterile Supply Unit) is situated between 16 general operating rooms and 11 obstetric delivery rooms, and serves both these areas as a major responsibility. It supplies goods to all wards, outpatient areas, the accident and emergency department, and to all the diagnostic and treatment areas.

The second unit (called Dental Sterile Supply Unit) is located in the Dental Clinical School and provides services to the 150 dental chairs and four operating rooms, as well as to the ancillary services such as X-ray and anaesthetics in the dental school.

The third unit (called Regional Sterile Supply Unit) is a free-standing unit used to perform production line sterilizing of single-use dressing packs and sterile linen for operating room use. Although designed with a capacity to handle the load for the entire Region, the full capacity has not yet been utilized and the unit is supplying only three hospitals.

In addition to these major units, significant sterilization or preparation of sterile products takes place in the Pharmacy for the production of Total Parenteral Nutrition fluids and intravenous fluid additives; in Radiopharmacy for the production of radionuclides for parenteral diagnostic use; and in Microbiology for preparation and end-sterilization of culture media.

Wherever economically feasible, disposable sterile products are used. An expert committee examines the need for, and cost of, all single-use materials before they are added to the approved stock list available within the hospital. There are at present 620 disposable sterile products approved for use in the hospital.

Use of sterile products creates demand, not only for the products themselves but also for the delivery systems, stock ordering and rotation systems, quality control, return of used goods, and reprocessing. In these enlightened days of disposable goods, it is almost necessary to have a training course for nurses to be able to recognize the disposable from the reusable.

All of these sterilizing units have been established as important service departments employing production-line methods and modern equipment to disinfect, clean, pack, sterilize, and supply all the demands and needs of this heavy workload.

The users have simple expectations – essentially that everything they want will be available as soon as it is needed. Quality control is expected to be 100%. Cost containment is not regarded as a valid excuse for failure to live up to any of these requirements.

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Q. by N. Hilmy – Indonesia

Dr Pritchard, my question is on the possibility of resterilization of disposable medical devices. We have problems in my country because we import a lot of disposable medical devices from several countries. We have two reasons why we would like to resterilize the devices. First, because of the value of the devices, and secondly, because they were bought and not used in time. We were approached to sterilize the devices by *gamma* irradiation. Of course this was rejected as we did not know what would be the effect of *gamma* irradiation on the devices. Subsequently, we were advised that the devices had been sterilized by another technique and that there were no complaints from the patients. We then carried out several studies in mice in our laboratory using the resterilized medical devices. There have been no problems encountered in the resterilization of devices. Would you comment on this.

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A. by R.C. Pritchard – Australia

I think, the basic principles in terms of resterilization of disposables are first the need to be able to clean the device. This excludes cardiac catheters and so on, because we do not know how. Secondly, whatever sterilization process you use should not degrade the materials; and thirdly, at the end of the process there must be some way of ensuring that the device still serves its original function. If you can satisfy these three principles, then resterilization is possible. The fact that one can get away with it once, or even one hundred times, is no evidence. One needs to look at resterilization as virtually an initial manufacturing process.

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Comment by F.E. Halleck – USA

I would like to comment on this also, as this very question comes up constantly in the US and other places in the world. I agree with Dr Pritchard here in what he said. The key element of any reprocessable item in a hospital is how well you can clean it. I did not discuss this in my my talk, but I should have; I had that in mind but time did not allow. We do not have cleaning standards that we can apply to medical instruments, or medical devices, or to whatever in the hospital or in any place for that matter. How do we define clean? We can define sterility by a mathematical term or some other definition but we cannot define clean. Clean is relative and an important worldwide issue that should be discussed and standardized in the future.

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Comment by J. Timmins – Australia

I would like to point out to Dr Pritchard that the report on the Working Party on Hospital Manufacturing referred to by him was, in fact, an interim report to the Health Commission of New South Wales, and that the final report has, in fact, changed a number of the statements that Dr Pritchard quoted. No doubt, the Working Party would have appreciated Dr Pritchard's comments on the initial report.

I would like to comment further on the criticisms of the 24-hour limit on TPN solutions. Earlier, they were regarded as bulk manufacture, which was taken from the definition of bulk manufacture used for the purposes of that report. I believe that we have to look at the aseptic preparation of IV solutions in hospitals as involving a certain risk of contamination, and by keeping the solution for 12, 24, 48, or 72 hours we are increasing the risk of heavier contamination that may well become significant. As such, one factor often forgotten is the check on refrigerated storage of these solutions, perhaps after they have left the manufacturing area. I am just referring to the 72 hours, as this time period can well be met by having solutions prepared on a Friday afternoon for administration on, say, a Sunday night, that may well still be being infused on Monday afternoon. So, that is three days, or 72 hours later, which is a significant time interval.

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Q. from the floor

Dr Amos, in the very busy areas of washrooms and equipment sterilization in your hospital, what sort of controls and how much control is practical, and who oversees these areas?

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A. by B.J. Amos – Australia

This is the very point that some of the other speakers have been talking about. There are no standards for sterilizing departments. There are standards in the US. The local council on hospital standards really dismisses sterilizing departments fairly briefly in amongst the environmental standards. However, I feel sure that this will be expanded. I believe that the answer is in proper management, training, and supervision of staff in the area. To a certain extent, there is no definition of clean, as Frank Halleck said. You have all sorts of equipment, automated dishwashing equipment, ultrasonic cleaners, hand washing, electronic dishwashers to clean tubing, and so forth. There must be a proper management structure in the area with supervision, leading hands to watch that the proper techniques are being observed and proper procedures are being followed, and that there are no breaches of those procedures. The keystone of all of that, I think, is in management, inspection, and supervision.

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Q. by E.R.J. Pavillard – Australia

I am interested in Dr Pritchard's comment that some of the legislation in New South Wales to improve standards has resulted from crisis management. We would like to think that good administration and good education would result in management without crisis. In fact, some states at the moment do not have these provisions, and we may assume that they are either doing very well, or that management is very imperceptive. We would like also to have standards in the neighbouring states to New South Wales for their hospitals. Could you make a comment.

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A. by R.C. Pritchard – Australia

The situation was one where an immediate action was called for by the Health Commission of New South Wales, and obviously it had to be a reaction that got the situation under control immediately. At this stage, the situation is being reviewed and there are signs that we are rolling back from extremely stringent standards. I would agree that it would be nicer to devise standards in a calm and controlled atmosphere, but in practice this is not what really happens.

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Q. by R. Edwards – Australia

I would like to ask the panellists comments or definition of the terms ‘disposable’ and ‘single use’, as my company is a ‘single use’ manufacturer and on our labelling we refer to ‘single use’ as a guarantee that the products are sterile, nontoxic, and nonpyrogenic at the time of opening the packet.

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A. by F.E. Halleck – USA

My definition is very simple. According to the manufacturers, once a device is opened and used on a patient, it cannot be reused after any reprocessing. I assume that most of the manufacturers in the world imply this with labelling of one-time-use sterile items. However, the biggest problem in hospitals is that many one-time-use items are opened, the sterility barrier breached and they are never used. This is an issue where waste is in hospitals and where we have to have some control on the reduction of cost. For example, you open up a cardiac catheter with a rather sizeable cost on it; just because you open the wrong size and throw it away, you cannot very well justify it. Most manufacturers do not want to take the responsibility of saying, you can resterilize, because they are concerned about the liability. It is what I call a vicious circle between the manufacturer who is trying to protect the liability issue, and the hospital that is trying to reduce the cost factor on these unused items. We were talking this morning about reprocessing items in hospital. There are those that are traditional, for example surgical instruments. You are not going to throw those away. There are a number of things that can reduce the cost in one-time-use items that are very costly. I do not see why we cannot resterilize. All it requires is repackaging and this is the problem. The manufacturer is not going to address that subject.

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A. by R.C. Pritchard – Australia

I have nothing to add to that. I would define a single-use item as anything, the manufacturer said was ‘single use’, although one need not necessarily follow that provision all the time.

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A. by B.J. Amos – Australia

I would agree with the other two speakers. Certainly, I believe that if the manufacturers specify 'Single-use', 'Destroy' or 'Throw away after the label is breached' then it is the responsibility of the user to follow those instructions. I think, if any user takes it upon himself to reprocess according to Robert Pritchard's guidelines for the reprocessing of goods, then the onus cannot be on the manufacturer any longer. It must be on the processor. All hospitals at the moment are beset by economic difficulties, and I believe, we are about to throw the idea out. It is just too complicated and too difficult to manage.

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Comment by S. Riley – Australia

I would like to comment that a couple of speakers have said that there are no cleaning standards or any standards set in hospitals. In most states in Australia, the Sterilizing Councils and the training and education programmes that are run do set standards. I find that there are problems with hospital administrations and some health authorities accepting these standards, because they come from a Sterilizing Department.

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Q. by L.F. Dodson – Australia

Dr Pritchard said that there was an adverse reaction to the labelling of bandages or dressings for 'Hospital Use Only – Sterilize Before Use'. Could you tell me what the objections were.

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A. by R.C. Pritchard – Australia

The particular problem that happened at our hospital was that cartons of unsterile cotton wool swabs were received labelled 'Non sterile – must be sterilized before medical use'. This immediately threw our Stores Department into a panic. The uses of cotton wool swabs around the hospital are many and varied. Some of them are definitely nonsterile uses, and some of them come up to microbiology to be packed in test tubes and we sterilize them. It was the all-embracing nature of the directive of the labelling that caused the problem. The manufacturer had put this on without informing the Stores Department. I think, had there been some communication beforehand, the problem would not have arisen, but it caused quite a deal of flurry before the matter was resolved. I believe this also happened with another manufacturer in another hospital.

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Comment by J.K Clarebrough – Australia

It usually behoves the chairman at the conclusion of such a session to make one or two comments and perhaps highlight what may have happened during his session. May I just briefly make the following remarks.

Dr Pritchard highlighted for us the problem of the production of the bulk sterile products within hospitals, the problem that it has led to, and the reaction of the New South Wales Health Commission, that is a reaction that must be looked at nationally as well. He pointed to the lack of proper guidelines for the control and supervision of Central Supply Departments and made a plea for increased training programmes for the people who used those departments. He finally made a plea for better communication between the hospital and the Commission.

Dr Halleck pointed out to us that there are controls for which operation manuals exist. But he did caution us on overcontrol and bureaucratic overinvolvement. He pointed also to the futility and possibly cost ineffectiveness in carrying guidelines to their ultimate. He emphasized the training in, and the observance of, aseptic medical practices, and isolation techniques, and critically analysed to their disadvantage ultraviolet light and laminar flow systems.

Finally, Bernard Amos took us on a very delightful tour of the Westmead Hospital. He again emphasized the complexity of a modern hospital, the importance of cost, the difficulty of maintaining standards in our current cost-restrictive situation, and again he pointed out the importance of the patient, the infringement and invasion of patient integrity, and the vast area of products that we are expected to supply.

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SESSION III

Conceptual Considerations for Sterile Products

Chairman
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Sterility Assurance vs. Safety Assurance

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To judge the safety of a sterile single-use medical product, emphasis has been placed upon the assurance of sterility afforded by the sterilization process, or the probability of the process producing a nonsterile item. This probability is generally expressed as 10^{-6} and has been given several connotations:

- less than one chance in one million that a contaminant will survive on a medical product;
- less than one nonsterile item in one million items;
- not more than one living microorganism in one million items.

These are attempts to express a theoretical concept in understandable terms. While probability can be determined quite precisely in industrial sterilization, it also looks at safety, which may result in a more rigorous sterilization process than is necessary. A neglected aspect is the probability of the one nonsterile item, or that one surviving organism in a million items, or in a thousand items, that causes an overt infection. This probability, although more difficult to define, must be much less than the probability of the process yielding a nonsterile item.

The discussion will be limited to sterile single-use medical devices.

It is generally acknowledged that sterile pharmaceuticals or parenterals that are sterilized by filtration and aseptically packaged rarely achieve a level of assurance of sterility as high as that of devices. When parenterals can be sterilized in their final container, the assurance is indeed 10^{-6}.

Before examining the possibility of sterile single-use medical devices serving as a potential source of infection, let us first examine some aspects of the infection process.

A vast amount of information is available concerning the many virulence factors that endow microorganisms with disease-producing capacity. More recently, it has been appreciated that altered host-defence mechanisms predispose the host to develop infections. There are many types of altered host-defence mechanisms, genetic and acquired, e.g. secondary to other diseases, and iatrogenic. It appears that a diminution in any host-defence system opens the door to microbial invasion and disease. This is true, irrespective of whether the microorganism is a ‘classic’ pathogen or a member of the host’s own normal flora with relatively low virulence under normal circumstances. The latter organisms have been called opportunistic pathogens.

Opportunistic infections, particularly in hospitalized individuals, have become major disease problems in recent years. Of the major sources of infection, the most important, by far, is man himself. Members of the patient’s normal microbial flora may seize the opportunity, when host resistance is lowered, to invade and multiply. On the other hand, certain microorganisms of potentially greater capacity to produce disease may be harboured only by a few persons, and be transmitted from these

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persons to other more susceptible individuals, in whom they may produce infection and disease.

Microorganisms may also be transmitted by inanimate objects, that not only permit survival and person-to-person transfer of opportunistic microflora, but may serve as numerical amplifiers of the microbial population. Distilled water and saline can support the growth of bacteria responsible for nosocomial infections. Devices, on the other hand, would at most only permit survival.

Consider now the probability of causing infection by a nonsterile medical device that has been derived from a sterilization process permitting no more than one nonsterile device in x number of devices. The device must contain at least one viable microorganism. The probability of having more than one organism is extremely low, as illustrated by an exaggerated example (Figure 1).

P (> 1)	4.6×10^{-3}	< 1 in 200
P (> 2)	1.1×10^{-5}	< 1 in 90 000
P (> 3)	1.7×10^{-8}	< 1 in 58 million
P (> 4)	1.9×10^{-11}	< 1 in 50 billion
Bioburden of 1000		
P (> 1)	4.6×10^{-6}	< 1 in 200 000
P (> 2)	1.1×10^{-11}	< 1 in 90 billion
[P (> 3)	1.7×10^{-17}	< 1 in 58 quadrillion]

Figure 1. Probability of survivors. Gamma radiation dose: 2.5 Mrd. Resistance of bioburden: D value = 0.3 Mrd.

If we assume, a single device is contaminated with one million microorganisms and that every one of those organisms has a very high order of resistance, a D value of 0.3, and then subject the device to the commonly used radiation dose of 2.5 Mrd, we can calculate the probability of survivors. The probability of one or more organisms surviving is 4.6×10^{-3}, or less than one chance in 200; of two or more surviving is 1.1×10^{-5}, or less than one chance in 90 000; of three or more surviving is 1.7×10^{-8}, or less than one chance in 58 million; of four or more surviving is 1.9×10^{-11}, or less than one chance in 50 billion (or in the British system, 50 milliard).

Assuming a more reasonable bioburden of 1000 microorganisms, and that they all have the same high order of resistance, the probability of one or more organisms surviving is 4.6×10^{-6}, or less than one chance in 200 000; and for two or more, one in 1.1×10^{-11}, or less than one chance in 90 billion (Br: milliard); and for three or more is beyond the ability to calculate, but is probably in the order of one chance in 50 or 60 quadrillion (Br: thousand billion).

Let us assume that one, or even a few, organisms survive on the device. What is the potential for causing infection? First, we must consider the intended use of the product. If the product is used to drape the patient, or gown the surgical team, an organism located somewhere on many square feet of fabric is unlikely to be dislodged and even less likely to gain access to a suitable environment for survival and growth. If the product is to be implanted or comes in contact with mucosal or subepithelial tissue, the probability of elution to a suitable environment is greater. However, the probability is very much greater in the case of an implanted prosthesis than for a wound dressing.

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Even if one or a few organisms drop off or are eluted from a device, what is the potential of causing an infection? Only a very few of the most virulent microorganisms are capable of initiating infection when a single cell gains access to subepithelial tissue, e.g. certain rickettsial, mycobacterial and *Pasteurella* species. With other organisms, a thousand or more may be required. Most bacteria require a critical initial number of cells even to initiate growth *in vitro* in a nutritionally ideal culture medium. This is especially true when placed in a new environment.

In addition to the number of microorganisms required to produce an infection, other questions should be considered.

- Was the bacterium injured by the sterilization process?
- Will the bacterium survive storage in, or on, the product?
- What is the probability that the bacterium in question will reach an appropriate site?
- What is the probability that it will multiply?
- What is the probability of encountering a host environment with impaired defence against invading bacteria?
- What is the probability that it will reach the minimum population level to result in infection?

We cannot answer all of these questions, but we do have an idea of the nature of the organism that can survive a sterilization process. This provides a level of sterility assurance. The organism may be injured, but also it may adapt to the rigours of survival in nature and endure the severe conditions of the sterilization process. Such microorganisms are rarely, if ever, the 'classic' pathogens. They are nevertheless, opportunistic pathogens or saprophytes poorly able to breach a host's defences, even if impaired. In the absence of defences, they may be able to survive and multiply, provided an adequate number of cells find an appropriate nidus and recover from injury.

The probability of an opportunistic pathogen causing infection is indeed difficult to assess. Nevertheless, the probability ranges from finite to insignificant. For products where the probability of a survivor causing infection is insignificant, the sterilization process may reasonably be designed to be less rigorous and provide a lesser degree of sterility assurance than the process for more critical products. Further, by our knowledge of bioburden and our ability to determine the level of sterility assurance during process validation, we may be able to reduce the severity of an 'overkill' cycle to more reasonable conditions.

Regardless of the criticality of the end-use of the product, the danger of a single-use medical device transmitting microorganisms to the patient lies neither with the sterility assurance of the sterilization process, nor with the probability of a lone survivor finding access to the host It is contingent more upon the integrity of the package against recontamination after sterilization through many stages of mishandling and misuse. The probability of introducing opportunistic pathogens by improper storage and handling is high.

The packages for sterile devices have been designed to prevent recontamination of the device when held in reasonable storage conditions. The manufacturer can control the sterilization process and the quality of the packaging of medical devices. He has exerted every effort to achieve a high order of sterility assurance and package integrity.

There is a need to educate hospital personnel in the use of good storage procedures, as well as in the proper handling and opening of sterile packages. The incidence of contamination of surgical

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materials in the operating theatre during surgery is often dismissed. Instruments and materials employed in surgery are generally not considered to be an important source of the organisms causing postoperative infection. Although there have been numerous microbiological studies made in an attempt to associate high bacterial fallout with possible instrument contamination, only one among the more recent studies, to my knowledge, has measured the contamination of the actual instrument during the course of surgery. The work was conducted by a graduate student in partial fulfilment of a Master of Science degree at the University of Minnesota, under the guidance of Velvyl W. Greene (1). The data are as yet unpublished, but the thesis is accessible. It is relevant enough to the subject of my discussion to review a portion of the work in some detail.

The study was conducted in two operating theatres set aside for cardiovascular surgery in the Mayo Memorial Hospital. Cardiovascular surgery was selected because it was a clean operation and because its long duration provided ample time for repeated sampling. Since occasionally other clean operations were performed in these rooms, they were included in the study. Thirty-two operations were sampled: twenty-one cardiovascular and eleven other clean operations. The average duration of the operations was four hours. The average number of metal instruments used in an operation was 169; the average number sterilized for an operation was 344. Therefore, there were plenty of unused instruments for sampling.

All sampling was done in the operating room by the rinse technique in culture medium and only the tips of the instruments were rinsed. The contamination was expressed as a contamination probability index (CPI) and defined as the ratio of the number of positive samples to the total number of instruments sampled. Quantitative results were derived from instruments and stainless steel strips. Other materials, such as used instruments, tubing, sutures, tapes, basin saline, and irrigation saline were sampled. These will not be discussed, since the data on unused instruments were sufficient for illustration.

As would be expected, contamination of the unused instruments increased with time of exposure. At the beginning of operation (zero time), the exposed instruments had a CPI of 0.017, or 1.7% of the instruments were contaminated, rather than the theoretical minimum of 0%. This could not be attributed to the failure of the sterilization process, but was most likely the result of unwrapping and handling, or to the sampling procedure.

After four hours, the average length of cardiac surgery, the exposed unused instruments had a CPI of 0.69 ± 0.08, which means that 70% of the sterile instruments were contaminated within four hours of operating-room exposure. The organisms found were mainly micrococci, diphtheroids, and bacilli, suggesting human contamination as a source. If so, the organisms were most likely disseminated as airborne particles by the operating room personnel or the patient. These observations were made during surgery conducted by expert surgical teams in modern, well-ordered surgical theatres.

The author concludes the thesis by the statements: ‘A lot of money, time and effort is spent in developing the proper sterilization of materials. It seems foolish to attain this initial sterility and then to allow materials to be unnecessarily exposed to the operating environment’.

In light of the microbiological fate of medical devices after sterilization, it would appear that less rigorous sterilization conditions may provide an adequate assurance of safety against infection in certain instances. This does not mean that we can be lax in our attention to sterilization, but that we may adjust our cycles to provide the needed level of assurance.

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For many years, physicians and regulatory agencies around the world have been searching for a

designation for 'level of sterility assurance' to replace the absolute term 'sterile'. As mentioned earlier, in most countries only one level of sterility assurance, 10^{-6}, is accepted as sterile. In Sweden, for example, drugs that are not sterilized in their final container, i.e. having a level of sterility assurance of less than 10^{-6}, may not be labelled 'sterile'. They are referred to as 'aseptically produced products'. Recently, the Swedish authorities have proposed that medical devices with an assurance level of 10^{-3} be called 'surgically clean'.

While many have been hoping for an expression to replace the word 'sterile', the late Ronald Campbell of the Bureau of Medical Devices of Canada has been most prolific in offering suggestions. Among his designations have been 'Clinical Hazard Index', 'Potential Hazard Index', 'Microbiological Safety Index', and finally 'Microbiological Survival Index' (MSI), defined as the absolute value of the logarithm of the probability that any one device is contaminated with a viable microorganism. Thus, for a product with a level of sterility assurance of 10^{-6}, the word 'sterile' on the label would be supplemented or replaced by MSI 6. While there are several products in the US and Canada that bear an MSI designation along with the word 'sterile', such labelling has met with strong opposition by both industry and the medical care professionals. The disadvantages would appear to outweigh whatever advantages there may be.

There are, however, a considerable number of products in the US today labelled 'sterile', that have been processed, with FDA approval, to levels of assurance less than 10^{-6}. The label in these cases does not include an MSI number.

If we examine the situation with respect to sterile consumer products (OTC or first aid products), we are confronted by the question of the actual need for sterility. While there may be a need for a high level of sterility assurance for some of these products, most are used on minor wounds of which most do not become infected if unattended. Their function is essentially to protect the wound from further insult or trauma. This situation was recently highlighted by the incident of nonsterility among sterile dressings imported from India and the Far East The dressings were imported by a number of countries where the reaction to the situation differed considerably, as did the assessment of risk involved in using such dressings.

In the UK, the public was advised to destroy any suspect dressings. Warehouse supplies were frozen and all Health Authority Supply Departments were informed. In Australia, the products were recalled to the consumer level. The risk was considered less serious in the UK than in Australia, even though the *British Pharmacopoeia 1980, Appendix XVI*, requires a dressing labelled 'sterile' to satisfy the conditions of the sterility test. The British found among the contaminating organisms staphylococci, streptococci, and clostridia associated with gas gangrene infections *(C. welchii, C. sporogenes*, and *C. sordellii)* and tetanus infection *(C. tetani)*. The clostridia were dismissed as being present from the soil and as they could be found in any nonsterile dressing. The Department of Health and Social Security further stated that any wound at the time of injury was likely to have been contaminated by bacteria in the air and on the skin, and that in first-aid treatment, a clean handkerchief might be used that could contain such organisms. The Department expressed the view 'that the risk from the dressings is slight, nevertheless, it is undesirable that a product labelled "sterile" should contain organisms as this could increase the possibility of use where sterility is of particular importance'.

Bandages, as well as dressings, were found contaminated with potential pathogens. The difference between a dressing and a bandage is somewhat puzzling. It is usual practice to use a dressing to dress

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or cover a wound and hold it in place with a bandage. However, the *British Pharmacopoeia 1980* and the *British Pharmaceutical Codex 1979* include bandages and swabs under the general category of surgical dressing, whereas *US Pharmacopeia* never mentions the word 'dressing' and uses only the word 'bandage'. Therefore, it appears that bandages are intended also as wound dressings. In the *USP*, a gauze bandage may be sterilized or not sterilized, in which case it must be so labelled. On the other hand, adhesive bandages are required to be sterile. Most countries do not have the latter requirement.

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Conclusion

The probability concept of sterility has lead many to question the need to process all surgical and first-aid materials to the same level of sterility assurance. In some cases, the need for sterility has been questioned. Should there be, in the judgment of the regulatory authorities and the medical profession, a need for sterility, then it should be possible to decide upon a level of sterility assurance based upon the 'end-use' of the product or product category. Should there be no need for sterility, but some high order of microbiological cleanliness, then requirements should be defined in a more meaningful way than a total microbial count and freedom from harmful organisms or freedom from pathogenic organisms. The latter requirement cannot be met, since potential pathogens and harmful organisms will be found on a product, if enough samples are examined.

In most cases, by setting a maximum microbial count per gram of product, the probability of these undesirable organisms being present will be reduced to a level at which the product will not endanger the public health. Not even by setting qualitative limits, expressed as freedom from specific organisms in a specific amount of sample tested by a defined test method, can we guarantee absolute safety of nonsterile products. We cannot guarantee absolute 'freedom from pathogens' or 'harmful organisms' for nonsterile products any more than we can guarantee '100% sterility' for sterile products.

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Educational Considerations

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Recent developments in the design and technology of sterilization processes, which commenced around 1950, have generated a new approach to the discipline. A need for more sophisticated education has been created than was previously considered adequate, when sterilization in hospitals was limited to steam and dry heat. The following examples, which demonstrate the need for increased basic knowledge and special training, are:

1. The reliability of steam sterilization in hospitals was upgraded by the establishment of central sterilizing departments and the development of prevacuum porous load sterilizers in Great Britain. This followed the revelation of serious technical faults and human errors during investigations into possible causes and remedies for epidemics of hospital-acquired infection by antibiotic-resistant staphylococci.
2. The use of heat-sensitive synthetic materials in the manufacture of complex instruments and for the production of a wide variety of medical devices necessitated the development of low-temperature sterilization processes using ionizing radiation or ethylene oxide. The latter is also used in hospitals.
3. The large scale commercial production of medical devices introduced the concept of sterility assurance and recognition that no process can be guaranteed to sterilize every article treated. The methods which are required to ensure an acceptably low frequency of unsterile items by minimizing initial contamination levels have been set out in codes of Good Manufacturing Practices that apply to the pharmaceutical and medical device industries.
4. Murrell and Scott published a paper of fundamental importance in 1966 (1), in which the relationship between water activity, or relative humidity, and the so-called dry heat resistance of bacterial spores was clarified. The findings explained the irregular results which had formerly been common in the determination of resistance to dry heat.
5. An important development, which is occurring at the present time, is the replacement of electromechanical systems by microprocessor technology for the automatic control of sterilization cycles.

As a result of these and other developments, the educational requirements for persons who design, supervise, or operate modern sterilization processes greatly exceed the teaching that is normally provided in medical and dental science, pharmaceutical microbiology, and in postgraduate, as well as basic nurse training. The engineers who have to service modern sterilizers also benefit from some knowledge of microorganisms and the conditions required to kill them.

This leads to consideration of the opportunities for specialized education that are now available in Australia. The initial step towards their establishment was the formation of the Sterilization

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Research and Advisory Council of N.S.W. Similar common-interest societies now exist in all Australian states and have played their part in the planning and operation of organized courses for hospital staff. The Australian Federation of Sterilization and Disinfection Societies has united them with the principal aim of co-ordinating education objectives.

The courses in New South Wales have been operating the longest and have trained the largest number of hospital sterilizing department employees. My own experience is limited to the Victorian course, that has produced 204 successful students during the past eleven years. It is conducted at Mayfield Centre, which is an educational facility of the Victorian Health Commission, and is organized by a specially appointed lecturer, who is a microbiologist with hospital diagnostic and university teaching experience. Other lecturers contribute in accordance with their special knowledge. Although the course does not assume a high level of prior knowledge, it is designed to reach the level required by hospital staff with consultative or supervisory responsibilities in the microbiological, technical, and engineering aspects of sterilization and infection control. Central Sterilizing Department (CSD) supervisors and their deputies, infection-control sisters and theatre sisters occupy most of the 24 places that are available each year. However, hospital microbiologists, pharmacists, engineers, and domestic service supervisors are also encouraged to enrol. This policy is intended to promote dialogue and mutual understanding between people who are involved in the different facets of infection control.

The course consists of the following units, all of which must be taken:

1. Microbiology
2. Heat sterilization
3. Bacterial filtration
4. Sterilization by ionizing radiation and chemical vapours; also chemical disinfection of inanimate objects and body surfaces
5. Middle management
6. Infection control.

Each unit consists of full-time attendance for one week at lectures given in Melbourne, followed by four weeks of study and assignments, and a two-hour written examination. A practical project is also undertaken with the consent of the hospital management. The Mayfield Centre certificate is awarded for a final aggregate mark of at least 60%.

I wish to select the microbiology component of the course for detailed comment. It is designed to assist the nurses, and any other students who are not already conversant with the subject, to understand the nature, distribution, and behaviour of the invisible organisms that are the target of all sterilization, disinfection, and aseptic procedures. It is regrettable that nurses who are appointed as CSD supervisors or infection-control sisters have no more than a superficial acquaintance with the subject. As the organisms can only be studied indirectly by use of the microscope and cultural methods, the subject should be taught by qualified microbiologists and some practical work should be included. The Mayfield course students spend three days in a teaching laboratory where they perform some basic techniques that enable them to become more familiar with the organisms at first hand. It is made clear to them that such a brief introduction does not qualify them as microbiologists, but it is pleasing to see their developing interest, and a desire for more time to be allotted to this part of the

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course is frequently expressed when they evaluate it.

The students who come to the course equipped with some knowledge of microbiology discover their limitations regarding sterilization practice in the next two units, as their degree or diploma courses are unlikely to have given sufficient time to this applied aspect of microbiology. On the other hand, the infection-control sisters sometimes feel that this part of the course is in excess of their needs. They are advised that they should know it, at least in principle, and that they might well need the information in a different job or at a different hospital. In the final unit, all students are exposed to the practical experience and sometimes different views of senior hospital microbiologists with regard to infection control.

The course which I have described is not designed for the needs of the main work force of the sterilizing department, as it requires a certain level of general education that prepares the student for classroom teaching and written examinations. In-service education is required to promote understanding of the tasks that are actually performed. However, an appropriate teaching manual would be required to ensure that what has been learnt will apply to all hospitals.

There are no special courses, outside the university and technical courses, that include applied microbiology for personnel in industrial sterilization. A course of the Mayfield Centre type is suitable but few places are available because priority must be given to hospital staff. However, occasional lectures and seminars provide up-to-date information on the topics selected. They also bring people with common interests together, with resultant sharing of knowledge and experience. All of us, who are at this Symposium, will benefit from the opportunity to view our own responsibilities in the context of the wider Australian and overseas scene.

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Sterility and the Law

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Introduction

The failure of a manufacturer to use proper procedures may cause him to run foul of various sections of Federal and State legislations, such as *The Customs Act, The Therapeutic Goods Act, The Pure Foods Act, The Sale of Goods Act*, as well as the advertising provisions of *The Trade Practices Act*. Added to this, common law sets out obligations imposed upon those who market drugs.

Similarly, there is a responsibility by hospitals and other institutions to ensure that products and equipment used by them do not cause damage to patients by using nonsterile products.

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Manufacturer's Liability

Common law liability which can arise by virtue of a manufacturer's failure to carry out adequate sterilization is based on the tort of negligence. The basic ingredients of this tort in laymen's terms are:

(i) a duty of care
(ii) a breach of the duty of care
(iii) damage consequent to the breach.

Until the 1930s a manufacturer did not owe a duty of care to a party with whom he had not contracted. However, in 1932 the whole situation changed.

Two old ladies visited a shop, and one of them purchased from the shopkeeper a bottle of ginger beer. The purchaser then handed the opaque bottle to her friend, who began to drink from it. After she had started to drink, she discovered a partially decomposed snail in the bottle. She subsequently claimed that this made her sick, and that she wished to sue someone for negligently allowing the snail to get into the bottle. As the retailer had not been responsible, and had no opportunity to inspect the bottle, and so it was not possible for her to sue him, she brought an action against the manufacturer. Ultimately, the House of Lords proclaimed what has turned out to be an introduction of the law of general manufacturer's liability.

In the words of the court, spoken by Lord Atkin, 'You must take reasonable care to avoid acts or omissions which you can reasonably foresee would be likely to injure your neighbour. Who, then, in law, is my neighbour? The answer seems to be, persons who are so closely and directly affected by my act that I ought reasonably to have them in contemplation as being so affected when I am directing my mind to the acts or omissions which are called in question'. This statement is rather imprecise and it does not provide an answer for every situation. It is sufficiently tight to cover a batch of products to pass through the manufacturing process without complying with sterility requirements and render the product safe for use. Specifically, the court held that 'a manufacturer of products which he sells in such a form as to show that he intends to reach the ultimate consumer in the form in which they left him with no reasonable possibility of intermediate examination and the knowledge that the absence of reasonable care in preparation of putting up the products, will result in an injury to the consumer's life or property, owes a duty to the consumer to take that reasonable care'.

Having shown that the manufacturer has the responsibility to the ultimate customer, the next question which must be answered, is the level of behaviour by the manufacturer, that renders him liable. Lawyers call this the 'standard of care'.

The standard of care is breached when the manufacturer acts in a way, in the production of his goods, that can be described as unreasonable. In most cases, it will be impossible for either the manufacturer or the consumer to ascertain exactly how a defective product came to be produced, viz. passed through the various processes including quality control and/or which member of the manufacturer's staff was responsible for the breakdown. The result of this problem has been to make the manufacturer the virtual insurer of his product. However, the law does not demand that the standard be so high, that liability will result, even for situations in which proper care could be eliminated. Defects that are practically undiscoverable are excluded, but once knowledge of the defect is gained, steps must be taken by the manufacturer, either to warn consumers of the likelihood of damage, or to rectify the procedure.

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The following is an example, in a rather bizarre way, that is applicable to the obligation imposed on hospitals. Mr. Roe was suffering from a damaged knee. He visited his medical practitioner who recommended that he had his cartilage removed. Mr. Roe subsequently entered hospital. Prior to his entering hospital, the medical practitioners at the hospital were concerned that a particular procedure was causing problems. The procedure involved spinal anaesthetic. The hospital staff had discovered that often, after spinal injections, infection appeared around the site. It was further discovered that the cause of the problem was the local anaesthetic, Nupercaine*, that was stored in glass ampoules on the shelves of the hospital pharmacy. In the pharmacy, the ampoules were not in a sterile environment, and investigation discovered that when taken into the operating theatre, the side of the ampoule came in contact with the needle, that was then inserted into the patient's back. The bacteria on the side of the ampoule caused an infection. To eliminate this problem, it was decided to store the ampoules in the pharmacy in a sterile environment. As the ampoules were made of glass, it was suggested that the best way to store them was to immerse them in a suitable solution. Phenol was chosen. When Mr. Roe entered hospital, it was decided to use Nupercaine and an ampoule was sent up from the pharmacy. The anaesthetist held the ampoule up to the light, to ensure that there had been no damage to it, and to ensure that no foreign matter had entered into it. Two weeks after a successful operation, Mr. Roe became a paraplegic. The cause of the paraplegia was an effect on the spinal cord by phenol. Investigation revealed, that some time between the date of manufacture and the time of removal to the operating theatre, a microscopic crack had appeared in the ampoule. This crack was not visible to the anaesthetist who examined the ampoule prior to the removal of the Nupercaine. Not finding anything apparently wrong, the contents of the ampoule were removed and the operation proceeded. It was subsequently discovered, that the presence of phenol in the ampoule of Nupercaine was a combined result, that the ampoule had sustained a microscopic crack, and that the composition of the phenol and Nupercaine enabled a free flow of liquid through the crack. Mr. Roe had received an injection of phenol and Nupercaine. Obviously displeased, Mr. Roe sued those he considered responsible for his new disability. The ultimate question, which the court had to decide, was whether the hospital had breached its duty of care toward Mr. Roe by not discovering the problem, or alternatively, by not realizing that the peculiar characteristics of the products used could render the procedure dangerous. In reaching his decision on the reasonableness, or otherwise, of the hospital's behaviour, Lord Denning stated:

'It is so easy to be wise after the event and to condemn as negligence that which was only misadventure. We ought always to be on guard against it, especially in cases involving hospitals and doctors. Medical science has conferred great benefits on mankind but these benefits are attended by considerable risks. Every surgical operation is attended by risks. Doctors like the rest of us have to learn by experience and experience often teaches in a hard way. Something goes wrong and shows up a weakness and then it is put right. That is just what happened here. Dr. Graham sought to escape danger of infection by disinfecting the ampoule. In escaping the known danger he unfortunately ran into another danger. He did not know that there could be undetectable cracks but it was not negligent for him not to know it at that time. We must not look at the 1947 accident with 1954 spectacles'.

In the concluding paragraph of his decision, Lord Denning stated:

'This man has suffered such terrible consequences that there is a natural feeling that he should be compensated. But we should be doing a disservice to the community at large if we were to impose

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liability on hospitals and doctors for everything that happens to go wrong. Doctors would be led to think more of their own safety than of the good of their patients. Initiative would be stifled and confidence shattered … We must not condemn as negligence that which is only misadventure'.

The third ingredient of the tort of negligence, namely foreseeable consequences, does not concern us greatly here. The law does not require the need for foreseeability of specific consequences (damage), but rather general consequences. That the failure to provide adequate (reasonable) sterility procedures could reasonably cause damage to the ultimate user of a product (the patient) is without question.

Within the medical supply industry, a number of specific questions arise, in particular, the effect of the manufacturer's instructions. It is not uncommon for manufacturers to label goods as being suitable for 'one purpose only' and not suitable for reuse. Hospitals have found that it is possible to resterilize some of the appliances. It is sometimes thought that the failure to carry out directions automatically renders the user liable, if subsequent damage is caused to a consumer. In fact, this is not the case. The test for negligence is provided by the courts, and depends on the reasonableness or otherwise of the actions of the individuals, or institution, or company accused of being negligent.

The existence, or otherwise, of a manufacturer's instructions will be no more than one extra element to be fed to the court, in order that it can determine the reasonableness, or otherwise, of the user's actions. It would be foolish, if this were not the case, as we would then be faced with the spectacle of a manufacturer determining the law, possible in a manner that has the inevitable effect of increasing sales. Nevertheless, it should be pointed out that where goods are labelled with a warning as to their use, or suitability, any person who wished to vary this use, should be prepared to justify the variation.

In a well-known case in Canada, a medical practitioner neglected to follow the instructions contained in a packet of neomycin, that if used on an individual over a protracted period, an audiogram should be taken at regular intervals. This, the medical practitioner failed to do, and, even though the patient's subsequent deafness could not be attributed to any mistake in diagnosis, or use of neomycin, it would have been prevented. Had the audiogram been taken, the problem would have been discovered and the treatment suspended. The court held, that the failure to heed the warning, which it accepted as scientifically accurate, constituted actionable negligence.

If we assume that the damage to the consumer/patient has been caused by the failure of the drug manufacturer, or institution, to provide a sterile product, it can be seen that the plaintiff must show that the manufacturer was negligent in not so providing. As is often the case, the quality of the goods is revealed well after manufacture or use, and then the defective goods usually come only from one batch. In a repetitive process, it is, of course, difficult for the plaintiff to identify the specific area of fault, as all previous and subsequent batches are up to standard.

This hurdle is overcome in the UK and the US by the use of a legal maxim *res ipse loquitur* that when literally translated means 'the thing speaks for itself', or put plainly, as a general rule, but for the fact of negligence, the product would be up to standard. In the UK and the US, when this argument is put, the onus of proof moves from the plaintiff to the defendant, whereupon the defendant (in our case the drug manufacturer or institution) would have to show that there was no negligence. If this cannot be done, and this is often the case, the presumption applies, and the plaintiff will succeed. In fact, the reason why the cause of Mr. Roe's paraplegia was ever discovered, was that a failure to

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ascertain the cause would have given rise to the application of the *res ipse loquitur* doctrine with the probable result of there being a successful plaintiff.

In Australia, the situation is different, and the doctrine comes forward only as 'evidence', and while the submission may be successful, the onus of proof still remains with the plaintiff. That is, the plaintiff must satisfy the court that the damage was caused by negligence.

It is possible for the plaintiff to do this by inference, as is pointed out in the celebrated case of Grant v. Australian Knitting Mills Ltd. In this case, Dr. Grant purchased a pair of underpants from a retailer and proceeded to wear them continually for six days. He subsequently suffered from a dermatitis, apparently caused by sulphur residues in the fabric. Although four million similar garments had been sold without complaint, the court held that 'if excess sulphites were left in the garment that could only be because someone was at fault. (It) is not required that the plaintiff specify who did wrong. Negligence is found as a matter of inference from the existence of the defects'. As already pointed out, however, this could be rebutted, if the defendant produced evidence, as in Roe's case, that demonstrated no negligence.

Difficulties have, to some extent, been overcome by the passing of the Federal *Trade Practices Act*. Under this Act, a manufacturer (but not a hospital) will be liable for loss or damage caused to an ultimate consumer, where:

(i) the goods were not reasonably fit for the purpose for which they were supplied (S74B)
(ii) the goods are not of merchantable quality (S74D).

There can be no doubt, that in manufacturing a pharmaceutical product that does not satisfy the requirements of sterility, it will not be of merchantable quality.

Traditional defences to common law actions in tort have been removed, and should a situation similar to that explained earlier in Roe v. Minister of Health arise, the matter would be handled differently. Assume that the drug company decided to store the ampoule in the sterile environment, and that when subsequently used by the hospital, the patient suffered damage. It would not be necessary for the plaintiff to prove that the decision to store the ampoule in the phenol was negligent. It would be sufficient for the patient to show that the ampoule, as supplied, was not reasonably fit for the purpose for which it was intended (as it clearly would not be) for liability to result. As distinct from traditional Sale of Goods legislation, the *Trade Practices Act* enables a successful plaintiff to obtain damages similar to those that he would have been able to obtain, had the action been one of negligence.

An extremely relevant clause in the *Trade Practices Act* is S74A(4). This section deems an importing corporation to be the manufacturer, so that a product manufactured overseas, not reasonably fit, marketed in Australia will be deemed to have been manufactured by the importer, and therefore the importer will be liable for any foreseeable damage to the consumer.

An interesting aspect of this provision of the Trade Practices legislation is that it has produced no decided cases. It is difficult to understand why lawyers would prefer the common law grounds of product liability, but it has been suggested that when the *Trade Practices Act* was first introduced, it concentrated mainly on the regulation of marketing and advertising, and the product liability sections, introduced later, have been largely neglected. Of course, it would be foolish for manufacturers to ignore its significance and the added responsibility it brings to them.

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DISCUSSION
SESSION III

Q. by L.F. Dodson – Australia

Dr Gaughran, I agree completely with almost everything you say about the risks associated with sterilization, the fact that infection requires pathogenic organisms, and that these organisms are pathogenic only in particular circumstances. I agree that Clostridium on an open wound is probably not of any great importance, but on an amputation stump or a compound fracture it is a dangerous organism. I am not sure what your proposition or what your comments would lead to. It seems to me that if we accepted what you have said, one may not be precise in a strict guarantee of sterility. It would have very distinct effects and could be coped with only with special regulations. If there was a product regulation system, one could regulate on the risk and on the product at the same time. As in the United States, all drugs of a certain kind have to meet a particular standard, and so, I am not quite sure how you could adopt your hypothesis. I think, what you have said relates to a remark I made, that when a quality control manager or a regulatory agent passes something or accepts a sterilizing process, he is in fact making a social judgment based on experience and not based upon calculations of bioburden, which I do not think are particularly accurate. What they are saying is that we have been doing something for a sufficient length of time to know what the community accepts, namely if there is something amiss we do not regard it as seriously as we should, as for example the high road toll. Judgments are really made in this way rather than on calculations of probability. This leads me to say that I do not believe, we can adopt your proposition unless we change to a system of regulations, and then if we do adopt the system, we have to wait another 50 years to know whether in fact it is acceptable.

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A. by E.R.L. Gaughran – USA

I do not mean to usurp the prerogative of the medical profession or second-guess the regulatory agencies. I thought that on a product to product basis, or category by category basis, an acceptable level of sterility assurance might be agreed upon by the medical profession and the regulatory agency, which would assure adequate safety at a particular level of sterility assurance, which you can have with industrial products and arrive at it quite accurately. This is now being done in the US and Canada. However, when it comes to a nonsterile product, some of the imposed conditions to obtain absolute freedom from contamination are just not achievable. What you have to do is set limits by way of numbers to provide assurances that they will not be a hazard to public health.

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Q. by J.F. Gardner – Australia

I would like to ask Brian Bromberger if the administrator or supervisor of a hospital sterilizing department is asked by the medical or nursing personnel to sterilize something by a method that is not ideal or to sterilize something with insufficient time available, who is responsible if anything goes wrong. That is, provided, of course, the user of the instrument has been told by the sterilizing department personnel that the method is not guaranteed.

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A. by B. Bromberger – Australia

I assume that as the result of the event somebody has suffered damage. If you have, as an example, a Jiminy Cricket sitting on your shoulder, telling you what is the best thing to do, and he says 'that is not how you as a professional should function', and despite this you do proceed in doing what you have been asked, then you take upon yourself the liability for your own action. We are all liable for our own action within a hospital environment, as an employer is also liable for this action. So if you do it, even though you say to somebody 'I don't think it is the right thing to do', you are still liable for having done it, as is, as I say, your employer. So the obvious thing for you to do, if you are asked to carry out a procedure that you do not feel is the correct procedure, you say 'I will not do it' and you let the Chief Executive Officer have a fight with the Medical Superintendent, who then will have a fight with the Matron, and when they have sorted it all out, they will dig you up and you will be right.

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Q. by R.A. Anderson – Australia

May I follow up on that last question. How do you stand with your conscience when the patient dies because some material is not available and it cannot be made available in a form which meets the normally accepted manufacturing standards? I can understand that an institution might have some responsibility, but the responsibility has been given to the individual who has to make a professional judgment that the item cannot be properly sterilized, and that if the item is available, in many circumstances it could be used quite satisfactorily.

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A. by B. Bromberger – Australia

There are many people who say the law is stupid, but nevertheless it is not a complete ass, and what it, in fact, says is based on 'reasonable' in the circumstances. This is on top of everything. Hence, what might be regarded as reasonable on the side of a road or in a bush nursing hospital, might be totally different from what might be reasonable in a large hospital, such as the Prince of Wales Hospital.

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Q. by G. Reeves – Australia

My question is directed primarily to the legal aspect. A week ago, I heard a physician ask the same question that has been asked here, namely, that if the manufacturer indicates that a device is for single use only, and if it is reused, and a problem occurs, where did he stand legally. A lawyer at the time said that the situation lay with the person who reused it, namely the physician or the institute authorising its use. As a manufacturer, when I went to a legal adviser and insurer, who made it quite clear that if I do not put 'For one-time use only' on the device, it was subject to the possibility of resterilization and reuse, then I was possibly condoning resterilization. In other words, there was no control over the way it may be resterilized. Therefore, this was my safeguard, and indeed their safeguard, because as I understand it, if the medical profession is sued, they will spray bullets at the manufacturer, and then the manufacturer ducks, hands it to his insurer who proceeds to take it from there.

I would like to ask a question, whether you still feel that the person who reuses a single-use item may not be liable.

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A. by B. Bromberger – Australia

The manufacturer does not make the law, and all the manufacturer can do is to recommend the correct use of his product. It may well be that the manufacturer who is very anxious to sell one million products will say, use only once, which means that a product that can be used ten times will not be used ten times. So, the manufacturer cannot stipulate law. If, however, a manufacturer of a medical or surgical appliance says 'Use this only once', then that obviously is an indication to the medical practitioner or hospital that it is intended to be used only once. If the user wants to use it more than once, then he has to take steps that are 'reasonable'. I am not a microbiologist, but if it is possible to resterilize the particular appliance, then the fact that the manufacturer has said 'Do not resterilize' does not make it actionable. It becomes actionable if the procedure that is involved is affected. So, as far as the ultimate consumer who is damaged is concerned, the 'Use-only-once' labelling is simply a warning to the person who uses the item or appliance.

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Q. by E.R. Pavillard – Australia

I would like to question Brian Bromberger further on this point, as we have left an important point floating. Mr Bromberger, you said earlier that the decision as to whether the item could be sterilized and used again safely was in the hands of the microbiologists, so to speak. One has to remember that those involved may not be microbiologists, and that many hospitals where this would be done may also not have a microbiologist, or if they do, he may not be directly in charge of the sterilizing department. Therefore if a problem occurred, the question of responsibility would be on shaky ground.

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A. by B. Bromberger – Australia

Well, this carries on really from the last question because if you did decide to go against the manufacturer's recommendations in an institution where you could not get expert opinion, and if damage occurs, then it would be evidence of your negligence. Just because the manufacturer says you cannot do it, it does not mean, it is negligent if you do so. It will only be negligent if it is unreasonable for you to do it. The unreasonableness will be decided by what the experts say is 'unreasonable in that circumstance'.

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Q. by T. Bozsan – Australia

Dr Gaughran, are there any new concerted efforts to reduce the CPI values, you quoted in hospital areas? I am referring to the probabilities of infection during the course of surgical operations.

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A. by E.R.L. Gaughran – USA

I find it hard to answer not being an expert in operating procedures, but certainly the number of instruments that lie exposed to air for long periods of time could be reduced. In one particular study, the data on sutures came out very much better when sutures were not allowed to lie exposed to the air for a length of time. All such things reduce probability of infection, but how practical such procedures are during the course of an operation is another question. I am unable to suggest how to improve operating room procedures, unfortunately.

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Q. by A. Perceval – Australia

On the last question, I would say that the quality of the air would be important. There are several points that I would like to make. One is that I get the feeling that the speakers from industry seem to be asking us to accept products that do not have a degree of sterility of one in 10^6. I just wonder, is this cheaper. It seems to me that you are going to need more labels and I feel that most of those in hospitals are confused enough with what is sterile and what is not sterile, to have things that have intermediate degrees of sterility. On the legal aspect, Mr Bromberger said that an action would not be successful if a process was known to be good before and after an incident. This morning Dr Pritchard said that random sterility tests of small batches or a single manufacture of articles, e.g. additives for parenteral nutrition fluids, are in fact useless. Therefore, I would like to ask, how can we show that the process of adding things to bags in a pharmacy was good after an isolated incident of a contaminated bag that somebody might sue us for.

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A. by E.R.L. Gaughran – USA

May I start at the beginning of the question. First of all, the air in the operating room does not appear to be the most important source of organisms; it is the operating team and the patient himself. Secondly, we are not asking that you accept different products that are not sterile. When we speak about levels of sterility assurance, they are all sterile. The difference lies only in how sure we are that they are sterile.

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A. by B. Bromberger – Australia

I do not wish to give the impression that you could not succeed if the batch before was alright and the batch after was alright. What I was endeavouring to point out is that it is often very difficult to prove, or to find out, exactly what caused the problem, because the batch before is usually OK and the batch after is usually OK. In the case of Mr Grant, no one else developed dermatitis, so how does this one man who wears this one pair of underpants and gets dermatitis prove that the company was negligent in not removing the impurities from the fibre. The problem that this gives rise to, is answered in the United Kingdom and in some jurisdictions in the United States by saying that only if it would have arisen by way of negligence, then there is a presumption of negligence. In Australia, however, we simply say that we can infer, which is not a presumption, that negligence may have arisen. It can be rebutted, as in the Rowe case, where it was shown how it did occur. This is what I was endeavouring to illustrate with regard to the batch before and the batch after.

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Q. by A. Perceval – Australia

Yes, I believe you. It is just that I was having a crack at a fellow microbiologist, Dr Pritchard, who said that random sterility tests are useless and the question is how, in fact, do we know that the batch before and after was any good when the batch before did not kill anybody.

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Q. by E.R. Pavillard – Australia

Perhaps we can leave that question open. I would like to ask Dr Gardner an important question. Do you know of any states in Australia where certification of individuals operating sterilizers is required and are these certifications equivalent to a license?

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A. by J.F. Gardner – Australia

I think that this question should be referred to Mr Frank Hebbard, as I believe that in New South Wales there might be such a requirement.

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A. by F. Hebbard – Australia

For ethylene oxide sterilization, a license is required.

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SESSION IV

Biological Control

Chairman
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SESSION IV

Biological Control

Introduction to Session
William G. Murrell

The scope of this session embraces biological control as related to good manufacturing practice, sterility testing, and the determination of adequate processes for sterilization of medical products. It concerns the species of spores used to determine sterilizing processes and the spore challenges needed to test and validate processes.

We will discuss the problems involved and review the various practices and new developments and, I hope, solve some of the problems.

Let me introduce this session by making a few remarks about the choice of the test or indicator bacterium, spore resistance, biological and chemical process indicators, and process quality control (QC).

The biological component most important in process determination is the basic resistance for which the process is designed, i.e. the species of bacterium most resistant to the particular processing conditions being considered. This species is usually fairly arbitrarily chosen to be the most resistant to inactivation under the conditions encountered in the sterilizing process. These conditions include a wide variety of complex physico-chemical conditions that occur and change during processing, such as the various gas atmospheres and pressures generated during processing, the range of water activities, and the supposedly inert organic materials or environment in which the spore is placed. There is no guarantee that some species other than the test organism may not survive some of the above conditions better than the test organism, as many have not been tested under such conditions. The test organism is usually not a pathogen and is usually much more resistant to the sterilization treatment than possible contaminant pathogens; hence some of the above uncertainties are balanced by safety factors of unknown size, often unrecognized. The use of the selected test organisms has probably not led to faulty process evaluation. However, when new products or processes are being introduced, the uncertainties in the choice of the test organism and the differing effects of the conditions of processing on test organisms should be recognized and evaluated.

The resistance of a particular species of spores to inactivation by heat or radiation is not fixed or constant. It varies with the way the spores are grown, with the physical and chemical conditions during processing, and with growth recovery conditions. The level of resistance needs careful

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monitoring, particularly during storage under recommended standard conditions, before being used to determine processes.

Biological indicators, usually spore strips, are often used to validate sterilization processes. Because of the variability in resistance of spores in such strips, e.g. in their F and z values, the preparation of spore strips likewise requires biological control at all stages: during sporulation, preparation and standardization of spore crops, during their shelf life and during the determination of their resistance. Each stage requires constant appropriate QC. We are concerned with a spore population that needs characterization in three ways:

(i) It is necessary to know the population size (n_o) and the stability of n_o. Because of the approximately exponential death or survivor curve, thermal death time increases with increase in n_o.

(ii) The decimal reduction time (D value) of the spore crop must be known.

(iii) The characteristic shape of the survivor curve needs checking since abnormalities may lead to errors in the calculated thermal death time (Figure 1).

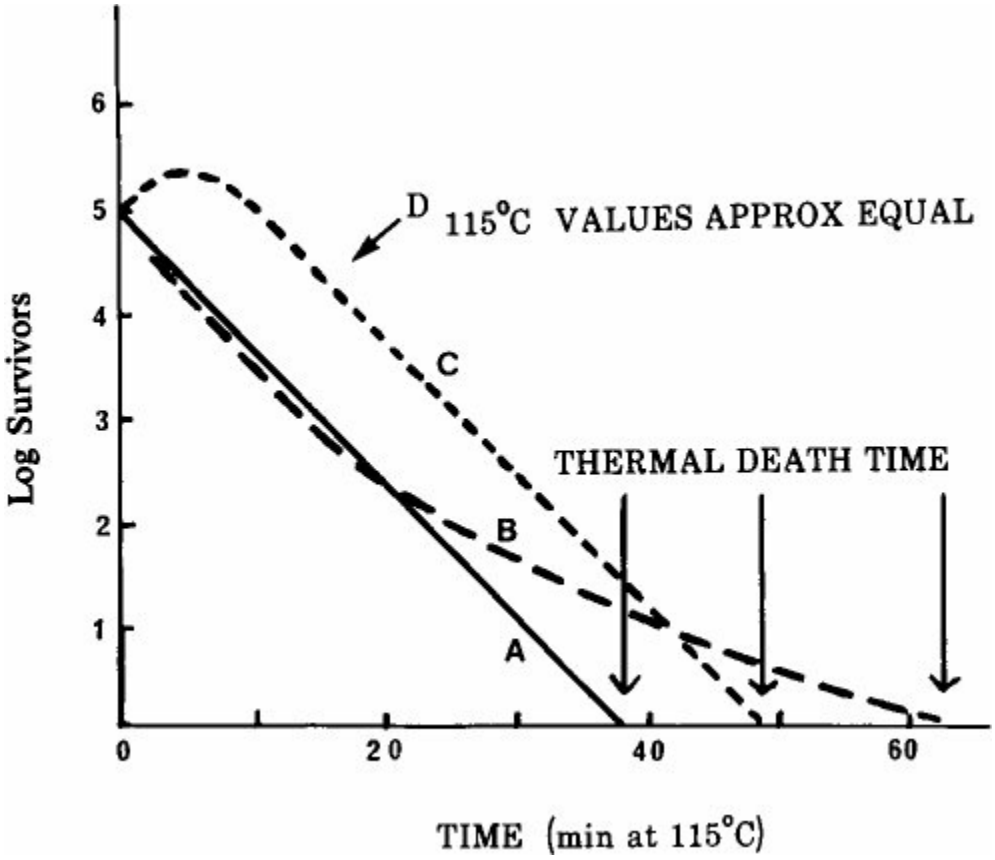

Figure 1. Effect of the shape of the survivor curve on the thermal death time (↑) for bacterial spores having similar decimal reduction times (D values) over most of the survivor curve.

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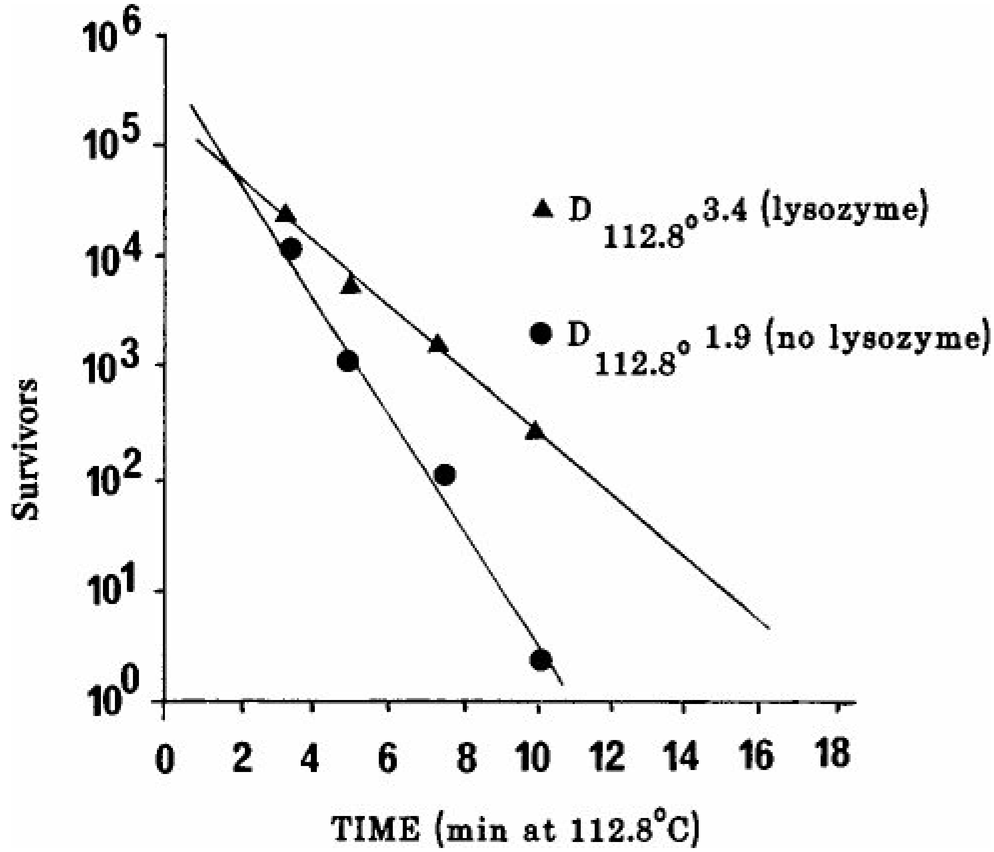

Figure 2. Effect of lysozyme in the growth medium on the recovery of heated spores of *Clostridium botulinum* 62A [Drawn from data of Alderton *et al*. (3)].

I cannot emphasize too strongly that QC of biological indicators requires a major effort and must be done correctly.

The number of contaminating spores, or the bioburden, should be monitored. If it becomes too high, the carefully evaluated process may be inadequate and understerilization may occur.

In addition to statistical and other inadequacies of sterility tests, the validity of sterility tests depends on the adequacy of the medium to detect survivors. Constant monitoring of the media and conditions used to test for survivors is therefore essential. The growth performance of media must be routinely checked with both standard and sublethally damaged test organisms. The risk that a patient provides better growth or survival conditions for unrecognized sublethally damaged organisms is always present. For example, lysozyme added to the medium enables the germination of many heat-damaged spores that would otherwise be considered nonviable (Figure 2). Germination is increased resulting in increased D values (1,2). Lysozyme in the recovery medium raised the heat resistance of Type E *Clostridium botulinum* spores by 1800-fold and Type A up to 3-fold (3).

The use of biological and chemical indicators as substitutes for process QC in validating processes has been frequently raised in this Symposium. The advantages and disadvantages of each have been summarized in Table 1. I would like to suggest strongly that, in view of the problems that I have indicated above, we are seriously deluding ourselves about the value of biological indicators and spore challenges. A good chemical indicator of the amount of sterilization treatment given is probably much more reliable, rapid, less expensive, and more readily automated. Better still, proper production controls supported by adequate records of process parameters provide far greater assurance of safety than either biological or chemical indicators. Production control is the most

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effective means of ensuring that safe products of the required quality and composition reach consumers. Appropriate QC records throughout production are essential to good manufacturing practice and their provision for regulatory authorities should be made mandatory.

Table 1

Comparison of indicators of severity of sterilizing processes

Biological	Chemical	Process quality control only
ADVANTAGES		
Apparent realism (emulation of practical situation)	Rapid Obvious results Inexpensive Readily automated	Essential to GMP No other monitoring needed
DISADVANTAGES		
False security if – wrong test species used – resistance unstable – challenge positioned wrongly – recovery conditions deficient – sterility tests faulty	Accuracy may be suspect (Chemical change not proportional to sterilant dose under some conditions)	If faulty process not recognized, it may lead to inadequate sterilization
Time consuming Heavy work load End product testing – statistically unsatisfactory – inadequate sampling – expensive		

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Biological Indicators – The World Standard for Sterility Monitoring

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Introduction

The paper will cover the current technology of biological indicators with special emphasis on some of the important scientific and technical issues that are involved in their manufacture and application. Rationale and scientific justification will be given for the selection of indicator organisms, spore carriers and packaging, and a critique of the varied biological indicator systems that are employed worldwide by industries and hospitals. Current US progress in developing biological indicator standards will be discussed as well as a worldwide plan for development of biological indicator standards that will alleviate problems worldwide when biological indicators are employed to monitor, measure, and evaluate sterilization processes. The concept of the biological indicator reference standard will also be discussed and basic recommendations and justifications for specifications of each reference biological indicator system given.

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Sterility Monitoring

One of the important quality attributes of many medical devices and drugs is sterility, because the total absence of microorganisms on medical items can immeasurably reduce the potential for treatment and nosocomial infections. Sterility is internationally generally accepted as an absolute quality attribute that guarantees complete absence of viable microorganisms even though sterilization technologists and scientists basically agree that sterility attainment is a probabilistic function (1). The use of the words ‘sterile’ or ‘sterility’ on products also has legalistic and government regulatory ramifications that have a direct impact on both domestic and international marketing. Thus, the assurance of the sterilization process to produce consistently a measurable degree of sterility attainment is paramount to the use of the sterility attribute on any labelled medical product. Scientists have recognized that the simple passage of packaged goods through a sterilizer chamber is not enough to guarantee attainment of sterility. Sterility attainment and maintenance are derived from the sum total of an integrated manufacturing process that includes product specifications and assembly, packaging, environmental monitoring, and validated and controlled sterilization processes (2). The varied procedures by which sterilization processes can be controlled and monitored include instrumental records of the physical and mechanical aspects of the sterilization process, sterilized product testing, and use of sterility monitors, i.e. biological and/or chemical indicators. It is not the purpose of this presentation to cite and discuss the technical debate over which system or systems are preferable to the other, but it is not debatable that worldwide biological indicators do serve as the foremost and most widely accepted monitors of sterility attainment. Since biological indicators are specially devised standardized, calibrated devices containing spores of recognized sterilization-resistant microorganisms, it readily becomes apparent why biological indicators are internationally recognized as the prime system of sterility attainment and measurement. A simple analysis by the quality control laboratory can easily detect and measure the destruction of these sterilization-resistant microorganisms (3).

Biological indicators are used in many industrial and hospital procedures such as new sterilization process development, product-sterilization cycle compatibility studies, sterilization cycle validations, and systematic or routine sterilization process monitoring (4). Since the destruction of the spores on biological indicators is the key element in determining sterility attainment, it is critical and mandatory to have reliable, reproducible, and exacting specifications not only to assure meaningful data but also to allow comparative studies and analyses of different, consecutive, and varied sterilization processes. Thus, it is imperative that biological indicators should be standardized and recognized by internationally acceptable specifications to allow for scientific, regulatory, and legal comparisons of sterility efficacy attainment. The requirements for internationally acceptable biological indicator standards include the following elements:

- Carrier
- Packaging
- Labelling
- Stability
- Organism selection
- Resistance measurement

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Microorganism Selection

Historically, many microorganisms and systems have been used as indicators of sterility attainment, such as bacteria, yeasts, and moulds, as well as microorganisms from products and garden soil. However, worldwide sterilization scientists have generally agreed that the use of spores of spore-forming bacteria are more suitable than other microbial forms, because they have a proven greater resistance and stability to environmental, chemical, and physical conditions. Perhaps, the first applications of spores for sterility attainment came from the evolutionary work done on food preservation and the continuous development of thermally processed canned foods. Marginally processed foods that were spoiled or causative agents for one type of food poisoning usually contained spore-forming bacteria, such as *Clostridium sporogenes, Clostridium botulinum*, and *Bacillus* spp such as *B. macerans*. Food processing scientists have made use of many of these organisms in their spore state to develop specific thermal processing conditions for low and high acid and proteinaceous canned foods (3).

Similarly, hospital and industrial scientists have used a variety of systems to measure sterility attainment. Most have come to the conclusion that spores of spore-forming bacteria are far superior to other microbial forms for this application since they have been convinced that product sterility tests are not satisfactory for sterility measurement. Table 1 shows a list of bacterial spore-forming microorganisms that are internationally recognized as having spores reproducibly and reliably resistant to specific sterilization processes. Where more than one bacterial species is listed for a sterilization process, the first microorganism is preferred or universally accepted for sterility monitoring. Although the genus and species of the spore former is shown, subspecies and strain variations are as common with these microbes as with other organisms. Commonly, the sterilization resistance factor is highly variable not only within different bacterial strains of a spore former but also within cultures of spores of a selected bacterial species. The organisms listed in Table 1 do not have strain or culture collection designations. This lack of culture designations from culture depositories has been of concern by various international groups in basic agreement with the principle of biological indicator standards. Based on experiences of some commercial biological indicator manufacturers, spore-forming cultures obtained from recognized culture depositories usually do not retain their sterilization process resistance through normal culture collection maintenance.

Table 1

Biological indicator organisms	
Sterilization process	
Pressurized steam	*Bacillus stearothermophilus*
	Clostridium sporogenes
	Bacillus macerans
Dry heat	*Bacillus subtilis* var. *niger (globigii)*
Ethylene oxide	*Bacillus subtilis* var. *niger (globigii)*
Formaldehyde	*Bacillus stearothermophilus*
	Bacillus subtilis

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Irradiation	*Bacillus pumilus*
Gamma electron beam	*Bacillus sphaericus*

Selective isolation of spores having high and reproducible resistance can be obtained. Proprietary or commercial biological indicators must be controlled to prevent loss of resistance from mass culture or spore crop procedures. Cultural conditions, harvesting procedures, purification of the spores, spore storage, and other factors play important roles in the quality of the spore crops that would be used in the manufacture of biological indicators.

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Spore Carriers

Spores of sterilization-resistant organisms are not in themselves biological indicators because the resistance is a sum total of all components of recognized biological indicators that include the spore carriers. Spore carriers may be of different types depending upon the specific application. Table 2 shows examples of materials and configurations that act as spore carriers. There are many variations of these that have been used by sterilization scientists and the large matrix of these configurations explains all too often why there is disagreement among sterilization scientists on the recognition of an international standard for biological indicators. Other controversial complications arise from biological indicators manufactured by direct inoculation onto components and onto simulated and finished products. Significant resistance variations are present between spores on different carriers and configurations. There may be good technical rationales for the use of one type of carrier over another but the lack of a specific spore-carrier standard is open to queries concerning the reliability of sterilization data. Selection of one carrier material and configuration for a standard is preferable to allowances for all the types shown in Table 2, as this would offer a base unit comparison for using other materials and configurations in sterilization processes where others are required (5).

Table 2

Spore carriers

Materials	Configurations
Paper	Strips
Textiles	Discs
Non-woven fabrics	Pellets
Glass	Cylinders
Metal	Product components
Plastics	
Products	
Simulated products	

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Spore Carrier Packaging

Although naked or unpackaged spore carriers can be used for sterility monitoring, it is more common and practical to have the spore carrier in some form of packaging. Table 3 shows a list of packaging options in which spore carriers are used in hospital and industrial products. Each of these packaging options is generically described and most types have variations depending upon the packaging material specification and the package dimensions. Obviously, these varied selections of packaging have brought a fair amount of confusion to individuals who are concerned with biological indicator standardization. Use of these forms of packaging also has merit as it relates to sterilization of specific products. For example, the earliest and still used packaging of spore strips consists of glassine envelopes.

Table 3

Biological indicator packaging options
Glassine envelopes
Preformed pouches
Plastic film pouches
Metal or metal/plastic laminate pouches
Glass vials or ampoules
Self-contained BI packaged systems
Inside the packaged product

All too often, users of such biological indicators do not know or recognize that the need for packaging the spore carrier is primarily to protect the integrity of the spore carrier after sterilizer processing, so that it can be transferred to the laboratory and analysed for sterility attainment. Of secondary importance in assigning resistance to a biological indicator is that which is contributed by the spore carrier and its packaging. In the case of the spore strip in the glassine envelope, the resistance of the biological indicator is determined by using the entire packaged spore strip and not the naked strip or spore carrier itself.

Use of other biological indicator packaging options is beneficial, advantageous, and necessary for other specific applications where sterility attainment must be ascertained. In wet environments such as washer-sterilizers, immersion water-filled sterilizers, and glass-packaged liquid products, the preferred biological indicator would be a glass vial or ampoule (2), wherein the destruction of the spores would be dependent upon thermal energy penetration through the glass packaged liquid to destroy the liquid-suspended spores. The resistance measurement of the glass packaged vial would be the total resistance of the biological indicator unit to steam or thermal energy exposure when directly placed in the chamber or suspended in the liquid phase of the packaged product itself. If the latter system is used, it is important to recognize that the thermal insulating effects of the packaged liquid will have a great influence on the total resistance of the biological indicator, much greater than the simply exposed unit.

The use of self-contained biological indicator systems is becoming established as the preferred method of routine sterility measurement because of technical and economic advantages. The self-

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contained units obviate the need for specific laboratory trained operators and microbiologists and offer ease of interpretation of the sterility attainment without microbiological training. The self-contained systems can be employed in almost any thermal or steam and gaseous sterilization process where the sterilant is dependent upon its direct penetration into the packaged product. The self-contained system may be employed in two ways, selective placement in the sterilizer chamber or in specially demarcated units of the packaged product (6). Since laboratory operations and technically trained personnel are highly costly to many hospital and industrial operations, the self-contained biological indicator system offers sterility measurement, reliability, and assurance at minimal expense.

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Resistance Measurement

The most scientifically disputed technical issues of biological indicators and their use arise from sterilization scientists and microbiologists as to the agreement by which method should resistance of biological indicators be measured. All sorts of handmade units of apparatus have been historically employed and many are technically not satisfactory because of nonreproducibility of results from one sterilization exposure cycle to another. Examples of resistance apparatus used are circulating oil baths, thermal death time chambers, and commercial sterilizers. Without going into specific technical aspects of the problems associated with these types of units, there is in the US a specially devised and controlled chamber, called a BIER vessel, containing the basic elements of control and uniformity that reliably and reproducibly measures the sterilant resistance of biological indicators. There are two standards for BIER vessels: steam or thermal units (7) and ethylene oxide gas units (8). The key specification for these standardized vessels is that all physical aspects of the sterilant gases are tightly controlled and thus yield reproducible resistance data from cycle to cycle on different batches and styles of biological indicators. The chamber controls and recording devices yield data that allow for direct comparisons. Although the BIER vessels may have different engineering designs, such as size, they do yield biological indicator resistance data that are equivalent and accurate. As the BIER units are unique analytical devices, there is no need for users of biological indicators to have them at their direct access because they are best used by commercial biological indicator manufacturers who can economically justify their use and operation.

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Proposed Biological Indicator Reference Standard

It is readily obvious from this presentation that industries and hospitals do require multiple types and designs of biological indicators but there must be a mechanism by which these different biological indicators can be compared and rated for their intrinsic resistance parameters. The establishment and acceptance of one type of biological indicator as a standard would cause immeasurable problems for all who must attest to the sterility of packaged products. Therefore, it is proposed that standards for commercial biological indicators be established with labelling specifications as shown in Table 4. Secondly, a primary biological indicator reference standard would be established similar to the reference standards that would be employed for chemicals and other physical items. The primary reference standard would use spores of *B. stearothermophilus* for steam, spores of *B. subtilis* for dry heat, spores of *B. subtilis* var. *globigii* for gaseous sterilants (ethylene oxide and HCHO), and spores of *B. pumilus* for irradiation processes. Table 5 shows the proposed primary reference standards for biological indicators for different sterilization processes. The common elements for all these biological indicators are the spore carrier and the packaging. These primary standards would serve as the base by which all other biological indicators are compared or related. The primary reference standards should not be used for routine sterilization operations but strictly for manufacturing of biological indicators and possibly for sterilizer operations that require a comparative data base. The data derived from standard biological indicators will also offer common international agreement of sterility assessment and attainment, and uniform and fair judgment of commercially available biological indicators by regulatory and other governmental agencies for all sterilization processes.

In summary, the multiplicity of generic biological indicators that are used in worldwide sterilization operations shows that biological indicators will continue to be the standards for assessment and measurement of sterility attainment. The establishment of and agreement on an international reference standard of biological indicators for specific sterilization processes will alleviate technical, legal, and regulatory disputes on the significance of the sterility labelling of packaged products.

Table 4

Labelling requirements for biological indicators

Name of manufacturer	Name of spore former
Trade name of product	Batch or lot number
Types of monitored sterilization processes	Expiry date

Other instructional and technical information

A. USP XIX resistance
B. D value
C. Spore counts
D. Cultural procedures and conditions
E. Results interpretation
F. Record keeping

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Table 5

Proposed primary standards for biological indicators

	Steam	Dry heat	Ethylene oxide and formaldehyde	Irradiation
Organism	*B. stearothermophilus*	*B. subtilis*	*B. subtilis* var. *globigii*	*B. pumilus*
Carrier	Paper strip	Paper strip	Paper strip	Paper strip
Package	Glassine envelope or paper pouch	Glassine envelope or paper pouch	Glassine envelope or paper pouch	Glassine envelope or paper pouch
Resistance measurement device	BIER steam unit	Dry heat oven	BIER gas unit	–

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Spore Resistance and the Basic Mechanism of Heat Resistance

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Introduction

The resistance of bacterial spores is responsible for the development of and determines many of the sterilization processes that are being discussed in this Symposium.

Spores are resistant to many drastic physical and chemical treatments that normally kill vegetative cells. Their most outstanding property, however, is their resistance to wet heat. The properties that determine their heat resistance are probably also involved in determining other properties, such as dormancy and their resistance to a variety of other adverse treatments. Heat resistance varies greatly both between and within species, and is a function of sporulation conditions, heating environment, and recovery conditions. The range in heat resistance of spores of all of the species of *Bacillus* and *Clostridium* is about 100 000-fold (1). However, in each species, the process of sporulation enhances the resistance by about 40°C above that of the mother cells of the same species (2).

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Structure and Composition

The bacterial spore is a unique biological structure resulting from a complex differentiation process within the vegetative bacterium. The following describes briefly the structure of the spore. The protoplast, or inner core, contains the cytoplasm, including the normally heat-labile macromolecules: DNA, RNA, ribosomes, enzymes, and other proteins. The protoplast is surrounded by a rudimentary germ cell wall. The next layer, the cortex, consists of peptidoglycan, a polymer of β1-4-linked amino sugar chains, cross-linked by short peptides of alanine, D-glutamic acid, and diaminopimelic acid. It differs from the peptidoglycan of bacterial cell walls in the nature of the cross-linking peptides, and in having less cross-links, since over half the muramic acid residues are modified to the delta lactam (2). The cortex is within a second cell membrane, and all this is surrounded by tough proteinaceous coat layers (Figure 8c). Finally, in some species, loose-fitting exosporia, appendages, and protein crystals are present.

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Heat Resistance Theories

Theories on the basis of heat resistance are numerous (3) and fall into two main classes. The first one proposes that resistance results largely from partial dehydration of the protoplast. It is known that drying greatly stabilizes proteins and some organisms to heat, and that the interior of the resistant spore has a characteristic high refractility, and hence most probably a relatively low water content.

Measurements of the density (4) and refractive index (5, 6, 7) of spores, while not giving a precise value, indicate that the water content of the protoplast must be low (<30%). Water adsorption isotherms of isolated spore cytoplasm show that water activities (a_w) of the order of 0.7-0.8 are required to achieve this water content (8) in agreement with the value suggested by the enzyme stabilization experiments of Warth (9, 10) (Figure 1). Such a reduction in water activity would require high pressures (40-50 MPa) which would have to be sustained by tension in the layers surrounding the protoplast (10).

Many workers have assumed, therefore, that partial dehydration could provide a general explanation of heat resistance. On the other hand, spores contain a very high concentration of calcium dipicolinate (CaDPA), together with lesser amounts of glutamate, phosphoglycerate, low molecular weight basic proteins, and possibly other substances that might stabilize spore components. The second group, therefore, proposes that stabilization depends on molecular rearrangements resulting from the presence of these substances, to give a 'general molecular stabilization' not dependent on partial dehydration itself. The theories are not mutually exclusive in that reduction in water implies an increase in interactions between the spore solutes, and that specific stabilizations, involving, for example, CaDPA may occur, in addition to a general stabilization due to dehydration.

Several pertinent examples of both classes of theory will be mentioned. For the partial dehydration mechanism, the theories dominating the literature are based on the mechanism by which the cortex may stabilize the spore protoplast.

(a) *Cortical Theories*

Four theories have been proposed:

(i) the contractile cortex theory (11)
(ii) the expanded cortex theory (12, 13, 14)
(iii) the osmoregulatory cortex theory (13, 14)
(iv) the anisotropic cortex theory (2, 10).

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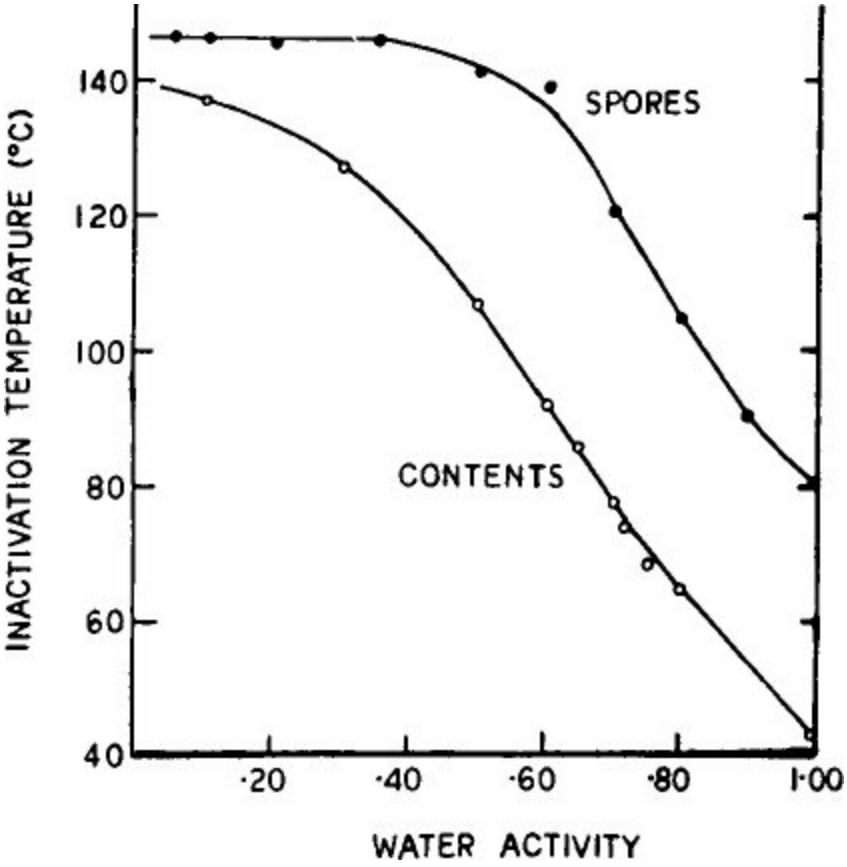

Figure 1. Heat stability of glucose 6-phosphate dehydrogenase at different a_w. Symbols: ○, spore contents; ●, intact spores. Inactivation temperature was the temperature at which the inactivation rate constant k was 0.01 min^{-1}. At a_w about 0.7, the enzyme had similar resistance to that in the intact spore at a_w 1.00 (9).

According to the contractile cortex theory, 'the protoplast is held dehydrated and shrunken by contraction of the cortex, which maintains the lowered water content by mechanical pressure on the protoplast'. There are no direct firm data to support this theory, but the polymeric chemical properties of the cortical polymer (1), the significant relation between level of heat resistance and diaminopimelate content as an index of the amount of cortical cross-linking (15), the calculations of Algie (16) on cortical strength and possible internal a_w, and the electron microscopic appearance (17) are compatible with the contractile cortex theory.

In the expanded cortex theory, partial dehydration is believed to be achieved by expansion of the cortex against the coats and application of pressure on the protoplast. The expansion is believed to result from the electrostatic repulsion of negatively charged groups of the cortical polymer. This repulsion is maintained by the absence of high concentrations of cross-linking cations. The evidence claimed to support this theory and the data that do not are presented in Table 1.

Table 1

Expanded or osmoregulatory expanded cortex Theory of Gould and Dring (13, 14, 18) and Gould (53)
Evidence not supporting:
Difficult to obtain the low ionic strength (17).

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Evidence claimed to support: Insufficient peptidoglycan to fill the cortical space (13). Coat strength adequate (11). 4M $CaCl_2$ reduced heat resistance of coat-defective spores by contracting cortex (13). Sensitized spores were stabilized by 3.6M sucrose (13, 14). Inward expansion of cortex in electron micrographs of disrupted spores considered to indicate that cortex is responding to expansive forces.	Retention of heat resistance by coatless mutants (54), coat-defective spores (55), and urea + mercaptoethanol-treated spores (13). Concentration of counterions in cortical polymer more likely to contract cortex than develop osmotic pressure (17). Dielectric data indicate absence of free ions (37, 38). Biophysical state of forespore developed before cortex and coat formation complete (27). Calculated pressure insufficient to dehydrate core (10). Uniform increase in *in vivo* heat stability of spore enzymes with redution in external a_w (10), i.e. no lag in response to lower a_w.

Gould and Dring (13, 14, 18) further developed their ideas and proposed that the loosely cross-linked cortical polymer, normally electrically neutralized by free positively charged counterions, would be osmotically active (osmotic pressure >3000 kPa) and take up water, causing the cortex to swell and partially dehydrate the protoplast. They used the same experimental evidence to support this osmoregulatory cortical model (Table 1).

Warth (2, 10) considers that the pressure necessary to dehydrate the protoplast results from cortical tension. He believes, the cortex is in tension in its concentric layers but in compression in the radial direction (Figure 2). There is, however, no evidence to indicate how these forces arise during sporulation. Three types of mechanism may contribute. During biosynthesis of the cortex, the concentric layers (probably long glycan chains) may be contracted by cross-linking reactions between the chains. Additionally, entropic swelling pressure may be developed in the radial direction by hydrolysis of certain cross-links. Thirdly, synthesis and accumulation of substances, particularly CaDPA, in the protoplast, after the cortex is partly formed, would displace some water and increase the turgor pressure.

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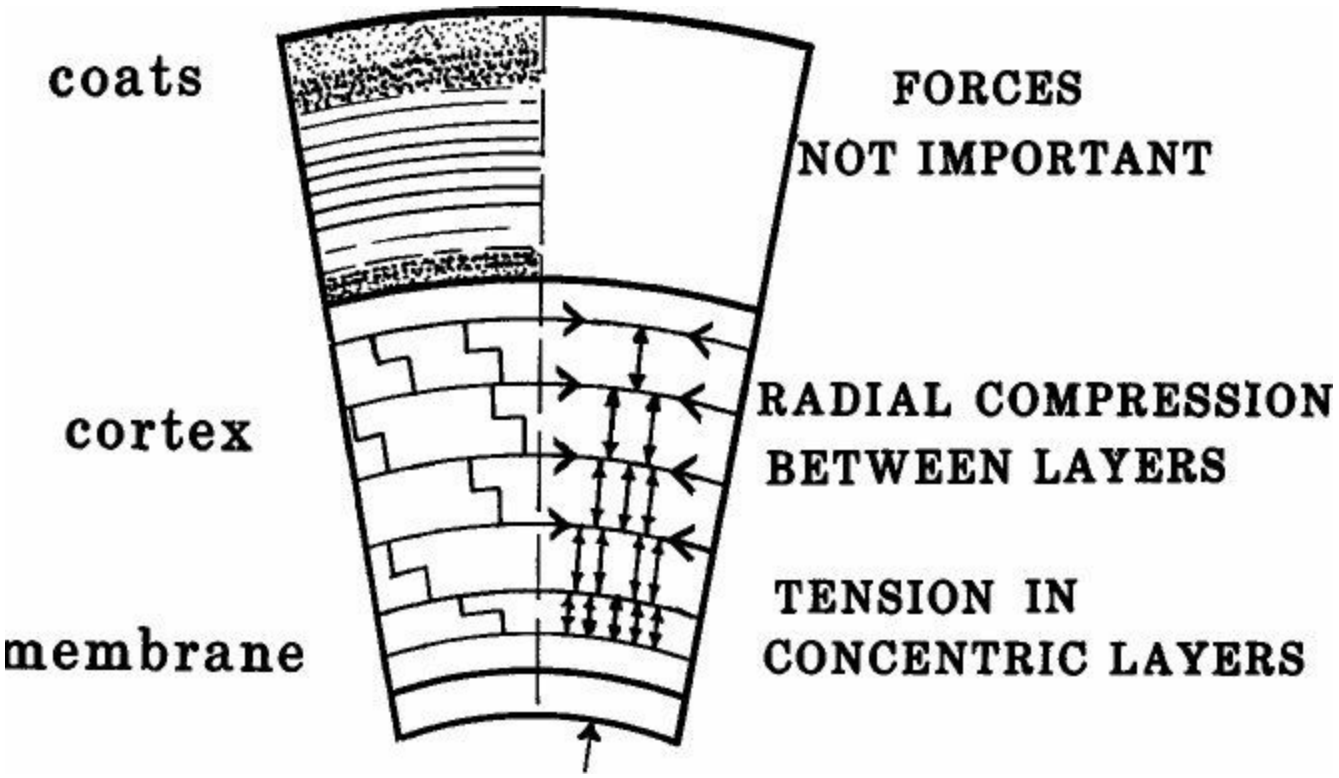

Figure 2. Forces in a model of a spore in which the pressure necessary for dehydration of the core is maintained by swelling of the cortex, by an anisotropic cortex in which layers parallel to the surface are in tension, while the swelling pressure is confined to the radial direction (10).

Warth (9, 10) then examined whether dehydration provided a general mechanism for stabilization of proteins, which he considered the most important class of heat-labile compounds. It has been known for many years that by drying, some proteins can be stabilized to heat (19). Assuming that the spore protoplast has a low water content, can dehydration account for all the stabilization required?

Warth's *in vitro* experiments with dried spore cytoplasm showed that reduction in a_w dramatically increased enzyme stability (9, 10). These results were obtained in the presence of any soluble endogenous compounds that may stabilize enzymes. Removal of the low molecular weight compounds had little effect on the stability of glucose 6-phosphate dehydrogenase in the higher a_w region. However, the addition of a variety of substances including the spore components CaDPA, Ca glutamate and sulpholactate did partially protect some enzyme systems at lower a_w (0.7), i.e. they restored some of the stability that was lost on partial purification of the enzyme.

Although there are problems with *in vitro* examination of enzyme stability due to the inevitable composition and structural changes that may occur during disruption of the spore, Warth's results do indicate that enzyme stability within the spore can be achieved simply by partial dehydration and that a protoplast a_w of not greater than 0.73 (20% water content) is required to give the stability encountered *in vivo*.

In considering the low water content of the protoplast, how could such a dehydrated system be attained and maintained? Warth (10) believes that for a spore in water with a partially dry interior, the existence of a pressure which may be called the turgor or osmotic pressure of the spore is required. This pressure is generated by the cortex, where the cortical peptidoglycan is assumed to be initially polymerized under strain-free conditions. Under these conditions, potentially very high

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swelling pressures exist and could be directed to compression of the interior, and fixed by cross-linking the glycan chains so as to bear the tension. In this situation, maintenance of the tension is possible since each successive layer of the cortex must transmit its pressure by compression of those beneath it. Warth's description of the system is not untenable, and certainly offers more of an answer than other dehydration models involving the cortex.

(b) *General Molecular Stabilization Schemes*

A number of the recent general molecular stabilization theories (Table 2) stem from the presence of large amounts of the spore-specific compound dipicolinic acid (DPA). Powell and Strange (20), Powell (21), and later Tang *et al*. (22) and Grecz *et al*. (23, 24) suggested that stabilization resulted from a chelate-cement matrix brought about by calcium ions chelating DPA, resulting in a waterproofing effect that trapped and protected the essential labile components. Bradbury *et al*. (25) suggested a solid support system of CaDPA (see below).

Table 2

Theories on mechanisms of heat resistance

Early theories	Ref.
Water-shielded protoplast	Cohn (56), Dyrmont (19)
Low water content	Lewith (57)
Uncoagulatable state	Esmarch (58)
Colloidal shrinkage	Daranyi (59)
Partial dehydration of protoplast theories (Cortex theories)	
Contractile cortex	Lewis, Snell, Burr (11)
Expanded cortex	Alderton, Snell (12)
	Gould, Dring (13, 14)
Osmoregulatory expanded cortex	Gould, Dring (13, 14, 18)
Anisotropic swollen cortex	Warth (2)
Reverse osmosis by centripetal cortex synthesis	Algie (16)
General Molecular Stabilization Theories	
Protein stabilization:	
Enzymes bound to protect active groups	Virtanen, Pulkki (60)
Masking of polar groups, 'bound protein'	Waldham, Halvorson (61)
Enzyme stabilization	Sadoff (62)
Insoluble thio-gel theory	Black, Gerhardt (31)
Chelation theories:	
Waterproofed system from CaDPA incorporation	Powell (20, 21)
Chelate cemented matrix	Tang *et al.* (22)
	Grecz *et al.* (23, 24)
Shrunken protoplast encased in structurally contracted cortex	Marshall, Murrell (8)
	Murrell (27, 28)

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In 1970, Marshall and Murrell (8) pointed out that after stage IV of spore formation, the protoplast is probably reduced to its minimum volume by the effect of metal ions or other gelation co-molecules that are being concentrated at this stage. This would result in exclusion of water. While the protoplast is in this minimum volume state, the cortex is built around it in a structurally contracted and stable cross-linked manner. Some residual water in the protoplast will possibly become chemically complexed with endogenous solutes such as DPA.

In subsequent studies concerned with the biochemical development of UV and heat resistance, electron microscopy of the developing spores revealed that the protoplast volume was actually halved at about this time (26). This reduction in volume was associated with marked changes in the cytological appearance of the DNA, UV resistance, the type of DNA photoproducts, and other biochemical events (27).

This volume reduction and associated changes have been likened by Murrell (28) to the phenomenon of synaeresis, proposed initially by Graham (29). The phenomenon may be described in the following way. ‘Under certain circumstances the particles of a gel may undergo a process of rearrangement or reorientation so as to produce a more stable system, a process which is accompanied by the separation or expulsion of a certain amount of fluid’ (30). This description does not imply a denaturation process, only a reduction in protoplast volume and hence molecular reordering, probably in the presence of various cations and basic proteins.

The observation of these changes in the protoplast has been followed by several studies that include much of our recent research which is aimed at understanding the biophysical state of the protoplast and its relation to heat resistance.

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Biophysical State of the Spore Protoplast

(a) *Water State of Spores*

This is of critical importance to a discussion on heat resistance, as the water content and water activity of the system can have a big influence on the heat resistance of spores and proteins. However, it is very difficult to describe quantitatively in terms of water properties or to state what is happening in the spore as it is not experimentally possible to determine the amount of water or its state in the various compartments of the spore (Figure 3). Although certain evidence indicates that the water content of the protoplast is probably low, spores are permeable to water (8, 31, 32, 33) and have a relatively high water content on a wet weight per volume basis (57-86% w/v) (8, 31). This suggests that the protoplast contains moisture. Permeability studies with labelled water indicate that water molecules virtually permeate all parts of the spore (8, 31). Further, studies on the heat resistance of spores equilibrated to various equilibrium humidities indicate that water can move in and out of spore protoplasts affecting their heat resistance (9, 34, 35) (Figure 1).

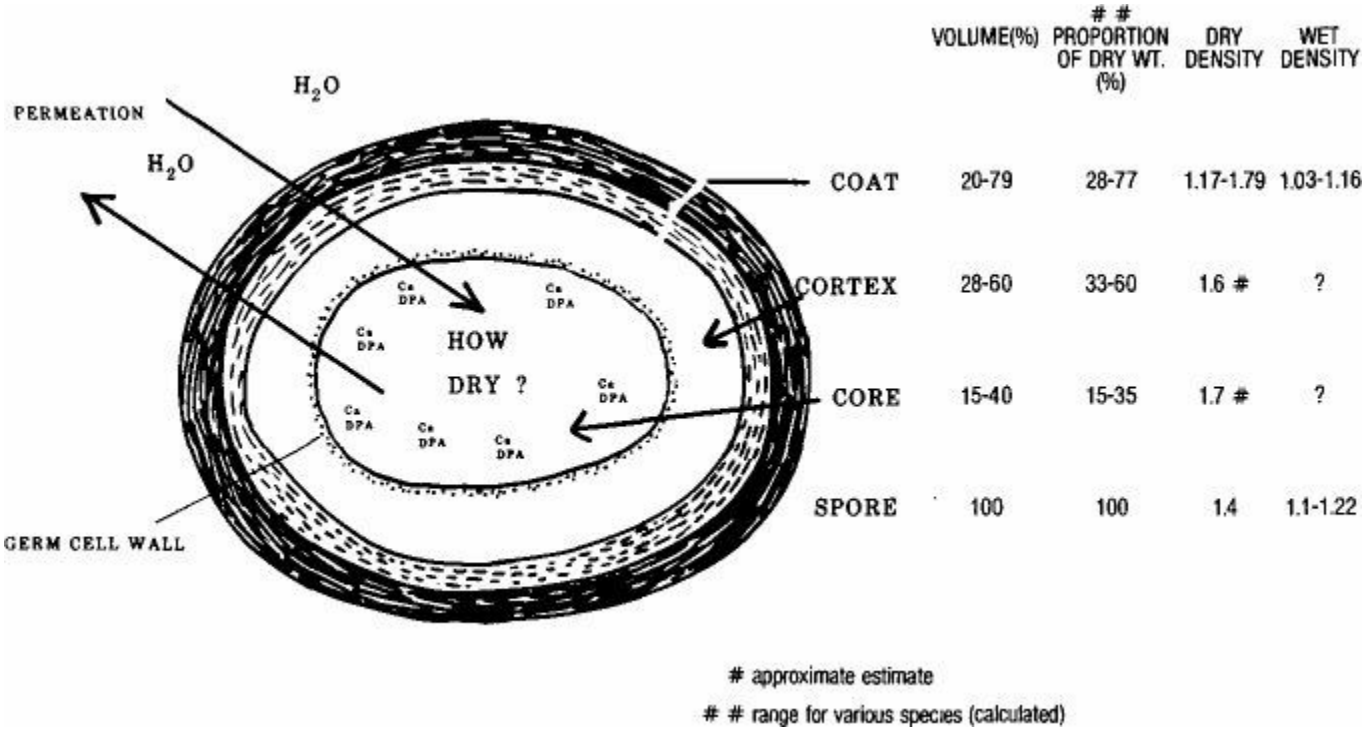

Figure 3. Diagramatic section of a mature spore indicating the various cytological compartments of the spore, their proportion of the whole spore, density, and permeability to water. Data from 4, 14, 8, and Murrell (unpublished).

These studies, however, do not rule out the idea that the amount of water in the protoplast region was reduced relative to the outer regions, as proposed by many of the theories. If there is some moisture in the protoplast and the amount of this water affects heat resistance, it would seem that there are two extreme states that can exist in the protoplast. At one extreme, it could be a moist gel of reduced a_w, and at the other, an insoluble macromolecular matrix containing a small amount of absorbed but relatively free water (high a_w). In the latter case, resistance would depend on the heat stability of the molecular matrix.

Since water can move freely in and out of the spore, Bradbury *et al*. (25) assumed that it was reasonable to extrapolate that the water bound by the different spore fractions, i.e. the coat, cortex,

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and protoplast, will have similar molecular mobility and sorption properties to that absorbed by the fractions isolated from disrupted spores. Based on this assumption, proton NMR studies on water mobility in spores and isolated spore fractions reveal that the water in the intact spore protoplast appears more mobile than that absorbed to the outer integument fractions (25). That is, the transverse relaxation rate ($1/T_2$) at a particular relative humidity was consistently less for intact spores than for isolated coat and coat + cortex preparations (25) (Figure 4). (The lower the relaxation rate, the more mobile is the water.) The molecular mobility of the water in the protoplast, however, is nowhere near as great as that in free water. Further, water sorption isotherms show that at particular relative humidities the isolated protoplast material absorbs as much as, and often more, water than does the isolated coat and cortex fractions (36). These observations have been interpreted as disproving the theory that the protoplast is drier than the outer regions of the spore (25).

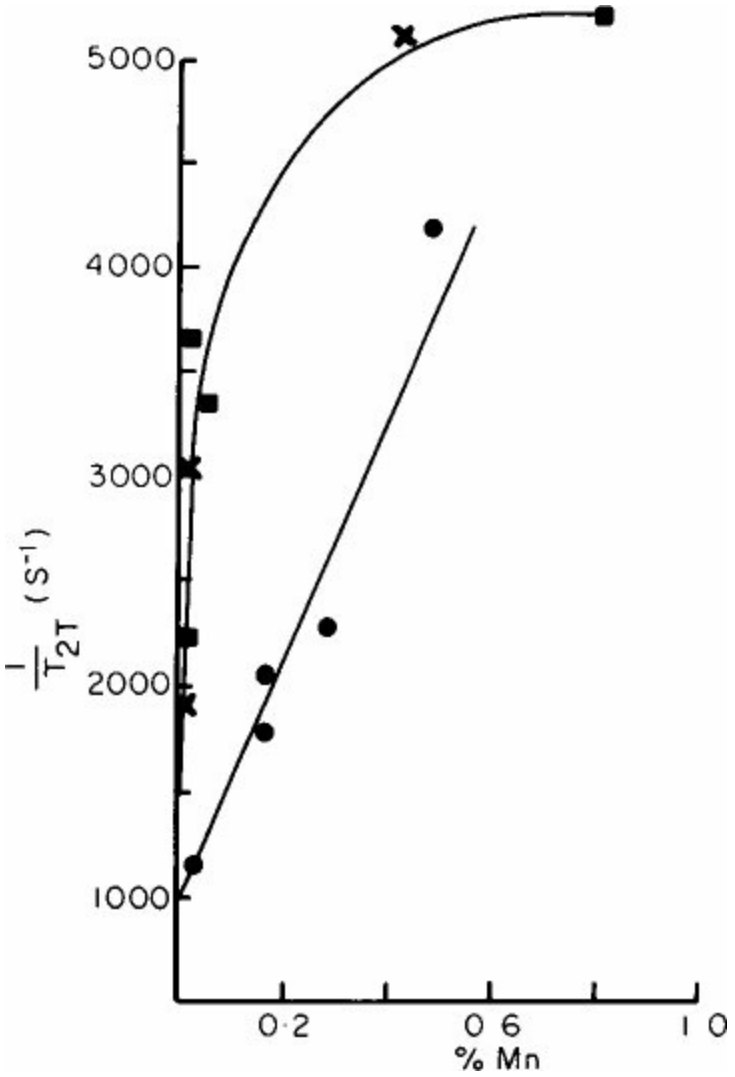

Figure 4. Graph of water relaxation rate ($1/T_{2T}$) for spores and spore components equilibrated at a_w of 0.98, against the % Mn: spores (●), coat (■), coat + cortex (X). The % Mn affects the $1/T_{2T}$ value; however, if the values for the spores or their components are compared at the same Mn content, the value for intact spores is less than that of its components at all Mn contents and a_w, indicating that the water in the spore is more mobile than that absorbed to the isolated fractions (25).

If there is a certain amount of water in the protoplast and even though it may be quite small in amount, what then is the molecular mobility, not necessarily solution mobility, of the low molecular

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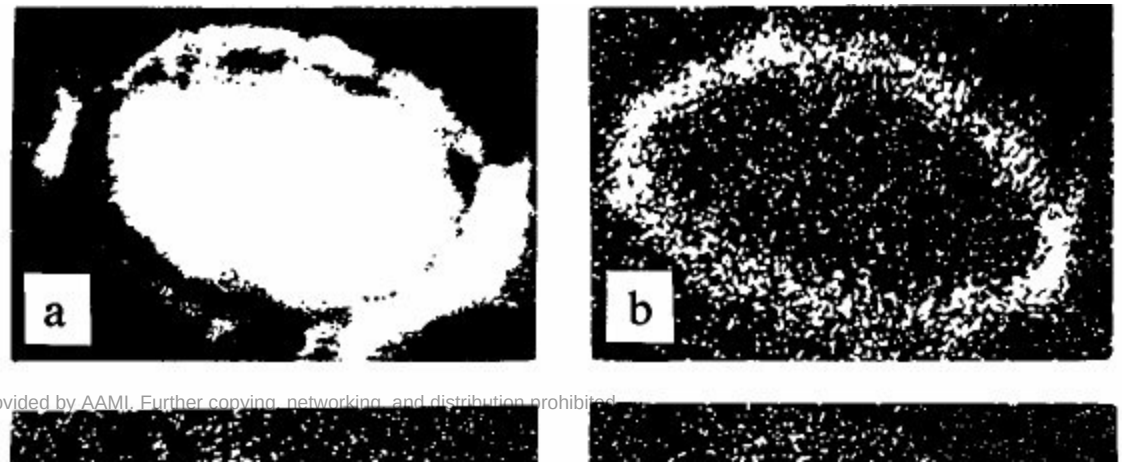
weight solutes and of the side chains and various hydrophilic groups of the nucleic acid and protein polymers? Are they free to rotate as in a solution and can the proteins and nucleic acids uncoil and denature when heated in solution?

(b) *State of Low Molecular Weight Solutes and Macromolecules in the Spore Protoplast*

Dielectric studies of Carstensen *et al*. (37, 38) measuring the electrical conductivity at high frequencies suggest that ions and small solutes within the spore are immobilized, but rapidly become mobile upon initiation of germination. Electron probe studies of freeze-sectioned spores reveal that most of the Ca, Mg, and Mn is located in the protoplast (Figure 5)(39). Photoproduct studies (40), β-attenuation data (41), and isolated forespore analyses (42, 43) indicate that most of the DPA is also present in the protoplast. Hence, the concentration of these solutes on a spore dry weight basis (2-3% Ca^{2+}, about 10% DPA) will be very high in the protoplast, i.e. about 6% Ca^{2+} and 30% DPA, insoluble, or in a supersaturated state.

The picture emerging from these results is that the constituents of the protoplast are in a highly concentrated medium in an insoluble, immobilized state, apparently with some 'free', 'mobile' water present. NMR studies on DNA and CaDPA mobility and the interaction of these constituents were, therefore, undertaken using radioactive-labelled components. A series of model systems were set up to try and evaluate the effect of various spore components such as DPA, Ca, and Mg on the nucleic acids DNA, RNA, and tRNA. Several biophysical techniques were used including ^{31}P-NMR, melting analysis (DNA strand separation), nucleic acid synthesis, X-ray analysis, UV photoproducts, UV spectroscopy, and fluorescent spectroscopy. Briefly, the conclusions from these experiments were that both DPA and its major chelate form, CaDPA, affected the mobility and stability of every nucleic acid component tested. For example, if DNA is hydrated to several a_w and is examined by ^{31}P-NMR, the normal T_1 (the spin lattice relaxation time, i.e. the time required for the molecules' normal motion to go through one phase or cycle) is approx. 375 ms, down to an a_w of 0.63. Below this hydration level, DNA undergoes a phase transition stabilizing at a_w 0.38 and a T_1 of 1300 ms (Figure 6). This change in T_1 is consistent with the change from the normal DNA B state to the very tightly coiled A state. In comparison, if DPA or CaDPA is combined with DNA in solution, the T_1 is lengthened to 1400 ms. Hence, the interaction of DPA/CaDPA with DNA induces a similar effect to that of reducing the normal torsional motion of the molecule, so that it appears to be in the A state. At higher concentrations of DPA or CaDPA, even at high a_w (0.98), no motion of the DNA is observed, consistent with the interpretation of the entire polynucleotide backbone being 'frozen' (44).



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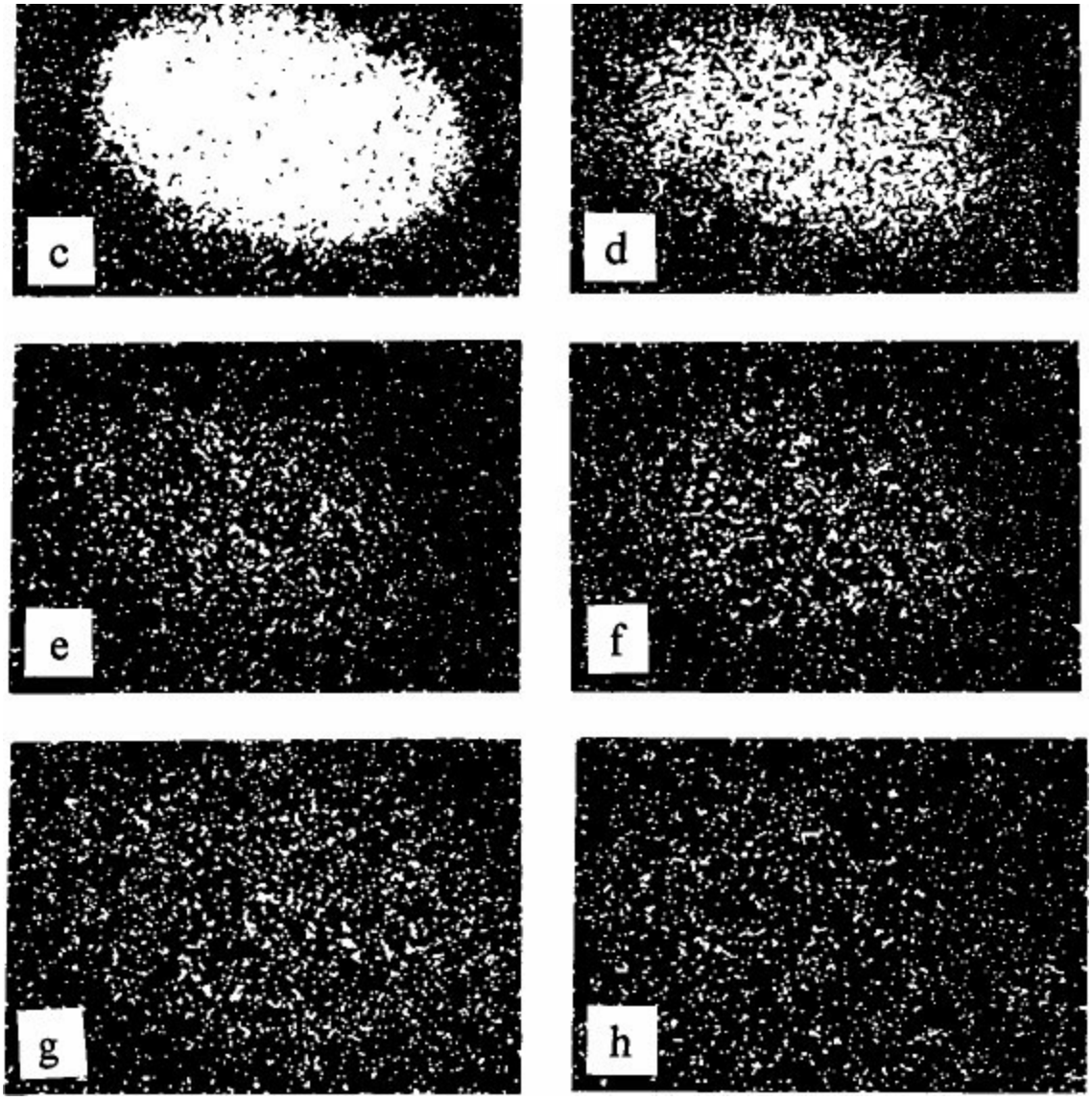

Figure 5. Elemental distributions in a cryosectioned wild-type *B. cereus* T spore. (a) Unstained scanning transmission electron microscopy (STEM) image, using dark-field divided by bright-field mode. The core (light area) and outer cortex/coat layer are easily discerned. (b) Silicon map. (c) Calcium map. (d) Phosphorus map. (e) Magnesium map. (f) Manganese map. (g) Sulphur map. (h) Continuum map. Note that the divalent cations, calcium, magnesium, and manganese are confined to the core region, along with most of the phosphorus. Silicon is strongly concentrated in the cortex/coat, whereas sulphur is distributed over the entire spore. The continuum map is featureless, indicating that the patterns observed in the other maps are not artifacts related to mass differences in the section (39). Bar indicates 500 nm.

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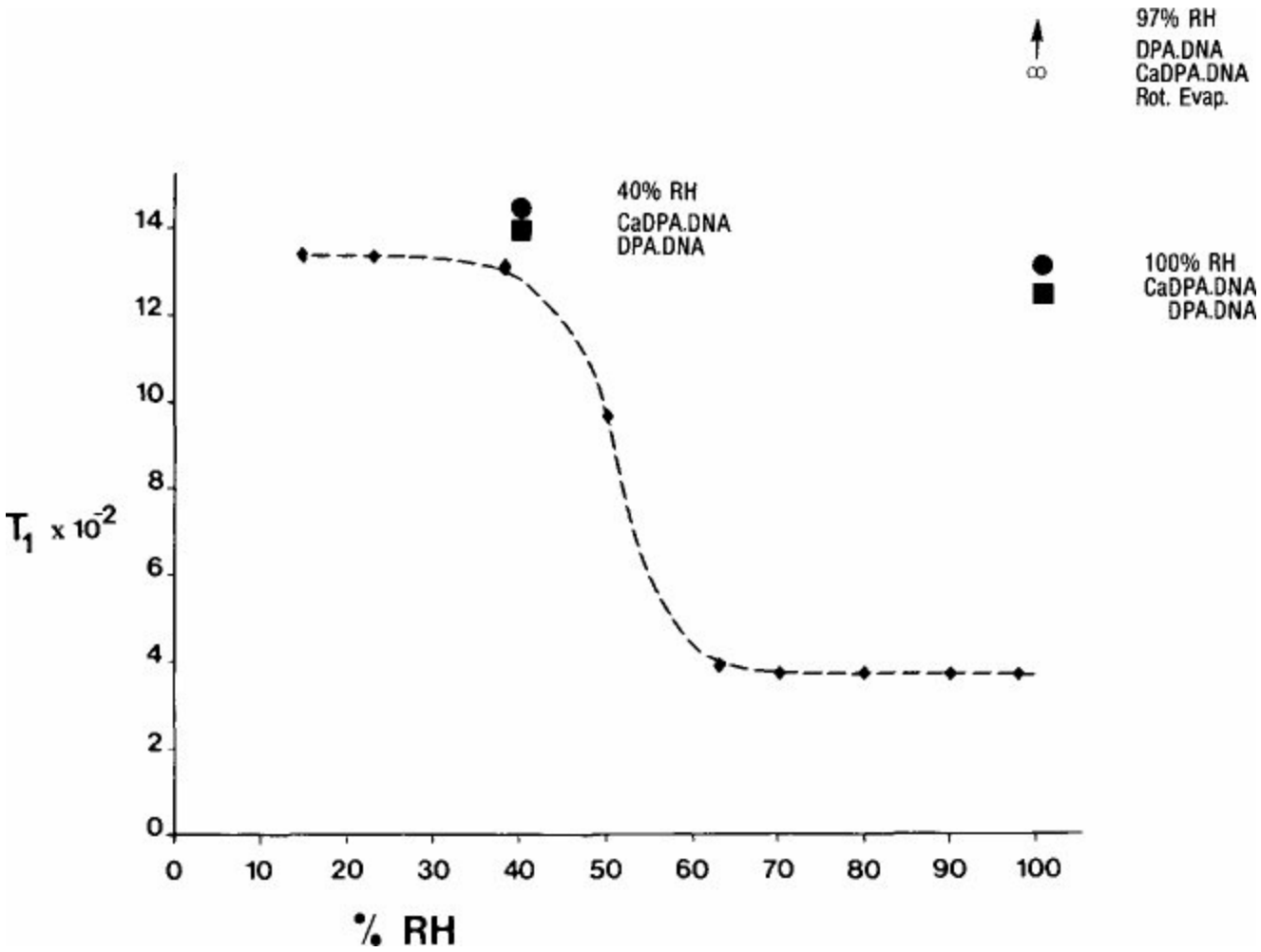

Figure 6. Effect of DPA and CaDPA on the $^{31}PT_1$ (molecular reorientation) motion of salmon sperm DNA at various equilibrium relative humidities. 300 mg of salmon sperm DNA (Calbiochem) was hydrated to the desired a_w by equilibration in a sealed evacuated desiccator using H_2SO_4/H_20 solutions. All solutions were degassed to remove molecular oxygen. Equilibration time was minimally 10 days. All a_w were checked after analysis by drying the samples and correlating water content to a_w by a water sorption curve. The error in a_w was less than 2%. Cornell and Lindsay (44). The symbols (●, ■, ◆) represent experimental points for the observed T_1 of DNA in the presence of DPA or CaDPA at the indicated equilibrium relative humidities.

The presence of DPA/CaDPA also increases the melting temperature of DNA by over 20°C (45). At very high ratios of DPA: base pair, the polynucleotide could not be melted. Additionally, DPA and CaDPA inhibit RNA synthesis (45).

Examination of UV-induced photoproducts from sporulating cells revealed that during irradiation of spores of *B. cereus* T DPA$^-$ mutants grown in the presence of exogenous DPA, a photoproduct was formed that on isolation and characterization by mass spectroscopy and NMR was tentatively identified as a combination of DPA and a thymine residue (Figure 7) (46). This photoadduct indicates the very close proximity of DPA to DNA in the spore as also indicated by earlier experiments where the presence of DPA was shown to affect UV resistance and the amount and type of photoproducts produced, presumably by the transfer of photochemical energy to the polynucleotide (40, 47).

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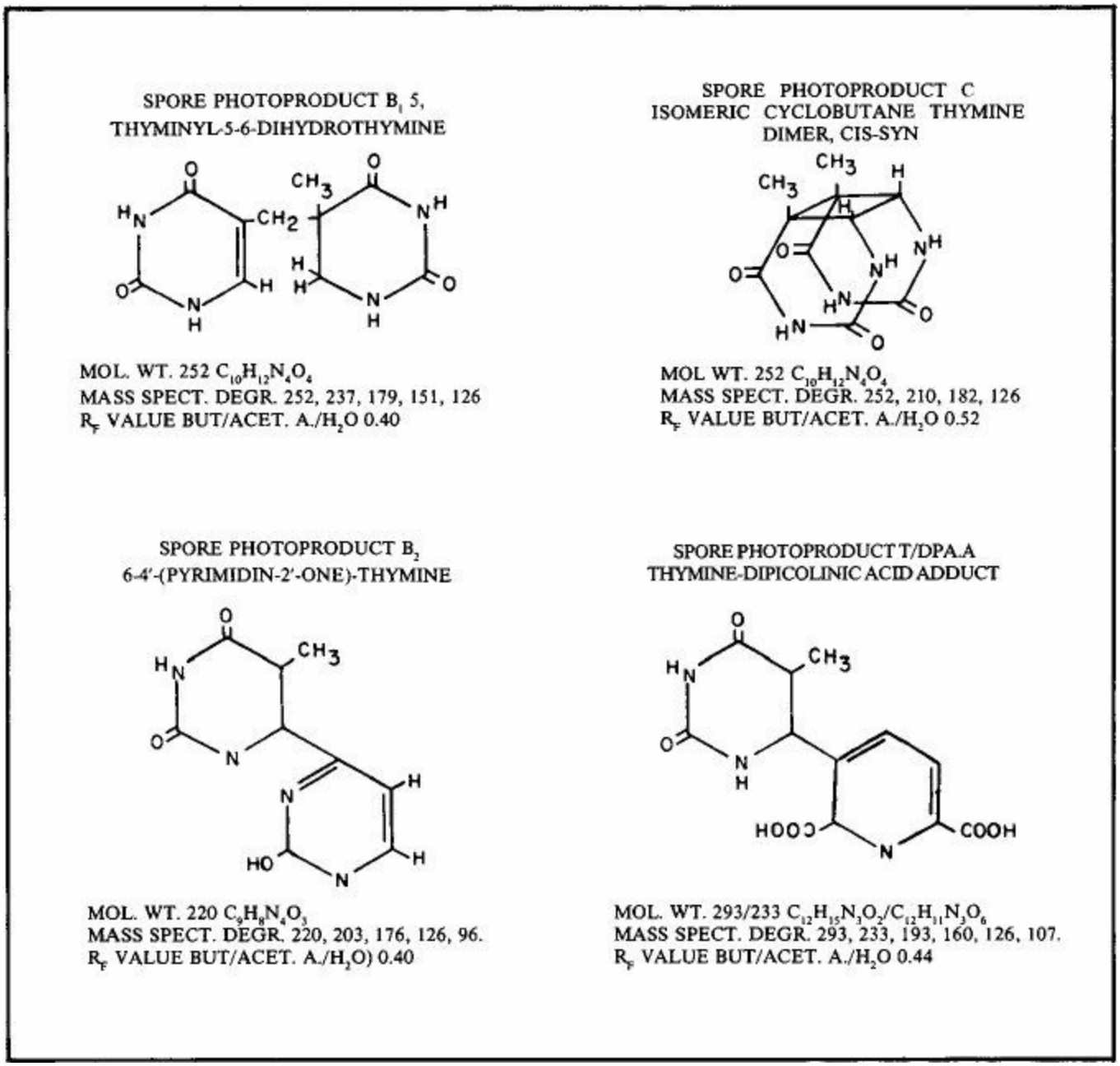

Figure 7. Photoproducts and thymine-DPA adduct formed during UV-irradiation (254 nm) of spores of *B. cereus* T HW1 DPA^- mutant grown with exogenous DPA (100 μg/mL) during sporulation. Lindsay and Murrell (46).

The question now arises, how can the interaction of DPA/CaDPA with nucleic acids be interpreted at the molecular level. The DPA molecule has an aromatic planar structure similar to other well-known intercalating substances such as the acridine dyes, for example ethidium bromide. The interaction of DPA with nucleic acids is therefore likely to be similar to that of an acridine dye or an antibiotic with planar ring structure binding to nucleic acids.

Our interpretation, therefore, of the *in vitro* and *in vivo* results is that during the sporulation process, which is analogous to a biochemical shutdown, DPA/CaDPA interacts with available nucleic acids effectively immobilizing their motion, inhibiting their metabolic roles, and increasing their stability. More importantly, the process of drug/dye and DPA/CaDPA binding involves hydrophobic forces (48). Thus, the incorporation of DPA in the developing forespore and its interaction with DNA could displace water from around the DNA and result in some dehydration of the protoplast.

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Furthermore, the amount of DPA/CaDPA far outweighs the amount of nucleic acids within the protoplast. Therefore, it would not be unreasonable to assume that any DPA or CaDPA not complexed with nucleic acids could aid in the stabilization of enzymes and proteins, either specifically or nonspecifically.

As a result of these studies, an understanding emerges of what is happening during spore formation and development of the biophysical state of the resistant spore.

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Formation and Development of the Biophysical State of the Protoplast of the Resistant Spore

Sporulation is a long, slow cellular differentiation process. Normal sporulation takes some 6-8 hours and is arbitrarily divided into seven stages (1). Stabilization of the forespore does not commence until stage IV in which a considerable number of biochemical changes occur. Cytologically, during this period there is a marked change in the appearance of the DNA; from the electron-transparent state of the vegetative cell, it becomes more fibrous and takes up a peripheral location in the forespore (Figure 8). Almost at the same time as the DNA changes occur, the volume of the protoplast (Figure 9) decreases by more than one-half (26). This major early reduction in protoplast volume occurs just before cortex synthesis and DPA formation. Nothing is known about the physiological mechanism responsible, but several explanations seem possible. First, a redistribution of cations between the protoplast and the surrounding 'exocellular' space between the two forespore membranes could reduce the volume by plasmolysis. Secondly, the germ cell wall may have an ability to contract. Relatively little pressure is required to remove the major part of the water as small changes in a_w are involved. Thirdly, this reduction in protoplast volume has been suggested as occurring by molecular reordering with the exclusion of water, a process of synaeresis (3, 27, 28). This process may result from the presence of various cations, and perhaps basic proteins (50, 51).

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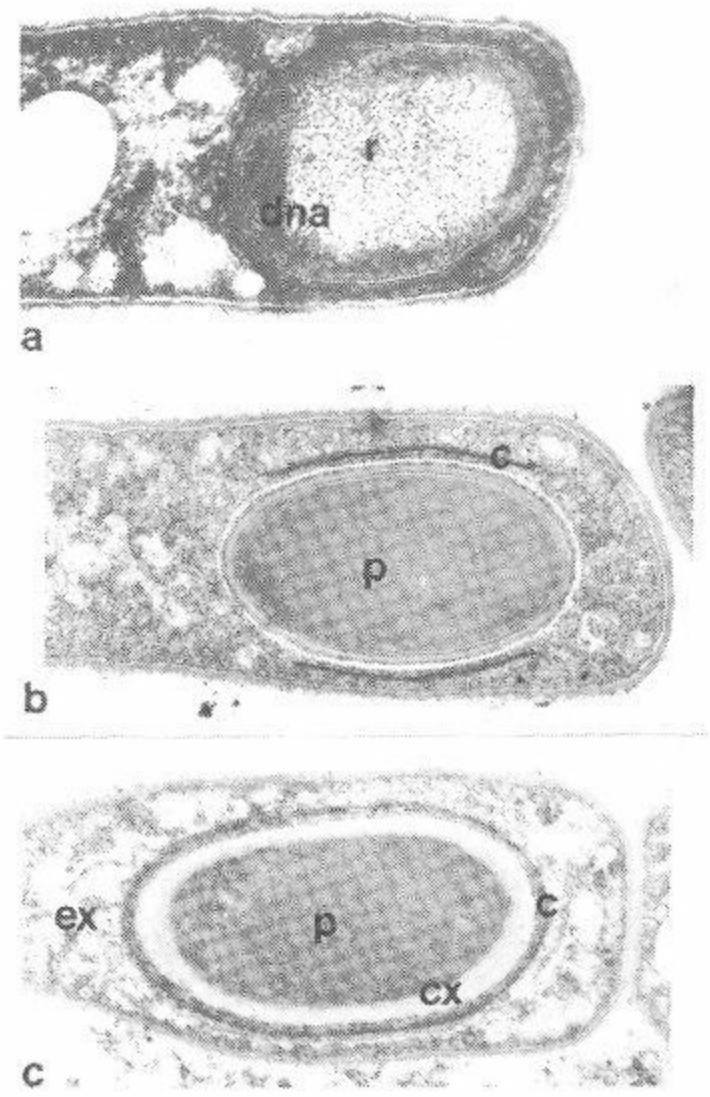

Figure 8. Electron micrographs of three stages in the development of the resistant spore of *B. cereus*. (a) Stage IV showing the DNA undergoing rearrangement and moving into a peripheral position (dna). The cytoplasm shows ribosomes (r) and heterogeneous appearance. (b) A spore at stage V with a homogeneous cytoplasm. The DNA is barely distinguishable. (c) A near mature spore, showing fully developed cortex (cx) and coats (c), still in the mother cell. p, protoplast; ex, exosporium.

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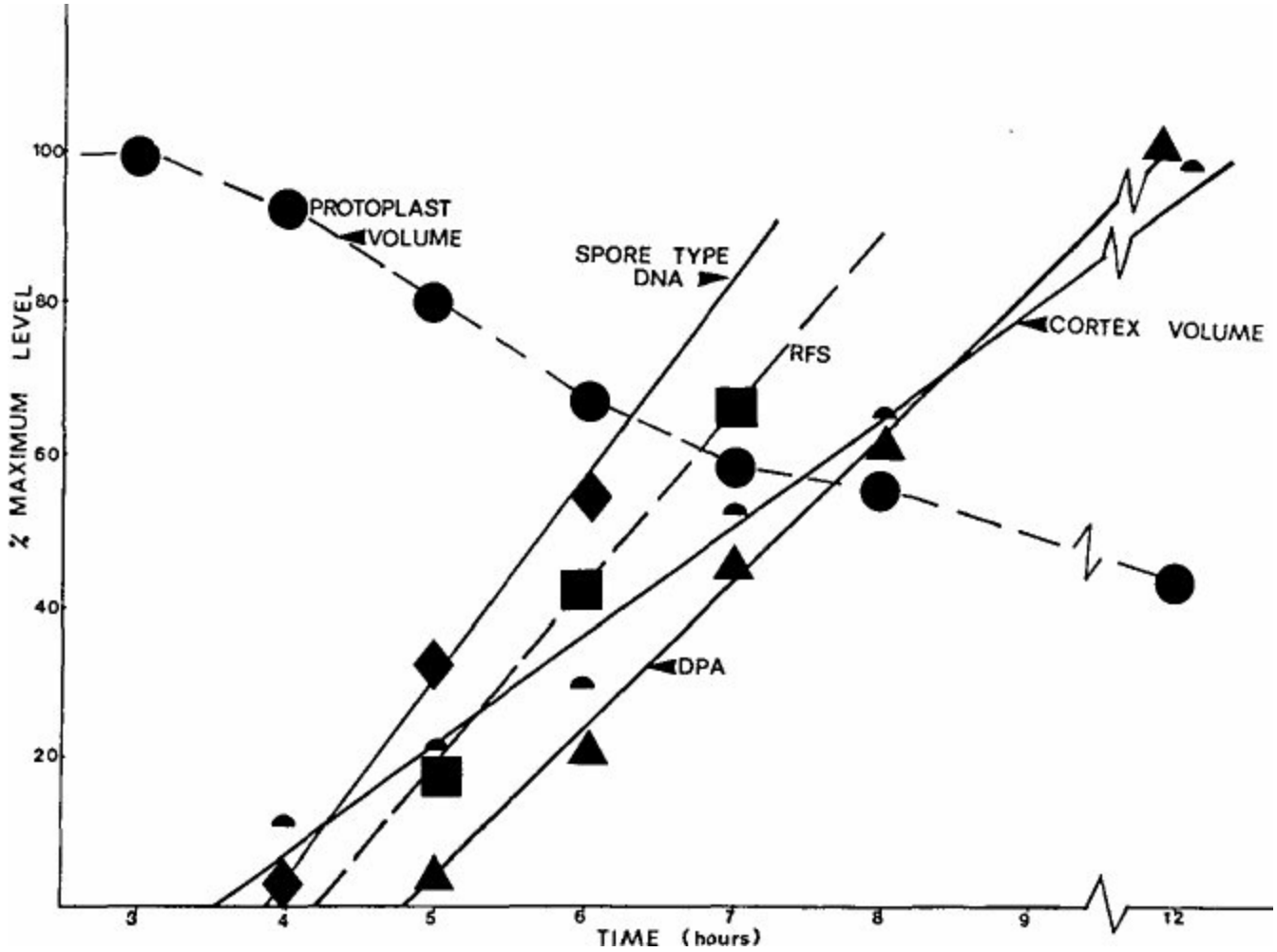

Figure 9. Reduction in protoplast volume in relation to other sporulation events in *B. cereus* T. Based on the data of Baillie *et al*. (49) and unpublished studies of Ohye, Warth, and Murrell (26). Refractile forespore counts (RFS), DPA, stages of sporulation, and photoproducts were determined as described previously (47, 49). The protoplast and cortex volumes of developing spores in the culture were obtained by summing the products of the proportion of sporulation stages in each sample (from electron micrographs) times the average protoplast or cortex volume of each stage. For example, at 6 h:

	Avg forespore protoplast vol (μm^3)	Relative protoplast vol in culture (μm^3)
10.8% cells with vegetative DNA	0.497	5.4
33.8% cells with transition DNA	0.402	13.6
55.4% stage V	0.255	14.1
100.0		33.1

Electron micrographs only of medial longitudinal sections were used for measurements. At least 60 suitable cell sections were examined in each sample. Cortex volume was the difference between the volumes enclosed within the inner and outer forespore membranes. Volumes were calculated by using the formula for a prolate spheroid (27).

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The reduction in protoplast volume is associated with several events, over a hundredfold increase

in cellular calcium content, DPA accumulation, and the formation of the cortex. There are difficulties in determining the exact relationship in time of all these events (27). The beginning of the reduction appears to precede Ca^{2+} uptake and DPA accumulation, and to be associated more with changes in the appearance of the DNA (27) and basic protein formation (50, 51).

Our interpretation is that from stage IV on, calcium and DPA specifically bind with available nucleic acids (giving this spore molecule a specific role) resulting in the stabilization of the nucleic acids, and presumably forcing the removal of water from around these molecules and out of the protoplast by hydrophobic reaction. The appearance of the forespore protoplast changes, becoming homogeneous, obscuring the DNA and ribosomes, before even the cortex and coats are complete (Figure 8b). It is around this transformed spore protoplast that the cortical peptidoglycan polymer is formed. Because this polymer, with an excess of negatively charged groups, is formed within the mother cell with presumably a relatively neutral cytoplasmic pH, and in the presence of the accumulating cations such as Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, it can be expected to be laid down in a structurally contracted and stable cross-linked state. The coats will be finally completed around the cortex and in this state protecting it from lytic enzymes released by lysing mother cells.

During stage IV and V, there is a gradual increase in resistance to octanol and other solvents, and heat resistance (52), and stabilization of enzymes to heat (Figure 10)(10). The final level of heat resistance is not achieved until the cortex and coats are fully developed.

Some of the residual water in the protoplast may become chemically complexed with metal chelates, protein, and polysaccharide polymer (2, 8). The protoplast will now be unable to take up water and swell as it is mechanically restricted by the cortex and coats. Possibly only a small amount of free solutes or polymer groups capable of exerting an osmotic pressure are present in the mature spore protoplast.

The water content of the protoplast in the mature resting spore will be determined by the amount of reduction in protoplast volume that occurs, and by the degree of contraction or closeness of fit of the contracted cortical envelope and perhaps coats. The water content of the protoplast, together with the solid support system generated by the interaction of the DPA/CaDPA and the DNA, may determine the degree of heat resistance.

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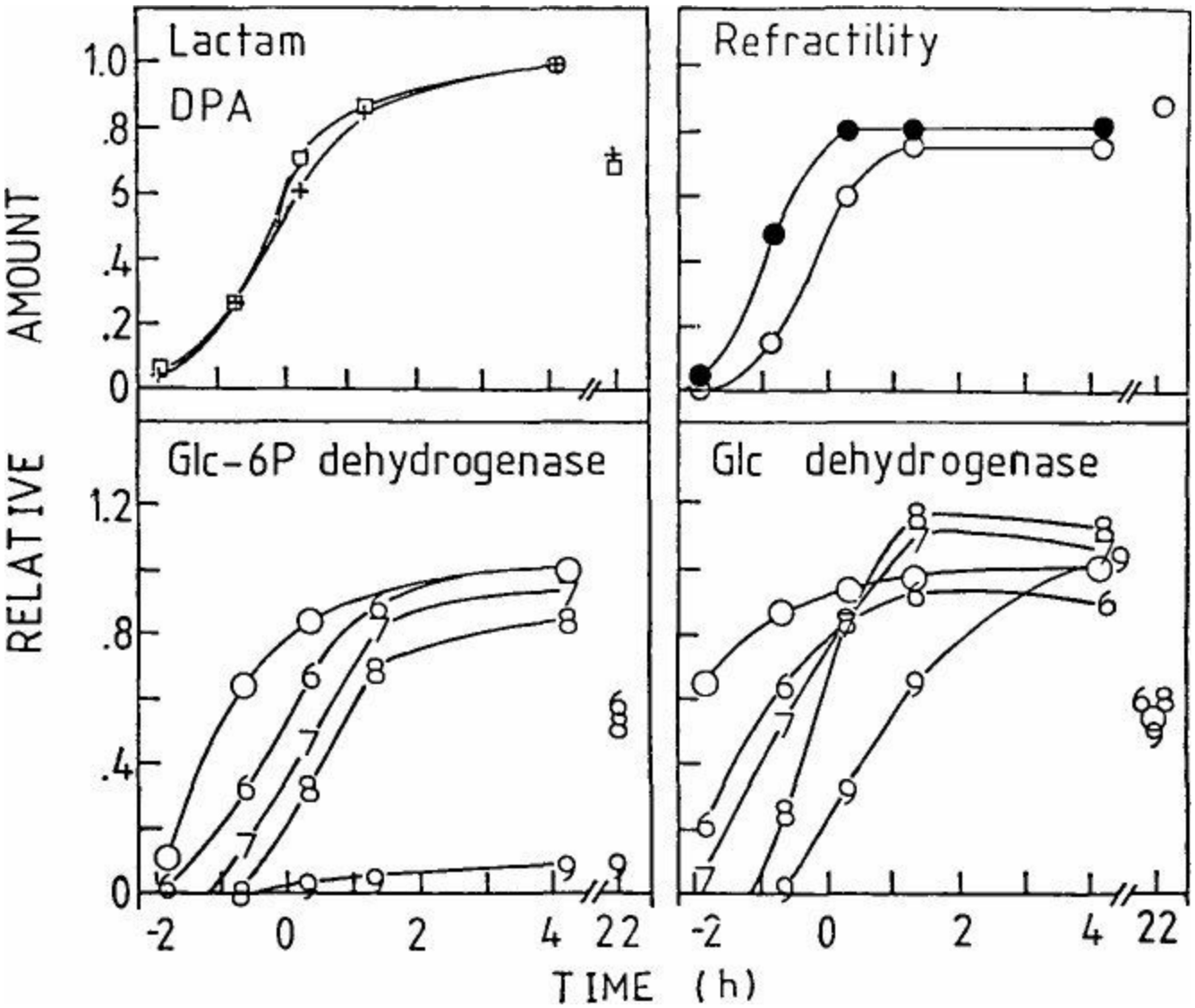

Figure 10. Development of heat stabilization of spore enzymes in relation to cortex and DPA synthesis during spore formation in *Bacillus cereus*. Symbols: □, muramiclactam; +, DPA; ●, phase-grey + refractile spores; ○, phase-white + refractile spores; 0, 6, 7, 8, 9, heated for 10 min at 25°, 60°, 70°, 80°, and 90°C respectively (10).

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Interpretation of the Molecular Mechanisms Involved in Resistance

Studies of the interaction of DPA and CaDPA with the various polynucleotide species with immobilization of these molecules suggest the type of molecular arrangement that will be present in the shrunken spore protoplast encased within the cortex and protected by the coats.

It is proposed that the essential labile macromolecules in the protoplast are heat-stabilized by the formation of noncovalent electrostatic and possibly hydrogen bond interactions with calcium dipicolinate, that acts as a 'solid support' in a similar fashion to that used to immobilize and stabilize enzymes in a charged polymer matrix, such as a concentrated polymethacrylic acid gel (25). Other small molecules, such as glutamic acid, sulpholactic acid, and 3-phosphoglyceric acid, when they occur in the spore protoplast in significant quantities, may play a similar role as their calcium salts, particularly in the case of DPA-deficient mutant spores that retain some heat resistance.

The stabilizing noncovalent bonds between the solid support and the macromolecule must be broken to denature the macromolecule and therefore, as compared with the native macromolecule in dilute solution, additional energy must be put into the system by heating it to a higher temperature to allow the molecules to uncoil and denature.

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Conclusions

It is believed that the resistance of the spore is developed and obtained by a number of biophysical changes that occur during sporulation. These involve:

(i) a reduction in the protoplast volume by over 50%
(ii) a reordering of the macromolecules
(iii) encasement of the reduced protoplast within contracturally synthesized cortex and the coat
(iv) concentration to a high degree of solutes with a concomitant reduction in water content in the protoplast
(v) maintenance of a lowered water content by development of pressure in the cortex
(vi) development of a solid support system of CaDPA with specific interaction of DPA/CaDPA with nucleic acids and possibly proteins
(vii) constraint of macromolecular motions which lead to denaturation.

The biophysical changes certainly result in considerable stabilization, but whether their contribution to stability is greater or less than that resulting from the associated partial dehydration may not be answerable. Partial dehydration adequately stabilizes proteins *in vitro*, but better evidence of the degree of dehydration of the protoplast in the intact spore, when immersed in water, is required to substantiate the partial dehydration theory.

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Acknowledgements

Grateful acknowledgement is made to the American Society for Microbiology for permission to reproduce Figures 1, 4, 5 and 6 from *Sporulation and Germination*, Levinson HS, Sonenshein AL, Tipper DJ, Eds., American Society for Microbiology, Washington, D.C., pp. 70, 72, and 250, and from *J Bacteriol* 1980, **143**, 486, and to *Biochimica et Biophysica Acta* for Figure 4 from *Biochim Biophys Acta* 1981, **678,** 160.

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Sterility Monitoring – Sterility Testing

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The process of sterility assurance covers not only the product, but also the whole of the manufacturing and testing process. Sterility assurance begins with controls and tests on raw materials and components, and covers the manufacturing and sterilization process through to control of stocks and sterility tests on the finished product. Aspects of the sterility test and considerations of its role, its limitations, potential for improvement, and possible future developments are discussed.

In describing some of the experiences in our own test laboratory, it is important to remember that the National Biological Standards Laboratory (NBSL) is the national control authority, and most tests are carried out on products already marketed.

The major problem is that sterility testing does not prove that the product is sterile. This follows from basic considerations of sampling statistics from which no amount of testing can prove that a batch of product is sterile (Table 1). Therefore, the objective of the test and what can be done to obtain the maximum value from the test are major considerations.

Table 1

Probability of acceptance of batches with different contamination rates

Sample size	% Contaminated			
	0.1	1	5	10
10	0.99	0.91	0.6	0.34
20	0.98	0.82	0.35	0.11
50	0.95	0.61	0.08	0.005
100	0.91	0.37	0.01	–

Whereas the sterility test may not be able to prove that a batch is sterile, it does detect grossly contaminated products. It is the last chance, the manufacturer has to prevent the release of a batch of a product where there has been a major breakdown in the normal manufacturing process. Such problems should be rare, but in our experience they do happen. Since 1975, we have encountered ten examples which I would classify as heavy contamination, where properly conducted sterility tests

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would have prevented the release of the products. It should be remembered that if NBSL finds a contaminated product, there are already two problems; not only a failure of the sterilizing process, but also the manufacturer's sterility test has failed.

Although the ten instances are a small percentage of 2500 samples tested, they may represent the tip of a substantial iceberg, since the Laboratory tests only a small fraction of batches that are released to the market. It is interesting to look at some examples.

In August 1981, we began a programme of testing 'sterile' wound dressings. It was immediately obvious, there was a major problem. In the initial survey, we tested 25 batches of imported dressings and found that 18 of them were nonsterile. In most cases, all items in the sample were contaminated, usually with a rich variety of microorganisms. Clostridia were isolated from about two-thirds of the contaminated samples, the most common isolate being *Clostridium perfringens*. In one sample, we found four species of clostridia, two types of enterobacter, as well as *E. coli* and *Yersinia* sp. These findings were substantially confirmed by extensive studies in England (1). We also tested a batch of needles that were part of the kit supplied with a vaccine. Of the 30 needles tested, 20 were contaminated. In both examples, the sterilization process had clearly been defective, and the importers, distributors, and users of the products had accepted them as sterile without further testing. The consequences of this unquestioned acceptance were potentially serious.

Unquestioned acceptance of products without a test for sterility can obviously result in major problems. Sterility testing is an essential part of quality control. However, even if a sterility test is carried out, it may also fail to detect problems, unless there is careful attention to detail, in particular, validation of test media and methods. For example, on one occasion a batch of a liquid preparation was found to be heavily contaminated (59 out of 60 containers showed growth of microorganisms) and on another occasion a batch of kidney dialysis units was heavily contaminated with a variety of moulds. In each case, the product was from a reputable source and had passed the manufacturer's sterility test. NBSL results were disputed. In the first example, the results were accepted when it became clear that inadequate steps had been taken by the manufacturer to inactivate preservatives and to demonstrate that the media were capable of supporting the growth of microorganisms in the presence of the product. The example of the dialysis units is also interesting. The manufacturer had tested the units by flushing media through the device and incubating the eluate, evidently expecting that any organisms present would be easily washed off surfaces to which they were attached. At NBSL, testing was carried out both by the flushing method and by filling the device with medium and incubating the filled unit. We detected contaminants only when the devices were filled with media and incubated. Flushing clearly has limitations in circumstances where microorganisms may be firmly attached to surfaces.

Some of the heavily contaminated products mentioned in these examples had obviously not been tested for sterility. In other cases, tests had been carried out, but the methods had been inadequate. Our results show that the test plays an important role, and attention must be given to the adequacy of the test methods. Heavy contamination is uncommon but relatively simple to detect. Much more difficult to pin down are intermittent, low-level contaminations that may arise from time to time. To detect low-level contaminations, it is essential that the test be as sensitive as possible and that the number of false positives be kept low. If this is not done, true positives will not be detected against high background of false positive tests.

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I would like to consider the problem of the sensitivity of the test and possible ways of increasing

it. Sensitivity could be increased by testing larger quantities of the product, but this would result in increased costs. It would be preferable if sensitivity could be improved without increasing either the complexity, or the cost of the tests.

Some of our results are reviewed, identifying some of the problems with existing tests. Table 2 shows the number of positive cultures in different medium/temperature combinations. At NBSL, standard media, SCD at 23°C and thioglycollate at 32°C are used. However, we also use an additional SCD medium at 37°C. The results shown in Table 2 do not include the results from tests on wound dressings, as the variety and number of organisms found was generally not typical of pharmaceuticals and devices. There were so many isolates that not all were characterized. The numbers involved were substantially greater than the total of isolates from all other tests in the past seven years and, if included, would obscure the results for pharmaceuticals and devices.

Samples were divided equally between the various media, so that similar numbers of positives could be expected if the media were of equal efficiency. The overall difference between SCD at 37°C and 23°C did not reach statistical significance. The numbers of bacteria were similar, but it is clear that the lower temperature favours growth of moulds. The difference between the numbers of moulds at 23°C and 37°C is highly significant ($P < 0.001$). The most obvious problem revealed by these results is the poor performance of the thioglycollate medium, that detected only one-third the expected number of positive results. The difference is highly significant ($P < 0.001$). This failing has an obvious effect on the efficiency of the test. In a conventional test, the sample will be divided equally between SCD 23°C and thioglycollate at 32°C. Based on the proportions in Table 2, the test detects only 65% of the possible contaminants.

Table 2

Sterility test isolates

Medium	Moulds	Bacteria	Unknown	Total
SCD 23°C	32	57	8	97
Thioglycollate 32°C	2	14	8	29
SCD 37°C	5	64	10	79

The efficiency is further reduced if incubation is limited to seven days, as it has been shown that about one-quarter of all contaminants show delayed growth (2). It can be predicted that a test based on the two media and a seven-day incubation period will detect less than half of all contaminants. This is obviously an unsatisfactory situation.

To identify the problem is simple, but to provide a solution is not so easy. Fluid thioglycollate medium has been used for many years and it was generally adopted as a sterility test medium following work carried out by Pittman (3). However, it has long been known that thioglycollate may be inhibitory (4, 5). An improved medium, dithionate-thioglycollate (HS-T) medium, was developed by Clausen (6) and was proposed as a replacement for thioglycollate medium. The apparent superiority of the medium was confirmed by others (7). Thioglycollate medium with haemin and vitamin K has also been recommended (8).

To assess the value of these media for sterility testing, we have used them in parallel with regular media for periods of up to one year. Results of these trials are given in Table 3.

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Table 3

Contaminants isolated in different media

	Regular			Experimental
1976-1977	SCD 37°C 43	SCD 23°C 32	THIO 32°C 10	HS-T 32°C 8
1978-1979	SCD 37°C 9	SCD 23°C 5	THIO 32°C 5	Enriched THIO 32°C 1

Although the number of positive results is small, neither of the alternative media shows any obvious advantages over thioglycollate medium, and all were markedly less efficient than SCD in sterility tests. These results are in conflict with the earlier reports (3, 6) of the superiority of HS-T medium. However, there is one major difference between the studies. In the earlier studies, media were inoculated with a variety of microorganisms. The inocula were small, but the organisms were from fresh cultures. By contrast, organisms surviving in pharmaceuticals could be debilitated by age, exposure to preservatives or to sublethal sterilization processes. A medium which is mildly inhibitory may permit the growth of healthy microorganisms, but may not favour resuscitation of damaged microbes (9, 10). Our own results were obtained during routine sterility tests, which may account for the differences between our results and the published reports on the efficacy of the HS-T medium.

Thioglycollate medium is not ideal for sterility testing and alternatives are needed. However, alternatives will need to be evaluated in routine test situations. This will be a very slow procedure. We are at present evaluating SCD incubated at 32°C in the hope that this will be as effective as SCD at 23°C and 37°C. We also plan to characterize further the bacteria isolated at the different temperatures. It is our hope that tests can be simplified by using a single incubation temperature. However, the major problem of the thioglycollate medium remains.

Finally, I would like to consider potential alternatives to the sterility test. While the test has its value, it also has limitations. Control over the sterilization processes is a more important factor than end product testing. There is some incongruity in the use of steam or gas sterilized items to test the sterility of other items sterilized by similar processes.

NBSL has for many years accepted the release of radiation-sterilized goods without sterility testing (11). This was considered to be justified by the reliability of the process, the ability to measure accurately the absorbed dose, and the relative lack of problems with the penetration of the sterilizing agent. In addition, there is often physical separation between the manufacturing plant and the sterilizing facility, that reduces the chance of mix-ups between sterilized and unsterile stocks.

In the past, there was lack of means to obtain accurate and reliable measurements of delivered sterilizing dose for sterilizing methods other than radiation. However, there are now available reliable multichannel recording thermometers that can not only record temperatures at many points, but can also calculate the total heat exposure at 121°C (F_0). The delivered sterilizing dose for heat sterilization can now be measured with an accuracy equivalent to that of radiation sterilization.

There seems no reason why the principles accepted for radiation cannot be applied to other methods of sterilization. All that should be required is knowledge of the bioburden, accurate and reliable measurements of the delivered sterilizing dose, integrity of the containers, and proper stock control to prevent mix-ups.

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There may also be alternative procedures such as chemical or biological monitors. Chemical indicators are not yet widely accepted, but there may be systems which could be used as an adjunct to physical monitoring of steam sterilization that, with suitable validation, may be able to substitute for sterility testing.

Biological indicators have been suggested as a means for controlling sterilizing cycles in a way which would permit the deletion of finished product sterility testing. However, there have been complaints to NBSL that some indicators have been of variable resistance. In our own experience, some commercial *B. stearothermophilus* indicators were killed by exposure for 3 minutes at 121°C and some gave no growth even when unheated. At the other extreme, some indicators survived exposure to F_0 values of 18.

Nevertheless, biological indicators have their value. This is recognized in the Australian *Code of GMP Appendix C – Guidelines on Tests for Sterility*. The Code permits a reduction in the sterility test sample size, where terminal sterilization is monitored with biological indicators. However, the indicators must be used in the manner specified in the Code, and the labelling on the indicators must specify storage conditions, expiry dates, and performance characteristics.

Product release based on control of the parameters of the sterilization cycle has obvious advantages. It is my view, that parametric release used in radiation process control can be extended to other methods of sterilization. However, each individual product and process will need to be carefully assessed on its merits. There will be no general or early acceptance of the deletion of the sterility test, even for products terminally sterilized.

By far, the most common method of sterilization is filtration and aseptic filling. A check of products tested for sterility by NBSL since 1975 has shown that approximately 70% of products had been aseptically filled. Finished product sterility testing will remain mandatory for these products. While there is a continuing need for the sterility test, our work will be in part directed towards modifying the test to improve its effectiveness. There is scope for substantial improvement and simplification.

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Good Manufacturing Practices – Overview

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Within the past decade, codes of Good Manufacturing Practices (GMP) for the manufacture of medical products have been written and implemented around the globe. These GMPs share the basic commitment to serve as industry standards, covering critical aspects in the manufacture, distribution and control of health care goods. Many of these GMPs apply directly or indirectly to the manufacture of sterile medical products, the primary focus of this overview.

There are differences in regulatory approaches to GMPs between Australia and the US, and even between the federal and the many state and local governments, as they evaluate and regulate manufacturers. Some regulators license and closely observe manufacturing operations, others require licensing or registration using occasional auditing of operations to assure compliance, and yet a third group requires only tacit compliance, where regulatory intervention occurs only if problems arise. The controlling GMP documents, whether the *Code of Good Manufacturing Practice for Therapeutic Goods* of the Commonwealth Department of Health or the *Good Manufacturing Practice for Medical Devices* of the US Food and Drug Administration, are intended to help assure that there are minimal product problems in the marketplace. What is desired by industry regulators worldwide is objective criteria upon which to evaluate and/or control the complex processes in the manufacture and delivery of safe and effective sterile medical goods.

It is unnecessary to review in detail the GMP publications worldwide which relate completely, or in part, to the manufacture of sterile medical products, because of similarities in the topics covered. It is also impossible to accurately evaluate the real or probable operating effectiveness of GMP documents that are currently in effect or proposed that deal with sterile medical products. Such judgements ultimately depend on who you are, whether from government, industry, or academia It is, however, appropriate to review some of the common subjects or concerns covered in these GMP documents. It is also timely to review a new approach to voluntary industry compliance in the United States involving industry guidelines developed by input from industry, academia, and government, relating to the sterilization of medical devices. This concept will hopefully find increasing support and acceptance by regulating bodies within the United States.

In the US, the writing of all official federal GMP documents is accomplished internally within the US Food and Drug Administration with little if any input from industry or academia The document when completed is published in the *Federal Register* as a proposed regulation open for comment by industry and academia At this time there is usually little chance for making any major changes in the philosophy or content of the document Because the FDA has to develop GMPs internally without outside input, the FDA has sometimes chosen to develop tentative documents such as the GMP for large volume parenteral solutions, which have not been, or may never be, proposed for official adoption. A reason for the FDA choosing this approach may be the result of a general consensus that the proposed GMP might not work as well as intended when applied to actual manufacturing

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operations, and by proposing such a tentative document, it allows a longer period for evaluation of content and need. Such tentative GMPs, however, may continue to serve as unofficial guidelines even though not officially adopted as a regulation. One can surmise that GMPs generated solely within a government regulatory body could result in a document that is difficult if not impossible for industry to follow. This may not be the result of any overt plan by the regulators to confuse or confound industry, but it may be because there is a genuine lack of knowledge and experience in the specifics and economics of industrial processes or controls on the part of those who compose the GMP.

The most effective government generated GMPs have been broad and general in their scope. These are the umbrella GMPs that cover the requirements of an entire group of products, such as medical devices and which are purposely not very detailed in the specifics of equipment, processes, and controls. Within the US the FDA has several umbrella GMP regulations in effect including the current *'Good Manufacturing Practice for the Manufacture, Packaging, Storage and Installation of Medical Devices'*. This document encompasses all aspects of medical device manufacturing. In this umbrella-type regulation the FDA did not include what it has repeatedly voices as necessary, the specifics for sterilization processes and related controls. This perceived need for specifics is best expressed in the supplementary Information at the beginning of the *Good Manufacturing Practice for Medical Devices*. 'The U.S. Food and Drug Administration (FDA) expects to publish additional GMP regulations applicable to specific types of devices. These future regulations will supplement the "umbrella" GMP regulation and will be of two types: One will contain requirements that will apply only to generic types of devices or classes of devices, e.g. pacemakers, eyeglasses, etc.; the other will contain requirements that will apply to certain devices or cross-class characteristics or processes, e.g. sterile devices, plastics, electrical properties, etc.'

Product sterilization has always been a difficult process for regulators to properly evaluate. Product sterilization is achieved by way of complex biological, chemical and physical processes, often employing rather elaborate pieces of equipment and involving related biological and/or chemical testing. As was expressed by the FDA when discussing the specialized GMP concept, spelling out specific details of sterilization processes and controls in cookbook like fashion was believed to be the way to insure effective control over the industry. Carrying this concept to its ultimate, meant telling manufacturers how to manufacture, sterilize and control their sterile products. It was presumed that regulators could write universally acceptable cookbooks for steam, ethylene oxide, dry heat, radiation and other chemical sterilization methods, and all that industry would have to do to comply is to follow the document in detail.

If only the world were so simple. Those of us who have worked with sterilization processes know that there is a myriad of sterilization processes, pieces of equipment, controls and testing procedures in use throughout industry worldwide. A cookbook approach would result in either a set of specialized GMP volumes that would in size rival a set of encyclopedias, or conversely, there could be but a few documents spelling out parameters for just a few approved processes. The latter would ultimately severely limit the types of sterile products commercially available to health care providers. Either of these two alternatives are basically unmanageable systems, unacceptable to a growing, dynamic business such as the sterile medical products industry, and certainly not cost effective.

The US medical device industry has repeatedly expressed to the FDA by way of various contacts that specifics for sterilization processes, and controls would stifle advances in technology. Many

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firms have developed highly individualized sterilization processes to meet particular product and packaging requirements. The argument is made that after all, the end goal or purpose of the sterilization process is to consistantly manufacture sterile products that are safe to use and, as long as this is accomplished, the specific methodology used should be left to the discretion of the manufacturer. Fortunately, this dialogue seems to have resulted in changes in approach to the evaluation and control of sterilization processes on the part of both regulators and industry.

The change began with a re-examination of the umbrella GMP to see if they are applicable to sterilization processes. Following is a list of general GMP compliance topics as found in the umbrella FDA document *Regulations Establishing US Good Manufacturing Practices for the Manufacture, Packaging, Storage and Installation of Medical Devices* along with questions raised that specifically relate to the manufacture and control of sterile products.

Buildings and Facilities:

1. Are the buildings of adequate size and design to accomplish the manufacture and control of sterile products?
2. Is there adequate space and facilities to conduct all phases of a sterilization process; as for ethylene oxide gas sterilization, the pre-sterilization conditioning, sterilization, post-sterilization aeration and quarantine operations?
3. Is the manufacturing environment controlled to prevent undesired product contamination?

Manufacturing Equipment:

1. Will manufacturing equipment unduly contaminate products, thereby taxing the ability of a terminal sterilization process?
2. Does the equipment properly protect the product from contamination, as in aseptic fill processes?

Components and Materials:

1. Are product and packaging materials and designs compatible with the sterilization process?
2. Are components sufficiently clean and free from microbial (and particulate) contamination?

Manufacturing and Assembly:

1. Is the manufacturing and sterilization equipment properly designed, and are manufacturing personnel sufficiently trained?
2. Is there proper production flow control to prevent confusion and mixups between sterile and non-sterile products?

Packaging and Labelling:

1. Are packaging materials compatible with the sterilization process?
2. Is the packaging designed to maintain product sterility until the package is opened and a sterile device presented for use?
3. Is the product properly labeled, as identifying what portions of the product is intended to be sterile or so the product can maintain sterility until use?
4. If resterilization is possible or indicated, are satisfactory resterilization directions given or available?

Measurement Equipment:

1. Are all sterilizer controls, gauges and charting equipment properly and routinely calibrated?

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2. Are biological and chemical sterility indicators and dosimeters appropriate to the process and the degree of control required?

Testing:

1. Is there biological testing of product and/or process indicators to assure product sterility or is there release of sterilized products permitted upon a review of validated process parameters?
2. Is there functional testing of product and packaging following sterilization?
3. Are component and product bioburdens determined?

Records and Reports:

1. Is there adequate documentation accounting for all products being sterilized?
2. Are there appropriate records documenting parameters for the entire sterilization and control process?
3. Are process indicators and/or sterility tests properly documented?

Reprocessing of Products:

1. Is there documentation to show product and packaging can withstand resterilization?
2. Are adequate records maintained regarding any reason for resterilization?

Calibration:

1. Are all sterilization process indicators, as dosimeters and biological indicators, tested and/or calibrated before use?
2. Are sterilizer gauges, recorders, thermocouples, etc., calibrated as scheduled using recognized standards?

Environmental Controls:

1. Is appropriate particulate and microbial filtration available where required?
2. Do employees wear proper uniforms and protective coverings?

Cleaning and Sanitation:

1. Is there a documented routine cleanup program?
2. Are cleaners and sanitizing solutions approved to assure effectiveness and to prevent harmful residues?

Personnel – Organization and Training:

1. Is there adequate staffing in numbers, training and experience to properly operate and control the sterilization process?
2. Does the quality control organization have the authority to make required quality decisions?

Complaint Handling:

1. Are records kept of all complaints relating to questions of sterility?
2. Is there proper investigation of all sterility complaints including laboratory sterility testing when appropriate?

Inspections and Audits of Processes:

1. Is there an audit program for sterilization processes and controls?
2. Is the audit team technically capable and sufficiently impartial to make judgements on the effectiveness of the sterilization process?

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Licensing:

1. Is there proper registration and/or licensing for the sterile medical product manufacturing operation?
2. Are all requirements for licensing routinely met?

These questions are examples of what can be derived from topics in the umbrella GMP to show that this document can be applied to specific sterilization processes and controls. As is shown, additional specific types of GMP are not needed in order to evaluate and control the manufacture of sterile medical products. General umbrella type GMPs, which are essentially 'what to do' not 'how to do' documents, adequately define what a manufacturer must do in order to comply while it does not unnecessarily discourage creativity in product and process design nor does it restrict opportunities for cost savings where possible and appropriate.

In practice, the umbrella GMP has probably been found to be much more useful both to the government and industry than originally expected. Because of this, the development of supplementary GMP has really become less and less critical an issue. The questions cited earlier relating to sterilization processing are typical of those currently being made by investigators who are utilizing the umbrella GMP. Instead of actively writing a specific GMP for sterile devices, the FDA has, at least for the interim period, undertaken evaluation of the appropriateness of new approaches to voluntary industry compliance. These include an in-depth educational program for its field investigators on sterilization processes, a recently revised detailed inspectional guideline covering umbrella GMP application to sterilization processes, equipment, and controls, and an increased dialogue with those outside the government, as working in open forums with industry and academia on developing guidelines and standards, to help foster better sterilization processing and controls.

Tying down manufacturers to detailed process-related GMP is becoming less and less desirable. More and more it is being recognized that when a government agency specifies sterilization methods, equipment, and testing, the responsibility for the success of the sterilization process moves from the manufacturer to the regulator. Wisely the FDA has historically avoided the temptation to dictate the mechanics of manufacturing and control operations, and has chosen to place full compliance responsibility with the manufacturer. Umbrella GMPs accomplish this without dabbling in 'how-to' cookbook regulations. It is hoped that the FDA and other regulatory bodies worldwide will continue to recognize the wiseness of this regulatory philosophy.

Along with this perceived change in GMP philosophy a desire is now being expressed by the FDA Bureau of Devices, to develop within recognized scientific and technical organizations process guidelines acceptable to industry and which the FDA could possibly utilize within their own regulatory structure in some yet to be determined manner. In the US, documents of this type, developed outside of the government, can as yet have no legal or enforcement significance and from a regulatory aspect but can be used only for reference or educational purposes. Time and experience may eventually result in a change to this approach.

Within the US there are now several examples of such guidelines relating to sterile products and manufacturing processes. These have been developed in the US by organizations such as the Pharmaceutical Manufacturers Association, the Health Industry Manufacturers Association, and the Association for the Advancement of Medical Instrumentation.

A very good example of this cooperative approach was begun about three and a half years ago at the Association for the Advancement of Medical Instrumentation (AAMI) in Arlington, Virginia A task force was formed to write guidelines for industrial ethylene oxide sterilization of medical

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devices. Industry, academia and government representatives working together developed a guideline under the auspices of this well recognized organization. A guideline was eventually published which individual companies within the medical device industry found they could readily adopt for their own internal use, and a document, the government could reference and use for training and even apply in auditing situations detailing basic sterilization GMP and process validation concepts.

The major concept put forth in the *AAMI Guideline for Industrial Ethylene Oxide Sterilization of Medical Devices*, and other related publications is installation qualification and validation of the sterilization process. This approach set forth in this and similar documents seems to answer the needs of both regulators and industry. It serves manufacturers as a guideline to establish and control the sterilization process and it provides regulators with a guideline and documented proof of an effective, reproducible sterilization process. The validation data generated provides something very tangible for the regulators to review. They will have detailed documentation of every step within the sterilization process. Following the guideline regulators can be assured as to whether the manufacturer has completed necessary validation work and if there are adequate controls assuring effectiveness of the sterilization process.

The AAMI Guideline serves the manufacturer as a base upon which to build processes and controls. By following qualification and validation concepts, sterilization can no longer be referred to as a mystical black box process used to effect a desired end result to a product Sterilization can now be expressed in objective, measureable terms. Some manufacturers have learned from validation data that their processes were unnecessarily stringent, the margins of safety so great, that specific process parameters could safely be reduced or shortened. This has proved to be a pleasant surprise for some companies by helping to reduce manufacturing costs and by delaying the need to purchase additional sterilization equipment because of pending capacity problems. Others, as some who experienced occasional sterility test failures when using traditional product sterility test methods, now know why they had these misses. They learned that they had processes which had inadequate margins of safety and that certain process parameters had to be increased The Guidelines are explicit in directing industry to take the initiative and to validate sterilization processes, to know exactly what is happening in sterilization processes and to develop a package of processing and control data to assure themselves, government agencies and, above all, their customers, of safe, sterile medical products.

A major concern within the AAMI Industrial Ethylene Oxide Sterilization Working Group was to make the Guideline as simple as possible while retaining the necessary criteria for assuring a good, meaningful validation program. It was hoped that by presenting sterilization validation in this manner, each company could efficiently and economically plan and execute its own custom validation program for specific equipment and products. Also, each company could evaluate the quality of any existing sterilization validation data, and hopefully be able to use it in their total validation program.

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This diagram found in the AAMI Guideline explains the flow of the entire validation program and provides to the manufacturer with an excellent foundation upon which to develop protocols. The AAMI Guideline further defines and explains each topic in this diagram.

Validation is directed to all new, or significantly changed, products, sterilization equipment, or process controls. The new product definition may include all existing products if a company is just beginning a validation program and has little or no existing product, equipment, or process data. The Guideline plainly defines the extent of a validation program: '1) Each production chamber should be qualified, 2) where process uniformity can be shown, new products can be qualified by one chamber in order to qualify all equivalent chambers, and 3) if a new product can be sterilized using a previously qualified cycle for a similar product, the new product can be qualified by equivalency.' Utilizing equivalency can be both technically sound and economically advantageous if properly exercised. 'The decision on equivalency should require a formal review utilizing professional judgement as to sterilization requirements.' If a product is judged to be equivalent, the company should have some documentation available to show how it arrived at that conclusion.

The Guideline also states that a validation program is not required for process release if process monitoring and microbial challenges for each sterilization cycle are equivalent to a performance run, and if the equipment used has undergone installation qualification. This approach may be used, for

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example, in instances where production qualities of product needed for validation runs are not available. However, the validation program is intended to be completed when the necessary quantities of product are routinely available.

It is important to note that the AAMI Guideline avoids prescribing specific numbers, processes, and equipment, except when absolutely necessary. This permits the manufacturer utmost flexibility in choices of process parameters, equipment, and controls. The ultimate responsibility for the effectiveness of the sterilization prcoess is upon the manufacturer. The manufacturer must prove to his satisfaction and ultimately to the satisfaction of the regulators, that there is in existence a documented validation program assuring that products labelled as sterile are, in fact, sterile. A manufacturer following this Guideline should be in compliance with the requirements of the FDA GMP for Medical Devices even though the Guideline has not yet received any official FDA recognition. We do know the FDA is using this document in its training of investigators and is referring to this document during facility inspections, recommending it to manufacturers who need to upgrade their sterilization operations.

Process validation has led to at least one major breakthrough for US manufacturers. Manufacturers, upon receiving appropriate FDA approval, may release sterilized product for sale upon review of documented sterilization parameters with no additional routine sterility testing being necessary. This is referred to as parametric release. A package of validation data must be presented to the FDA proving the reliability and reproducibility of the sterilization process before this practice is permitted To date a number of manufacturers have their radiation, ethylene oxide and steam autoclaving processes approved for parametric release.

In summary, GMP documents can be beneficial to regulators and industry as guidelines in the manufacture and control of sterile medical goods. To be most effective, the scope of GMP documents should be directed to the manufacture and control of a broad class of health care products, as medical devices, and should have nearly universal application within that category of products. GMP for specific subgroupings of products or involving unique manufacturing and control procedures, such as sterile medical products, may dictate specifics in processing equipment and controls. In doing so, these documents may stifle product and process innovation, result in increased costs for the manufacturer and consumer, and may remove, at least in part, the responsibility for the success or failure of a manufacturing operation from the manufacturer and to the regulator. This is a responsibility regulators should want to avoid. The manufacturer, through the use of proper equipment and a qualified staff must assume ultimate responsibility for the development, maintenance and control of a manufacturing operation for sterile medical goods. The preferred method by which a sterilization process can be developed and evaluated is through completion of a process design and validation program Such a program is described in the AAMI Technology Assessment Report No. 1-81, *'Industrial Ethylene Oxide Sterilization of Medical Devices.'* Completed programs provide both industry and its regulators with documented, objective data which can be used in constructing and evaluating the effectiveness of a sterilization process and related controls. Finally, a suitably designed, validated, controlled and documented sterilization process offers greater confidence in the success of a sterilization process; better than that offered by employing only the traditional sterility testing of relatively small numbers of product or biological controls.

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DISCUSSION
SESSION IV

Q. from the floor:

Dr Halleck, this question is actually not related to industry but to in-hospital sterilization. Could you tell me what purpose, other than to validate the design function of a steam sterilizer or the presence of ethylene oxide, the value of a routine use of biological indicators has in hospital sterilizers? This question is asked because of the uncertainty of the cleaning capabilities of the staff, the quality of the equipment, and the packaging and the placement of the items, and so forth. To me, at best, biological indicators only show that the equipment is working.

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A. by F.E. Halleck – USA

I agree with what you say. There is value in the use of biological indicators in hospital processes. The purpose of this is first an early detection of equipment malfunction, because sterilizers, like automobiles, have things that will break down, and it will show early enough that there is a problem. You have a certain amount of security, based on the higher resistance of the biological indicator rather than just a routine maintenance programme, to see whether you have a problem with the sterilizer. In the US, the use of biological indicators in hospitals is not mandatory, except that the Joint Commission of Hospital Accreditation states that all steam sterilizers should be checked once a week with a challenge test pack to determine the effectiveness of that sterilizer, and to monitor the chamber itself. The challenge test pack and the procedure have been specified in a standard that is available from the American Association for the Advancement of Medical Instrumentation.

The Joint Commission also states that if you have a steam sterilizer with more than one cycle, you run a biological test using two biological indicators inside a challenge pack in that sterilizer once a week. There are several kinds of challenge packs, but the standard one consists of 13 folded towels in a certain configuration of sizes and shapes with double wrappers of muslin or linen.

For ethylene oxide sterilizers, the Joint Commission recommends that the hospitals use for every cycle two indicators inside some type of package. They feel that the parameters of ethylene oxide sterilization involving integration of the humidity control, the packaging material, the gas concentration, and time are so interrelated, that they are not comfortable with once a week monitoring.

Thirdly, if a product used is an implant, they recommend that a biological indicator be used in a simulated product with that implant, whether it is steam or gas sterilization.

However, under no circumstances in any cycle, either the ethylene oxide or the steam sterilizer cycle, are products held in quarantine till the biological indicator test is done. Even though you can get results on the biological test in 48 hours with some degree of assurance, we recommend, as does the *USP* not to hold the product in quarantine. If you wish, you may continue to incubate for seven days. Now the question is, how safe is that procedure. The procedure is based upon the judgment of a large number of people, not myself necessarily, and is based on statistical data and evidence that the resistance of the biological indicators is far greater than that of the potential bioburden on the product. Therefore, the chances of a patient getting a nonsterile item is very remote. However, when the biological indicator is ‘positive’, it is an indication that the sterilizer must be immediately shut down, and a programme of service be addressed to get it in proper working order.

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Q. from the floor:

I would just like to add that it still does not indicate that all items in the load are sterile, if you do not get growth.

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A. by F.E. Halleck – USA

I agree. But remember, a biological indicator is a monitor to confirm that the process is working. The indicator in the chamber does not replace the biological indicator. You must put it in a package. It only gives you a degree of a relationship to the packaged product that you are sterilizing. In hospitals, you have an entirely different problem, because you have a multiplicity of packages. It is always a mixed load. However, if you use a 'challenge pack', as the Joint Commission recommends, and AAMI has developed as a guideline, I think you will rarely find a sterilizing process that shows contamination, particularly if you use the recommendation of the Joint Commission as well as the Association of Operating Room Nurses Guidelines.

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Q. by S. Riley – Australia

I have a two-part question to put to Dr Halleck. You suggested that the self-contained spore strips can be handled effectively by Sterile Supply Department staff. After hearing Dr McKay's statement on some of the problems with biological monitors and media, is it your opinion that:

1) Those staff who are not skilled in microbiology, such as most hospital sterilizing personnel, should carry out spore monitoring tests?
2) If you agree with this, could it not be subject to operator misuse?

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A. by F.E. Halleck – USA

First and foremost, I do not recommend that the Central Service Department do any kind of microbiological testing, such as the implantation of spore strips. Generally (I am talking about the US), people working in the Central Service Departments, even nurses, are not qualified to do microbiological testing and aseptic transfer, or have the laminar-flow bench, where it is needed, to transfer the spore strips into the test-tube test. This is usually done by the Pathology or Microbiology Departments in the hospital. What I recommend is, that because you do not have the capabilities of a microbiologist in the Central Services or instrument processing rooms, where sterilizing is taking place, there are available worldwide today self-contained indicator systems. There are others, that work equally well, that can be used with confidence by those who are not trained as microbiologists. If there is a 'positive', I would recommend that the 'positive' samples be sent to the microbiology laboratory for confirmation. Generally, for routine analysis, such as in the US, these self-contained biological indicators are almost fool-proof, as we have specialized incubators available for them. You can have small ones, which are desk-type, and you do get a feeling of comfort with them.

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Q. by E.R. Pavillard – Australia

Dr McKay, you made a statement that, I think, is very disquieting. It relates to the fact that when you tried to elute microorganisms out of a haemodialyser coil, you got negative cultures. When you filtered the coil with culture media and incubated it, you got positive results. A similar case was mentioned by Dr Dodson. Now, if, in fact, you cannot remove microorganisms from a sample of an item, then how can you measure the bioburden? If you cannot measure the bioburden, then what we have talked about today may not be as valuable as we think it is.

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A. by D. McKay – Australia

I think, measuring the bioburden of, say, a liquid preparation may be a relatively simple process, but measuring the bioburden of something complex like a kidney dialysis unit, or a piece of skin grafting material, or something of that nature, may be almost impossible. I think, when problems like that are found, then the only solutions are the fractional exposure techniques described in the AAMI document on validation of radiation and sterilization. Some of these techniques, fortunately, do not require an estimation of bioburden, but they do require an estimation of the resistance of the bioburden. This is done not by counting, but by giving graded doses in the sterilizing process, and by looking for fraction positive results. However, I agree, it is almost impossible to quantitate the number of microorganisms in some samples.

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Q. by G. Nelson – Australia

I would like to ask Mr Nygard, or the panel, their views regarding good manufacturing practice, as to whether a similar sort of practice should apply to the manufacture of the primary packaging materials, that are there to ensure retention of sterility of the device and whether there is any move to legislate on this.

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A. by J.E.W. Nygard – USA

To answer your latter question first, unfortunately I am not aware of any legislation, but at the same time, I am certainly not privy to what goes on inside regulatory operations within the US. It would appear to me that the question of sterility barrier is one that has been discussed repeatedly, certainly within the US. It has resulted in numerous meetings, and professional organizations have been formed concerned with the issue of sterility barrier for sterile products. At this time, in the US, the responsibility for the adequacy of the sterile barrier rests with the manufacturer of the item. He provides the assurances that the packaging system used is in fact a sound one, or one that will retain the sterility of the product. The responsibility lies with the manufacturer of the sterile item and the packaging supplier, with tests to determine the quality of the materials and seals of the package used.

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A. by F.E. Halleck – USA

I would like to add more to that subject. Perhaps you are not aware, but you should know that there is a programme being instituted which is to begin in 1983 in the US, on standards, specifications, and guidelines to be applied to primary packets for sterility barriers. This will be based on package materials that are available and used in hospitals. This study is to be undertaken by The Applied Paper Institute, or TAPI, in co-operation with AAMI and the Health Industry Manufacturers Association. We will, hopefully, devise and arrive at specific test methodologies by which we can evaluate the biobarriers of various packaging materials. We foresee right now (although we are not sure until the tests are done and it will be a year's study or longer) that there will probably be a specific test methodology for making a judgment on a packaging material. There will also be different tests for different types of packaging material. For example, there will be one type for a textile type of wrap, there will be another type for paper or paper-type products, there will be one for nonwovens, and there may be quite a different one for films or plastic films, and so on.

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SESSION V

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Sterilization Process Technology – Part 1

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SESSION V

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Sterilization Process Technology – Part 1

Introduction to Session

Ronald A. Anderson

In addressing this subject of sterilization, aspects of heat sterilization of medical products, as well as some factors affecting the maintenance of sterility, will be considered.

There are methods that can eliminate high levels of microbial contamination. However, one of the difficulties associated with the sterilization of some medical products and many pharmaceuticals is that they, along with the contaminants, are adversely affected by some or all of the agents used. Very often, the process that is finally chosen takes account of potential product instability, as well as the extent and nature of the bioburden.

With some pharmaceutical products, we can take into account the effect that components of the product may have on the contaminants. Some pharmaceutical formulations will augment kill by heat and possibly reduce the severity of the treatment required. The *British Pharmacopoeia* recognizes that some antimicrobial chemicals augment the effects of steaming to such an extent that a 30-minute exposure at 98-100°C is used for some products. The process called 'Heating with a Bactericide' is permitted for some products that will not satisfactorily withstand the higher temperatures used in autoclaving cycles. It is well recognized that if this process is used, the bioburden must be low.

The increasing tendency to apply a heat treatment, based on knowledge of the bioburden and on other factors which may affect the efficiency of the process, is satisfactory when properly validated cycles are applied with proper controls.

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Heat Sterilization – Process Development and Validation

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In the United States, the need to validate heat sterilization processes has become formalized as a result of federally mandated Good Manufacturing Practices. In response to this, groups of technical experts have drafted documents that detail various methods appropriate for developing and validating heat sterilization processes. These groups include Health Industry Manufacturers Association, Parenteral Drug Association, Parenteral Manufacturers Association, and Canadian Environmental Health Directorate – Health Protection Branch. The input from these experts has in a very rapid fashion brought together and defined state-of-the-art information, which is a highly useful tool in designing optimal cycles.

The term 'sterility' has more recently been approached on the basis of probability, as conventional methods of sterility assessment, in reality, offer a very low level of assurance. This concept permits design of heat processes that meet particular applications, while providing a high level of sterility assurance.

Two basic concepts have come to the forefront as approaches to the development of sterilization cycles. They lend themselves very well to heat sterilization processes. The first concept is generally referred to as 'overkill', a method in which a high, calibrated level of resistant micro-organisms is used to ensure that a significant sterilization dose has been applied. The second concept involves investigation of the resistance of the naturally occurring microorganisms or bioburden associated with the material being sterilized. The information is then utilized to define a calibrated biological indicator, thus assuring delivery of an effective sterilization dose. The former technique utilizes lot-by-lot sterility testing, while the latter may employ this type of testing, but also permits the use of dosimetric testing.

Materials sterilized by heat processes generally fall into two broad categories, those that are heat stable, and those that are affected by heat. An example of the former would be metal hypodermic needles and of the latter, various parenteral solutions. Sterilization of nonheat-labile materials lends itself to either the overkill or the bioburden approach. However, the overkill is favoured, because it requires a less rigorous approach, and, as such, it is more cost-effective. Materials that are degraded in a heat sterilization process can be sterilized by defining a minimal dose which results in less product degradation, while still providing a significant level of sterility assurance.

Overkill involves use of an appropriate spore in the form of an inoculated carrier such as a filter disc or chromatography paper, or may be directly inoculated on or in the product or material being sterilized. By use of resistant spores, we can ensure the delivery of a significant dose that is adjudged sufficient to render the product sterile. Typical resistance factors or D values for the more commonly used spores to monitor steam or dry heat processes are presented in Table 1.

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Table 1

Spores for monitoring heat sterilization processes			
Spore	D value	Temp °C*	Process monitor
B. stearothermophilus	1.5	121	Steam
B. subtilis var. *niger*	1.0	170	Dry heat
Clostridium sporogenes	0.7	121	Steam
Clostridium sporogenes	3.5	112	Steam

*Temperature at which the D value was determined.

The bioburden approach requires an in-depth analysis of the product bioburden, the inate resistance to steam or dry heat of that bioburden, and the definition of a biological indicator capable of reflecting a degree of resistance beyond that found for the product bioburden. In this manner, we are able to bring into our equation a statistical factor and define a probability of nonsterility. This probability of nonsterility is generally greater than that which is obtained from a standard sterility test, such as that defined in the *US Pharmacopeia*.

Bioburden determination must be done to define both numbers and types of organisms. However, in this analysis, the types that we are interested in are generally only the spore-forming organisms, or others that may have a significant resistance to the particular heat process used. Generally, non-spore formers are of little consequence. In some instances, it may be appropriate to isolate and propagate particular species and understand their particular resistance to the heat sterilization mode, and use them as the biological indicator for monitoring the process.

The exposure temperature chosen for a process may be the standard 121°C in steam, or 170°C in dry heat. However, in many cases lower temperatures are used, especially where the material is heat-labile, and reduced temperatures result in less product degradation.

Processing at lower temperatures has long been practiced in the food canning industry, and more recently this technology, largely as a result of commercial preparation of the parenteral solutions, has been more often used in the treatment of medical products.

When employing lower temperatures, either in steam or dry heat, we are increasing the range of organisms that may potentially survive the process. It becomes, therefore, even more important to understand the thermal resistance characteristics of the bioburden, to increase the frequency of monitoring, and establish more rigorous standards, intended to exclude microorganisms from the manufacturing environment.

The method used in assessing the resistance of the bioburden, or the indicator microorganisms used in monitoring a process, requires the determination of the D value. D value determination of non-heterogenous populations, as is the case of the bioburden of most products, is prone to error, because we are dealing with inactivation rates that differ for the various microorganisms making up the total population. However, the need to understand this resistance pattern outweighs the error introduced, and adjustments can be made in our calculations to overcome the error factor.

D values are best determined using either the direct enumeration technique to plot the rate of microbial kill over time, or the fraction negative technique of Halvorson and Ziegler, in which

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positive and negative data from sterility test assessments are used to calculate the D value. The mathematical expressions are shown in Table 2.

Table 2

D value assessment

Direct enumeration	Fraction negative (Halvorson-Ziegler)
$D = \frac{t}{\log N_o - \log N_u}$	$D = \frac{t}{\log N_o - \log [\ln (\frac{r}{q})]}$
t = exposure time	t = exposure time
N_o = initial population	N_o = initial population
N_u = population at time t	r = number of samples tested
	q = number of samples sterile

Both methods can be very effectively used to determine a D value for homogenous populations, as in the example of organisms used for biological indicators. However, the direct enumeration technique is not so effective when determining bioburden resistance, because we may be working with a low population which in itself represents a recovery problem, and regression analysis of the data will not properly compensate for the nonlinearity generally associated with a survivor curve for a nonhomogenous population. The fraction negative technique is more appropriate for data for the nonhomogenous population, because we are calculating a D value at discrete points. It permits us to discard the data at shorter exposure times, where the population of the nonresistant organisms present has had a large influence on the D value obtained. It is possible, using either technique, to select the thermal resistant fraction of the bioburden by means of a heat shock procedure and thus deal only with the more resistant organisms.

In thermal processing, a linear relationship has been shown when D values are determined at different temperatures. A plot of D value vs. temperature can be used to establish a constant or z value, defining the temperature change necessary to alter the kill rate by a factor of 10 or one log value. This is shown in Figure 1.

This information is useful, because it permits us to extrapolate the lethal rates at higher or lower temperatures using actual data at known temperatures. Also employed in heat processes is the F value concept. F_T^z may be defined as the lethal effect at some temperature as compared to a reference temperature. For steam sterilization, the usual reference temperature is 250°F, and F in this case is referred to as F_o if z = 18.

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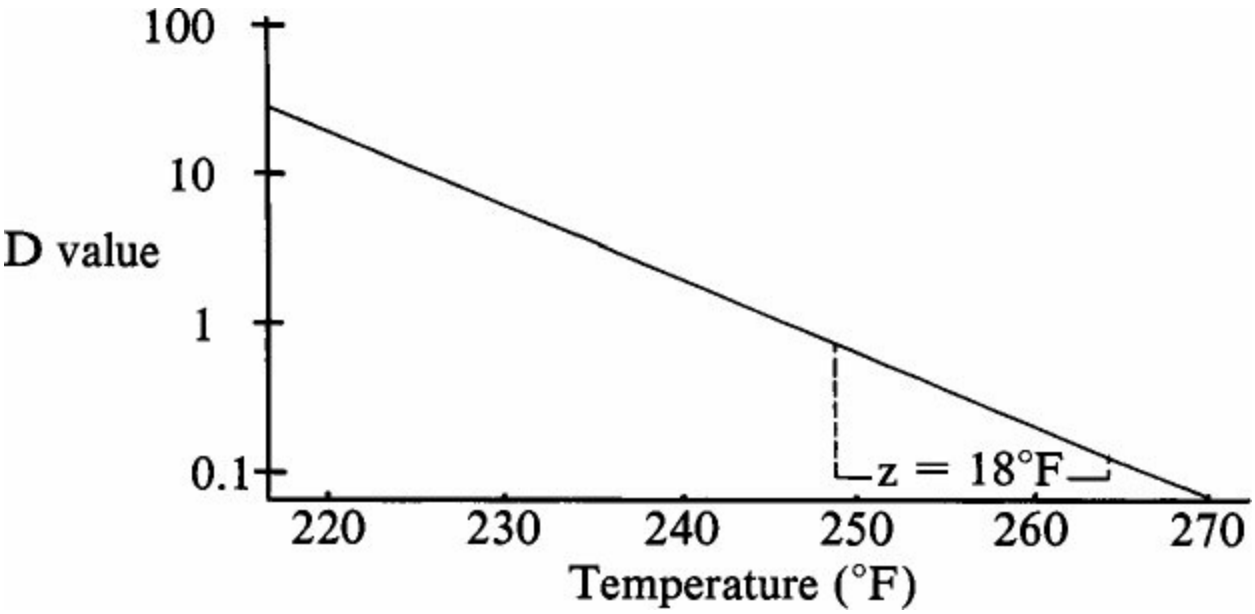

Figure 1. z value determination.

F values are calculated as follows:

$F = \Delta t \cdot \Sigma L$

Δt = time interval between temperature measurements

$$\log L = \frac{T - 250}{z}$$

L = lethal rate

By definition, in this equation, if T = 250 then L = 1. Thus, for temperatures higher than 250°F, L > 1 and for temperatures less than 250°F, L < 1.

An example of the F value concept is shown in Figure 2. A thermal plot of temperature vs. time has been made and the lethal rate (L) at two-minute intervals calculated to give an F value for the process. Thus, we have calculated the total sterilization dose including the effect at all temperatures above 200°F, and equated this to the effect of sterilization at the single temperature of 250°F. In the example, exposure at 250°F is for about four minutes. However, the total exposure is equivalent to exposure for 7.2 minutes at 250°F, including the heating and cooling time.

A similar analysis of dry-heat processes can be made, and the commonly accepted reference temperature is 170°C and z=20.

The use of these techniques permits temperature data, collected from each sterilization cycle, to be converted to a known reference standard, or to a standard that has been developed for the particular material being processed. This provides further assurance of achieving a minimal dose.

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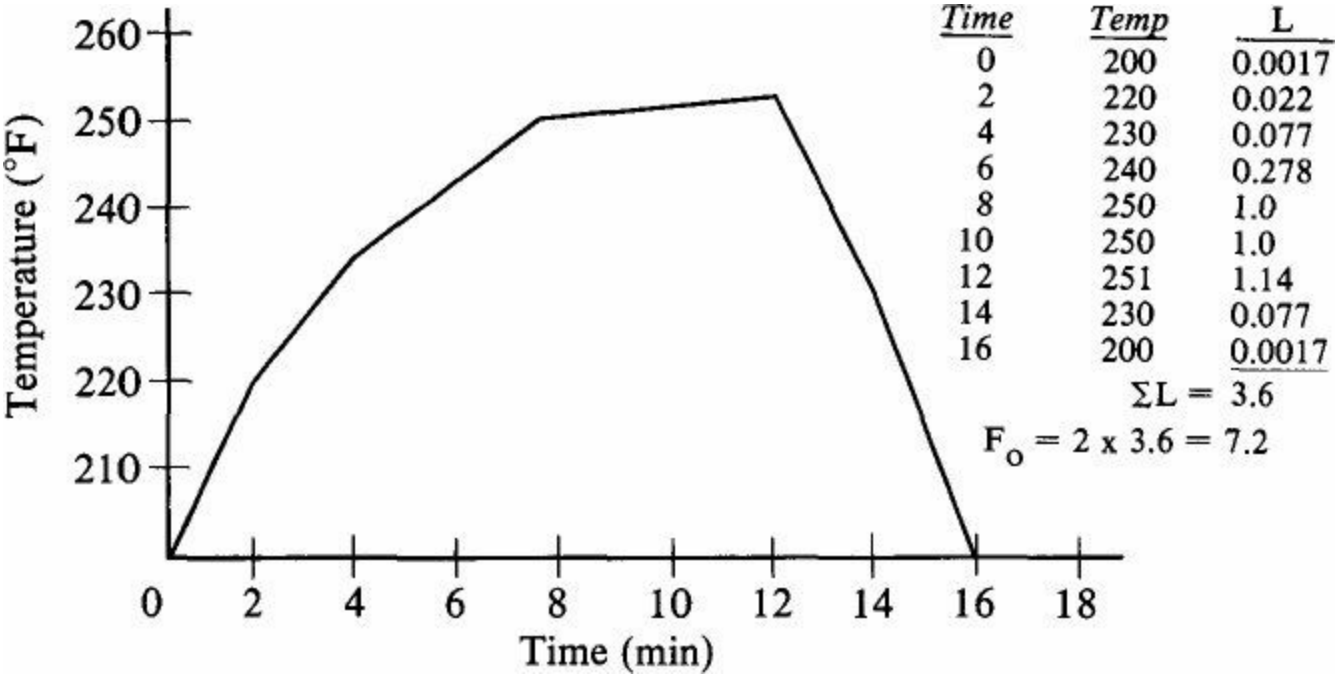

Figure 2

The information gained in the analysis described permits us to establish a sterilization cycle appropriate for the product application studied. If we have determined the resistance of the bioburden, or are using a resistant spore form, the sterilization time or cycle length can be approximated using the following equation:

$t = D(\log M + X)$

t = sterilization time

M = average population or bioburden + 3 SD

X = desired safety factor

The sterilization time begins when the temperature at which D was determined is reached, and is found through thermal mapping. The thermal mapping must be done in a manner that will identify the slowest to heat and the coolest areas in the load. The contribution from heating and cooling, or temperature excursions around the process temperature, that is the F value, can be assessed, and factored in, to adjust the total sterilization cycle time.

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Validation

The primary function of a validation programme is to determine if an effective sterilization process can be routinely and reproducibly carried out. The validation of heat sterilization processes is similar in many respects, whether it is steam or dry heat, done in a closed chamber or in a heat tunnel. There are some important aspects that must be evaluated in a rigorous programme designed to qualify all critical phases of the heat process.

The installation, or equipment qualification phase, is the logical first step in assuring that the process can be conducted effectively, and that it is reproducible. Equipment lacking the capability of reliably performing within established parameters will not permit a process to be run in a controlled manner.

In the following listing, important areas to be covered in the installation qualification are specified.

Installation Qualification

Item	Steam	Dry-heat ovens	Dry-heat tunnel
Equipment manufacturers literature	X	X	X
Utilities required for operation	X	X	X
Boiler capability	X		
Maintenance programme and log	X	X	X
Physical condition of equipment	X	X	X
Calibration programme	X	X	X
Drawings of equipment and facility	X	X	X

During the installation qualification, pertinent aspects are reviewed including all available literature from the manufacturer to ensure that the equipment is capable of performing the desired process. Any needed changes or upgrading should be done as a part of validation. The utility requirements necessary to provide all energy needs should be specified. For steam processes, the boiler capacity and makeup capability should be assessed to ensure delivery of sufficient quality steam. A preventative maintenance programme and a log documenting maintenance activity should be provided. The physical condition of the equipment should be assessed and, as noted previously, necessary changes should be made. This will ensure than no change will be necessary during the validation study, that will influence the results of individual tests. A calibration protocol to be conducted during the validation tests should be prepared. There should be included calibration of monitoring and sensing equipment, such as pressure gauges or temperature gauges, timers, thermocouples, etc., and the calibration should be traceable to a NBS reference. Drawings of equipment and facilities should be on file, to be referenced for maintenance purposes or modification in the system.

Following completion of the various aspects of the installation qualification, work on the second phase of validation, the performance qualification, is ready to begin. Generally, the performance qualification is considered to fall into two categories: equipment validation and microbiological validation.

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Validation of Steam Sterilization Processes

Equipment Validation

During the validation cycles for a steam process, the minimal exposure parameters are used to establish cycle effectiveness. A temperature profile is made, one designed to map effectively the thermal variations in the chamber, as well as to delineate the heating and cooling rates of the load. All monitoring and sensing equipment is calibrated at the beginning and the end of each validation cycle. At least three repetitive runs are made to establish performance consistency.

Microbiological Validation

Challenge microbial spores are used in each of the validation cycles. Generally, 20 samples are used and positioned in a manner providing challenge to the cooler or slower heating zones in the load. Consistent kill, or reduction of spore population, should be demonstrated in each cycle.

Validation of Dry-Heat Ovens

Equipment Validation

In the performance qualification of hot-air ovens, the equipment must be extensively monitored to determine the heat distribution profile. Sites found to heat more slowly, or to be at lower temperatures, will serve as the reference points for measuring time at temperature and the location of biological indicators. This should include heat-penetration studies of the material being processed, to define further the reference time at temperature and biological indicator locations. If the system is equipped with a forced-air capability, the flow rate during validation should be the minimum defined in the process parameters, and the loading configuration should reflect the 'worst case' situation.

Microbiological Validation

Microbiological studies are included during validation to provide additional assurance of the delivery of the required heat dose to all portions of the product. Placement of the biological indicators must represent 'worst case' load size and configuration. A geometric pattern for placement of the biological indicators should be followed, with emphasis given to cooler or slower to heat areas.

Validation of Tunnel Systems Employing Heat

Equipment Validation

Heat tunnels are more complex than ovens and require a study and validation of more variables. It will generally be necessary to conduct prior to validation an extensive heat profile of the system, to establish the process parameters that will then be used in the actual validation programme. This will be necessary for each specific throughput rate and temperature to be used. For a given tunnel process, monitoring during validation should include:

1. Hood temperature at maximum line speed
2. Hood temperature at minimum air flow rate
3. Hood temperature at coolest burner rate specified for the process
4. Heat-up profile of the material being processed
5. Geometric location of thermal probes within the product to include the full width of the conveyor system.

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Microbiological Validation

Microbiological studies to confirm the efficacy and uniformity of the tunnel operation should consist of biological indicators placed in a geometric pattern in a manner to assess the operation under minimal temperature and maximum line speed.

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Sterilization – The Hospital Environment

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In Australia, we are faced with a number of problem areas that are generally not applicable in the United Kingdom and in the United States of America. These affect in many ways the methods and materials used for sterilizing procedures in most hospitals.

There are approximately 290 public and 80 private hospitals (excluding nursing homes) in New South Wales that carry out patient care. They all require sterile items.

In the total number of 370 hospitals, only 30 are equipped with 64 prevacuum steam pressure sterilizers which is approximately 8% of all hospitals. A similar situation exists in hospitals in other states.

We shall compare Australia with the UK and US, from which we purchase, both directly and indirectly, a large proportion of items that must be sterile when used. In the UK, the land area is 87 818 square miles with a population of 55 000 000. In Australia, the land area is 2 974 579 square miles with a population of 15 053 000. The breakup for states, excluding Tasmania, the Australian Capital Territory, and the Northern Territory, is as follows:

	Land Area	Population
New South Wales	309 433 square miles	5 260 000
Victoria	87 884 square miles	4 000 000
Queensland	667 000 square miles	2 000 000
Western Australia	995 000 square miles	1 250 000
South Australia	380 070 square miles	1 250 000

England, Scotland, and Wales would fit into Australia 34 times and into New South Wales 3.5 times.

Mainland United States (48 states) has a land area of 3 022 260 square miles and a population of 231 480 000.

The distance between Australian cities and towns, and the small population inhabiting inland areas is such that technical expertise related to specialized equipment is often beyond the capabilities of available maintenance staff. Maintenance services are mostly from capital cities. There can be both a delay and a high cost in providing services. A service call could cost $A500 or more to rectify a minor fault. Before the serviceman returns to base, another fault could occur. Sophisticated equipment in many locations could be unreliable, and lengthy breakdowns could be detrimental to patient care. In the UK and US, because of shorter distances and much higher population density, expert technical service is usually only a short distance away.

Shortages of water can be serious, especially where it is used in large quantities for the operation of equipment such as vacuum pumps in prevacuum steam pressure sterilizers. Even in Sydney,

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regulations require that water used for this purpose be recycled. This is usually done by passing water through expensive chiller systems and then reusing it.

There are some differences between country and city hospitals. Most country hospitals are small in size, and surgical procedures are of a simple type and require general type surgical equipment. Sterile-item requirements in such hospitals are relatively small. Such a small workload would not justify an expense of installing high-output complicated equipment such as prevacuum steam pressure sterilizers.

New South Wales country regional centres have been, or are being, developed to provide many sterile items for patient care from a central service, and sterilizing facilities in all hospitals are subject to this rationalization. Such rationalization is related to the type of hospital and the type of patient being treated. Where a hospital has one or more operating theatres, the aim is to provide, where possible, all sterile equipment other than items such as surgical instruments, holloware (stainless steel or plastic), and anaesthetic equipment. Sterile items to be supplied include theatre textile packs, swabs, sponges, dressings, and single-use items, e.g. syringes, needles, catheters, and devices. Thus, sterile items could either be prepared and processed at a regional or subregional supply unit associated with a regional textile service or purchased as sterile from commercial sources in bulk and distributed in small quantities to user-hospitals.

To provide this type of service, it is very costly because of distances between hospitals, poor road conditions, and small stock quantities involved. One service functioning in northern NSW has, as an example, one supply route for which the round trip is 560 km. This trip caters for only seven hospitals on a three-times-a-week basis.

It has been found that it is not practical to supply sterile packs of surgical instruments and holloware to such hospitals from a central supply unit, because of the number of surgical procedures that could be carried out between deliveries. Possible delays of deliveries may be due to natural hazards such as floods and fires. Very large numbers of instruments are required and costs of providing such a service are prohibitive. For these reasons, these hospitals are provided with sterilizing facilities suitable for their needs. Most hospitals carrying out surgery are equipped with downward displacement steam pressure sterilizers, hot air sterilizers, and ancillary equipment including ultrasonic cleaners, drying cabinets, respiratory equipment, decontaminators, etc.

Hospitals in cities may have similar equipment to those in country areas, depending on their size and workload. However, finance has not been available to equip all city hospitals with the prevacuum type steam pressure sterilizers. Where hospitals have a large surgical workload and carry out sophisticated surgery, the sterilizing and ancillary equipment is comparable with the most modern hospitals anywhere overseas. Equipment installed includes prevacuum steam pressure sterilizers, downward displacement steam sterilizers, hot air sterilizers, ethylene oxide sterilizers, aeration cabinets, drying cabinets, ultrasonic cleaners, washer decontaminators, and drying equipment for respiratory items.

The low temperature steam-formaldehyde process is at present not being used in NSW hospitals, and will not be accepted until the present design is further improved.

In NSW, for many years, hospitals have been strictly controlled with regard to the quality, design, and type of sterilizing and ancillary equipment. Management of hospitals has been required to refer requests for new or replacement equipment to the Health Commission. Each request is then investigated. If approved, only equipment that meets relevant Australian standards, Health

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Commission, and Public Works Department specifications, is purchased. On completion of manufacture, each item is checked before delivery for compliance to specification and performance-tested in the factory with a hospital-type load. After installation, a further check of satisfactory operation with a hospital-type load is made before the unit is handed over to the hospital for routine use. All companies wishing to market new sterilizing and ancillary equipment for use in NSW hospitals must first submit technical data to the Health Commission and Public Works Department. If the data are satisfactory, a unit is installed for exhaustive 'in-use' testing in a busy city hospital. If satisfactory, the unit is then considered suitable for competitive tender for public hospitals or for purchase by private hospitals. Ethylene oxide sterilizers are installed only in some of the larger city or regional base hospitals and are further subject to occupational health authority regulations, including the licensing of operating staff.

Hospital staff operating sterilizing equipment in NSW falls into two classifications, nursing staff and lay staff. The majority of country hospital equipment is operated by nursing staff. However, most large country hospitals and almost all city hospitals are equipped with a sterile supply unit, either of the general type, or specifically for operating theatre supply. Training of staff for these areas has been carried out in NSW since the late 1960s. The early courses were organized by personnel working in sterile supply units who had formed The Sterilizing Research and Advisory Council of Australia (N.S.W. Branch). After a number of years, financial assistance was sought from the Health Commission, who then appointed a full time tutor. Two courses have been in operation for some years:

- Course 1 – Sterilizing Technology
  This course is of twelve-month duration and consists of part correspondence, part live-in tuition, and is concluded by an examination and issue of certificates. Student intake each year is approximately 70.
- Course 2 – Sterile Supply Management
  This course is of six-month duration and is of a similar type to the Sterilizing Technology Course (correspondence and live-in). A prerequisite for this course is a successful completion of Course 1.

Students attending live-in sessions received leave-with-pay and also travel and sustenance allowances. Financial restrictions have delayed the commencement of additional courses, including one for maintenance personnel. Similar courses are conducted in most other states in Australia with course content approved by a federal body as being of a comparable type.

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Packaging Materials

It has been found that many types of packaging materials that appear on the Australian market were designed for use in prevacuum steam sterilizers and/or ethylene oxide sterilizers. Whilst these packaging materials may have improved resistance to the recontamination of pack contents after sterilization, and be suitable for the prevacuum steam sterilizer and ethylene oxide process, they are of little use if the pack contents cannot be sterilized in the first place. It is to be noted that in the UK it is most unusual to see a downward displacement porous load steam sterilizer in use. I have visited many hospitals over a number of years and can recall seeing only one unit of this type.

The type of sterilizing equipment installed in hospitals determines many aspects of procedural methods, and requires selection of items and other materials that are compatible with this equipment. Most Australian hospitals must retain their surgical instruments and holloware within the hospital and reprocess them wrapped and sterilized for elective use. The majority of steam sterilizers installed in Australian hospitals are of the downward displacement type, and the location of most hospitals requires equipment that is simple to operate, relatively easy to service and maintain, with spare parts being readily available.

It is to be remembered that removal of air from packages and their contents is much more difficult in the downward displacement type unit. If steam in its downward movement passes under packages before air is displaced, that air remains in the pack and is compressed towards the centre of the pack, with possible survival of organisms in that area. For this reason, we have used packaging materials that will allow within time limits air to be readily displaced, steam to reach all surfaces to be sterilized, and drying of the pack and contents. The packaging must also have resistance to recontamination when handled and stored correctly within time limits. Surgical instruments may be in sets of up to 100 items, small multiples to a pack, or as a single item.

To facilitate drying and to spread condensate produced during the sterilizing stage, many hospitals first wrap instruments in textiles. This wrap is then usually used on the operating theatre instrument table. Covering such a wrap is either a double-thickness wrapper of textile, which when the package is complete provides a multilayer of textile around the contents, or, in addition, an outer multilayer wrap of paper. The paper used is bleached bag Kraft GSM49 type manufactured to an Australian standard. This standard is basically similar to the British Ministry of Health Specification TSS/S/330004. Holloware is made into packs with strict limitations of the quantity and types of items in each pack. These packs are wrapped in textile wrappers of double thickness, and the pack is multilayered. Some hospitals wrap in multiple layers of paper.

Textiles used in operating theatres as wrappers for items to be sterilized are now standardized with regard to material, dimensions, method of fold, etc. The material is polyester-cotton 50/50 with a yarn of 33 Tex weft and weave, and with a weft and weave of 23 yarn/cm. This material has been investigated by microbiological advisers to the Health Commission and found to be comparable with other materials in use in the UK and the US. After use, textiles are laundered and inspected over illuminated tables for holes, lint, fluff, stains, etc. Holes are repaired with adhesive patches. Other types of packaging are paper bags of bleached bag Kraft, manufactured to an Australian standard, similar to the British Ministry of Health Specification TSS/S/30006. Aluminium foil of a suitable quality, sealed glass jars, and metal containers are used for items to be subjected to the hot air

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sterilizing process. Various other types of specialized packaging from commercial sources may be used if suitable for the type of sterilizing equipment installed.

Over recent years, there has been a shift to the purchase of many prepacked sterile porous and nonporous single-use items from commercial sources. This was resisted for many years by many small hospital authorities on the basis of cost. In many instances, labour costs for the preparation of these items within hospitals was regarded as nil. Staff who were on duty had sufficient time to carry out such work without affecting other duties. Since rationalization of sterilizing equipment within hospitals has been effected, it has been found more economical in many instances not to replace old equipment, thereby changing this situation.

A number of problems do occur in some hospitals with the provision of some types of commercially packaged sterilized items. A hospital may be required to have on hand items used very infrequently. Some of these problems are:

(a) The marketed pack may contain too many items to be used within a reasonable time. If, after a period of time, excess items are discarded, costs rise. The nursing staff regards the commercially sterilized packs as sterile forever, whereas they know that hospital-sterilized items have a limited shelf life.

(b) In some areas, for instance subregional stores in a base hospital, purchase items such as Foleys catheters (ten to a box). The box is opened for distribution of one or more of each size to each user, and this may, or may not, be carried out in ideal conditions. For some years, I saw in a hospital general store all stock stored alphabetically, and there were syringes stored alongside the sugar.

(c) Transport and storage of commercially prepared items are not always carried out in the same conditions as those items processed within the hospital. It is usual to have in most hospitals sterilizing facilities adjacent to the main use point and to provide suitable facilities and methods of transfer to other use points. Commercial items are often transported by rail with poor handling conditions or in vehicles for long distances in wet or very dusty conditions, then dumped in a general hospital stores area, where all types of other items, including food, are also delivered. Storage in hospital main stores may be adjacent to food items or, even if in a separate section, be vulnerable to insects and vermin. In addition, the store staff is very often not trained in the correct handling of such items.

Such are some of the problems involved with sterile products in the hospital environment.

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Sterility Maintenance – Porous Packaging Materials

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The primary function of a package enclosing a ‘sterile’ medical or surgical item is to be an absolute barrier towards microorganisms. The protective function of the package with respect to preventing mechanical damage to the contents, although important, is secondary to its action as a barrier.

Generally, the function of the package as a barrier operates from the time that the item is exposed to the sterilization treatment until it is used. It follows that the material(s) constituting the package must permit the sterilization treatment to operate effectively, yet present a total barrier to potential microbial contaminants during poststerilization storage, handling and transport. With ionizing radiation as the sterilizing agent, these requirements can be readily met; high-energy photons or electrons traverse barriers known to be impenetrable in respect of microorganisms, e.g. metal foil and polyethylene film, essentially without reduction in lethal effectiveness. In such circumstances, the principal operational concerns associated with the package are ensuring the effective function of seals and minimizing structural defects. In contrast, with sterilizing treatments which require the translocation of the inactivating agent across the barrier, e.g. saturated steam and ethylene oxide, the packaging material must permit the ready passage of the agent while preventing the penetration of microorganisms. Also important with these sterilizing treatments is the need for adequate permeability of the barrier towards air, as evacuation is often a part of the treatment cycle, and if air cannot pass freely across the barrier, the package is liable to burst. In practice, these requirements are met by fabricating the package, at least in part, from so-called ‘porous’ web materials, such as paper or spun-bonded polyolefin. It is also worth noting that, for a variety of reasons, porous webs may be included in the package of items destined for radiation sterilization.

The ability of porous packaging materials to act as barriers to microorganisms is clearly a prime consideration in sterility maintenance.

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Direct Tests of the Penetrability of Porous Packaging Materials

Test Design

Documented test procedures aimed at measuring directly the ability of a material, packaging or otherwise, to stop particulate matter have certain features that are common to all.

The essence of these test procedures is that a sample of the material under test, taken at random from bulk, is located as a barrier in a fluid flow, gaseous or liquid, containing a dispersion of test particles (cellular or nonbiological particulate matter). The stopping power or efficiency of the barrier is determined by comparing the size and numbers of particles contained in the fluid on each side of the barrier.

The performance of such tests involves several crucial operations:

Setting up

The system must have a minimum of two compartments separated by the barrier under test. One compartment must allow introduction of the fluid to the barrier in a uniform manner. The type, size, shape, density, charge, etc. of particles must be similar to those likely to be encountered by the barrier in practice, as should be the fluid carrier. Particles must be uniformly dispersed in the fluid at an appropriate concentration.

Passage of the fluid

There must be a driving force for the fluid flow between the two compartments, which in turn is responsible for movement of the particles towards the barrier. Commonly, either reduced or positive pressure is this force. With liquid fluids, capillary action or gravity can provide the necessary force.

Sampling

In its simplest form, this can be done by placing a second barrier of known appropriate stopping efficiency downstream relative to the test barrier and examining it for the presence of particles after allowing passage of a given volume of fluid. More sophisticated sampling devices are possible, including those that monitor continuously during fluid flow.

Analysis

The analytical method must be specific for the chosen test particle and ideally it should be sufficiently sensitive to detect the presence of a single particle. Generally, methods of analysis based on chemical means of detection cannot meet this ideal. Physical methods, on the other hand, for example detection of isotopically labelled particles, although not reported in routine use, could well be valuable in this regard. The ideal can, however, be approached when viable cells constitute the test particles, since a single viable cell cultured under proper conditions will produce a visible colony that can be readily scored.

U.K. Test

At present in the UK, the Department of Health and Social Security (DHSS) specifications (1, 2, 3) relating to papers used to fabricate drapes, or to make bags for sterilization purposes, include a requirement for compliance with a test for methylene blue penetration. This is the sole requirement in

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these specifications concerned directly with standards on particle penetration. The test method is that of British Standard 2577 (4), devised to permit the assessment of the protection afforded by respirator canisters against particulate clouds.

The implication behind the DHSS specification on penetration, as applied to sterilizable papers, is that it provides a measure of the protection afforded by those papers against microbial penetration. In other words, a limit on penetration of a barrier by a nonbiological particulate dispersion is used to indicate penetrability towards viable microorganisms. The validity of this action has to be questioned. Present limited data do not allow the efficiency of a barrier in respect of nonbiological test particles to be equated with that in respect of microorganisms. A measurement of the stopping power of a barrier relates only to the conditions under which the measurement is made, and therefore, in determining the penetrability of materials towards microorganisms, appropriate viable organisms must be the test particles.

Typical Test Procedures Employing Microorganisms

Published tests of the penetrability of packaging materials towards microorganisms may be divided into two general categories, those employing gaseous fluid (usually air) as carrier of the test organism and those using a liquid (usually water). The test employing air is intended to simulate the challenge to materials encountered with normal storage conditions, whereas that employing liquid is regarded as simulating the worst conditions that materials may encounter.

Typical of the tests using a liquid carrier is the one described by Harbord (5). A sample of packaging material of specified size is floated on the surface of an aliquot of sterile water contained in a Petri dish. A drop of a suspension of viable *Staphylococcus saprophyticus* cells is carefully located on the upper surface of the material and left in contact with it for up to two hours. The material, together with the cell suspension, is then removed from the dish and the extent of penetration by cells is determined by culturing the aqueous contents of the Petri dish in nutrient medium. In principle, the test is similar to those laid down in the Pharmacopoeia Helvetica (6) and in the Pharmacopoeia of the German Democratic Republic (7) for the impenetrability of plastic containers towards microorganisms. The time of contact between the barrier and the culture of test organism in the container tests is, however, appreciably longer than two hours, presumably with the intention of allowing time sufficient for growth to penetrate across a continuous column of liquid joining the two sides of the barrier.

A test of similar design has recently been officially adopted in Germany for use in specifying ‘sterilization paper for bags and tube packings’ (8). The DIN test method uses *Staphylococcus aureus* cells in water suspension ($\lesssim 10^7$ cm^{-3}) as the microbial challenge. Five drops of cell suspension are placed individually on each of five replicate sterilized samples of paper (5 cm × 5 cm) and allowed to dry in air under normal laboratory conditions. The underside of each paper sample is then placed in contact with the surface of a blood agar plate for a period of five seconds and microbial penetration is assessed by observation of surface growth following appropriate incubation.

In common with other tests of this same general type, the DIN ‘wet’ test findings are greatly influenced by the wetting properties of the test material and, in effect, the test may well measure water-repellancy properties of the material rather than microbial penetrability. In practice, running the test has also revealed a number of technical difficulties that are not catered for in the official test description. An example is the variability in the contact between the underside of the sample of

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material and the agar surface because of deformation of the sample during sterilization and drying.

The test described by Hunter, Harbord, and Ridett (9) is illustrative of those done on materials using a gas as carrier of the test organism. In this test, a 'mist' of particle size varying between 1 and 10μm, prepared from an aqueous suspension of *Chromobacterium prodigiosum* is generated in air on one side of a test sample of material (10 cm dia.). A negative pressure is applied to the other side of the material, thereby drawing the contaminated air against the test barrier. Those organisms that penetrate it are seeded on the surface of a nutrient agar plate by means of a slit sampler and are subsequently detected as colonies after appropriate incubation.

The above example serves to point out two design features common to several other similar tests. Firstly, the test organism is easily recognized, so that incorrect interpretation of results due to chance contamination is minimized, and secondly, cells of the test organism are relatively small, thereby, on the face of it, presenting a rigorous challenge to the material under test. However, the challenge here, and in many other tests too, is actually a 'mist' containing the test cells, which can only mean that the stopping power of the barrier under test is set by the properties of the 'mist' droplets (dimensions, charge, shape, etc.) and not those of the microorganisms. Furthermore, the existence of a 'mist' implies that the atmosphere being drawn through the barrier is saturated with water vapour, a condition generally unlike that encountered under normal storage.

It is worth mentioning here that certain of the test methods aimed at examining *product* integrity are also open to question on similar grounds. Often these tests involve tumbling or vibrating sealed packaged products in atmospheres contaminated with cells mounted on talc or some other insoluble support. Penetration is then scored by testing the contents for sterility in the usual way. (Alternatively, a tray of culture medium, enclosed in a simulated package, is exposed to a dispersion of contaminated talc in circulating air; penetration is then scored directly with little risk of obtaining 'false positive'.) Again in these tests, the challenge is somewhat unreal since the penetration of the package is to a large extent a function of the properties of the support and not of microbiological contaminants.

The recent German standard for 'sterilizationpaper' also includes a so-called 'dry' test which is said to complement the test employing cells in water suspension outlined above (8). While not actually locating dried microorganisms in a gaseous fluid for challenge purposes, the test may be considered here, because of similarities in design, with those aimed at examining product integrity. A circular sample of paper (41 mm dia.) is located between appropriate gaskets over the mouth of a standard laboratory glass bottle using a screw cap in which a 31-mm circular opening has been machined. After steam sterilization of the assembled bottle, a fixed quantity of silica powder of specified particle size, contaminated with spores of *Bacillus subtilis* ($\leqslant 10^6$ g^{-1}), is spread over the surface of the test sample of paper. The assembly is then warmed to 50°C and cooled to 10°C to generate pressure differences across the test sample. This warming and cooling cycle is repeated a further four times. Following the fifth cycle, the bottle is appropriately incubated. Ten such assemblies form a single DIN 'dry' test. Microbial penetration is indicated by the presence of *Bacillus subtilis* colonies on the surfaces of the nutrient agar medium. Again, the test is not fully defined in the DIN specification and, in practice, this could result in wide variations in the performance of critical procedures from laboratory to laboratory.

Recently, Schneider (10) has presented a critical review of the principal microbiological tests that are performed on porous packaging materials. It is clear from this review that most tests are simplistic in design and empirical in regard to choice of test conditions. Consequently, they are not

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necessarily discriminating in respect of assessing the barrier efficiency of materials towards the passage of microorganisms, which in turn means that they are of unknown value in indicating the performance of the packaging material in maintenance of product sterility.

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A Quantitative Approach to Assessing Microbial Penetrability

The present status of tests of penetrability of packaging materials has led us to the belief that there is a real need for an improved evaluation method. On grounds of relevance alone, it is evident that the test method must employ as its challenge airborne microorganisms.

Design Considerations

A microbiological test must, by necessity, be destructive and it has therefore to be performed on only a fraction of the material under examination. The fraction has to be a representative sample of the whole material. Furthermore, the test must be designed for use with relative ease and rapidity. Clearly also, the design must encompass variables associated with the conditions challenging the packaging material in practice. Certain specific problems associated with the design of a quantitative test may be considered under the following headings:

Nature and production of the challenge

In view of the diversity of microorganisms present in the environment, it may be necessary to employ more than one type or species of organism. Certainly during development work, comparison will have to be made between the penetration achieved by different organisms under given test conditions. In choosing an organism, due regard must be given to nonpathogenicity, ease of production and recognition, type, viability, average cell size and size distribution. An airborne challenge may consist of microorganisms contained within droplets of aerosol, located on solid particles, or as a dispersion of discrete cells in air. A challenge made up of droplets or solid particles may be rejected on the grounds outlined above. For a given species of organism, a dispersion of discrete cells is made up of particles of the smallest possible dimension and is very likely the most rigorous challenge that can be devised. Logically, such a dispersion is the challenge of choice. The production of a dispersion can be achieved by aerosolisation of a liquid suspension of cells into droplets of sufficiently small size that on expansion into the gaseous fluid, 'instantaneous' evaporation of the liquid droplet occurs, leaving the discrete cells suspended in the gas. Dispersions so formed are referred to as 'dry'. Nebulisers restricting droplet size and so giving rise to dry dispersions have been described (11).

There is some evidence to suggest that cell concentration in the challenge is important in penetration measurements, so that the influence of this variable may also have to be examined in detail.

Presentation of the dispersion

In any determination of penetrability, there is a need for a driving force to present the dispersion to the sample of material under test. The easiest driving force to control and reproduce is pressure difference.

Ideally, in using difference in pressure as the driving force, the size of sample under test should be variable, since this will permit variation of the overall flow rate through the test material for a given pressure difference. The design of the test method may be such that pressure difference is held constant, and so, for a given material and sample size, the overall flow rate through the material is effectively fixed. However, for different materials, because of differences in resistance to gaseous fluid flow, different flow rates will occur. If flow rate is a determinant of penetrability,

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then clearly such differences are critical. In such circumstances, the test should be designed to allow variation in pressure difference in order to exercise control over flow rate.

Estimation of extent of penetration

Generally, in order to estimate the extent of penetration, determinations have to be made of the numbers of microorganisms in the dispersion both upstream and downstream of the sample of material under test. A critical requirement is then the ability to recover accurately and with a high degree of precision microorganisms from a known volume of the gaseous carrier. In practice, the collecting device must be able to recover microorganisms from a gas flowing over a wide range of rates and containing a wide range of concentrations of microorganisms. The characteristics, specifications and performances of various devices for collecting microorganisms have been detailed (12) and the all-glass impinger would appear to be particularly valuable in airborne microbiological testing where flow rate is a variable.

Apparatus and Methods

A previous communication gave a schematic of the rig, designed and constructed in our laboratories specifically to measure the penetration of different types of porous packaging materials by airborne microorganisms (13). The challenge to the sample of packaging material is a dispersion of spores of *Bacillus subtilis* NCIB 8056 in air, presented to the material under rigorously controlled test conditions. To determine the extent of penetration, estimates are made of the number of spores per unit volume of the spore dispersion directly upstream and downstream of the test sample. With the present rig and methodology, amongst the experimental variables that can be changed at will are concentration of spores in the challenge dispersion, volume of dispersion drawn through the test sample, the temperature and relative humidity of the dispersion, the type and size of the test sample and the pressure difference across the sample. Penetration of a particular sample of material for a given set of experimental conditions is derived thus:

$$\frac{\text{concentration of spores downstream of the sample}}{\text{concentration of spores upstream of the sample}} \times 100 = \%\ \text{penetration}\ (P)$$

To date, our work has focussed on the study of the relationship between penetration and the rate at which the spore dispersion is presented to the porous packaging material. For convenience, this rate has been designated 'challenge flow rate'. Challenge flow rate has been varied between $1 cm^3\ min^{-1}\ cm^{-2}$ and $1 dm^3\ min^{-1}\ cm^{-2}$ material sample by changing the pressure difference across the test sample; other experimental variables have been held constant.

Typical Findings

Over the past three years, a substantial number of different porous packaging materials have been examined using the methodology based on challenging with an airborne spore dispersion. Challenge flow rate has been shown to have a marked effect on the extent to which spores penetrate a given packaging material. For all medical grade papers tested to date, curves relating penetration (P) and flow rate (V) exhibit certain common features that are typified by the findings presented in Figure 1. Over relatively low challenge flow rates, penetration decreases progressively with increasing flow rate; at flow rates greater than a critical rate, which is specific for the paper under test, penetration

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increases with further increase in flow rate. These findings clearly demonstrate that such papers are not absolute barriers to airborne microorganisms, and they also show that there are some very subtle effects of the rate of presentation of the spore dispersion to the test material on the extent of penetration. The different effects of changing challenge flow rate on penetration, seen over different domains of flow rate, can be generally explained if we consider the material under test as a filter, fibrous or porous, opposing the flow of a dispersion of particles of given mass.

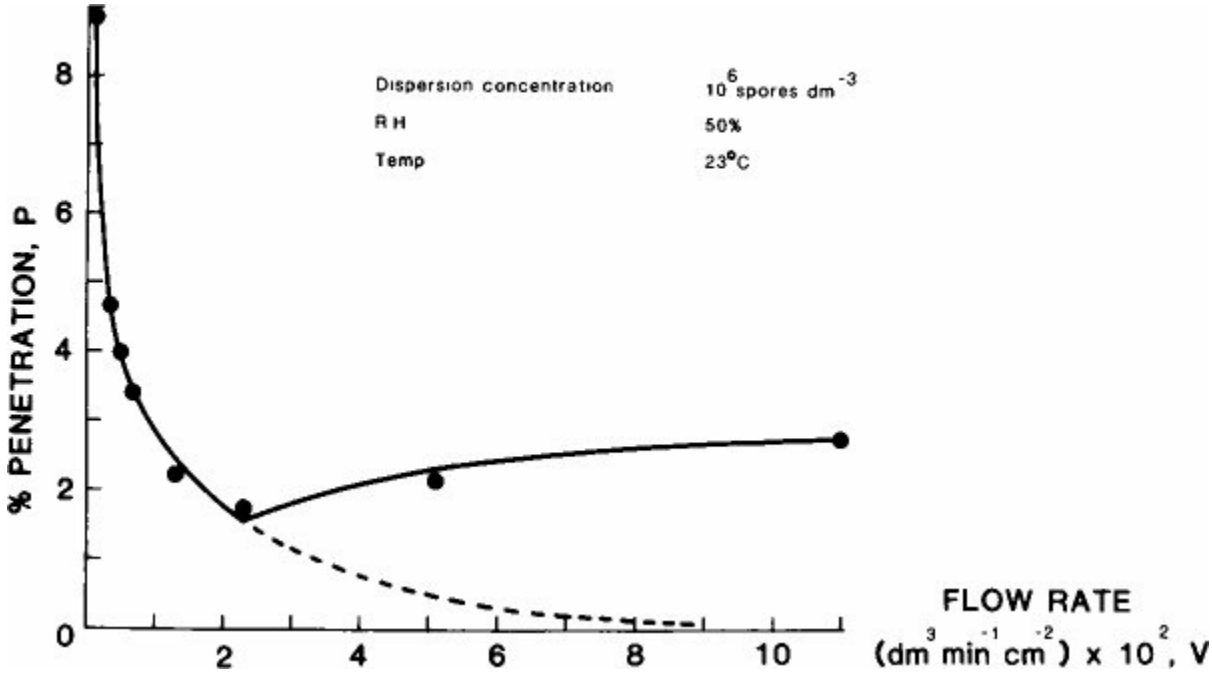

Figure 1. Typical relationship between penetration (*P*) and challenge flow rate (*V*) for a medical grade paper examined under fixed test conditions.

In general, fibrous filters are relatively deep with inter-fibre distance large in comparison with fibre diameter. A reasonable approximation of the arrangement of fibres within a fibrous filter is the so-called 'staggered array' (14), depicted in Figure 2 (l.h.s.). Parallel fibres of circular cross-section can be thought of as lying in an orderly fashion, perpendicular to the fluid flow. The structure of a porous filter, in contrast, is approximated by a capillary arrangement (15), consisting of circular capillaries spaced equidistantly and running parallel through the depth of the filter [Figure 2 (r.h.s.)].

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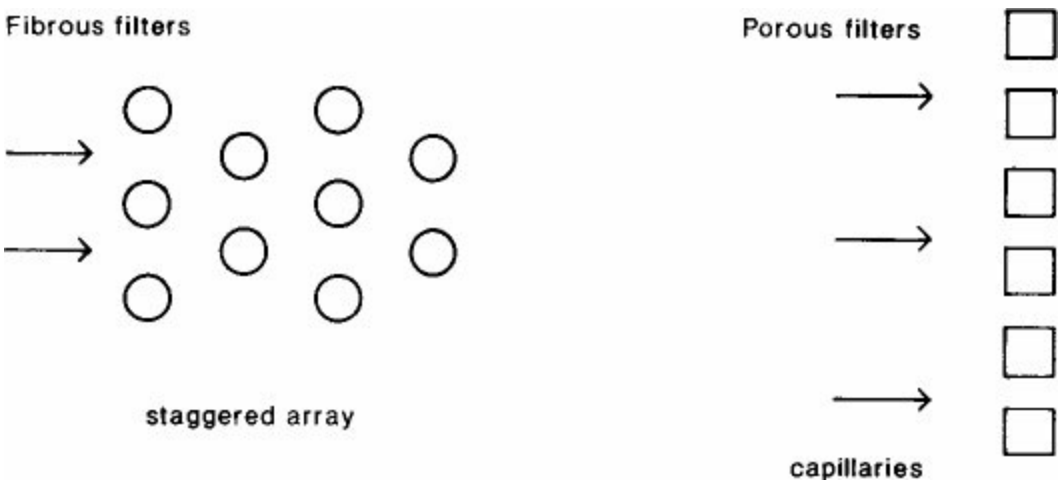

Figure 2. Models of the structure of fibrous and porous filters. Arrows depict the direction of fluid flow.

In considering the capture of flowing particles in a fibrous filter, it has been proposed that each individual fibre may be regarded as an independent entity isolated from the rest of the filter (16). This has allowed the behaviour of the filter under conditions of fluid flow of varying rates to be analysed in terms of flow around a given fibre. The flow of a fluid around a single fibre is defined by streamlines as shown in the upper diagram of Figure 3. For particles following streamlines that fall outside the boundary of the potential diameter of a given fibre (Y), there is essentially no likelihood of capture on that fibre, irrespective of flow rate. For particles in streamlines within the boundary of the fibre diameter, capture occurs when the particles, driven by inertia, leave the fluid flow, impact upon the surface of the fibre and are retained there. In these circumstances, the probability of particle capture depends upon flow rate, and the maximal distance separating streamlines, which possess particles that leave the streamlines and collide with the fibre, defines the effective diameter of the fibre (y). For a given flow rate, this diameter is determined by the so-called critical trajectory of the particle, which is defined as the limiting pathway taken by a particle resulting in collision of the particle with the fibre. Obviously, the nearer the value of y/Y is to unity, the greater the likelihood of particle capture, which in turn will result in lower levels of penetration. As flow rate increases, this ratio approaches unity (lower diagrams in Figure 3).

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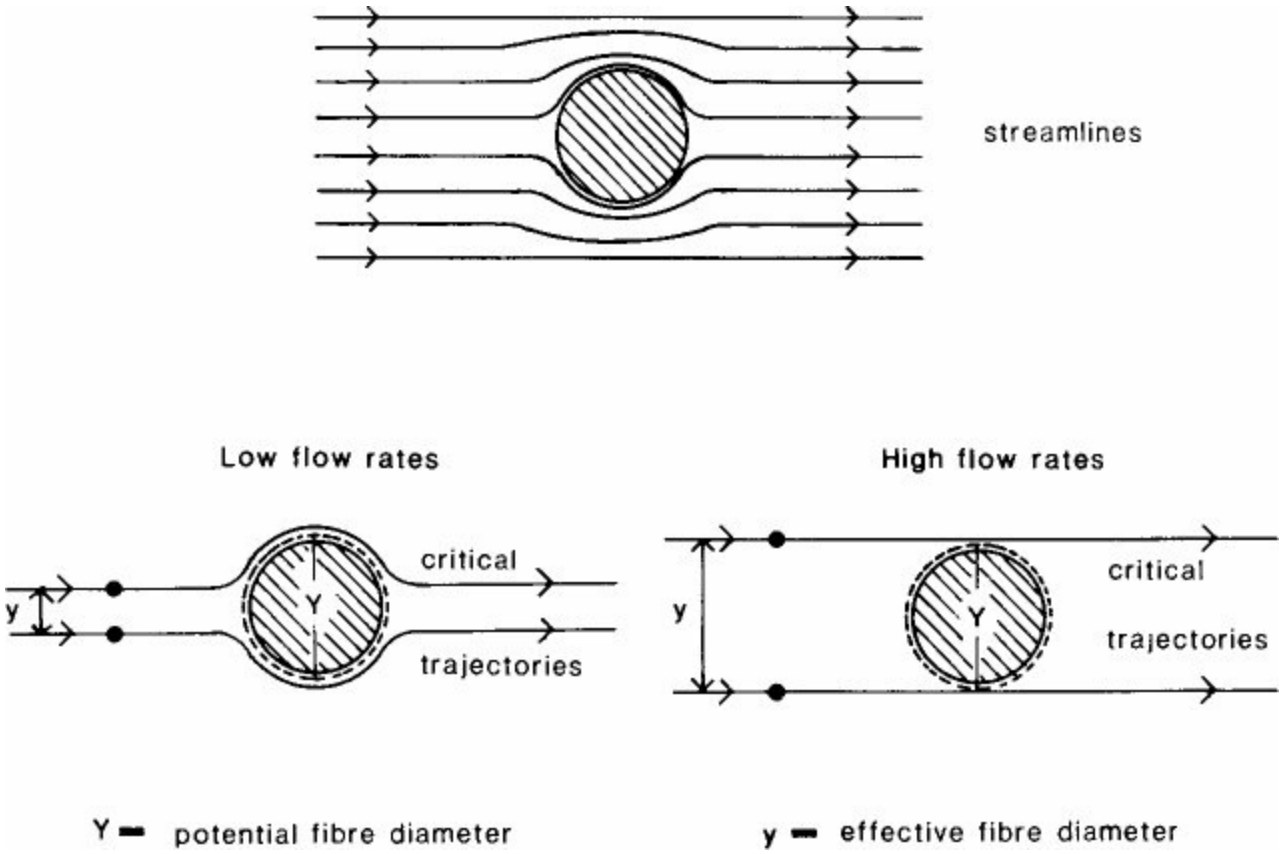

Figure 3. Diagrammatic representation of the process of particle capture from a fluid dispersion flowing around a single fibre.

The process of particle capture on a porous filter is represented in Figure 4. Particles, driven by inertia, fail to keep to the streamlines of fluid entering the individual capillaries and are deposited on the solid intercapillary surface of the filter. On increasing flow rate, the radius of the critical trajectory tends towards the effective radius of the pore, and the value of y/Y correspondingly approaches unity. Increasing flow rate results, therefore, in increasing particle capture.

The events described above for particle capture in fibrous and porous filters are clearly the basis of an explanation of *decreasing* penetration of paper materials by dispersed spores with increasing flow rate, seen at relatively low flow rates (Figure 1).

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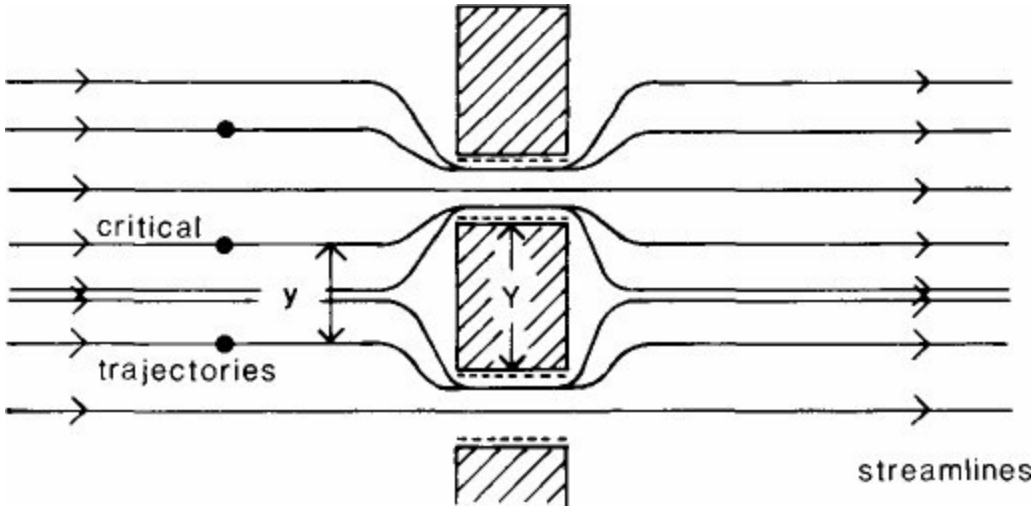

Figure 4. Diagrammatic representation of the process of particle capture from a fluid dispersion flowing through a capillary within a porous filter.

The explanation of the *increase* in penetration seen at flow rates above a critical level is sought in terms of the energy of association between the particle and the paper fibres. At relatively high flow rates, inertial forces associated with the fluid may be increased to a point where they exceed the forces of adhesion maintaining the particles on the surface of the fibre. Previously captured particles will then return to the fluid flow and, in so doing, will be free again to penetrate the paper. This phenomenon, called re-entrainment, is known to occur in the paper/spore system. Alternatively, the inertial forces associated with the particles at high flow rates may be large enough to cause particles to impact on and 'rebound' from the surface of the fibre, thereby re-entering the fluid flow. Both these events, connected with high flow rates, could lead to increased particle penetration. They could then be a basis of a rationalisation of the abrupt change, seen at a critical flow rate, from an inverse relationship between per cent penetration (P) and challenge flow rate (V) to a direct relationship (Figure 1).

The form of the curve relating P and V at relatively low flow rates can best be represented by the expression

$$P = P' \,.\, V^{-K} \tag{1}$$

A logarithmic transform of the latter yields a simple linear equation

$$\ln P = \ln P' - K \ln V \tag{2}$$

in which K and P' are constants. The value of-K is the slope of the plot of ln P vs. ln V at low flow rates and P' is an estimate of the per cent penetration when the challenge flow rate takes a value of 1 $dm^3\ min^{-1}\ cm^{-2}$ sample. P' is derived from the intercept of the y-axis and the extrapolate of the line (Figure 5).

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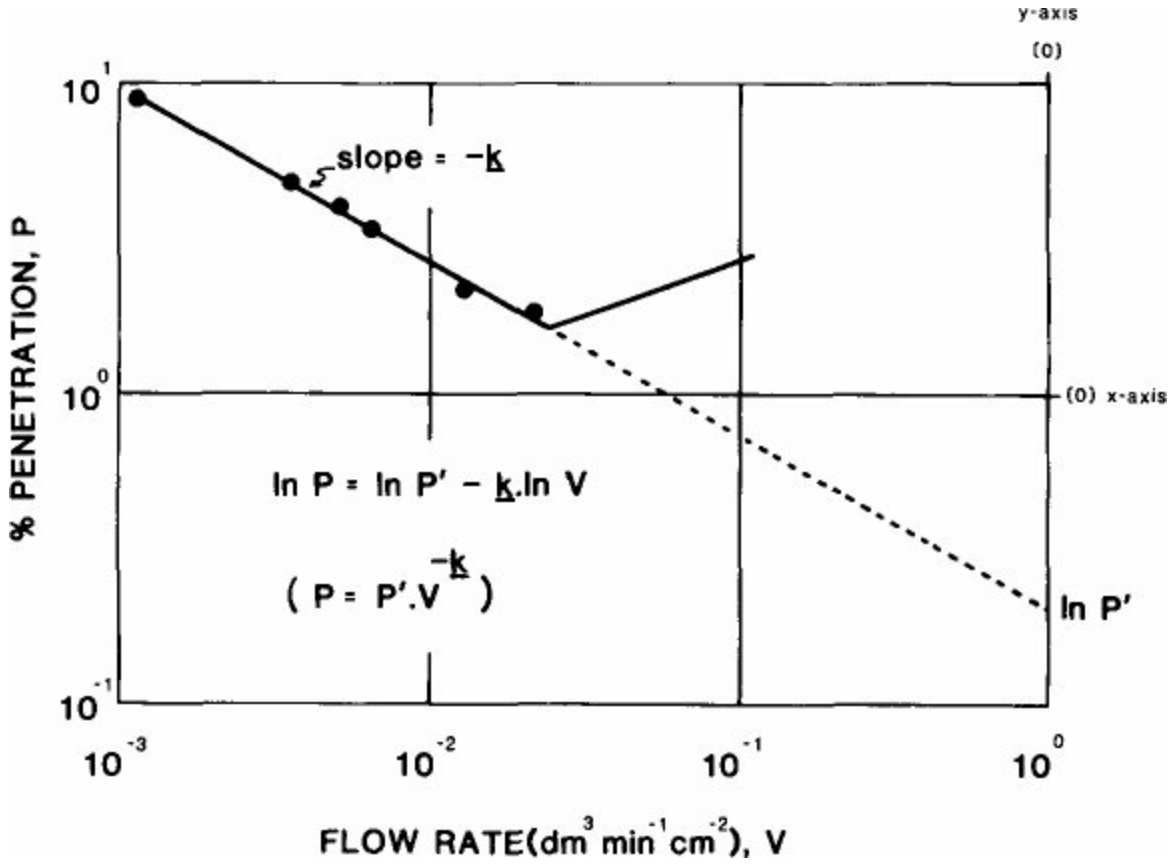

Figure 5. Plot of ln *P* vs. ln *V* for data presented in Figure 1, showing an inverse relationship between *P* and *V*. The plot demonstrates the derivation of constants K and *P′*, given in equations (1) and (2).

The constant K is a measure of the changing rate of decrease in penetration with increasing flow rate, a high value of K being indicative of a structure conducive to efficient barrier properties. The name given to K is the *penetration rate constant*. The constant *P′*, being the predicted level of penetration for a given paper at a fixed flow rate, is designated the *specific penetration*. The value taken by *P′* is low when the packaging material acts as an effective barrier. A recent communication gives values of K and *P′* for a range of commercially available medical grade papers (13).

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Parallel Studies of Microbial Penetrability

The usefulness of the quantitative approach described above is illustrated by the findings presented in Table 1. Here, values of penetration rate constant (K) and specific penetration (P') are listed together with DIN test findings for a range of different medical grade papers (*a* to *f*). The table also gives the results obtained with two papers that were included as 'Controls'.

These Controls were used to standardize the DIN test methodology. Control 1 was a paper that, when examined in the laboratory where the DIN test was originally devised, passed the test, whereas Control 2, when examined in similar circumstances, failed. The tabulated findings for these two papers demonstrate that our DIN test methodology is comparable to that of the standard laboratory. It is noted, however, that the failure of Control 2 in the 'wet' test depended solely on the presence of an 'uncountable' area of growth appearing at a single location on only one test sample of paper. The remaining 24 locations gave no growth and therefore it would appear that the defect responsible for a breakdown in the barrier properties of this paper, and its failure, occurs erratically and not regularly across the paper. Also, Control 2 passed the 'dry' test, a fact that supports the above notion. Nonetheless, the responses of Controls 1 and 2 encouraged us to proceed with the examination of the other papers by the DIN test methods.

Table 1

Findings from parallel microbiological penetration studies performed on six different medical grade papers

Web designate	Manufacturer	Specification	Grammage ($g\ m^{-2}$)	DIN Test Results		Airborne spore challenge	
				'wet'*	'dry'†	Penetration rate constant (K)	Specific penetration (%) (P')
Control 1	A	DIN	60	Pass	Pass		
Control 2	B	–	60	Fail (uc)	Pass (3)		
a	C	006	100	Pass	Pass	1.15	2.96×10^{-3}
b	C	006	60	Pass	Pass	0.20	1.78×10^{0}
c	D	006	60	Pass T(1); R(0)	Pass (4)	0.53	8.60×10^{-1}
d	E	006	60	Pass	Pass	0.55	2.20×10^{-1}
e	B	DIN	60	Pass T(4); R(5)	Pass (11)	0.11	1.57×10^{1}
f	B	DIN	60	Pass	Pass	1.92	3.18×10^{-8}

(n) indicates the total number of colonies, otherwise no growth observed.

(uc) uncountable

T = first test

R = retest

DIN = meets German Standard 58 953

006 = meets DHSS Specification No. TSS/S/330.006

Interpretation of DIN test results: * With no growth, the paper passes and with >5 colonies in total, it fails; with ⩾5 colonies in total on retesting, the paper passes.

† With ⩾15 colonies in total and no one sample giving >5 colonies, the paper passes.

All six test papers, manufactured to either DHSS specification for 'paper for bag, sterilization' or DIN specification for 'sterilizationpaper', passed both the 'wet' and 'dry' DIN tests. However, it seems that papers *a*, *b*, *d* and *f* met the pass criteria more readily than *c* and *e*. In fact, paper *e* was

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just on the limit of compliance and it is possible that it was used to set the arbitrarily chosen pass/fail level.

A major inference that may be drawn from the DIN test results ('wet' and 'dry') for the six test papers is that several of them (*a*, *b*, *d*, and *f*) might be thought of as being absolute barriers to microorganisms. They show no growth which suggests zero penetration. This inference is not in keeping with the findings obtained on challenging the papers with an airborne dispersion of spores; penetration is seen at all values of flow rate examined. Furthermore, the different papers take different values of the constants K and P'. We note that an inverse correlation exists between the value of the penetration rate constant (K) and the specific penetration (P'), and that the combination of a high value of K and low value of P' is indicative of effective barrier properties. When this combined measure of effectiveness is applied, the barrier efficiency of the test papers ranks in the order *e<b<(cd)<a<f* over a wide range. On the other hand, ranking efficiency according to the response to the DIN test would put the papers in the order *e<c<(a b d f)*. These two rankings are clearly not relatable, particularly in view of the position of paper *b* in the group showing zero penetration when actually *b* exhibits poor barrier properties to a dispersion of airborne spores.

The set of findings presented in Table 1 demonstrates the insensitivity of the DIN test ('wet' and 'dry'). It fails to separate the six test papers in respect of their microbiological barrier properties. The papers were made to either DHSS or DIN specification, and all passed the arbitrarily chosen pass/fail levels of the DIN tests. The same six papers, however, showed markedly different barrier properties when challenged with a dispersion of airborne spores. The different behaviours under test are in accord with known differences in paper structure that could affect barrier efficiency (e.g. grammage, thickness, fibre density, fibre diameter). In our view, present findings illustrate the promise of airborne challenge testing for quantitatively describing barrier properties of porous packaging materials. Such descriptions are measures of the ability of materials to stop the passage of microorganisms, which, in turn, could define their effectiveness to maintain product sterility in the field.

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DISCUSSION SESSION V – Part 1

Q. by R.A. Anderson – Chairman

May I put a question to Prof. Tallentire. The question is one that was put to an earlier speaker, concerning responsibility for the quality of the packaging materials, viz. whether the responsibility lies with the supplier, or whether it is the user or manufacturer of that packaging material. Do you have any additional comment?

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A. by A. Tallentire – UK

As an academic, I am not really concerned with the subject, but I would say that it should be the supplier and not the user. The supplier has a responsibility to sell material of a correct quality for an intended use.

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Q. by F.E. Halleck – USA

Prof. Tallentire, the DIN method (the ‘wet’ and ‘dry’ test methods) and the methods you have developed in your laboratory for evaluating papers are based on single-web materials. How does that equate to the standards in hospitals, where you should have double layers in order to deliver the product aseptically to a sterile field? The practice in hospitals is to use double layers of packaging materials, so as to deliver the product aseptically to a sterile field. The outside layer is usually discarded. How does your test method relate to that practice?

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A. by A. Tallentire – UK

It is not a test, and I am not suggesting that what we are doing is a test. What I am suggesting is that we are characterizing the penetration properties of materials to microbial challenges, but it is not a test. What we can do is that we can grade papers. At the moment, we have not chosen to set a particular standard of penetration. That is number one priority. If we wish, we could readily do the test that was mentioned. We could, in fact, put two layers of material in our experimental rig and I would predict that the extent of penetration for a low challenge would, in fact, be equal to the penetration of the first layer of web, provided that the penetration rate was the same.

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Q. by F.E. Halleck – USA

With your knowledge of packaging, which is based on testing, do you feel that the industrial manufacturers of medical devices are doing a disservice to hospitals by providing only a single layer as a sterile barrier, which is the outside layer, whereas the hospital must provide two layers for their packaging?

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A. by A. Tallentire – UK

It is time we got better webbed materials for packing commercial sterile medical devices.

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Q. by B. Evers-Buckland – Australia

Prof. Tallentire, I have two short questions. First, have you done any work with coated papers, or have you done any work with any type of coated papers where you are going to get a resistance to your flow rate?

The second question is: with an increase in humidity for your test method, what would you expect the graphs to do, say if you raised the humidity from 50% to 60%?

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A. by A. Tallentire – UK

We have done work with coated papers. You get differences in flow rates with coated papers, but the flow rate that I am plotting is, in fact, the real flow rate, i.e. the rate of penetration of the test material.

I do not know the answer to your second question, because we have not done such a test, but I think that I could make a prediction. As I pointed out, we are in the very early days in this whole exercise, and I would suggest that with an increase in relative humidity there would be an increase in fibre diameter. In those circumstances, the web would become a more effective barrier.

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Q. by J. Nisbet – Australia

I would like to ask Frank Hebbard a question. As contract sterilizers, we are concerned with minimizing ethylene oxide residue in therapeutic devices after treatment. Could you outline the methods by which hospitals ensure minimum levels. How are these levels measured and what safe limits are acceptable?

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A. by F.L. Hebbard – Australia

I cannot really answer your question satisfactorily. We are concerned with minimum levels. As I stated earlier, ethylene oxide sterilizers are approved for use in hospitals and are restricted in their location and are fitted with aeration systems. The hospitals are advised, but we have no control of what they do. However, we do advise hospitals to aerate these units and/or the load. We have no methods of testing within hospitals to measure actual residues. We do have legislation and regulations related to occupational health concerning aspects of ethylene oxide residues in loads after removal from sterilizers, which are being enforced.

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Q. by J. Brewer – Australia

I have a question to Prof. Tallentire. When you are plotting flow rates vs. permeability, or cell counts, you get that inflection point. What is physically happening at that point, and is it consistent from sample to sample?

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A. by A. Tallentire – UK

Yes, very much so. It is consistent with each paper, but its location varies from paper to paper. The reason is twofold. One is the process of entrapment. At low flow rates, you get organisms adhering to the surface of the fibres, and that is where they are captured. At high flow rates, you tend to get inertial forces of gaseous fluid exceeding adhesive forces of the organism to the fibre surface, which results in pulling them off. So, it is really an artefact. We have actually proved that this occurs. The other possibility is that forces of inertia exceed adhesive forces, and as organisms impact onto the surface of the fibres, they rebound.

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Q. by R. Croft – Australia

Prof. Tallentire, just following an earlier question on relative humidity, you said, you were in the embryonic stages of the studies. Have you any proposals to do a ‘worse case’ study, viz. with higher temperature and humidity?

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A. by A. Tallentire – UK

About four years from now, I believe.

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Q. by R.A. Anderson – Australia

There was an earlier question which I believe was to Dr Dodson, who queried how well one can determine the nature and extent of bioburden on some of these materials. Mr Whitbourne referred to this as a fairly central part on one of the validation schemes, and you did not comment on that. Can we get a comment from you.

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A. by J. Whitbourne – USA

Well, the bioburden is, if you are talking about a plastic material, difficult because you will never get more than perhaps 10-20% of what is there. However, if we are talking about a sterilization process, we are of course trying to kill the microorganisms present. Therefore, it does become important to measure as much as possible the bioburden level. I cannot really give you a quick answer, because there are a lot of things that need to be discussed relative to your question. However, in my opinion, a great deal more emphasis needs to be given to many sterilization processes, ethylene oxide in particular, radiation to some extent, and certainly low temperature heat processes, regarding the innate resistance of the product. I have seen a number of sterilization processes where the resistance of the product was far greater than that of a biological indicator. Although I recognize that there is a certain amount of disagreement regarding bioburdens, and because we do not always get exact numbers, it nonetheless is of great importance.

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Q. by F.E. Halleck – USA

Mr Whitbourne, you made a comment that the overkill method was more economical than the bioburden, which I agree with whole-heartedly. I would like to have you expand this subject.

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Q. by J. Whitbourne – USA

Bioburden studies are done to look at the manufacturing environment. They give information on how clean the environment is. The overkill method is certainly much cheaper and depends on the type of processing, especially with steam or with dry heat. If you can use the high temperature, and can use a spore strip that has *B. stearothermophilus* on it for steam sterilization, or *B. subtilis* for dry heat, the overkill method is a much simpler, easier, and cheaper to measure the process performance.

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Q. by G. Nelson – Australia

A question to Prof. Tallentire. It was fascinating to see the work that had been going on to try and define test methods for the barrier properties of paper. I suspect, the work has probably taken a long time. It was most interesting on two points. First, to be absolutely specific that certain papers are better than others as a barrier for microorganisms. Secondly, that the degree of penetration was higher at the low flow rates. I really would not have anticipated this on thinking about it prior to your results. I would like to go on to ask two basic questions. One is related to time. In Australia, there is a big problem, as there is worldwide, in trying to define a physical property of the paper to put in a standard that will give a satisfactory measure of barrier properties. I would ask you, how long do you think it would be in continuing your work, for this to be achieved.

The second question is related to future development of improved papers. Do you think, it will be possible from the work you are doing to define all the properties, or the majority of the properties of paper, to be able to tailor-make an even better paper in the future?

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A. by A. Tallentire – UK

In answer to your first question, I retire in 15 years time and I believe, this will just keep me going, so I have no idea. I am going to continue on at the present rate and enjoy what we are doing.

To answer your second question, the whole purpose of our programme of work is to get to a point where we will be able to go to the papermaker and say that this is how to make paper if it is to be an effective barrier.

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SESSION V

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Sterilization Process Technology – Part 2

Chairwoman
Anna J. Skopek

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SESSION V

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Sterilization Process Technology – Part 2

Introduction to Session

Anna J. Skopek

The following papers will deal with aspects of *gamma* irradiation sterilization.

Gamma rays have been successfully employed for sterilization of medical supplies and devices for more than twenty years. As a sterilizing agent, *gamma* irradiation offers a number of advantages. Some of these are:

- ability to control the process by physical means
- compatibility with a wide range of materials
- enormous penetrating ability
- freedom in utilization of packaging materials showing thermal instability
- low chemical reactivity
- high reliability and easy adoption for continuous processing.

Various international and national codes and guidelines have been developed to specify regulatory requirements and to provide technical guidance in this field. The activity of the International Atomic Energy Agency resulted in the formulation of a Recommended Code of Practice for the Radiosterilization of Medical Products. One of the statutory tasks of the International Atomic Energy Agency is to 'accelerate and enlarge the contribution of atomic energy to peace, health and prosperity throughout the world'. In accordance with these objectives, the Agency has contributed to the development of the radiation sterilization technology. Current programme activities, as well as future plans of the International Atomic Energy Agency in this field, are outlined by Ramendra Mukherjee.

Appendix A of the *Code of Good Manufacturing Practice for Therapeutic Goods* in Australia covers special provisions concerning the procedures to be applied in radiation sterilization of therapeutic goods. The radiation sterilization provisions are based on the principle that the primary manufacturer bears the responsibility for the quality of goods, including sterility.

The operator of the radiation facility bears the responsibility for delivering the required dose of radiation. The provisions are based on the use of a prescribed minimum absorbed radiation dose of 2.5 Mrd. Experience has shown that this dose is satisfactory for sterilization of medical products. Nevertheless, it is the responsibility of the primary manufacturer to validate the prescribed absorbed radiation dose. When validation has been carried out, and when the factors involved are consistently under control, routine sterility tests need not be carried out and products may be released on dosimetry data.

Gamma sterilization process development requires a multidisciplinary approach: microbiological validation, dosimetry, and product and package integrity evaluation. Microbiologists have been engaged in determination of the inherent radiation resistance of microorganisms and in investigations of the influence of a variety of environmental factors on microbial radiation resistance.

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Knowledge of the microbial burden prior to sterilization is a necessary prerequisite in the

determination of the margin of safety and efficiency of a commercial sterilization process. An accurate assessment of this safety factor becomes increasingly important when sterility release is based on dosimetry data. In order to validate the sterilizing dose, many workers have studied the inactivation of microorganisms by depicting dose-survival curves. The inactivation of bacteria by irradiation is a negative exponential process. At higher radiation doses, the fraction of survivals approaches zero. Theoretically it never becomes zero.

The sterilizing dose will therefore depend on the initial degree of contamination and the final probability of survival that can be tolerated.

Pamela Wills will discuss investigations related to process development and validation carried out by the Australian Atomic Energy Commission, Australian industry, and research institutes in South-East Asia and the Pacific region.

Until a few years ago, a sterilizing dose of 2.5 Mrd had been generally accepted in many countries, including Canada and the United States. This dose was initially based on experience showing that products could be safely and effectively sterilized with that dose.

Recent scientific advances and accomplishments in the field of *gamma* irradiation in North America have suggested that irradiation doses other than 2.5 Mrd may be equally acceptable. Advances in understanding of the kinetics of the *gamma* sterilization have supported changes in the process validation methodology, and have reduced problems associated with sterility testing. The advances have arisen out of the efforts of individual scientists as well as the committee of the Association for the Advancement of Medical Instrumentation (AAMI). The committee was formed in 1976 to study dosimetry, process control, and methods for dose determination, and to develop voluntary guidelines for the irradiation sterilization of medical devices. During a four year period (1976-80), new dose-setting strategies were developed and statistically evaluated using computer simulation. The mathematical models are based on a natural D_{10} resistance phenomenon of microorganisms, the nature of the article to be sterilized, and a desired sterility assurance level.

The new strategies involve dosimetry release, and are free from difficulties involved in subculturing of individual bioburden organisms and in individual determinations of D_{10} values. They are accurate, precise, provide reproducible results, and do not require microbiological experimentation and techniques beyond the capabilities of present industrial microbiological laboratories.

In 1980, the somewhat arbitrary 2.5 Mrd dose was, in some instances, reduced as a result of the application of new scientific dose-setting procedures. John Masefield will discuss the current North American practices in *gamma* sterilization.

A generally accepted practice is to apply a single sterility assurance level of 10^{-6} to all medical devices. In North America, this single sterility assurance level has been substituted by a range of levels, depending on the end use of the product. In Canada, this principle is known as the Microbiological Safety Index (MSI). It has been argued that products which do not come in direct contact with a patient, or do not compromise the natural defence barrier of a patient, do not require the same degree of sterility assurance.

A more flexible approach to a sterilizing dose would lead to a broadened application and improved productivity of *gamma* sterilization processes. While the future rate of expansion and growth of *gamma* irradiation sterilization will vary from country to country, it is obvious that the main potential for growth will remain in the medical products area.

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The Role of the International Atomic Energy Agency in Sterilization Controls

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Introduction

The programme activities of the International Atomic Energy Agency (IAEA) for over fifteen years have contributed to the development of the technology and the practices for radiation sterilization of medical products in its member states. Particular emphasis has been placed in these IAEA programmes to meet the needs of the technologically less advanced countries to help upgrade the standards of their existing health-care services through an improved provision of sterile medical supplies for safe clinical use. These programme activities have been implemented through: (a) support of relevant research to generate necessary information for suitable practices in keeping with the local conditions, (b) organizing scientific meetings and training courses for free dissemination of technical expertise and information, (c) providing technical assistance to the developing member states in the form of advisory services by experts, pre-investment surveys, installation and commissioning of irradiator facilities, and the build-up of the technical manpower and infrastructure for a sustained operation of the sterilization practices, (d) issuing scientific publications, manuals, and guidebooks in the fields concerned, (e) particular assistance in the preparation of a recommended international Code of Practice to facilitate standard sterilization processes that could lead to high-quality, safe, sterile medical products.

The parameters involving an accurate delivery of the recommended sterilizing radiation dose to the product are among the most important factors involving sterilization control. The IAEA-sponsored, internationally co-ordinated high-dose intercomparison/calibration programme to provide dose assurance aims to fulfil this objective. In addition, this programme has developed a series of dosimeters that can be used in the product during normal operation of an irradiation facility, in parallel with an operator's routine system, to serve as a mutual control standard.

Promotional activities that help to contribute towards the attainment of a controlled sterilization method will be detailed in the paper.

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IAEA Objectives

It is one of the statutory tasks of the IAEA to accelerate and enlarge the contribution of atomic energy to peace, health, and prosperity throughout the world. In keeping with these broad objectives, and with particular regard to the welfare needs of the developing member states, the Life Sciences programme of the IAEA has been designed to promote the development and extension of relevant nuclear techniques and technologies to foster early beneficial returns in the fields of medicine, public health care, and related industries.

There are grave inadequacies in the public health-care standards of most of the technologically less advanced countries of the world. The situation in these densely populated developing countries may be better illustrated when their unfavourable doctor-to-population ratios of some 1:100 000 is compared with that in some of the most advanced countries with one doctor for every 500 people, or less. These developing countries experience other adverse health-care situations in having a limited number of hospitals with inadequate facilities and support staff for sterile medical supplies for clinical services. A great majority of the population has to rely upon mobile dispensaries and/or camp health centres. In such circumstances, the patients are likely to encounter nosocomial diseases through cross-infection from nonsterile supplies. Problems such as this could be largely overcome by the provision of ready-to-use sterile medical supplies.

The development and beneficial applications of large radiation sources for industrial use has long been another objective of the IAEA-sponsored programme for the member states. Progressive advances since the early sixties in the technology of the large irradiator sources using ^{60}Co for sterilization of medical supplies is a relevant area in the programme's activities. At present, radiation sterilization of medical products constitutes next to power generation one of the most important industrial uses of large radiation sources (1). Moreover, it is indeed heartening to note that the host of this important Symposium has been among the pioneers of the beneficial application of atomic energy from as early as the late fifties. The continuing trends have resulted in a worldwide inventory of large commercial-scale *gamma* irradiators engaged in the service of the sterilization of medical supplies. These facilities are distributed throughout 36 countries and have an estimated installed capacity of about 100 million curies of ^{60}Co (12). The high level of efficacy of radiation sterilization methods, combined with ease of handling and control, reliability, a unique suitability for use on substances susceptible to heat and chemical agents, and, above all, the hygienic advantages inherent in prepacked hermetically sealed sterile items, achieves the objectives.

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Rationale for Promotional Efforts in the IAEA

A recognition of the potentials of radiation sterilization technology and the practices to cater for the problems and pressing needs of health-care services in the developing countries have led the IAEA to become involved in promotional activities over the past 16 years. The programmes have contributed to the development of the operation and control of radiation sterilization practices in the member states who have sought IAEA assistance. The introduction of this technology to as many as ten developing countries in Europe, the Middle East, and Asia is attributed to such IAEA-sponsored activities. IAEA-sponsored programmes have also played a supplementary role in the introduction of the technology in many other national developments.

The basic nature of the approach of the IAEA programme consists of a transfer of relevant technical experiences for adaptation to the socioeconomic environment of the recipient countries in order to foster an early beneficial return. An integral part of this is the aim to establish an infrastructure of local trained technical personnel.

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IAEA Research Support and Co-ordination

The early phase of the IAEA programme for development of the practices of radiation sterilization of medical supplies in the member states primarily dealt with institutes in European countries. This period spanned the late sixties and the early seventies. In co-ordinating research efforts, a number of the leading countries in the field of application, such as UK, USSR, Federal Republic of Germany, France, and the Scandinavian countries, collaborated with several European countries that were new in the field. Table 1 lists the countries in Europe that received technical assistance under the IAEA-supported programmes. Most of these countries have further elaborated the sterilization practices under their national development programmes and have now acquired commercially operating irradiator facilities with defined national criteria and regulations. The international recommendations, as formulated by the joint IAEA/WHO Working Group of experts (7), have facilitated these developments.

Table 1

IAEA-supported activities on radiation sterilization practices in Europe and in the Middle East

Country	Large-scale irradiation facilities	Research support and coordination	Expert assistance on technical, economic, and marketing services	Training of personnel and fellowships
Bulgaria				+
Czechoslovakia		+	+	+
Denmark		+		
Egypt			+[b]	+
Greece		+	+	+
Hungary	+[b]	+	+	+
Israel			+[a]	+
Poland		+		+
Turkey				+
Yugoslavia	+[b]		+	+

a – UNDP/IAEA-supported expert assistance project

b – Co-60 gamma irradiator and the associated services provided under the UNDP/IAEA project

Encouraging developments in technology in North America, Europe, and Australia, together with the positive impacts of the IAEA-co-ordinated activities, have stimulated interest by countries in Asia and the Far East during the early seventies (Tables 2 and 3). Following the successful commissioning of the ^{60}Co radiation facilities in India and South Korea, both through the support and joint venture of UNDP, IAEA, and the governments concerned, interest in this field of beneficial nuclear applications has extended to the Philippines, Indonesia, and Thailand. In addition, some small scale applied research activities relevant to the practices and process control have also been triggered off in other countries of the region, such as Pakistan and Bangladesh, under the IAEA Regional Co-operative Research Agreement (RCA) programme.

Table 2

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IAEA-supported activities on radiation sterilization practices in Asia and in the Far East

Country	Large-scale irradiation facilities	Research support and coordination	Expert assistance on technical, economic, and marketing services	Training of personnel and fellowships
Bangladesh		+		+
Burma		+		+
India	+[a]	+	+	+
Indonesia		+	+	+
Korea (South)	+[b]	+	+	+
Pakistan		+		+
Philippines		+	+[d]	+
Thailand	+[c]	+	+	+

a, b – Co-60 gamma irradiators for sterilization of medical products under the UNDP/IAEA project

c – Co-60 gamma irradiator used mainly for food irradiation

d – Detailed pre-investment market survey of potential medical products has been completed under the UNDP/IAEA project

While introducing the technology in geographical areas, such as tropical countries of Asia, best possible use should be taken of the resources of knowledge (4) (Table 2). Nevertheless, striking differences in the ecological and the environmental hygienic conditions of the tropics, together with the types and priorities of the medical supplies, may, however, impose certain limitations on the feasibility of a direct extrapolation of available technical information and criteria for use (2). The situation may, therefore, necessitate generation of some relevant technical information under local conditions to ensure the establishment of effective and safe practices relevant to their local medical manufacturing industries and the environment. The Agency's regional co-ordinated research programmes on this subject (Tables 1 and 2) have been designed to meet these objectives.

The IAEA-co-ordinated programme of research on radiation sterilization practices suited to local medical supplies for countries in Asia, the Far East, and the Pacific carried out surveys on the role of bioburden characteristics on the nature of the sterilization practices. Furthermore, a comparison of the involved radiation facilities at the institutes of the participating countries, with regard to the efficiency of sterilization and the standardization of the methods for radiation sensitivity evaluation (D_{10} value), was undertaken as a part of the calibration process. This exercise served as a helpful guidance for investigators relatively new in the field. This co-operative study was made possible by the generous help extended by the Australian Atomic Energy Commission at Lucas Heights, especially with the assistance of Pamela Wills, one of the co-investigators in the project.

Table 3

Gamma-irradiators for sterilization of medical products established by the joint support of IAEA, UNDP, and governments

Country	Operator	Designer	Capacity (MCi) initial	Capacity (MCi) maximal	Date of commissioning
		ISOMED, Bhabha Atomic			

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India	Research Centre, Bombay	H.S. Marsh NE Ltd.	0.12	1.00	1974
Korea (South)	KAERI, Korea Atomic Energy Research Institute, Seoul	A.E.C.L.	0.10	1.00	1976
Hungary	Debrecen	A.E.C.L.	0.25	1.00	1976
Yugoslavia	Vinca	French CEA	0.20	1.00	1978
Egypt	NCRT National Center for Radiation Technology, Cairo	A.E.C.L.	0.40	1.00	1978
Indonesia	Badan Tenega Atom Nasional, Jakarta	^{60}Co supplied by A.E.C.L., equipment by Marubeni (Japan), shielding pool (local source)	0.22	0.35	1983
Thailand (Planning stage)	O.A.E.P., Bangkok		0.20	0.30	1983-84

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Development of Gamma Irradiator Plants and their Process Control

So far, through the support and involvement of IAEA programme activities, commercial scale *gamma* irradiation facilities for the sterilization of medical supplies have been established in India, South Korea, Hungary, Yugoslavia, Egypt, and Indonesia (Table 3). The setting up of demonstration facilities in these countries is expected to enable them to intensify further their relevant research, training, and upgrading of health-care services, thereby enlarging the scope of beneficial nuclear applications (3).

Upon request, expert assistance has been provided to a number of facilities in member states for the development of the microbiological control of radiation sterilization (6), including the advice on monitoring the hygienic standards of the production sites. Assistance, including training of the technical staff involved, has been given on the microbiological assay (initial counts) of medical materials prior to sterilization. The microbiological efficiency of the ^{60}Co sources was investigated and calibrated using standard preparations of *B. sphaericus* strain C_1A and *B. pumilus* strain E601 (6). Based upon requests from the member states, technical justification, and availability of funds, some equipment was provided for the various service control laboratories, including microbiology.

Through the services of IAEA experts, as well as the co-ordinated research programmes, steps have been taken to control the sterilizing efficiency of several irradiation facilities in member states (5). Such expert services included the development of suitable physical and chemical dosimeters, their applications to the calibration of the radiation source, and relevant mechanical devices needed for smooth operation of the facility. Training of local technical staff in the routine dosimetry procedures is also undertaken through these expert services.

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Dose Assurance for High-Level Radiation Facilities

The correct use of radiation in sterilizing medical products affects the quality and safety of the finished products direct, which in turn affects patient health. This, therefore, warrants research and development to establish the efficiency of the radiation dose, so that the processor can use radiation dosimetry measurements during commissioning and routine operation as a form of quality control to guarantee the safety and/or reliability of the irradiated product.

Studies carried out under the IAEA-co-ordinated research programme of the Dosimetry Section on the above objective have helped in the development and evaluation of a series of dosimeter systems to meet the requirements of high-dose application fields. Some of the promising systems include alanine (ESR), radiochromic dye, ethanol – chlorobenzene, ceric-cerous sulphate, glutamine (lyoluminescence), and clear Perspex* (PMMA). Research has also been carried out on the possible effects of environmental variables, such as temperature, humidity, and storage duration on the stability and accuracy of the dosimeter readings and their correct interpretation. The availability of such comparative data on dosimeter systems is a technical base for international dose-assurance service. This dose-assurance service could create a situation where public health authorities or regulatory authorities could be furnished with data that serve as a guidance for a decision concerning an approval of irradiated products.

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IAEA Publications

An essential role of the IAEA towards the promotion of the radiation sterilization practices in the developing and the developed member states is served through publications of the proceedings of meetings, as well as reports and technical manuals (Tables 4a and 4b). Meetings have encompassed diverse problems of practice, which have served as a reference source and a guidance for workers in the field and for advanced students in universities and training courses. The recommendations for the relevant manufacturing practices have been utilized by a number of the member states while formulating their own national regulations and code of practice (7, 8).

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Role of the IAEA in the Regulatory Aspects of the Radiation Sterilization of Medical Products

The promotional roles of the IAEA have been briefly enumerated in the previous sections of this paper. The regulatory authority for the safety of the radiation-sterilized medical products belongs to the Health Department of a country's government. Accordingly, products should follow specific controls according to the country's national pharmacopoeia (7).

However, to facilitate the necessary upgrading of the existing specifications, in the light of the current developments in the technology and relevant experiences the IAEA organized several working-group meetings of experts (7, 8). The recommendations of the experts have framed the basis for an international code of practice that has been published (Table 4a) and is intended for use by workers in the field of application in member states (7). Furthermore, in view of the rapidly expanding use of radiation-sterilized medical products in countries beyond the national boundaries of their production, periodic upgrading of the document might become necessary in the future. The IAEA wishes to remain responsive to such needs of member states and to developments in the practices concerned.

Table 4a

Scientific meetings and publications on radiation sterilization of medical products organized by the IAEA

Title of meeting	Date and venue	Date of publication
*Application of Large Radiation Sources in Industry [C]	27-31 May 1963 Salzburg	1963
Radiosterilization of Medical Products, Pharmaceuticals and Bioproducts [P]	17-19 January 1966 Vienna	1967
Code of Practice for the Radiosterilization of Medical Products [P]	5-9 December 1966 Vienna	1967
Radiosterilization of Medical Products [S]	5-9 June 1967 Budapest	1967
Radiation Sterilization of Biological Tissues for Transplantation [P]	16-20 June 1969 Budapest	1970
*Utilization of Large Radiation Sources and Accelerators in Industrial Processing [S]	18-22 August 1969 Munich	1969

[C] = Conference; [P] = Panel meeting; [S] = Symposium.

*Meetings partly related to radiation sterilization

Table 4b

Scientific meetings and publications on radiation sterilization of medical products organized by the IAEA

Title of meeting	Date and venue	Date of publication
Manual on Radiation Sterilization of Medical and Biological Materials [M]		1973
Revision of the IAEA Recommended Code of Practice for the Radiation Sterilization of Medical Products [W]	5-9 June 1972 Riso, Denmark	1973

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Radiation Sterilization of Medical Products, Pharmaceuticals and Biological Tissues [R]	22-23 November 1971 Riso, Denmark	
Radiation Sterilization of Medical Products, Pharmaceuticals and Biological Tissues [R]	15-16 February 1973 Budapest, Hungary	
Ionizing Radiation for Sterilization of Medical Products and Biological Tissues [S]	9-13 December 1974 Bombay, India	1975
Effects of Sterilizing Radiation Dose upon the Antigenic Properties of Proteins and Biological Tissues [P]	27 Sept – 1 Oct 1976 Athens, Greece	
High-Dose Measurements in Industrial Radiation Processing [A]	25-29 September 1978 Vienna, Austria	1981

[A] = Advisory group meeting; [M] = Manual; [P] = Panel meeting;
[R] = Research coordination meeting; [S] = Symposium; [W] = Working group meeting

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Conclusion

The diverse activities under the IAEA programme have attempted to help generate timely awareness of the authorities in the developing member states of the availability of improved practices for sterilization of medical supplies. Motivated by the need to help upgrade existing health-care services, necessary steps have been taken in a number of countries to provide ready-to-use sterile medical supplies. The example of these few countries and the preliminary encouraging results will undoubtedly stimulate similar interests in other countries. The IAEA programme instrumentality will continue to remain responsive and will continue to provide as far as practicable the necessary back-up support in co-operation with WHO.

This Symposium has significantly helped to forward such aims to improve health-care services in a global context. Through a review of the current status and state-of-the-art, and by exchange of information on the future promotional steps, we are sure to proceed further. The opportunity afforded me to participate in this Symposium, the benefit gained from the information disseminated, and the development of contacts are gratefully acknowledged and very much appreciated.

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Gamma Sterilization – Process Development and Validation

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Abstract

Radiation sterilization process development involves microbiological validation, dosimetry, and assessment of product integrity. These are discussed, as well as relevant investigations carried out by the Australian Atomic Energy Commission (AAEC), Australian industry, and research institutes in South-East Asia and the Pacific region as part of an International Atomic Energy Agency (IAEA) co-ordinated research programme.

Study of the radiation dose needed to sterilize cotton balls and other cellulose materials processed in Australia, Bangladesh, Indonesia, the Philippines, and Thailand gave sterilization dose estimates ranging from 11-40 kGy (1.1-4 Mrd). Differences in estimates were more attributable to differences in method than in bioburden. The sterilizing dose for these 'natural' products depended more on the frequency of radiation-resistant organisms per unit than on total presterilization bioburden levels or the types of organism initially present.

The use of *Bacillus pumilus* spores as a biological dosimeter and ceric-cerous sulphate solution as a chemical dosimeter for an international calibration of cobalt-60 sources was also investigated. Dose rates in the various countries differed by up to 25% from the true value, causing variations in dose estimates or decimal reduction dose values for *B. pumilus* spores of up to 35%.

Results of these studies suggest that some sections of the appendix on radiation sterilization incorporated into the Australian *Code of Good Manufacturing Practice for Therapeutic Goods* or the International Atomic Energy Agency's *Recommendations for the Radiation Sterilization of Medical Products* are neither relevant nor appropriate for regulating the use of this technology. With increasing commercialization of radiation sterilization in Asia and a probable expansion of international trade in medical products from these countries, revised recommendations would provide valuable guidelines for regulatory authorities, manufacturers, and operators of contract or 'in-house' radiation facilities.

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Introduction

Radiation sterilization has been practised commercially in Australia since 1960. Three industrial cobalt-60 radiation plants have been built, one of which was declared obsolete and dismantled several years ago. Johnson & Johnson Australia Pty. Ltd. operates an 'in-house' plant in Sydney and thus has facilities and technical and professional expertise to develop new radiation-processing applications. Ansell International Pty. Ltd. now owns the second remaining industrial plant initially constructed for Tasman Vaccine Laboratories (NZ) in Melbourne. Excess radiation sterilization capacity is made available to other companies under contract. Process development is carried out in conjunction with the customer or by subcontracting analytical work to National Association of Testing Authorities (NATA) registered laboratories. At the AAEC's Lucas Heights Research Laboratories, limited ^{60}Co facilities are available to undertake irradiations and process development under contract or in consultation or collaboration with AAEC staff.

Development of a radiation process to sterilize a product involves the same steps as those required for other terminal sterilization techniques such as heat and ethylene oxide. Three basic questions need to be answered:

How much treatment is required?

What assurance is there that the product has received the required amount of treatment?

Is the product and its packaging adversely affected by the treatment? Radiation sterilization process development therefore involves biological validation, dosimetry, and assessment of product integrity. I shall emphasize the first two aspects. Relevant microbiological and dosimetric investigations carried out by the AAEC, Australian industry, and at institutes in South-East Asia and the Pacific region are described and discussed in the context of the Australian *Code of Good Manufacturing Practice for Therapeutic Goods (ACGMP)* and regulatory practices in other countries.

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Microbiological Validation of Gamma Sterilization

Medical products are routinely sterilized in some countries by radiation doses of 8 to 45 kGy (1, 2). The rationale for the use of different doses is discussed by John Masefield in these proceedings. In Australia, a minimum absorbed dose of 25 kGy has been used routinely for 22 years, its use having been legitimized in the 1971 edition of the *ACGMP* (3). Although the *ACGMP* provides for the use of higher or lower doses, 25 kGy has come to be accepted in Australia, as in many other countries, as THE sterilizing dose.

It is pertinent to recall how 25 kGy came to be selected as the optimum sterilization dose. When, in the 1950s, different species of microorganisms were tested by Ethicon, Inc., USA, for radiation resistance, spores of a common non-pathogenic environmental contaminant *Bacillus pumilus* were found to be the most resistant (4). This result was confirmed by Darmady *et al*. (5) in the UK, who irradiated 100 discs, each loaded with 10^5-10^7 organisms from different species, at doses up to 25 kGy. Again, *B. pumilus* spores were the most resistant to radiation; a few survived 20 kGy, but all were inactivated by 25 kGy. The sterilization dose was therefore set at 25 kGy on the assumption that it would include a large safety factor because the radiation resistance of the natural bioburden should be less than that of *B. pumilus* spores. Much relevant scientific information has been accumulated since these investigations were carried out, and a reappraisal of the continued use of 25 kGy as the optimum dose is now warranted.

The duration of the treatment, or the radiation dose, required to ensure that processed material meets a predetermined level of sterility, depends on the number of organisms present and their resistance to radiation. This is because the radiation inactivation of microorganisms occurs exponentially, as does inactivation by heat and most other sterilizing agents. Thus all biological validation programs, irrespective of the methodology, aim to relate microbial numbers and resistance to dose.

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Biological Validation Programme in Australia

The biological validation programme for radiation sterilization set out in the *ACGMP* is similar to recommendations made in 1974 by the IAEA (2). It has five main requirements, whether the dose to be validated is 25 kGy or some other dose:

(a) To estimate the presterilization microbial contamination level and identify the types of microorganism present.
(b) To estimate the radiation resistance of the contaminants to assess whether they will be sterilized at the given dose.
(c) To ensure that the bioburden levels of subsequent production batches do not exceed the initial estimate.
(d) To make sterility tests on irradiated items.
(e) To contaminate specific items deliberately with appropriate microorganisms and test for sterility after irradiation.

If bioburden estimates of subsequent batches are substantially different, the whole procedure has to be repeated.

Because of the microbiological effort required to meet the *ACGMP* guidelines, it is not surprising that biological validation attempts on Australian manufactured goods have been quite limited, being restricted to companies with access to radiation facilities.

The continued use of 25 kGy as a standard sterilizing dose in Australia is based on the assumption that levels of radiation-resistant microorganisms in raw materials and manufacturing environments do not exceed those reported in the Northern hemisphere (6-11). We have attempted to test this assumption for one product prepared in three different manufacturing environments. The main result of this investigation and the summarized results from similar investigations in neighbouring countries are presented.

Validation of Radiation Sterilization Dose for Cotton Balls Processed in Australia

(i) Experimental

Cotton or cotton-rayon balls manufactured and/or packaged in Sydney or Melbourne between 1977 and 1979 were investigated. Samples from two to six batches processed in three locations were examined for aerobic bioburden numbers and types of organism present. Anaerobic organisms were not investigated because they were not present in at least one of the brands tested. From estimates of the radiation resistance of the microbial contaminants, radiation sterilization doses for each brand of cotton ball were calculated.

Membrane filtration was used to assess bioburden levels. Isolates were broadly classified by their reaction to gram stain, morphology, colony appearance, and presence or absence of spores. Substerilizing-dose (1 to 5 kGy) treatment (12) was used to isolate selectively the more prolific or more radiation-resistant organisms. To distinguish between these categories, pure cultures of isolates were prepared and frozen or dried aliquots irradiated at one or two screening doses (5 to 20 kGy); the fraction of organisms surviving irradiation were then determined. Organisms requiring a dose of more than 20 kGy to achieve an 8 log cycle reduction in numbers were considered resistant to

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radiation. These resistant isolates were tested further to assess accurately the radiation parameters D_{10} (decimal reduction dose) and Dq (quasi-threshold, or lag dose).

(ii) Results and Discussion

Bioburden – Mean bioburden levels on cotton or cotton-rayon balls processed in the three environments varied from 23 to 1156 colony-forming units (CFU) per gram (Table 1). Within each environment, bioburden ranged from 7 to 50 CFU/g over six production batches and from 60 to 2247 for two batches prepared by another manufacturer.

The percentage of occurrence of different types of organism differed between batches both before and after low doses of radiation (Table 2), with no apparent correlation. For example, for one brand the percentage of spore formers, which ranged from 15 to 61% before treatment, either increased or decreased after doses of up to 3 kGy, forming 6 to 35% of the total survivors. For the other two brands tested, spore formers made up either 41 to 82% or 56 to 75% of the isolates (Table 3).

Table 1

Bioburden levels of cotton balls processed in Australia

Batch	Colony-forming units per gram Manufacturing environment		
	A	B	C
1	50	2247	15
2	34	60	39
3	19		158
4	7		
5	11		
6	17		
Mean	23	1156	71

The three most radiation-resistant organisms isolated from Brand A cotton balls were a gram-positive rod, a coccobacillus, and a spore former. They wer not identified further. In general, very radiation-resistant isolates are difficult to classify and culture, sometimes taking a week to grow at 32°C. Because of this slow growth, their presence could easily be missed in validation methods based on growth/no growth techniques.

Table 2

Distribution of main types of bacteria on four batches of Brand A cotton balls, before and after low doses of radiation

Type	Percentage of total bioburden	
	After manufacture	After low-dose irradiation
Spore formers	15-61	6-35
Gram-positive rods	4-23	13-63
Gram-positive cocci	3-46	14-56

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Table 3

Range of distributions of organisms on different production batches of cotton balls treated with low doses of radiation

Type	Percentage		
	A	B	C
Spore formers	6-35	41-82	56-75
Gram-positive rods	13-63	0-6	0-22
Gram-positive cocci	14-56	0-53	15-40
Yeasts	0-20	0-18	0
Fungi	0-31	0	0-14
Unidentified	0-25	0	0

Radiation resistance of bioburden – Microbial contaminants from cotton balls were classified as radiation resistant if a dose of 20 kGy was required to achieve 10^8 inactivation. *B. pumilus* spores were inactivated by doses up to 25 kGy under the experimental conditions used in this investigation. For the three brands tested, at least 0.5 to 2.6% of the bioburden organisms were resistant to radiation, approximately half being more resistant than *B. pumilus* spores. Spore formers made up the highest proportion of resistant organisms, the remainder being gram-positive rods, cocci, coccobacilli, or yeasts (Table 4).

Table 4

Distribution of radiation-resistant bacteria on cotton balls

Type	Percentage of resistant* bacteria Manufacturing environment		
	A	B	C
Spore formers	48	85	63
Gram-positive rods	46	5	16
Gram-positive cocci	4	5	21
Coccobacilli	2	0	0
Yeasts	0	5	0
Percentage of PSC	2.6	0.5	2.0

* Resistance to radiation equal to or greater than that of *B. pumilus* spores

The frequency of the resistant bacteria, grouped in classes according to the dose needed to inactivate 10^8 organisms, is shown in Table 5. From samples of Brand A cotton balls with a total bioburden level of 2186, three very resistant organisms were isolated that required doses of 35 to 40, 40 to 45, or 55 to 60 kGy to achieve 10^8 inactivation. Equally resistant organisms have been found at comparable frequencies in Poland (1 in 2000) (7), Sweden (1 in 3000) (8), and Denmark (1 in 1400) (11), but have not been detected in similar studies performed in the USSR (9) and Canada (10).

Although the bioburden on Brand B cotton balls was high, most of the additional presterilization contaminants (PSC) were sensitive to radiation; only 0.5% were resistant to radiation compared with

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2.6% of the PSC on Brand A cotton balls. None of the organisms isolated from Brand B cotton balls had resistances as high as those found on the other two brands. However, high resistance organisms would possibly have been detected if more samples had been tested to compensate for the very high percentage of the bioburden that was sensitive to radiation.

Comparisons between batches, brands, and products of the extent of contamination with radiation-resistant organisms can more readily be made if the number of resistant organisms present is expressed as the number in a specified quantity or volume of production units, rather than as a proportion of the total bioburden. For example, assuming a unit size of 100 × 1 g packets of cotton balls, the number of organisms with a resistance to radiation similar to that of *B. pumilus* spores would be 38 for Brand A or 372 for Brand B (Table 6), corresponding to the experimentally determined frequencies of 0.016 or 0.003 respectively (Table 5). If Brand B organisms were proportionately as resistant as Brand A organisms, we could expect 1900 organisms in this resistance class, not 372. Total bioburden levels *per se* are therefore not necessarily useful nor appropriate for predicting contamination levels of radiation-resistant organisms in materials.

Table 5

Minimum frequency of radiation-resistant organisms in presterilization bioburden of cotton balls processed in Australia

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Class interval*		Frequency Batch						
No.	kGy	1	2	3	4	5	6	Overall
				Brand A				
1	20-25	0.00314	0.00287	0.04722	0.01370	0.06599	0.00394	0.01647
2	25-30	0.00524	0.00287	0.01111	0.01370	0	0	0.00823
4	35-40	0	0	0	0	0	0.00394	0.00046
5	40-45	0	0.00287	0	0	0	0	0.00046
8	55-60	0	0	0.00278	0	0	0	0.00046
Sample Bioburden		954	348	360	73	197	254	2186
				Brand B				
1	20-25	0.00229	0.01189					0.00322
2	25-30	0.00025	0.00959					0.00115
3	30-35	0.00025	0					0.00023
Sample Bioburden		3938	417					4353
				Brand C				
1	20-25	0.02326	0	0.01017				0.01030
2	25-30	0.04651	0	0.00226				0.00412
3	30-35	0	0	0.00452				0.00412
5	40-45	0.02326	0	0				0.00103
Sample Bioburden		43	43	885				971

* Dose range for inactivation of 10^8 organisms

Inactivation dose for microbial contaminants isolated from cotton balls – From calculations based on the frequency of occurrence of the most resistant isolates and their resistance to radiation as determined under laboratory conditions, doses of 34.6, 20.8 and 28.6 kGy are required to ensure not more than one survivor from $10^6 \times 1$ g packets of Brands A, B and C cotton balls respectively (Table 7). For Brands A and C, these doses, calculated from organisms forming only 0.05 to 0.1% of the natural bioburden, were more than adequate to eliminate all other contaminants. Thus, the sterilization dose for Brand A was set by one very resistant strain of bacteria with an average contamination level of only 1 per 100 packets. However, the dose for Brand B, based on the most resistant isolate, was insufficient to inactivate all bacteria in the adjoining resistance class, or all bacteria in the predominant class of sensitive organisms. Under the experimental conditions used in this

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investigation, organisms in the second highest resistance class needed a dose of 22.9 kGy for their inactivation. This dose ensured at least a 10-fold safety factor for organisms with other resistances.

Table 6

Estimate of number of resistant organisms in 100 (1-g) packets of cotton balls

Radiation Resistance	Number per 100 packets Manufacturing environment		
	A	B	C
Less than *B. pumilus*	2 240	115 068	6 962
Equal to *B. pumilus*	38	372	73
More than *B. pumilus*	22	160	65
Total	2 300	115 600	7 100

Estimation of sterilization dose for cotton balls – Inactivation doses reported in the previous section were calculated from radation parameters obtained experimentally from isolates tested under conditions known to protect bacteria from the lethal effects of irradiation. Organisms would be expected to be more sensitive when irradiated *in situ* and correspondingly the sterilization dose would be lower.

The D_{10} and D_q values for the most resistant isolate from Brand A cotton balls were lower when determined from a suspension of the isolate dried onto cotton balls and irradiated in air (Table 8). Using these values, the sterilization dose for Brand A cotton balls was reduced from 34.6 to 21.3 kGy. Other work in progress suggests that organisms are about 15% more sensitive when dried on cotton balls and irradiated in air than when tested under the conditions used in this investigation. On this basis, Brands B and C cotton balls would be sterilized by doses of 20 and 24 kGy respectively.

Table 7

Inactivation doses for bioburden on a million (1-g) packets of cotton balls

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Class (No.)	Bioburden in 10^6 packets	D_{10}[a] (kGy)	D_q (kGy)	Inactivation dose[b] (I.D.) (kGy)	Safety factors[c] I.D. for most resistant	Highest I.D.
			Brand A			
Total bioburden	2.3×10^7					
Most resistant (8)	1.06×10^4	5.96 ± 0.16	10.6	34.6	1	1
Second highest (5)	1.06×10^4	4.61	5.4	24.0	2.0×10^2	2.0×10^2
Third highest (4)	1.06×10^4	4.0	5.6	21.7	1.7×10^3	1.7×10^3
Most sensitive	2.24×10^7	<2.5	–	18.4	$>3.2 \times 10^6$	$>3.2 \times 10^6$
			Brand B			
Total bioburden	1.2×10^9					
Most resistant (3)	2.76×10^5	3.81 ± 0.08	0.12	20.8	1	3.5
Second highest (2)	1.30×10^6	<3.75	–	22.9	$>2.7 \times 10^{-1}$	1
Third highest (1)	3.84×10^6	<3.12	–	20.5	>1.2	5.7
Most sensitive	1.19×10^9	<2.5	–	22.7	$>1.8 \times 10^{-1}$	1.2
			Brand C			
Total bioburden	7.1×10^7					
Most resistant (5)	7.31×10^4	5.04 ± 0.11	4.08	28.6	1	1
Second highest (3)	2.93×10^5	<4.38	–	23.9	$>1.2 \times 10^1$	1.2×10^1
Third highest (2)	2.93×10^5	<3.75	–	20.5	$>1.4 \times 10^2$	1.4×10^2
Most sensitive	6.96×10^7	<2.5	–	19.6	$>4.0 \times 10^3$	4.0×10^3

a Radiation resistance determined either experimentally on serum broth suspension of isolate dried onto polyethylene strip; irradiation *in vacuo* or theoretically calculated as a maximum value from upper value of class interval.

b Inactivation dose: D_{10} (log No/10^6 packets) + D_q

c Safety factor: $\text{antilog}\left(\frac{\text{inactivation dose} - d_q}{D_{10}} - \log \text{No}/10^6 \text{ packets}\right)$

The use of 25 kGy in Australia for sterilizing cotton balls manufactured or packaged in three different environments has therefore been validated, assuming that industrial conditions remain relatively stable.

(iii) Relevance of Results from Cotton Balls to Validation Programmes

The results reported here show that

- there is little correlation between total presterilization microbial contamination levels and the radiation dose estimated for sterilization;
- there is no correlation between the types of microorganisms present and the estimated radiation sterilization dose;
- radiation sterilization dose depends solely on the extent of the bioburden contamination by organisms in the highest class of radiation resistance or in the highest and second highest resistance

class; and

– radiation sterilization dose depends on the frequency of these resistant organisms in products rather than on their frequency in total bioburden.

Table 8

Influence of irradiation conditions on resistance of most resistant isolate from Brand A cotton balls

Support	Atmosphere	D_{10} (kGy)	$\frac{D_{10}P.E.}{D_{10}C.B.}$	D_q (kGy)	Sterilization dose for 10^6 cotton balls
Polyethylene (P.E.)	*In vacuo*	5.96		10.6	34.6
Cotton balls (C.B.)	Air	4.44	0.74	3.4	21.3

Validation programmes should therefore be less concerned with total bioburden and identification of contaminants and concentrate on detecting the frequency or increases in frequency of very radiation-resistant organisms in product units, rather than in total bioburden.

Substerilizing dose treatments of the product for the quantitative determination of contaminant survival, using membrane filtration or the AAMI incremental dose method (13, 14), can be valuable techniques for this purpose. Once the sterilization dose is set, it must be monitored periodically to check its continuing validity. For example, the results from the cotton balls suggest that for sterilization at 25 kGy, a suitable quality control programme might involve subjecting 100 units to an 'audit' or 'challenge' dose of about 10 or 12 kGy (14, 15). The absence of survivors would indicate that manufacturing conditions are under control and that the processing of 10^6 units at 25 kGy should ensure not more than one survivor.

Validation of Radiation Sterilization Dose for Cellulose Materials Processed in Several Countries in Asia and the Pacific Region

(i) Experimental

Between 1976 and 1982, participants in an IAEA co-ordinated research project examined radiation sterilization practices related to local medical supplies and manufacturing conditions for countries in Asia and the Pacific region. During this project, bioburden levels of several cellulose products were assessed in the country of origin, and sterilization doses estimated from radiation parameters acquired under different irradiation conditions (Fig. 1). These investigations were facilitated by IAEA research contracts with the countries involved.

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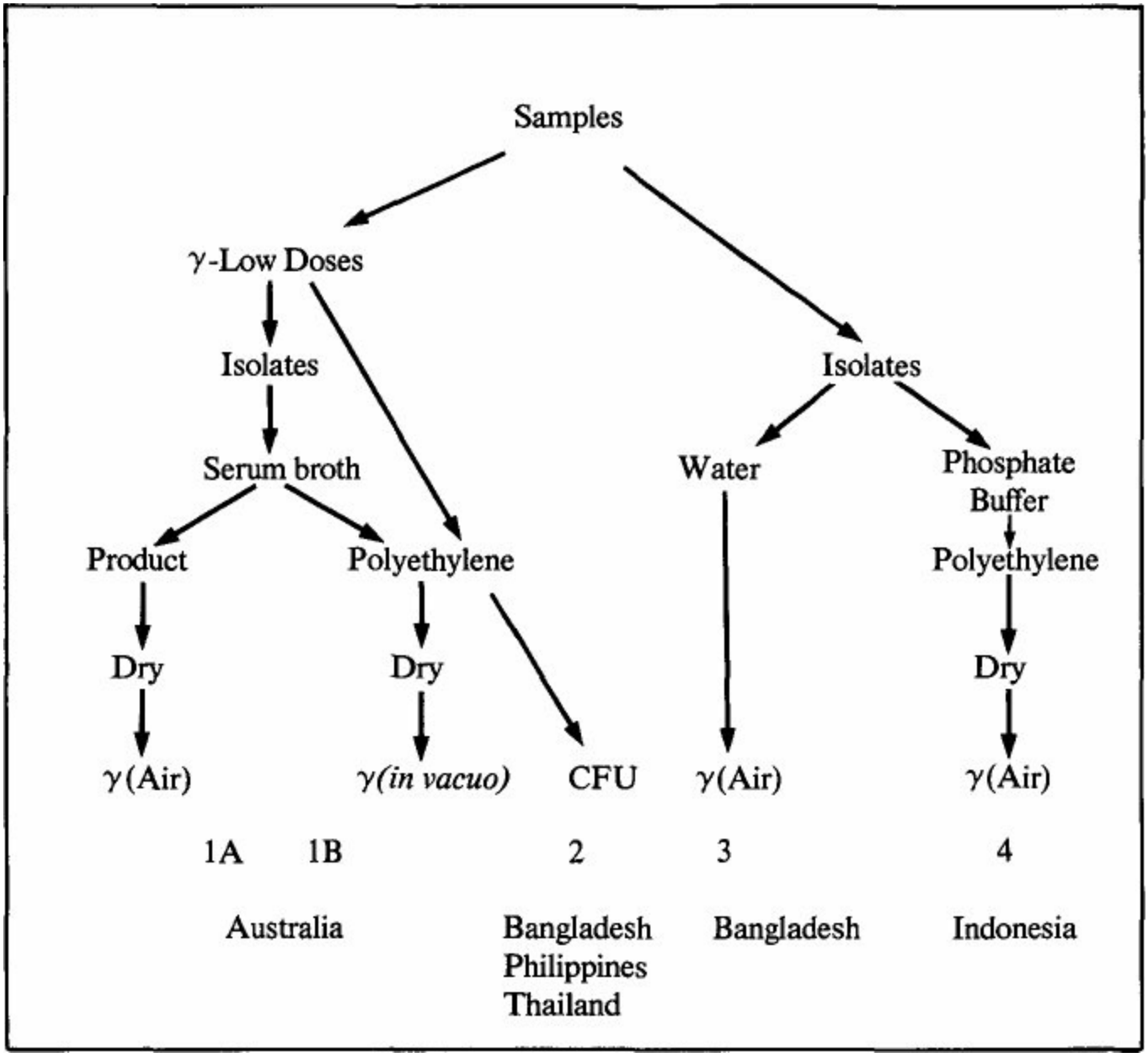

Figure 1. Sterilization Dose Validation Methods.

(ii) Results and Discussions

The results presented in this section were extracted from reports given at IAEA research co-ordination meetings by scientific investigators from Bangladesh (A.K. Siddiqui, Institute of Food and Radiation Biology), India (N.G.S. Gopal, Isomed), Indonesia (N. Hilmy, Centre for the Application of Isotopes and Radiation), the Philippines (C.C. Singson, Philippine Atomic Energy Commission), and Thailand (U. Navanugraha-Yuthamanop, Office of Atomic Energy for Peace). In some instances, basic data have been reinterpreted to obtain sterilization dose estimates.

Mean bioburden for cotton balls ranged from 120 (Thailand) to 460 000 (Bangladesh) CFU/g. Sterilizing doses of 37.6 and 26.0 kGy, respectively, were calculated (Table 9). Incomplete data for dressing gauze, surgical bandages, and swabs are shown in Table 10. Material processed in Bangladesh had the highest bioburden of up to 10^6 CFU/g, giving sterilization dose estimates of 36 and 39 kGy for bandages and gauze, respectively.

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Table 9

Estimates of sterilising dose required for a million (1-g) packets of cotton balls processed in South-East Asian and Pacific Region countries

Country	Validation method (Fig. 1)	Mean PSC/g	Sterilising dose (kGy)
Australia	1A, 1B	23-1 200	2.1-2.4
Bangladesh	2,3	460 000	2.6
Indonesia	4	1 200	1.2
Philippines	2	350	2.1
Thailand	2	120	3.8

In general, sterilizing doses based on data obtained by direct irradiation of material were higher than doses calculated from data obtained by irradiating bacteria isolated from the product. The sterilizing-dose estimate of 37.6 kGy for cotton balls produced in Thailand (average PSC of 120 CFU/g) was obtained by this method following an extensive study of the quantitative determination of survivors after irradiation at substerilizing doses of large samples collected during six months of commercial production.

Levels of Sterility or Decontamination

Setting radiation sterilization or decontamination doses requires a judgment to be made by regulatory authorities about the degree of assurance needed to show that an article is sterile or decontaminated. In many countries, radiation doses are either based on a judgment that the dose should ensure that not more than one organism will survive in a million treated units, or they are set at 25 kGy or some other predetermined dose on the assumption that this level of sterility will be achieved or exceeded. Such decisions can have the effect of ‘overkill’ with a consequent waste of radiation sources, increased process time, excessive microbiological effort, and the possible degradation of the product. Two examples of overkill are given below.

Table 10

Estimates of sterilizing dose required for one million (1-g) packets of cellulosic products, excluding cotton balls, processed in South-East Asian and Pacific Region countries (data incomplete)

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Country	Brand	Bioburden frequencies				Radiation parameters		
		CFU/g						
		Range	Mean	Aerobic spores (Anaerobes)	Resistants	D_{10} ± S.E. (kGy)	Sterilizing dose (kGy)	Validation method (Fig. 1)
Dressing Gauze								
Bangladesh	1	10^3-10^6	370 000	0.53 (0.002 5)	0.059	3.82 ± 0.37	39.5	2
Indonesia	1	80-7 300	530		0.025	1.85 ± 0.05	15.1	4
	2	230-3 600	2 100		0.021	2.35 ± 0.15	18.9	4
Philippines	1		$>10^6$					
Surgical Bandage								
Bangladesh	1	10^4-10^6	150 000		0.061	3.59 ± 0.039	36.1	2
Various								
India	many	low-80 000						

The AAEC manufactures two types of lyophilized reagents for clinical use as injectables with the radioisotope technetium-99m. These reagents are produced under very clean manufacturing conditions in small batches of about 1000 × 1 mL units that are *gamma* sterilized at 25 kGy. Analysis of bioburden data collected from all batches manufactured over the past five years shows that the average pre-sterilization total microbial count per 1000 units is 4 organisms for one product and 50 organisms for the other. Assuming that the resistance of contaminants equals that of the most resistant organism found on cotton balls, and that current production rates and conditions continue, sterilization at 25 kGy guarantees that only one organism would be expected to survive in 400 years' production of one product, or 40 years' of the other.

In recent years in North America, the concept of variable levels of sterility or decontamination according to the end use of the product has been developed for medical products (16), and sterilization at different doses is now widely practised in this region. Sterilization doses based on annual production volumes may be more appropriate for low volume, low contamination materials than doses designed to ensure sterility in a million items. It is pertinent to recall that the processing conditions for canning foods, which are designed to ensure inactivation of *Clostridium botulinum* spores, were initially based on one year's production, that happened to be, at that time, a million cans.

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Dosimetry for Gamma Sterilization

Chemical Dosimetry

In many countries, the absorbed dose achieved in material irradiated in commercial sterilization facilities is routinely monitored using clear Perspex* or red acrylic dosimeters. For Australian irradiators, the accuracy of the plastic dosimeters is checked by parallel irradiations made periodically with dosimeters containing ceric-cerous sulphate solution. Absorbed dose is calculated from the Potentiometric change induced in the ceric-cerous sulphate solution (17), measured under contract at the Lucas Heights Research Laboratories. This dosimetry calibration service is also used by a commercial radiation plant operating in Malaysia.

However, in an IAEA sponsored interlaboratory comparison of different dosimetric techniques suitable for calibrating radiation sterilization facilities, which was carried out in several northern hemisphere countries, ceric-cerous sulphate dosimetry did not perform as well as some other methods (18). In contrast to this experience, ceric-cerous sulphate dosimetry was successfully used to calibrate radiation research facilities in several Asian and Pacific countries. This work was carried out by the AAEC under an IAEA Technical Contract as part of an Agency-co-ordinated research project on radiation sterilization of medical products in the Indo-Pacific region.

Bulk ceric-cerous sulphate dosimeter solution was prepared and dispensed into 2-mL ampoules. Dose rates determined for an AAEC ^{60}Co facility, using this solution or primary standard Fricke dosimetry, agreed within 2%. Five replicates, each containing six dosimeters, were forwarded to participants in the Agency's program. One replicate was intended as a spare, in case of breakages in transit (a very rare occurrence). Three replicates were separately irradiated at a dose nominated by the participant, usually 20 kGy. The irradiated dosimeters were returned to the AAEC for measurement, together with an unirradiated replicate to be compared with an untravelled replicate, should the measured dose of the other replicate deviate considerably from the nominated dose.

Five countries participated in this study: Bangladesh, India, Indonesia, the Philippines, and Thailand. The measured doses for each country agreed well within and between replicates. However, for two countries, the measured dose differed significantly from the nominated dose (Table 11). For the same nominated dose, there was a 35% difference between the lowest and highest dose. This is equivalent to setting a sterilization dose of either 20 to 27 kGy to achieve the same degree of sterility for identical numbers and types of organism. The deviations between measured and nominated doses were not attributable to changes in the dosimeters, induced by travelling or by exposure to higher ambient temperatures, as untravelled and travelled dosimeters performed similarly when irradiated at Lucas Heights at a dose rate comparable to that used by the participant.

Table 11

Calibration of radiation facilities in South-East Asia and Pacific Region countries by chemical (Ce^{4+}/Ce^{3+}) or biological *(B. pumilus)* dosimetry

Country	AAEC estimate of dose rate / Country estimate of dose rate	
	Chemical	Biological
A	0.925	0.982

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B	1.023	Not available
C	1.030	0.976
D	1.060	1.120
E	1.250	1.231

All dosimeters for this calibration project were irradiated in facilities surrounded by air, as occurs with most commercial facilities, except for those irradiated in under-water rigs located in the fuel element storage pond at Lucas Heights. Because the radiolytic conversion of ceric to cerous ions is known to be slightly temperature-dependent (19), the time – temperature relationships in one rig were investigated over one year. Depending on the season and the dose rate, the temperatures during irradiation varied by up to 30°C, with a consequent variation in G value for a 10mM/10mM Ce^{4+}/Ce^{3+} solution of up to 6%. These values show that the temperature during irradiation must be known for the accurate calculation of absorbed dose in ceric-cerous sulphate solution, and suggest an explanation for the discrepancies found for this dosimeter in the original IAEA intercomparison study.

Ceric-cerous sulphate dosimetry is not mentioned in the appendix on radiation sterilization in the *ACGMP*. As the reliability and value of the Ce^{4+}/Ce^{3+} system for measuring absorbed dose has been demonstrated unequivocally over many years, it should be included among the other dosimetry systems suggested in Section A.7.1 of the relevant appendix of the *ACGMP*.

Biological Dosimetry

Biological dosimeters can be used to estimate absorbed dose. The dosimeter is irradiated and the fraction of organisms surviving irradiation determined. Dose can be assessed by reference to a calibration curve constructed from a semi-logarithmic plot of survival ratio versus dose. Because of the time, effort, and uncertainty involved in biological survival determinations, it is generally considered that dose can be more accurately monitored by other parameters such as the speed of the conveyor system or the use of chemical dosimeters. Nevertheless, because the lethal effect of radiation on organisms is the *raison d'être* for radiation sterilization, there is some justification for using biological dosimetry to calibrate new or modified radiation facilities.

Each participant in the IAEA's S-E Asian and Pacific Region Radiation Sterilization Research Project prepared a biological calibration curve for his or her radiation facility using *B. pumilus* ATCC 27142 spores supplied by the AAEC. Serum bottles containing spores lyophilized from a stock serum broth suspension were prepared by the AAEC and four replicates each of 36 samples were distributed to all participants. For each replicate, six samples were irradiated at four doses, generally 3, 6, 9, and 12 kGy, and survival determined by the method normally used by each participant. The fourth replicate was returned unirradiated to the Lucas Heights Research Laboratories for testing by the same method. The ratio of the mean D_{10} value for *B. pumilus* spores estimated by the AAEC to that determined by each participant was used as an index of the accuracy of the participant's assessment of dose rate in their radiation facility.

For three participants, D_{10} estimates agreed well with those determined by the AAEC (Table 11). For the fourth participant, the high deviation from the AAEC D_{10} estimate corresponded with a similar discrepancy in dose rate as determined by chemical dosimetry.

The use of different techniques and media by the participants did not influence postirradiation

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survival determinations. However, survival increased when irradiated samples were stored at 32°C before testing, increasing the D_{10} value by 20% (Table 12), which is equivalent to raising the 10^6 inactivation dose for *B. pumilus* spores from 20 to 24 kGy.

Table 12

Influence on postirradiation storage at 32°C on D_{10} of *B. pumilus* spores

Time at °C before testing (days)	D_{10} (kGy)
0	3.36
1	3.61
3	3.82
7	4.02
10	3.96
14	3.91

If postirradiation recovery at 32°C is a general phenomenon applicable to other types of bacteria, this has considerable practical significance for countries with high ambient temperatures. Sublethal injury and the possibility of repair during storage should be taken into account when performing validation studies.

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Summary and Conclusions

Gamma radiation is an effective sterilizing or decontamination treatment for many medical products and raw materials. Process control involves calibration of dose rates in radiation facilities with a reliable dosimeter such as the ceric-cerous sulphate solution and biological validation aimed at estimating the frequency of radiation-resistant organisms on material before treatment. Various techniques for process control are described.

Basic data obtained in Australia and from some Asian manufacturing environment are presented, with particular emphasis on cellulose products. Up to 1% of bioburden organisms on cotton balls processed in Australia were more resistant to radiation than *B. pumilus* spores. The frequency or organisms with the highest, or next-to-highest, resistances determined the radiation sterilization dose, even though the contamination level for these organisms may have been only 1 in 2300 organisms or 1 in 100 units.

Protocols for the microbiological validation of radiation sterilization of medical products as set out in the IAEA Recommendations (2) and the *ACGMP* (3) are labour-intensive, time-consuming exercises beyond the logistic, financial, and microbiological resources of most sectors of the industry. In addition, much of the data required is irrelevant, as illustrated in the cotton ball study. The method is also open to criticism because of its requirement for radiation resistance studies on pure cultures of bioburden under laboratory conditions, rather than studies of resistance *in situ*. Alternative methods involving product irradiation at low doses have been investigated in several laboratories, and a few techniques developed to overcome this problem have been adopted.

Worldwide commercial and regulatory experience in the radiation sterilization of medical products gained over the past twenty years means that the technological data base is considerably broader now than when the IAEA Recommendations and the *ACGMP* were formulated. If we are to take advantage of these accumulated experiences and new developments, and possibly increase the flexibility of radiation sterilization technology and its control, it is time to review and revise, where necessary, the relevant sections of the *ACGMP* and the IAEA Recommendations.

Although the use of 25 kGy as a routine sterilizing dose for medical products has served Australia and many other countries well, this dose should not be regarded as sacrosanct and immutable. Overkill is an inefficient use of limited resources. If acceptable levels of sterility or decontamination can be achieved with doses less than 25 kGy, financial savings from treatment at lower doses could partially offset the rising cost of medical products. On the other hand, manufacturers and regulatory authorities should be aware that doses above 25 kGy may sometimes be necessary.

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Current North American Practices in Gamma Sterilization

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Abstract

The first commercial application of cobalt-60 radiation sterilization took place in Australia in 1960. By 1982, the process was being used worldwide, primarily for the sterilization of medical products.

Widescale adoption of the technique was stimulated in the 1970s by improved process economics, by the development of new radiation-stable plastics, and by the use of new scientific dose setting procedures leading, in many instances, to sterilizing doses that were lower than the traditional but arbitrary 2.5 Mrd.

These methods, which do not require the laboratory determination of organism resistances (D_{10}), accurately account for the natural resistance of heterogenous microbial populations on medical devices.

Efforts are now on the way to gain international acceptance of the dose setting procedures in order to foster trade in products sterilized at these lower doses.

In order to deliver a variety of doses to a wide range of products efficiently and accurately, a new type of computer controlled incremental-dose irradiator has been developed in North America.

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Current North American Practices in Gamma Sterilization

Following the demonstration by the United Kingdom Atomic Energy Authority in the late 1950s that cobalt-60 irradiation sterilization was feasible and practical, Australia in 1960 established the world's first *gamma* irradiator in Dandenong for the destruction of anthrax bacillus in goat hair (1). Shortly thereafter, Johnson & Johnson adopted cobalt-60 irradiation in the United Kingdom and the United States for the sterilization of medical products.

Between 1962 and 1977, shipments of industrial cobalt-60 by Atomic Energy of Canada Limited (AECL), mainly for medical product sterilization, rose from a few hundred thousand curies per year to 5 million curies per year. Between 1977 and 1980, this doubled to 10 million curies per year and is expected to double again to an amount in excess of 20 million curies by the end of 1982 (Figure 1) (2).

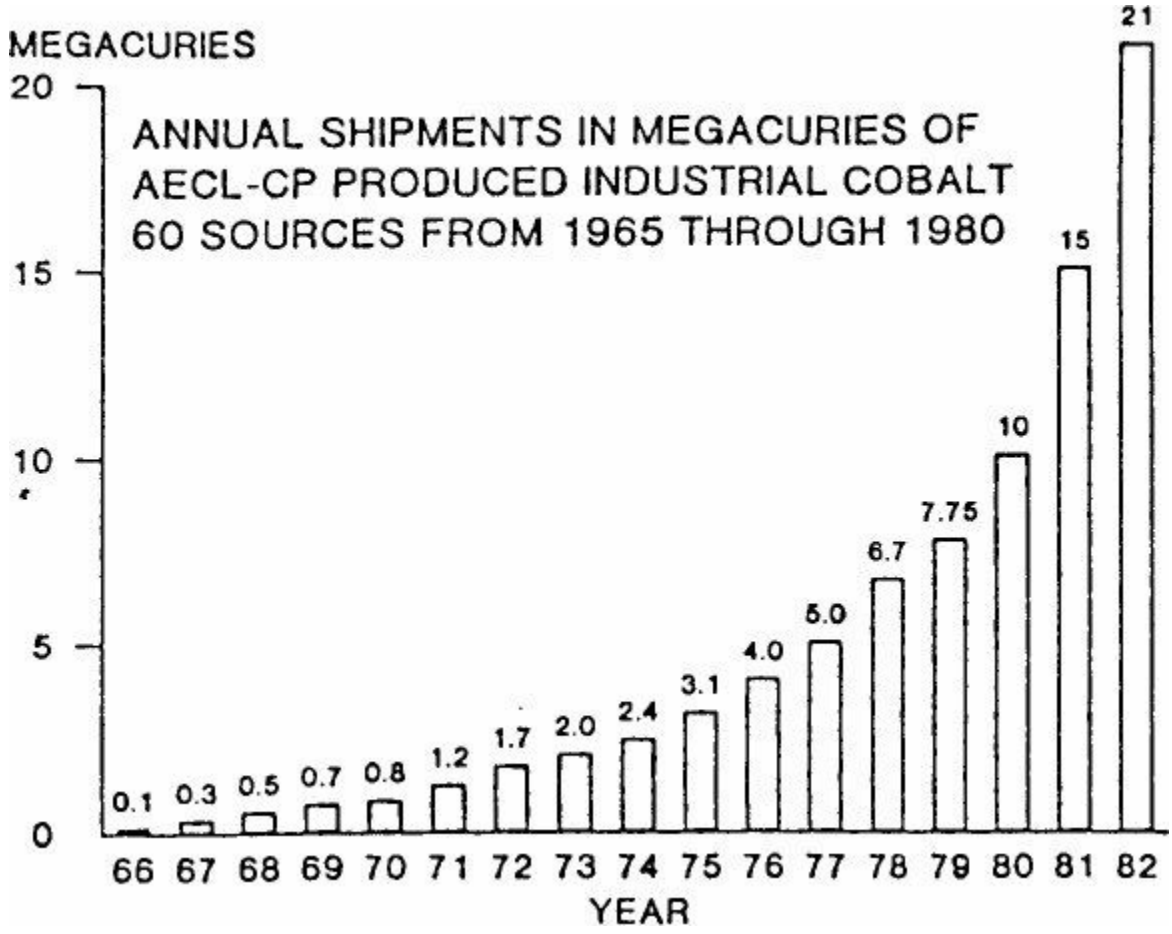

Figure 1.

There are now 125 commercial irradiators in 36 countries on six continents, containing 66 million curies of cobalt-60, out of a total design capacity in these units of 190 million curies (i.e. an average of 35% utilization of design capacity).

Of these, North America has 34 irradiators with 30 million curies of cobalt-60 installed out of a design capacity of 88 million curies (i.e. 38% utilization).

This means that North America currently has 45% of the world's installed cobalt-60 processing capacity, sterilizing between 20 and 30 million cubic feet of medical products per year. When fully charged with cobalt-60, these units will be capable of processing between 90 and 100 million cubic feet per year.

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The rapid growth of cobalt-60 sterilization in recent years can be attributed to the following factors:

1. Growing recognition of the inherent reliability of the process.
2. The increasing availability of inexpensive radiation-stable plastics commonly used in the manufacture of medical devices.
3. The economics of the process became competitive with those of alternative sterilization processes (3) as a result of:
   Relatively stable cobalt-60 prices.
   Economics of scale resulting from the establishment of high capacity, high throughput sterilizers.
   The elimination of poststerilization product testing and associated product quarantine.
   The wide use in North America of sterilizing doses lower than 2.5 Mrd.

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Availability of Radiation-stable Plastics

The effects of ionizing radiation on a wide range of organic materials have been extensively investigated and documented, and include changes in physical and chemical properties that result in strength loss, colour change, and odour generation (Table 1) (4, 5).

Table 1

Radiation effects on materials at 2.5 Mrd

Material	Colour	Physical properties	Odour
Cellulosics	*	*	*
Polyvinyls	*	*	
Polyethylene			*
Fluoroplastics		*	
Polypropylene	*	*	
Acrylics	*		
Polycarbonate	*		
Nylon	*		
Glass	*		

* Denotes adverse effect

Levels of colour change and odour generation that were generally considered as an acceptable part of the sterilization process in Europe were rejected in North America, a problem which seriously hampered the growth of the process during the 1960s and early 1970s.

Of particular importance were the discoloration of polyvinyl chloride, cellulose and cotton, and the odour generated in the cellulose – polyethylene laminates used in high-volume production hospital procedure packs.

These problems have been alleviated by:

1. The growing commercial availability of radiation-stable grades of polypropylene and polyvinyl chloride.
2. A general lowering of sterilizing dose in North America below 2.5 Mrd.

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Guidelines for Determining Sterilizing Dose

The shift in North America away from using the fixed but arbitrary sterilizing dose of 2.5 Mrd, first recommended by Artandi and Van Winkle (6), resulted in large measure from work conducted by the Radiation Sterilization Subcommittee of the Association for the Advancement of Medical Instrumentation (AAMI).

This committee, formed in 1976, was charged with the task of formulating industry guidelines for control of the radiation sterilization process, including recommendations for the establishment of sterilizing dose (7).

The results of the committee's work are described in detail in *Sterilization of Medical Products*, Vol. II (8) and are presented as practical guidelines in the AAMI document entitled: Process Control Guidelines for Radiation Sterilization of Medical Devices (Proposed – RS-P 10/82). Following is a brief review of the four dose setting methods (B1 to B4), plus an audit procedure (B5), contained in this document.

Before describing these methods, I want to stress that though the authors of this paper are responsible for the words, our colleagues on the committee, with particular reference to the very talented statistician Ken Davis of Ethicon, Inc., are responsible for most of the work.

Hypotheses: All four recommended dose setting methods are based upon internationally accepted hypotheses, namely that:

1. Product bioburden comprises a mixture of homogenous populations, each of which behaves in a D_{10} fashion (9).
2. 'The choice of dose should depend upon the microbial contamination to be found on the item and the margin of safety required when considering the end use of the item'. (IAEA Recommended Code of Practice 1967)
3. Conventional sterility tests on small numbers of finished sterilized product are totally inadequate as a means of verifying the attainment of a preselected Sterility Assurance Level below 10^{-2} (Table 2) (10).

Table 2

Probability of acceptance of nonsterile product		
Sample size	Fraction nonsterile	Probability of acceptance
20	0.001 6	0.999
	0.060	0.50
	0.168	0.05
1000	0.000 03	0.999
	0.001 2	0.50
	0.003 7	0.05
3 685 000	0.000 001	0.05

The committee was further influenced in its approach to dose setting by work reported in 1972 by Tallentire on the use of substerilizing doses on product samples as a means towards developing new

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approaches to microbial control. Tallentire stated that 'the best evidence of the effectiveness of a process must arise from experiments with naturally contaminated production items themselves, rather than with contrived situations produced in the laboratory.' (11).

This is confirmed by the fact that the D_{10} values of microbes determined after separation from product followed by culturing and exposure to substerilizing doses vary widely with changes in laboratory technique (Table 3).

As such, the relationship between the laboratory determined resistance and that of the microbe as it naturally occurs on the product is not always known.

Tallentire also wisely observed that 'we envisage that sterility testing of items given sub-sterilization doses could reveal the frequency of radiation resistance organisms and, if desired, the types of these organisms.' (11).

Since this approach to dose setting offered the potential of eliminating the need for manipulating organisms in the laboratory, it was selected as the most promising. What was subsequently achieved was the development of acceptable formulae for extrapolating from observed sterility-test results on substerilization-dosed product samples to the dose required to achieve any selected sterility assurance level between 10^{-2} and 10^{-6}.

Table 3

Difference in D_{10} value when organisms were dried on Kaycel* material or on glass

D_{10} value Mrd	
Dried on Kaycel*	Dried on glass
0.04	0.11
0.05	0.09
0.09	0.18
0.21	0.30
0.10	0.26
0.09	0.22
0.17	0.33
0.13	0.43
0.05	0.14
0.17	0.28
0.17	0.45
0.31	0.35
0.27	0.32

* Trade Mark

Computer simulations were used to analyze the behaviour of microbial populations of varying numbers and radiation resistances at defined increments of dose.

More than 30 000 experimental design simulations, using 90 different populations of microbial contamination, were used to create the substerilization test data and to verify the formulae.

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Summary of Methods

Method B1. Dose Setting Using Bioburden Enumeration

The sterilizing dose in this method is determined by reference to a derived microbial population (Table 4) that represents a more stringent challenge to the sterilizing process than that likely to be provided by natural product bioburden or by conventional biological indicators (such as *B. pumilus*).

Method B1 requires four stages of activity:

Stage 1. Determine the average bioburden per entire device, using samples from at least three different production lots.

Table 4

A standard table of microbial D_{10} resistance					
Resistance D_{10} Mrd	0.10	0.15	0.20	0.25	0.28
Frequency	0.654 87	0.224 93	0.063 02	0.031 79	0.012 13
Resistance D_{10} Mrd	0.31	0.34	0.37	0.40	0.42
Frequency	0.007 86	0.003 50	0.001 11	0.000 72	0.000 07

The population in Table 4 (8) was selected after studying microbial-resistance frequency distributions reported in the literature, including that shown in Table 5 plus those obtained from a number of North American companies (Table 6).

Table 5

Microbial distribution resistances (Population reported by Czerniawski and Stolarczyk (12) that was derived from the environment rather than from medical devices)					
Resistance D_{10} Mrd	0.10	0.15	0.20	0.22	
Frequency	0.603 7	0.207 4	0.058 1	0.052 7	
Resistance D_{10} Mrd	0.28	0.33	0.39	0.46	0.52
Frequency	0.045 0	0.019 5	0.009 4	0.003 7	0.000 5

Table 6

Microbial distribution resistances (Population representing microbial resistances likely to be found on medical devices of a cellulosic nature, derived from Whitby and Gelda (13))
Resistance

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D_{10} Mrd	0.08	0.10	0.15	0.20	
Frequency	0.550 9	0.262 6	0.131 6	0.035 1	
Resistance D_{10} Mrd	0.25	0.27	0.29	0.31	0.33
Frequency	0.011 0	0.003 9	0.002 5	0.001 6	0.000 8

Stage 2. Select a convenient sample item proportion (SIP) for sterility testing purposes and determine the dose that will provide a Sterility Assurance Level (SAL) of 10^{-2} for the chosen SIP from the bioburden versus SIP Table 7 (14).

Procedures for interpolation between both bioburden and SIP values as given in the following table are provided in the AAMI Guidelines.

Table 7

Gamma sterilization verification dose setting table given average bioburden per device and sample item proportion (Verification dose given in Mrd)

Average bioburden per entire device													
DEVICE SIP	2	5	10	50	100	500	1000	5000	10^4	$5x10^4$	10^5	$5x10^5$	10^6
1.0	0.36	0.46	0.52	0.71	0.80	1.00	1.10	1.32	1.42	1.66	1.76	2.01	2.12
0.1	**	**	**	0.46	0.52	0.71	0.80	1.00	1.10	1.32	1.42	1.66	1.76
0.01	**	**	**	**	**	0.46	0.52	0.71	0.80	1.00	1.10	1.32	1.42
0.001	**	**	**	**	**	**	**	0.46	0.52	0.71	0.80	1.00	1.10

** Verification doses for these SIPs are not recommended. An alternative dose setting strategy (B2, B3, or B4) should be used.

Stage 3. Irradiate 100 product samples of the chosen SIP at the SAL 10^{-2} dose selected in Stage 2. The delivered dose must not exceed the target dose by more than 0.05 Mrd, or 5%.

Perform sterility tests on the 100 irradiated samples. If no more than two positive results are obtained from the tests, the SAL 10^{-2} dose may be considered verified.

Stage 4. Select the SAL appropriate to the end use of the product and use Table 8 to select the sterilizing dose that provides the selected SAL.

Table 8

Gamma sterilization dose setting table given average bioburden per device and SAL (SAL dose given in Mrd)

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Average bioburden per entire device													
Log (SAL)	2	5	10	50	100	500	1000	5000	10^4	$5x10^4$	10^5	$5x10^5$	10^6
-3	0.60	0.72	0.80	1.00	1.10	1.32	1.42	1.66	1.76	2.01	2.12	2.37	2.49
-4	0.88	1.09	1.10	1.32	1.42	1.66	1.76	2.01	2.12	2.37	2.49	**	**
-5	1.19	1.33	1.42	1.66	1.76	2.01	2.12	2.37	2.49	**	**	**	**
-6	1.52	1.67	1.76	2.01	2.12	2.37	2.49	**	**	**	**	**	**

If this table does not provide dose, then an alternative strategy (B2, B3, or B4) should be used.

For cases where the actual bioburden and/or the SIP fall outside certain bounds of those given in Tables 7 and 8, interpolation is allowed. Interpolation in the tables is not required if the average bioburden of the device and the SIP are within 20% of the tabled values.

Method B1 is the least difficult to perform, is the least expensive, and provides the most conservative sterilizing dose estimate. As such, it is the most frequently used method.

Method B2. (DS Method) *Dose Setting Using Fraction Positive Information From Substerilization Dosing of Representative Product Samples*

This method is based upon analysis of the measured response of natural bioburden on representative product samples exposed to preselected incremental substerilizing doses of radiation.

Sterility testing of the irradiated product samples provides:

1. The lowest dose at which some sterile samples are obtained – First Fraction Positive dose (FFP).
2. The first dose at which all samples become sterile (FNP). The difference between the FNP dose and the FFP dose defines a standardized dose setting (DS) window in the experimental sterility test data that is used to determine conservatively the more resistant components of the natural microbial contamination (Figure 2).

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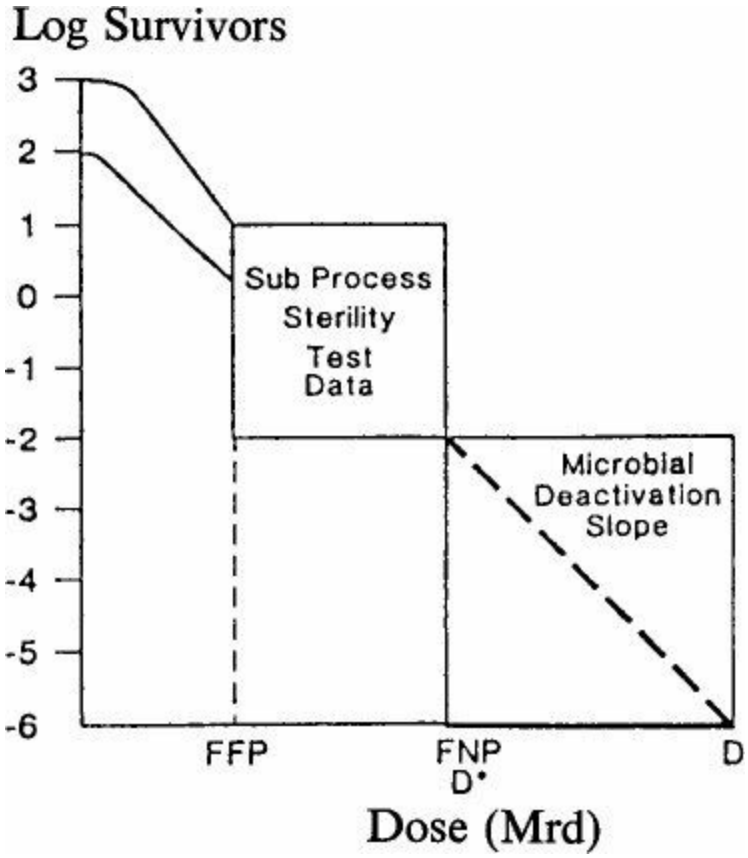

Figure 2.

Formulae are provided which use this information to determine the sterilizing dose that will achieve any required SAL.

It should be emphasized that the method requires neither enumeration of bioburden nor the resistance determination of microorganisms.

While the actual methodology in the AAMI Guideline provides for variability in the delivery of the designed incremental doses, the following example in the interest of clarity assumes that the delivered dose equals the target dose.

The method requires four stages of activity:

Stage 1: Sampling

Randomly select 280 samples of a convenient but representative sample size (SIP) from each of three independent production lots.

Stage 2: (Experiment 1) First Substerilization Irradiation and Sterility Testing Equipment

(1) Perform a substerilization dose experiment in which 20 samples are irradiated at each of nine incremental doses, from 0.2 to 1.8 Mrd as shown in Table 9 (100 items from each lot are saved for Stage 3 – Experiment 2).

Table 9

Number of samples required for the experiments

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Dose Mrd	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	Hold samples for Stage 3 experiment	Total samples required
Lot 1	20	20	20	20	20	20	20	20	20	100	280
Lot 2	20	20	20	20	20	20	20	20	20	100	280
Lot 3	20	20	20	20	20	20	20	20	20	100	280

(2) Perform sterility tests on the irradiated samples and record the number of positives observed at each dose as shown in Table 10.

(3) Determine the first fraction positive dose (ffp) for each of the three lots that, in this case, is Lot 1, 0.4 Mrd; Lot 2, 0.4 Mrd; Lot 3, 0.2 Mrd.

The primary First Fraction Positive (FFP) dose for the experiment is the median of the three lot ffp doses adjusted downwards to the theoretical dose where 19 out of the 20 samples would be positive (Table 11). In this example, the FFP dose is adjusted to 0.305 Mrd by deducting from it the augmentor dose (A) obtained from Table 11.

Table 10

Positive sterility by incremental dose

Dose Mrd	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8
Lot 1	20	12	2	0	0	1	0	0	0
Lot 2	20	7	3	0	0	0	0	0	0
Lot 3	19	9	0	1	0	0	0	0	0

(4) Determine the d* dose (the first incremental dose at which all samples are sterile) for each of the three lots. In this case: Lot 1, 0.8 Mrd; Lot 2, 0.8 Mrd; Lot 3, 0.8 Mrd.

The primary D* dose for the experiment is the median of the three lot d* doses, provided no individual lot d* dose exceeds the median d* by 0.5 Mrd or more, in which case D* is equal to the maximum d* dose. In this case D* is equal to d* in Lot 1 or 2 which is 0.8 Mrd. Lot 1 is therefore defined as the CD* lot.

Stage 3: (Experiment 2) Second Substerilization Dose and Sterility Testing Experiment

(1) Irradiate the 100 remaining samples from the CD* lot (Lot 1), determined in Stage 2 (Experiment 1), at the D* target dose of 0.8 Mrd. The actual dose delivered at this experiment

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which may vary from the target dose is denoted as the DD* dose.

(2) Perform a sterility test on the 100 samples irradiated at DD*. The number of positives resulting from this test is designated as CD*. In this example we will assume one positive.

(3)Establish the First No Positive (FNP) dose for the experiment:

(a) If CD* is zero, FNP is equal to DD*.

(b) If CD* is greater than zero and less than 10 positives, FNP is equal to DD* + 0.2 Mrd.

(c) If CD* is greater than 9 positives and less than 16 positives, FNP is equal to DD* + 0.4 Mrd.

(d) If CD* is greater than 15 positives, D* should be redetermined.

In this case, FNP = DD* + 0.2 Mrd = 1.0 Mrd.

Table 11

The dose setter augmentor (A)

Number of positive sterility tests at FFP	A Mrd
19	0.000
18	0.013
17	0.022
16	0.031
15	0.038
14	0.045
13	0.052
12	0.058
11	0.065
10	0.072
9	0.079
8	0.087
7	0.095
6	0.105
5	0.115
4	0.128
3	0.143
2	0.165
1	0.200
0	0.200

Stage 4: Sterilizing Dose Calculation

(1) Calculate the resistance (DS) of the population surviving the DD* dose.

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When FNP – FFP is less than 1 Mrd, DS = 0.2 Mrd + 0.2(FNP – FFP).

When FNP – FFP is 1 Mrd or greater, DS = 0.4(FNP – FFP).
For the Example, FNP – FFP is 0.64 Mrd, hence
DS = 0.2 Mrd + 0.2(0.64 Mrd) = 0.328 Mrd.

In this case, FNP – FFP = 1.0 Mrd – 0.305 Mrd = 0.695 Mrd. Therefore, DS = 0.2 Mrd + 0.2(0.695 Mrd) = 0.339 Mrd.

(2) Calculate the dose (D**) that will provide a measurable SAL of 10^{-2}.

D** = DD* + [log (CD*/100) + 2](DS)
1.0 Mrd + [log (1/100) +2] 0.339 Mrd
= 1 Mrd + [−2 +2] 0.339 Mrd
= 1.0 Mrd

(3) Calculate the final sterilizing dose required to achieve the SAL appropriate to the end use of the device.

Dose = D** + [− log (SAL) − log (SIP) − 2) (DS)]
= 1.0 Mrd + [− (−3) − 0 − 2] 0.339 Mrd
= 1.339 Mrd

In this instance, the dose required to obtain a SAL of 10^{-3} is 1.339 Mrd.

To provide an interface between the B1 and B2 dose setting methods and the more traditional methods, and to permit the use of historically valid microbial resistance data, two additional methods (B3 and B4) are included in the AAMI Guideline (12). These two methods require a high level of microbiological expertise in that they necessitate a laboratory determination of microorganism resistance. In practice these methods are seldom used.

Method B3. Dose Setting Using Method B2, Substituting Maximum Known Organism Resistances

This method follows exactly the protocol outlined in the first three stages of Method B2.

The final sterilizing dose determination (Stage 4) is modified to allow for the substitution of known maximum bioburden resistance values for the DS value in Method B2:

Dose = D** + [− log (SAL) − log (SIP) − 2](Max D_{10})

The Maximum D_{10} values may be from historical bioburden data or may be derived from the incremental dose experiments conducted in the first three stages of the method.

Method B4. Dose Setting Using Natural Product Bioburden Resistance

In this method a substerilization dose experiment is conducted to determine the dose at which not more than 10 out of 20 samples tested will be positive in a sterility test. Sufficient samples are then dosed at this level to obtain 200 microbial isolates and the radiation resistance D_{10} of each isolate is determined.

The dose required to reduce a population of the observed distribution and resistance to the required SAL is then calculated. This dose is then added to the initial 50%-sterility dose to give the

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sterilization process dose.

This method suffers from the disadvantages that it cannot be readily conducted by most companies because of the extensive nature and high cost of the microbiological effort required. Also, the D_{10} values determined may not be representative of the actual resistance of the microbe as they occur on the product.

Method B5. Sterility Dose Audit

Audit procedures have been developed that are applicable to all four dose setting methods. These procedures are designed to detect changes in the product bioburden that would require an increase in the previously established sterilization dose.

When adjustments are indicated, the audit procedure specifies the magnitude of such adjustments to the dose.

In certain circumstances, the procedure recommends that the dose be re-established rather than augmented.

The audit procedure is performed as follows:

(1) Select 100 representative product samples using the same SIP upon which the sterilizing dose was determined.

(2) Irradiate the 100 samples at the D** dose determined in the original dose setting experiment. (In the case of Method B1, D** is equal to the verification dose given in Table 7.) The delivered dose must be less than D** + 0.05 Mrd or 1.10 D**, whichever is less. If the delivered dose is less than 0.9 D**, the audit may be repeated.

(3) Test for sterility the 100 samples to determine the number of positive samples.

(4) The proper audit action is determined by consulting the Action Criteria versus Number of Positives (Table 12). The proper action associated with the number of positives and the chosen SAL and SIP is explained in the legend for this table.

Table 12

Action criteria for the established dose audit
(criteria based on 100 tests at D**)

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Degree of extrapolation		Action criteria for quality audit at D**				
Log (SAL)	SIP	0+	1+	2+	3+	> 4+
3	1	A	A	A	C	R+ (0.24)
4	1	A	A	A	C	R+ (0.32)
5	1	A	A	C	C+ (0.36)	R+ (0.36)
6	1	A	C	C	C+ (0.40)	R+ (0.40)
3	0.1	A	A	A	C	R+ (0.32)
4	0.1	A	A	C	C+ (0.36)	R+ (0.36)
5	0.1	A	C	C	C+ (0.40)	R+ (0.40)
6	0.1	A	C	C+ (0.38)	R+ (0.44)	R+ (0.44)
3	0.01	A	A	C	C+ (0.36)	R+ (0.36)
4	0.01	A	C	C	C+ (0.40)	R+ (0.40)
5	0.01	A	C	C+ (0.38)	R+ (0.44)	R+ (0.44)
6	0.01	A	C+ (0.33)	C+ (0.41)	R+ (0.48)	R+ (0.48)
3	0.001	A	C	C	C+ (0.40)	R+ (0.40)
4	0.001	A	C	C+ (0.38)	R+ (0.44)	R+ (0.44)
5	0.001	A	C+ (0.33)	C+ (0.41)	R+ (0.48)	R+ (0.48)
6	0.001	A	C+ (0.35)	R+ (0.44)	R+ (0.52)	R+ (0.52)

Legend

A = Accept original dose as valid.

C = Caution – original dose requirements may have increased. Check GMPs.

C+ = Caution – original dose requirements have changed; increase dose by the value given in parentheses in Mrd. Check GMPs.

R+ = Re-establish dose – increase dose immediately by the value given in parentheses in Mrd and then re-establish dose. Check GMPs.

Log (SAL) = Log of the Sterility Assurance Level

SIP = Sample Item Proportion

D** = The audit D** is the larger of D* + 0.2 Mrd or D* + [2 + log (CD*/100)](DS + A), rounded up to the nearest 0.1 Mrd, or an audit-augmented D**. When sterilization dose is augmented, augment D** by the same Mrd value.

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Impact on the Health Care Industry of Implementation of Scientific Dose Setting Procedures

As a result of the utilization of these scientific dose setting procedures which accurately account for heterogenous microbial populations and product end use, many products manufactured in North America today are being safely sterilized at doses significantly lower than the arbitrary 2.5 Mrd level. Some of these are included in Table 13.

Table 13

Typical dose ranges for *gamma* sterilized medical products

Product	Dose Range (Mrd)
Diagnostic strips	<0.8
Electrodes	0.8 – 1.5
Saline solution	
Blood collection tubes	
Cotton balls/swabs	
Plastic laboratory ware	
Surgeons' gloves	
Specimen containers	
Disposable thermometers	
Ophthalmic ointments	
Grounding pads	
Syringes	1.5 – 2.0
Infant wear	
Hospital packs	
Surgeons' gowns	
Surgeons' gloves	
Packaging materials	
Catheters	
Empty I.V. solution bags	
Culture collection systems	
Gauze sponges	
Surgeons' scrub brushes	
Bovine serum	
Bandages	> 2.0
Orthopaedic prostheses	
Glove powder	
Stockinette	
Orthopaedic mixing bowls	

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Water-filled syringes
Hypodermic needles
Surgical blades
Surgical sutures
Vascular grafts
Surgical marking pens
Needle counting systems

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Incremental Dose Irradiator

Adoption of scientific dose setting methods in North America by industry with the endorsement of government prompted the design of a commercial irradiator that could deliver a wide range of sterilizing doses efficiently and economically.

The new type of irradiator described delivers an increment of the total dose during each cycle, enabling various levels of sterilization to be achieved in the irradiator at the same time.

Product sterilized in the incremental dose irradiator receives a multiple of a preselected incremental dose. For example, if one product carrier requires a 0.8 Mrd dose, and the second carrier 2.4 Mrd, each receives 0.8 Mrd during the first cycle. The first carrier exits after the first pass. The second carrier passes through the chamber two additional times to receive another 1.6 Mrd dose.

A computerized control system for the incremental dose irradiator has been designed by Isomedix, Inc. This control system ensures that each carrier receives the required number of increments to achieve a preprogrammed dose. Furthermore, the system monitors all stages of the irradiation process and provides documentation.

The progress of each carrier is traced through the irradiator until it arrives at the unloading station. To accomplish this, each carrier is assigned a permanent binary identification number which is read by light sensors strategically located at the product loading station, the irradiator entrance and exit, and the product unloading station (Figure 3).

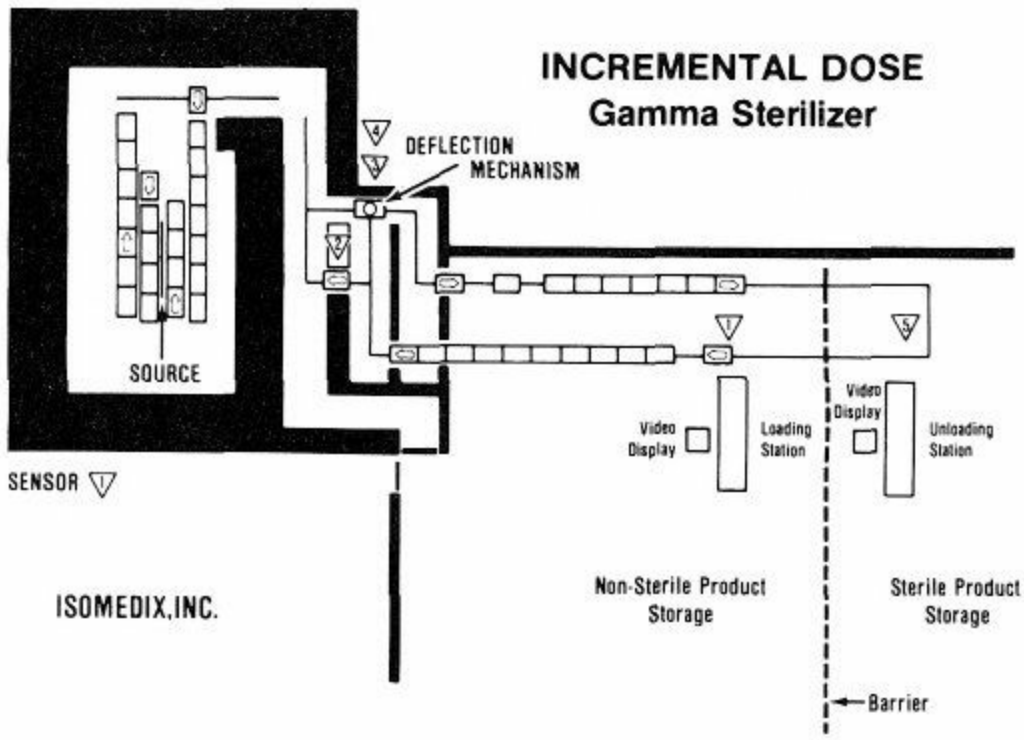

Figure 3.

As the first carrier in a production lot moves into the loading station, Sensor No. 1 places its binary identification number into the computer memory. A video monitor displays the customer name, product lot number, number of cartons to load into the carrier, number of cartons comprising the lot, number of cartons remaining, and placement of dosimeters, if required (Figure 4).

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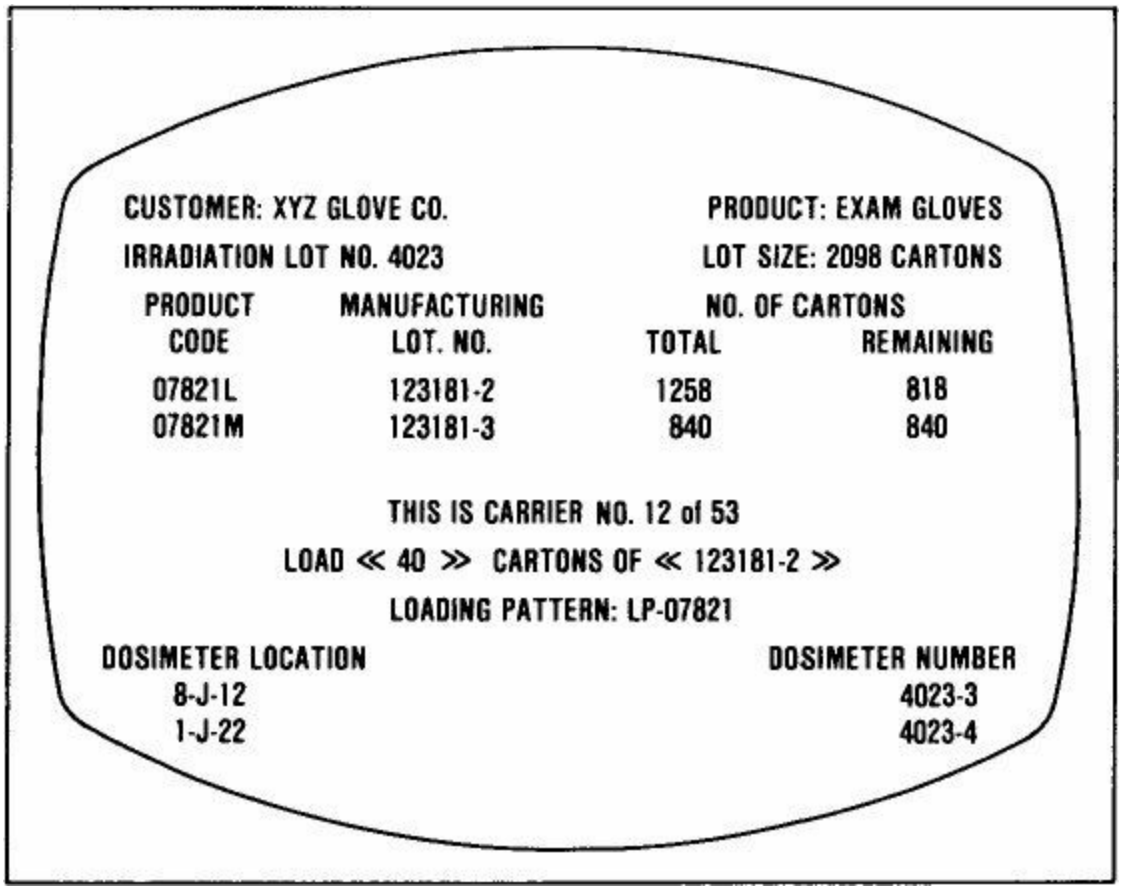

Figure 4.

As the carrier proceeds into the irradiator, Sensor No. 2 reads the assignment number and displays the carrier position, number, programmed number of passes and number of pass currently running on the video monitor located at the control console (Figure 5).

At the exit to the irradiation chamber, Sensors No. 3 and No. 4 read the carrier number. The computer compares the number of cycles completed with the number of cycles required for the total process. If the number of cycles required and the number of cycles completed agree, the Carrier Return Control discharges the carrier into the unloading station on the sterile side of the warehouse. If the comparison does not agree, the control returns the carrier to the chamber for another incremental dose.

At the unloading station, a video monitor displays the same information as was displayed at the loading station for that carrier (Figure 4). This display informs unloading personnel what product is in the carrier and the location of dosimeters in the carrier, greatly speeding up the handling process.

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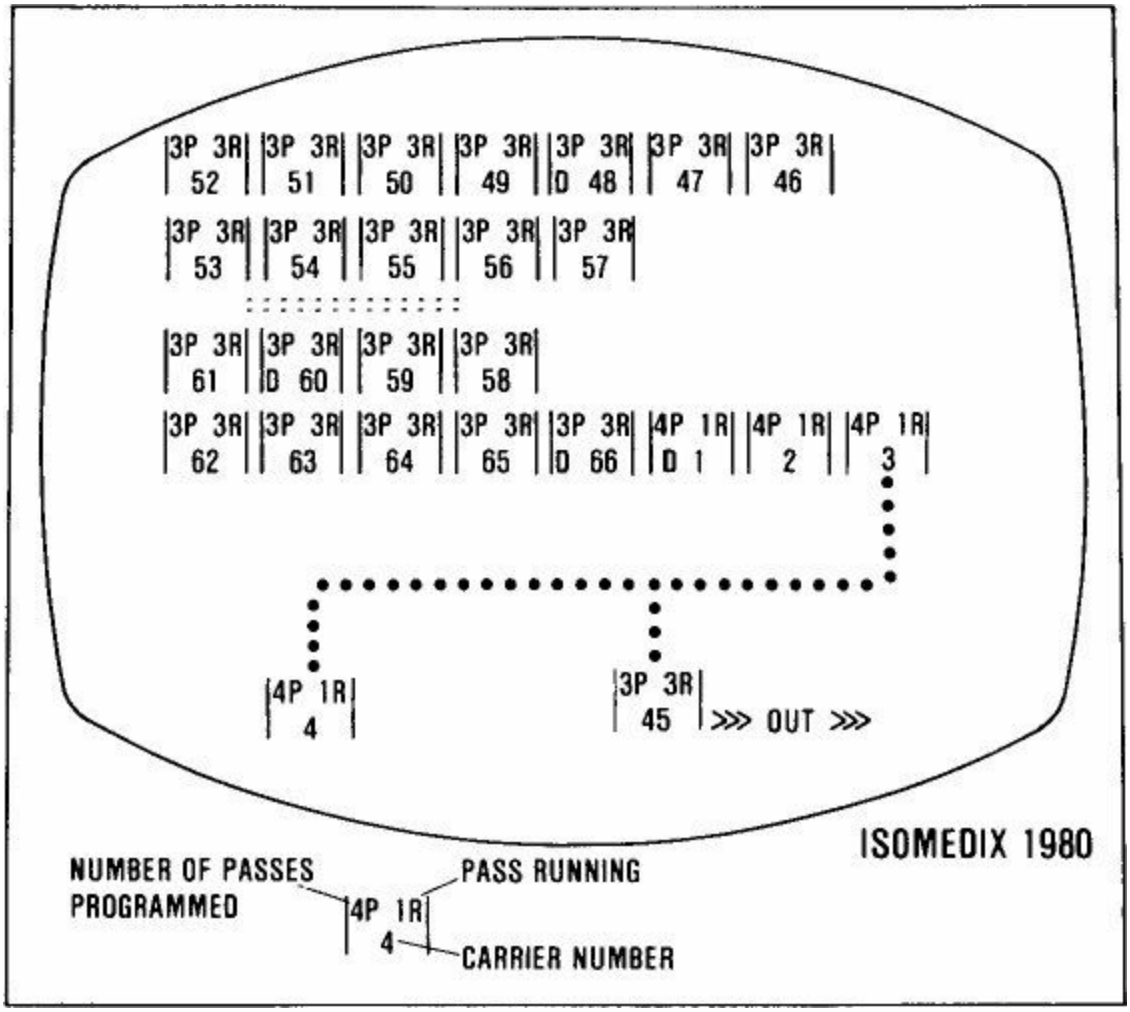

Figure 5.

As a final measure, the computer prints out hard copy documentation of all the information relating to the product.

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Conclusion

I would like to leave you with a thought expressed by Dr Van Winkle of Ethicon, Inc. at a meeting of experts on radiation sterilization held in Vienna in 1966. ‘Legislation which sets arbitrary figures for irradiation dosage, source design or other manufacturing parameters can never assure safe or efficacious products, and needlessly hampers future research and development.’ (15).

The health-care industry and the governments of North America agree and, accordingly, no longer require the delivery of arbitrary, fixed sterilizing doses. The dose setting methodologies described herein have played an important role in making this possible. We hope that you will give them your earnest consideration.

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Glossary of Terms for Gamma Radiation Dose Setting and Auditing

NOTE: *Notation is in lower case when it refers to results for samples from single lots, and upper case when it refers to a summary for all three lots*.

Bioburden. The total of all viable microbes on a packaged item or unit immediately prior to radiation sterilization processing.

*CD**. The number of positive sterility tests from 100 samples irradiated at D*.

D_{10}. The radiation dose required to kill 90 per cent of the organisms of a homogenous microbial population. It is defined on the basis that the death of microbes follows first order kinetics.

*d**. For each lot of incrementally dosed samples, d* is equal to the minimum dose of (1) or (2) below:

(1) the first incremental dose at which 0/20 positives occur, immediately followed by 0/20 positives;

(2) the first incremental dose at which 1/20 positives occur, immediately preceded and followed by 0/20 positives.

*D**. An initial estimate of that dose of irradiation that will provide a SAL of 10^{-2} for a sample. Subject to some exception, it is the median of the three d*s.

*D***. The sterilization audit dose at which no more than 1 in 100 samples are expected to be nonsterile.

DS + A. An effective D_{10} value assigned to the population of microorganisms surviving a dose of D* Mrd. Both DS and A are obtained from tables on the basis of the sterility test results of incrementally dosed samples.

Fraction Positive: A quotient with a number of positives in the numerator and the number of samples in the denominator.

first fraction positive (ffp). The lowest incremental dose for a given lot at which at least one of twenty samples is sterile.

First Fraction Positive (FFP). The median value of three lot ffps.

First No Positive (FNP). The lowest dose in an incremental sterilization dose series at which 100 samples of a lot are expected to be sterile.

Incremental Dose. Irradiation doses ranging from 0.2 Mrd to 1.8 Mrd, delivered in increments of 0.2 Mrd.

Sample. The experimental unit that is either the whole item or unit, or a proportional part as determined by weight, volume, or surface area, chosen so as to represent validly the bioburden.

Sample Item Proportion (SIP). The proportion of the item that was sampled for dose setting procedures. When small items such as sutures are sampled, an entire item (SIP = 1) should be taken for testing; whereas when large items such as surgical gowns are sampled, it may be necessary to select only a portion of the item, e.g. 1 per cent (SIP = 0.01).

Sterility Assurance Level (SAL). The expected maximum probability of an item or unit being nonsterile after exposure to a valid sterilization process. SALs range from 10^{-3} to 10^{-6}, depending on product use.

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DISCUSSION SESSION V – Part 2

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Q. by B. Graham – Australia

Mr Masefield, I have the impression that the method you cite may critically depend on the number of organisms of high resistance at the upper end of the distribution and if so, do you regularly do any work to verify that the distribution is reasonably constant or do you depend on audit procedure?

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A. by J. Masefield – USA

The audit procedure is the technique. Naturally, in the case of the B1 Method where a resistant distribution has been selected, there is a consistent attempt to compile additional data in a general way to make sure that the distribution is more stringent than what is found in practice.

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Q. by F.L. Hebbard – Australia

Mr Masefield, you have referred basically to commercially sterilized products and the basis of bioburden to reduce dosage. Let us take an example. If I were an importer and I was receiving a large shipment of densely packed items with gross soil and a heavily contaminated resistant spore-bearing organism, how would the persons in the radiation field to whom these items may be sent assess what dosage should be given, and is there a method to assess bioburden levels?

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A. by J. Masefield – USA

I would recommend that you carry out one of the dose-setting strategies on representative samples of the lot of the product being imported. The beauty of these methods is, in my view, that they are not complicated and not expensive. You need access to an irradiator where you can deliver incremental doses but that is all, you require. The rest is a few sterility tests and a calculation. It is, therefore, a very practical proposition to carry out a dose-setting experiment to know where you stand in any situation with resistant organisms.

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Q. by A. Tallentire – UK

One cannot help but admire the enormous amount of work which this group has put in in order to get to this level of sophistication. I think, we owe them a great debt of gratitude for pushing forward the frontier of radiation sciences. You mentioned my name on several occasions, John, and I would like to include Frank Lahey and John Dwyer, who were in on the early hypotheses that form a part of the AAMI guidelines. I would now like to ask you a question. Both the B1 and B2 Methods require the recovery of microorganisms. The B1 Method requires a bioburden determination on unirradiated microorganisms. The B2 Method uses a sterility test technique to detect microorganisms that have been irradiated and my question is: do the AAMI guidelines in any way give guidance regarding optimization of recovery conditions and hence give a conservative estimate of the dose to be set?

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A. by J. Masefield – USA

No, AAMI have not optimized methods that should be adopted to test sterility. They do not make recommendations on culture media and leave that to the judgment of the manufacturers. I should, perhaps, explain one thing. Voluntary guidelines in North America are not intended as rigid documents. The situation is dynamic, and the guidelines are being constantly upgraded. The most recent issue enables some interpretation. For one thing, when it says to deliver, say, 0.8 Mrd, one does not deliver 0.8 Mrd, as it usually comes out to be 0.76 or 0.84 Mrd. We have to do a great deal of work to understand how to handle interpretation in the real world. This subject is covered in the most recent edition. The first edition failed to do this.

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Q. by N. Hilmy – Indonesia

I would like to ask John Masefield to comment on how we see the AAMI guidelines in my country. We have had difficulties in irradiating 100 samples together, especially if the density of the material is high, such that we could not get a minimum dose homogeneity. If we irradiate at the minimum dose for the verification experiment, it means that the maximum doses are higher than the dose stated in the verification experiment; that is not fair for the initial contamination. Our source is not very large, so dose rates are not very high, and it is a problem to get the dose homogeneity in 100 samples. I would like also to ask if the aim of the AAMI guidelines is to get a more practical and, of course, an acceptable method. I would also like to ask about Method 4. If you still use the D_{10} value of the most resistant microbes, it means that the guideline is complicated. I am not an experienced microbiologist, but to isolate and determine the D_{10} of the most resistant microbe is not easy, especially in our laboratory.

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A. by J. Masefield – USA

I will answer your last question first. I do not mean to be facetious, but you will notice that on the AAMI Committee we had microbiologists, statisticians, regulatory people, and experts in radiation. The only time I have seen Method B4 used, it required the D_{10} determination of 200 isolates, and it was considered by the company who did it that it was the first and last time that they would go through with it. The method is extremely difficult and requires very sophisticated facilities to get a representative answer.

Concerning your first point, it is very important that you divide your samples into packages where the dose for the incremental dose experiment is uniform throughout. You can put them into one thousand packages, provided you can calibrate. You mentioned that you have a small source; therefore, you have a low dose-rate which means that you can deliver the dose very accurately, as you have a long time exposure. Therefore, you do have the opportunity to do it accurately. It is worth remembering that having set a dose, we are talking about a dose that will be delivered in the low dose zone of the irradiator. This really means that there is a very substantial added safety factor that in practice is being applied. It is only a small percentage of the volume of the irradiation container that receives the low dose, everything else is receiving 20%-30% more. You know, dose uniformity is typically 1.3 to 1. Really, you have a 20-odd per cent overdose every time you put something in the irradiator.

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SESSION V

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Sterilization Process Technology – Part 3

Chairman
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Silver Spring, Maryland, USA

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SESSION V

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Sterilization Process Technology – Part 3

Introduction to Session

Richard J. DeRisio

Ethylene oxide (EO) has gained wide acceptance in industry and hospitals throughout the world as a sterilant for medical devices. It is the simplest epoxide, a cyclic ether, and a highly reactive alkylating agent. At room temperature and atmospheric pressure, EO is a colourless gas with a characteristic ether-like odour having a widely variable threshold in humans. The mean lowest detectable concentration in air is approximately 700 ppm which is well above the maximum permitted exposure level for workers.

Ethylene oxide has several advantages as a sterilant:

1. The levels of relative humidity and temperature ordinarily used cause little deterioration of many component materials of medical products that would be destroyed at the high moisture and temperature levels employed with steam sterilization.
2. Some materials that are irreversibly damaged by radiation through discoloration or embrittlement can be sterilized by EO.
3. The equipment is not so expensive as to preclude performing sterilization in one's own plant.
4. When properly used and validated, EO is a very effective sporicidal agent capable of delivering a high degree of lethality to a product load in a reasonable time.
5. The high diffusivity of EO permits sterilization of products in their final primary and secondary packages.

There are disadvantages to the use of EO, however.

1. Ethylene oxide sterilization involves several variables. Moreover, sophisticated process instrumentation providing accurate measurement of chamber parameters such as temperature, gas pressure (and/or concentration), and relative humidity may lull some users into a false sense of security. We are in fact interested in the values of these parameters at the spore contamination sites on the device rather than in the chamber space external to the load where the measuring and control instruments are customarily located. Validation must provide a measurable observed correlation between chamber variables and the actual lethality delivered to the devices themselves.
2. Preconditioning for temperature and relative humidity may be required, depending upon the packaging, storage history of the device, and perhaps climate. It is particularly desirable that loads sterilized under contract be preconditioned so that in mixed loads there is no mass transfer of water vapour from one portion of the load to another during the early stages of the

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process cycle. Preconditioning products sterilized under contract can normalize loads that due to an unknown history in shipping, storage, and handling may have variable relative humidity levels.

3. Residues of EO and its reaction by-products, ethylene glycol and ethylene chlorohydrin, may be objectionable for certain devices; thus there must be a means for aeration of the lot according to a validated procedure that reliably effects the desired residue levels.
4. The hazards associated with exposure of workers to EO gas warrants controls on equipment design, the manner in which the cycle and equipment are operated, and the conditions under which the devices are aerated.

The properties of EO that render it such an effective sterilizing agent are also responsible for its toxicity to humans. Accordingly, it is appropriate to include a session on the subject of EO in this Symposium because of the continuing interest by regulators and the industry in its use in two major areas: effective cycle development and validation, and the safety of workers exposed to EO. This afternoon's session will address specifically these two issues and it is fortuitous that our speakers are internationally recognized as experts in their respective areas.

Regulatory authorities in the US and in other countries are concerned with two aspects of EO toxicity in humans. One is patient exposure to residues of EO and its reaction products, ethylene chlorohydrin and ethylene glycol, that may be present on the device after sterilization. The other is the exposure of the hospital or plant employee to EO gas during sterilizer operation and related activities. These are independent concerns and each must be addressed separately.

The control of EO residues is a matter subject to FDA jurisdiction. Under the device GMP regulation, EO is considered a manufacturing material, that is, a substance used to facilitate the manufacturing process but which is not intended to be included in the finished device. The US GMP regulation requires, in section 820.60 (d), that such materials be removed from the device or reduced to a level that does not adversely affect the device's fitness for use. In addition, records must be kept documenting the removal of manufacturing materials. FDA considers it acceptable for a firm to perform a one-time study of device aeration (including reprocessed loads if applicable) with the development of residue data in the form of dissipation curves, for example. Thereupon, a firm need only demonstrate that devices from each sterilizer load were aerated under the same conditions as those used in the study. Of course, one presumes that the sterilization cycle has not changed.

In 1978, FDA proposed a regulation entitled 'Ethylene Oxide, Ethylene Chlorohydrin, and Ethylene Glycol: Proposed Maximum Residue Limits and Maximum Levels of Exposure'. Although this document has never been finalized, the residue levels proposed for several types of medical devices have been used as guidelines by the industry and FDA. For example, in the review process for a new medical device sterilized with EO, the FDA scientific reviewer will use the proposed levels as a guideline in evaluating the firm's residue levels. During FDA inspections, the proposal is used by investigators when reviewing a manufacturer's actual residue level data. However, the Office of Medical Devices in the review process may require lower residue levels than those in the *Federal Register* announcement. A subject of intense interest now is the development of standard methods for residue determination, an issue currently being considered by a committee of the Association for the Advancement of Medical Instrumentation (AAMI).

Perhaps one of the most effective means of reducing product residues (and unnecessary device

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outgassing in the workspace) is through conscientious cycle development that provides adequate process lethality without excessive overexposure to the degree that residues attain unreasonably, and perhaps hazardously, high levels. Intelligent cycle development can help minimize product residues.

Users of ethylene oxide sterilization have traditionally employed overkill cycles that are developed using challenges consisting of a large number of microorganisms that are highly resistant to the sterilant. This approach permits accommodation of fairly large fluctuations in natural product bioburden without jeopardizing product safety. Another aspect of the overkill cycle is that it provides for a safe product even if there have been unpredicted and perhaps unnoticed fluctuations in sterilization parameter values in the chamber or, more pertinently, at the contamination sites on the device. A disadvantage with overkill cycles is that they may result in unnecessarily high residue levels.

On the subject of the work environment, earlier this year, the US Occupational Safety and Health Administration (OSHA) published an advance notice of proposed rulemaking. Based upon scientific data summarized in its *Current Intelligence Bulletin #35*, the US National Insititute for Occupational Safety and Health (NIOSH) recommended that ethylene oxide be regarded as a potential occupational carcinogen and that OSHA's present exposure limit of 50 ppm be re-examined. In fact, several companies had already lowered their internal employee exposure limits to levels substantially lower than 50 ppm. NIOSH estimates that approximately 75 000 health-care workers in sterilization areas in the US are potentially exposed to EO and that 25 000 others are incidentally exposed.

The American Conference of Governmental and Industrial Hygienists (ACGIH) is an internationally recognized organisation that sets workspace exposure levels for hazardous substances. Earlier this year in their 1982 Guide, ACGIH proposed and published an EO level of 1 ppm, measured as an eight-hour time-weighted average. Following the customary two-year comment period for such proposals, that level will become an ACGIH standard.

In January 1982, OSHA published in the *Federal Register* a request for comments concerning its intent to propose rulemaking. Citing the activities of organizations such as ACGIH, and summarizing experimental findings concerning the effect of ethylene oxide on worker health, this notice requested comments concerning the effect of lowering of worker exposure levels from the current 50 ppm eight-hour time-weighted average to any of the several levels under consideration, namely 0.5, 1.0, 5.0, and 10.0 ppm. Comments on several very specific issues were requested. To generalize, these issues related to actual human and animal data concerning exposure to EO, the adequacy of the present 50 ppm level, the risks associated with the lower levels subject to proposal, suitability of current clinical methods used to assess the effects of EO exposure, the practicability of several engineering alternatives for controlling worker exposure, and certain economic and environmental data related to commercial use of EO.

It appears that significant improvements in the workspace environment could be made through an educational programme directed toward health-care and industrial users of EO. Such training might include, in part, a presentation of actual observed workspace hazards such as those reported in the literature by Dr Glazer. The following situations were encountered in a limited field study:

- a sterilizer exhaust vent passed through a window and ended within one foot of the intake for an air-conditioning unit;
- a floor level sterilizer drain discharged directly into the room where the sterilizer was located;

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the local EO concentration measured one foot above the drain during a cycle was 8000 ppm;

- immediately upon opening the sterilizer door, an EO concentration of 1200 ppm was observed in that area;
- EO concentrations above and behind aeration cabinets venting directly into the room in which they were installed ranged from 300 to 500 ppm.

There are literature reports of the physiological effects of such worker exposure, and clearly exposure levels can be reduced significantly by good design. I believe, we should understand its importance and be made aware of the safety of workers exposed to ethylene oxide gas.

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Ethylene Oxide Sterilization Process Development and Validation

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Introduction

Of the approximately 7,000 million pounds of ethylene oxide (EO) produced annually in the United States, less than 0.5% is used by the medical device and diagnostic product industry for the purpose of delivering health-care products free from microorganisms. Although on a relative scale, the amount of ethylene oxide used for sterilization purposes may seem trivial, it represents a quantity capable of processing 10 to 12 thousand million items on an annual basis (10).

In many countries, ethylene oxide is under intense scrutiny by manufacturers, users, and regulatory agencies due to its toxic properties. It is considered by the National Institute for Occupational Safety and Health (NIOSH) as a mutagen and a potential human carcinogen in the workplace. Nonetheless, this highly reactive alkylating agent is considered essential to the international health-care system. Indeed, in the vast majority of cases, there are simply no practical alternative methods for the sterilization of high-volume single-use medical products. It is for this reason that approximately 65% of the US medical device firms recently inspected by the FDA use an ethylene oxide process (8).

In addition to considerations for safe handling and limiting worker and environmental exposure, successful application of ethylene oxide as a gaseous sterilant in the health-care profession requires a thorough understanding of concepts and procedures covering product and package compatibility, equipment selection, cycle development, validation, certification, and routine operation.

Unlike steam and irradiation processes, EO sterilization requires accurate control of many critical process variables (Figure 1). An understanding of the interrelationship of these variables and how they impact on process reliability, product functionality, and product sterility assurance has been a concern of the Association for the Advancement of Medical Instrumentation (AAMI). This professional association recently published a document entitled *Guideline for Industrial Ethylene Oxide Sterilization of Medical Devices*, the contents of which is the theme of this presentation (1).

	STERILIZATION PROCESS				
CONTROLS REQUIRED	DRY HEAT	LIQUID/ CHEMICAL	EO	STEAM	RADIATION
Time	*	*	*	*	*
Temperature	*	*	*	*	
Pressure			*	*	
Vacuum			*	*	
Humidity			*	*	
Packaging	*	*	*	*	
Concentration		*	*		
pH		*			

Figure 1. Controls required for various sterilization processes (13)

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Historical

Unlike the more familiar thermal methods of sterilization, the use of ethylene oxide is a relatively recent development. Initially its pesticidal properties were limited to the killing of insects (4). In 1933, Gross and Dixon (9) filed a patent describing a method of sterilization based on the microbiocidal properties of ethylene oxide. Somewhat later, ethylene oxide was found to be an excellent gaseous fumigant for treating pepper and other spices (3).

It has been almost 35 years since Phillips and Kaye (16) published a series of now classical papers (11, 17) in which the parameters for inactivating bacterial spores using ethylene oxide were described. As a result of these and subsequent reports, notably by Bruch (2), Ernst and Shull (5), Ernst and Doyle (6), and Kereluk, Gammon, and Lloyd (12), the practical use and application of this agent as a sterilant has been established in a wide variety of fields ranging from food processing to interplanetary space vehicles and probes. Truly large scale industrial use for medical products has occurred only in the last 20 years.

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Process Development

There are four broad phases that characterize process development using gaseous ethylene oxide:

Equipment Selection
Cycle Development
Product/Package Functionality
Aeration

By far the most demanding phase is that of cycle development.

Equipment Selection

The major equipment components of the sterilizing system include the preconditioning chamber (where necessary), exposure vessel, ethylene oxide feed system, aeration facility, steam supply, utilities, and process control and monitoring hardware. Equipment must be shown to function reproducibly while maintaining calibration tolerances, and provide adequate documentation of process parameters. Physical location of equipment within the manufacturing facility can also be important. For example, the location of preconditioning should facilitate timely transfer of product from the preconditioning chamber to the sterilization vessel.

Equipment should be selected to provide a chamber control temperature range during gas exposure that is less than or equal to ±3°C about the nominal cycle temperature during gas exposure. Heat transfer and temperature control equipment should be capable of limiting product temperature variation. A temperature range across the product load of less than or equal to 10°C during gas exposure is common.

Sterilizers now incorporate microprocessors and computers for process control. Such computerized systems can provide more accurate process control over the electro-mechanical controls found in most sterilizers currently in use (15).

Cycle Development

Cycle development studies should be carried out on a product in its final design and package configuration whenever possible. The two phases of cycle development are: (a) physical process parameter evaluations and (b) microbial challenge systems.

(a) *Physical Process Parameters*

The AAMI Guideline (1) recommends that several physical process parameters be investigated during cycle development, including product load characteristics, chamber humidification, chamber air content, and EO addition.

1. *Product load density and geometry* are important, since changes in product density, number of cartons, or pallet configuration may significantly affect the ability of the sterilant to penetrate the product and destroy viable microorganisms. Large, dense loads may hinder penetration. For routine processing, a standard load configuration is extremely important.
2. *Chamber humidification* refers to humidification within the sterilization chamber, as opposed to "preconditioning" in a separate, environmentally controlled facility *before* the product is placed in the sterilizer. Humidification is accomplished by the addition of steam, which simultaneously raises the temperature of the load and provides the moisture necessary for effective kill. The relative humidity and dwell time must be sufficient to moisturize the driest

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product that is anticipated during routine processing.

3. *Chamber air content* is an important parameter. Evacuation of the chamber removes the air barrier, thus facilitating the penetration of gas and heat and allowing the injection of EO to concentrations appropriate for sterilization without excessively high pressures. When ethylene oxide 100% is used, air removal is important because of the explosive properties of EO/air mixtures. Evacuation by means of vacuum pumps or steam ejectors is common practice; air removal by displacement is less common. The so-called 'air-displacement cycle' requires specific purging conditions to ensure that the required ethylene oxide concentration is reached. The *rate* of evacuation can also impact sterilization effectiveness, since slow draw-downs may remove excessive amounts of moisture and dry out the product.
4. For the *gas addition* phase of the cycle, liquified EO taken from a storage cylinder must be converted into a gas prior to injection into the vessel. This is accomplished by heating the liquid in a volatilizer or heat exchanger. The temperature of the gas entering the vessel should be at or above chamber temperature. However, high inject gas temperatures can result in product damage and in drying of the load, which will adversely affect microbial inactivation. Low inject tempteratures will cool down the load, causing the exposure temperature to drop below the minimum needed, and will increase the risk of gas stratification.

– *Ethylene Oxide Exposure Cycle*

The four critical parameters to be studied during cycle development are:

Ethylene oxide concentration
Relative humidity
Temperature
Exposure time

1. *Ethylene Oxide Concentration*

   Ethylene oxide concentrations in excess of 400 mg/L are commonly used. As the concentration of EO is increased, lethality also increases within certain limits. The use of very high EO concentrations, to enable a corresponding reduction in exposure time, must take into account the possible negative impact on EO residuals. From a practical standpoint, increased concentration will also mean an increase in pressure, which can become especially significant with EO/chlorofluorocarbon mixtures. As mentioned previously, inadequate moisture can retard sterilization; however, excessive moisture can react with EO and thereby reduce its effective concentration. Excessive moisture can also result in product damage. To prevent stratification of EO mixtures within the chamber, circulation fans may be necessary. As in any dynamic chemical system, the concentration of the reactants has a profound effect on the outcome of the reaction.
2. *Relative Humidity*

   The effect of moisture, in the form of water vapour, on EO sterilization has been the subject of numerous reports in the literature (7, 11). There is no question that moisture is an important factor in achieving sterility. Desiccated bacterial spores are extremely difficult to kill with EO; however, when the spores are exposed to adequate relative humidity, this resistance can be eliminated. The AAMI Guideline (1) notes that relative humidity in excess of 30% is common practice. Moisture in the chamber is not enough – it must reach the site to be sterilized.

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3. *Temperature*
   Temperature affects the permeability of gaseous EO through packaging materials and through microbial cell walls. The classic studies of Phillips and Kaye (16) showed that sporicidal activity increased by approximately threefold for each 10°C rise in temperature. Within limits, the cycle time can be reduced if the temperature is elevated. Product temperature should be controlled by correlation with chamber temperature. When the product does not contain thermocouples during routine processing, the specification of minimum/maximum chamber temperature is necessary. When conditioning takes place within the chamber, heating may be necessary to achieve minimum product temperature before exposure time is initiated; again, relative humidity should be maintained at a level greater than 30%. Temperature also influences pressure and, since gas concentration may be determined by pressure readings, the temperature must be specified.
4. *Exposure Time*
   Developmental studies must determine the minimum exposure time needed to achieve the desired margin of safety. Exposure cannot be initiated until all minimum process specifications are met. In practice, this means that all thermocouples must come up to temperature. Significant factors affecting exposure time are the degree of humidification of product and the nature of the packaging material used. Low EO concentration and/or low temperature frequently result in longer exposure times to achieve sterility.

Postexposure Process Parameters

Upon completion of the exposure phase of the cycle, two processing steps remain: evacuation and chamber ventilation.

1. *Evacuation*
   After the exposure phase of the cycle, the chamber must be evacuated to remove the sterilizing atmosphere. A sharp drop in pressure should be avoided, however, since product and/or package damage may result. The most common example of this type of damage is the 'ballooned package', a result of the limited permeability of many packaging materials. To prevent such damage, a maximum evacuation rate must be specified. This is the point in the cycle when materials are most susceptible to damage, since the product and package have been subjected to elevated temperatures for the longest period of time.
2. *Chamber Ventilation*
   Safety procedures and precautions are necessary to ensure that the sterilizer operator is not exposed to EO when opening the door and unloading product. For example, operators could be instructed to open the sterilizer door slightly and wait for a specified period of time before unloading the chamber, or to wear approved gas masks for protection. Ventilation systems should be designed in such a way as to provide air-flow patterns and velocity sufficient to protect manufacturing personnel in the vicinity of the sterilizers as well as sterilizer operators.

Conditioning

The discussion at this point should perhaps return briefly to a consideration of product humidification prior to EO exposure, since some consider this to be the most important parameter for achieving success.

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There are two methods of conditioning in current use: external conditioning outside the chamber in an environmentally controlled facility, and conditioning inside the chamber. Frequently, both are used. The method of choice depends on the nature of the materials and the type of cycle. Hard surfaces such as glass, metal, and plastic may require a relatively long period of time to absorb moisture, and external conditioning for significant periods may be necessary. On the other hand, cellulosics, paper, and cloth readily pick up moisture and generally do not require long humidification times; conditioning inside the chamber is generally more than adequate. Some cycles make use of a specialized conditioning step within the chamber prior to exposure, where steam is introduced and vacuum simultaneously applied. Such a procedure forces moisture deep into product sites within a relatively short period of time.

It is important to design a conditioning process that can handle even the driest product. Excessive drying may occur, for example, when pallets are stacked high above the floor, near heating ducts. Seasonal variations may also affect the ambient moisture of many raw materials.

The critical role that moisture plays in achieving satisfactory results with EO processing is best exemplified by what can be called the EO-Carrier Effect (Figure 2). Ethylene oxide is able to carry moisture with it through films that are normally impermeable to moisture, such as polyethylene. It is this characteristic that enables EO to sterilize items that cannot be readily sterilized even with steam, such as mating surfaces like plungers fitted into barrels of syringes, and where plastic tubing parts are connected (7).

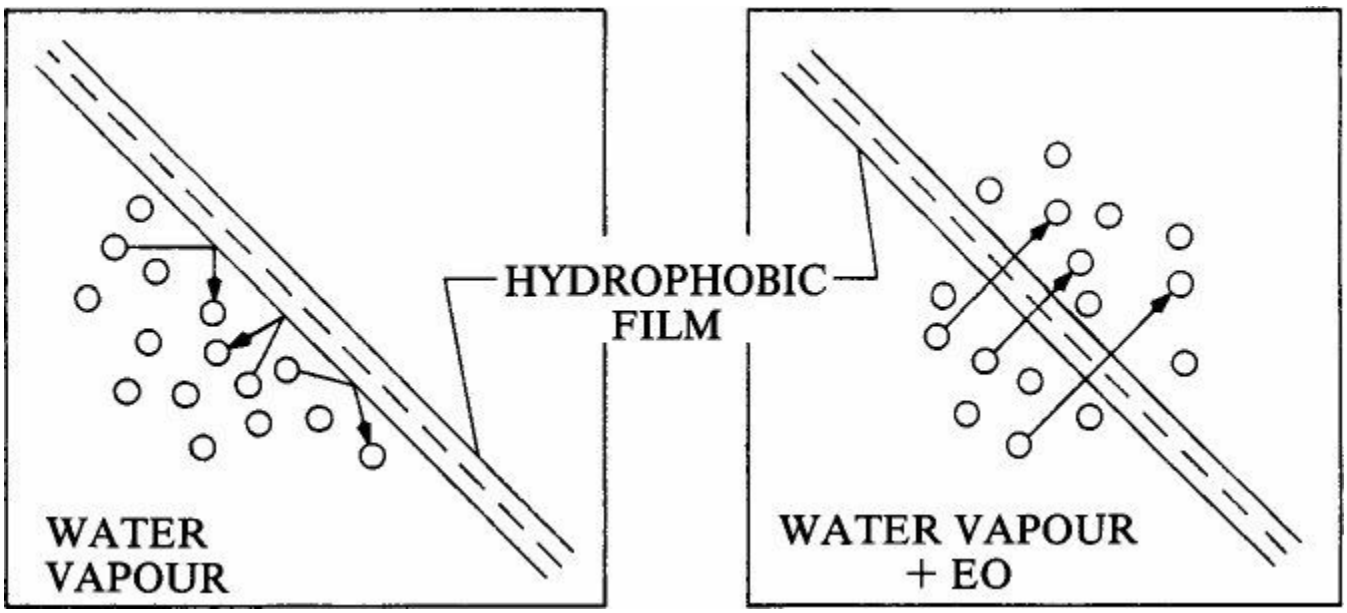

Figure 2. Ethylene oxide carrier effect. Transmission of moisture through a hydrophobic film (7)

Problem Areas

Among the practical problem areas that may be encountered when using EO are the following:

1. *Load Geometry*
   The gas must be able to penetrate the load, yet excessive EO absorption must not take place. Standardization of load geometry is important.
2. *Vacuum Rates*
   A low vacuum rate frequently results in the removal of the water that was added by the preconditioning step and must be avoided.

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3. *Optimum Moisture*
   Whether moisture is controlled by humidity sensors or by the pressure differential from added steam, the appropriate quantity for a given situation should be determined experimentally rather than derived from values reported in the literature.
4. *Ethylene Oxide Superheat*
   Inlet gas temperature should be controlled; this parameter has been frequently overlooked in the past. An excessiely wide temperature range must be avoided to prevent superheating within the vessel.

It must be remembered that physical parameters such as temperature, time, humidity, and gas concentration cannot be considered to be independent – they are, in fact, *interdependent*. If one parameter is arbitrarily changed, the others may no longer be valid.

(b) *Microbial Challenge*

There are two generally accepted microbiological approaches for challenging the effectiveness of the physical parameters previously described: the 'Overkill' method and the 'Bioburden' method. In using these two methods, four types of microbial challenge tests may be used.

1. *Inoculated Product* – Inoculation of actual product or product parts with a suspension of spores resistant to EO.
2. *Inoculated Simulated Product* – Use of a specialized device that contains spores, either by direct inoculation or by means of an adventitious carrier that is known to be equally as difficult or more difficult to sterilize than the natural product.
3. *Inoculated Carrier* – Use of an adventitious carrier bearing EO-resistant spores (e.g. a paper spore strip) with correlation to inoculated product or inoculated simulated product.
4. *Natural Product* – The product with its naturally occurring presterilization bioburden can also be used to establish microbial death rates and sterilization cycles.

– *Overkill Method*

Traditionally, overkill methods have been used to establish EO sterilization cycles. This approach is based on the concept that the sterilization process will inactivate the microbial challenge, plus an additional safety factor. The microbial challenge consists of selected numbers of EO-resistant spores, typically *Bacillus subtilis* var. *niger*, without necessarily relating the challenge population to the presterilization bioburden. This method provides an overkill because the cycle conditions established to kill the microbial challenge, plus an additional safety factor, are more severe than those required to kill the presterilization bioburden.

– *Bioburden Method*

The bioburden method is employed when product or package functionality would be adversely affected by the rigors of an overkill process. It is somewhat analogous to the F_0 approach to calculate cycle lethality for steam sterilized parenteral solutions. The method requires the collection of presterilization microbiological data and the correlation of the numbers and/or resistance of bioburden to the indicator microorganism. This permits cycle selection by establishing the challenge microorganism population (indicator microorganism) with a safety factor added above the anticipated bioburden. Bioburden resistance can be determined by exposure of actual product samples to fractional exposure-time increments at proposed cycle conditions. The presterilization bioburden load on medical products has been shown to span an extremely wide range (Figure 3).

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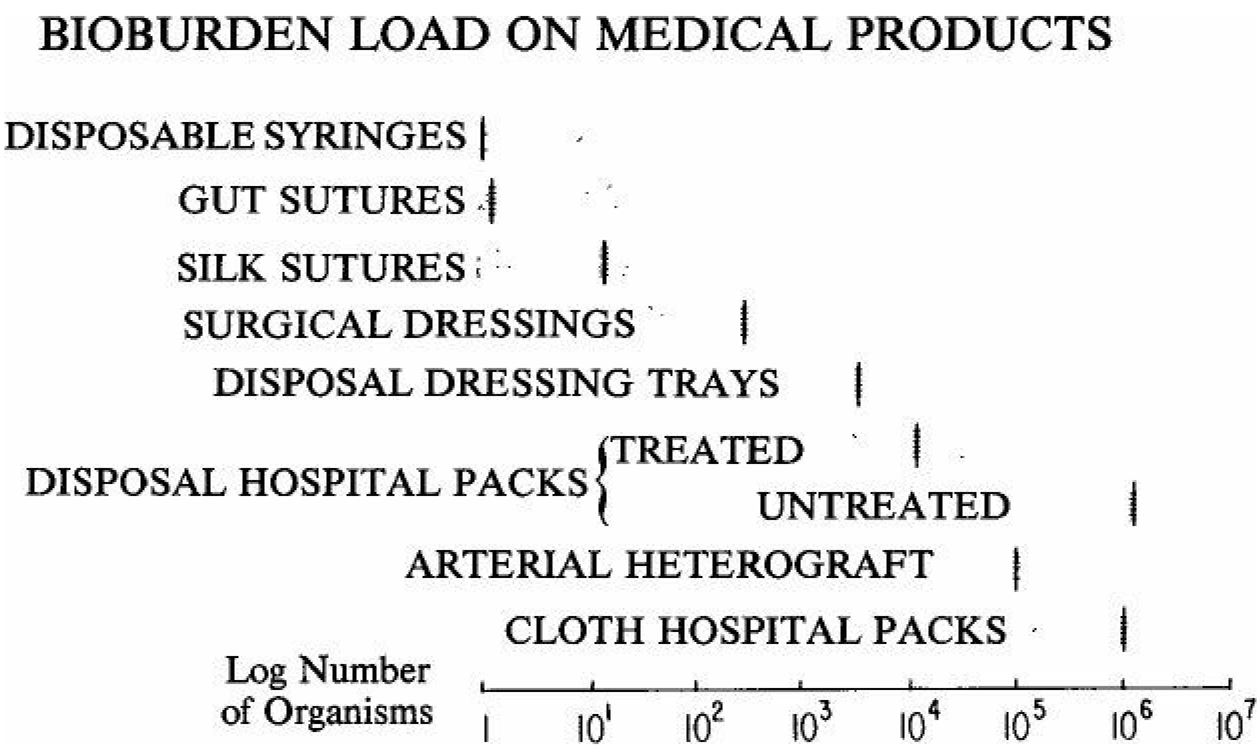

Figure 3. Presterilization bioburden load on a variety of medical devices. Vertical bar identified the arithmetic mean (14)

Safety Factor – After the necessary data are generated via the overkill or bioburden methods, the level of sterility assurance (safety factor) is set at either 10^{-3} or 10^{-6} depending upon the end use of the product, e.g. a 6-logarithm microbial reduction for products intended to come into contact with compromised tissue (i.e., tissue that has lost the integrity of the natural body barrier), or a 3-logarithm microbial reduction for products not intended to come into contact with compromised tissues.

Product/Package Functionality

An integral part of process development is the determination of product and package functionality after the items have been subjected to the selected sterilization conditions. Product performance and sterile-barrier integrity must be acceptable after sterilization. Product/package functionality testing should also be conducted after the maximum authorized number of sterilizations.

Aeration

Process development should also include a determination of EO residues. Studies must be conducted, or prior information must be available, to establish a procedure to ensure removal of product residues to specified limits. The primary conditions that affect aeration efficacy in reducing EO residues are temperature, dwell time, air convection, and load configuration.

Validation

Validation is a term that describes the overall programme used to demonstrate that a particular product can be reliably sterilized by the designed process under actual production conditions (Figure 4). The elements of validation are:

a. An *installation qualification* of equipment (empty);

b. A *performance qualification* of the process, using specified product in the qualified equipment;

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c. An administrative *certification* procedure to review and approve documentation of (a) and (b).

Figure 4. The overall validation programme as presented by **AAMI** (1)

New products and new sterilization equipment or process conditions must be validated. Each production chamber must be qualified. Where process uniformity between chambers can be shown, however, new products may be qualified by one chamber in order to qualify all equivalent chambers. If a new product can be sterilized using a previously qualified cycle for a similar product, the new product may be qualified by equivalency.

A validation programme is not required for product release if process monitoring and microbial challenges for each sterilization cycle are equivalent to a performance qualification run and if the equipment used has undergone installation qualification. This approach may be used, for example, in instances where production quantities of product, needed for validation runs, are not available. The validation programme should be completed, however, when the necessary quantities of product are routinely available.

During the *installation qualification*, the sterilization process equipment, including the sterilizer chamber, preconditioning chamber, and ancillary systems, should be reviewed and tested to verify

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satisfactory operation within the specifications required by the sterilization process. This evaluation should be documented. Also an empty vessel temperature distribution test should be completed using a minimum of three temperature sensors for chambers less than 100 cubic feet and an additional sensor for each 200 cubic feet. Examples of equipment which should be verified and documented to ensure that they are compatible with process specifications include chamber and piping construction; seals and connections to maintain pressure and vacuum; instrumentation with adequate accuracy, precision, and range; air, steam and water supplies; gas circulation systems; and electrical power supply. A formal maintenance programme must be developed and established for all applicable parts of the installation. Also a documented metrology and calibration programme for instruments must be established, consistent with *Good Manufacturing Practices*.

The performance qualification provides rigorous microbiological and physical testing, beyond routine monitoring, to demonstrate the efficacy and reproducibility of the equipment and the lethality of the sterilization process. Criteria for acceptance should include conformance with operating specifications for sterilization process parameters, for microbiological challenge, and for product functionality.

1. *Number of Runs*

The performance qualification should include a minimum of three successful, planned qualification runs, in which all of the acceptance criteria are met. If any one of these runs shows that sterility assurance and/or product functionality requirements cannot be met, or if process parameters cannot be maintained within limits, additional qualification runs are required after appropriate modifications are made. Qualification runs must be carried out at maximum intended chamber loading or with the product mix and loading that are considered most difficult to sterilize.

2. *Product Temperature Sensors*

Product load temperatures should be monitored with a minimum of three temperature sensors, for chambers of less than 100 cubic feet, and an additional sensor for each additional 100 cubic feet of product, up to a maximum of 24 sensors. Temperature sensors are preferably located adjacent to biological indicators at centre, top, and bottom positions distributed throughout the load.

3. *Product Temperature Spread*

A temperature range across the product load of less than or equal to ±3°C during gas exposure is common.

4. *Biological Indicators*

Sufficient microbial challenge systems should be distributed throughout the load to provide at least one per 100 cubic feet of sterilizer product load. No fewer than 10 locations per load should be used; these locations should be documented.

5. *Biological Indicator Location*

The microbial challenge must be located in the most difficult-to-sterilize product sites intended to be sterile, or at convenient product locations that have been correlated with these sites in development studies. In performance qualification runs, the maximum sterilizer product loading to be produced must be utilized. The microbial challenge systems should be placed at the most difficult-to-

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sterilize locations in the chamber load, including top, centre and bottom.

6. *Ethylene Oxide Concentration*

Common practice is to have the concentration of EO in excess of 400 mg EO/L.

7. *Relative Humidity*

Relative humidity in excess of 30% is common practice to avoid load desiccation and facilitate sterilization.

Certification

It is essential that a controlled file of validation programme documents be established and maintained. This file should include initial documents, as well as updated documents, filed by system and equipment. In addition, an approval system consisting of a certification for the initial validation programme and subsequent requalificaitons must be established.

Production Sterilization

Having completed all of the prerequisites, we are now ready to move into routine manufacturing. During routine sterilization, there are five areas that must be controlled:

1. *Gas Certification*

A certification of feed gas identification and analysis should be obtained from the gas supplier or performed by the user; certification must be traceable to each delivered batch of EO feed gas.

2. *Process Parameter Monitoring*

Accuracy and repeatability of routine sterilization instrumentation must be sufficient to permit reliable control of the process parameters that were developed and validated.

3. *Microbiological Testing*

This includes bioburden monitoring or the use of biological indicators. Normally, the number of biological indicators needed for routine sterilization is smaller than that required for performance qualification. When sterilization process development and validation have been accomplished, terminally sterilized products may be released without sterility testing of finished products, following satisfactory biological indicator testing.

4. *Reprocessing*

When a product fails to be processed within specifications or fails testing and release criteria, written reprocessing procedures must be followed.

5. *Requalification*

Performance requalification is recommended, when changes are made in the equipment, process, product, or packaging that could affect EO sterilization efficacy. Also in the absence of known change, a requalification should take place once a year. The following are examples of those changes that may necessitate performance requalification.:

a. *Product Tolerances*

A significant change in product design tolerances that may affect the ability of gas to penetrate the product.

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b. *Product Venting*

A significant change in sterile barrier venting, e.g. from a vented to a nonvented end, or form one type of filter or filter porosity to another.

c. *Product Design*

A significant change in product design, including product materials composition or thickness where gas penetration is required, may affect EO sterilization efficacy.

d. *Packaging*

Changes that could affect microbial barrier efficiency; substantial changes in package design, e.g. from a blister package to a pouch; changes in corrugated case design that may create significantly higher chamber loading density or heavier paper loading; changes in vendors that may have a significant effect on materials properties.

e. *Gas Specifications*

Changes in diluent concentration or type.

f. *Equipment*

Changes that affect the ability to maintain specified operating parameters or that substantially change the rate of heat transfer or gas penetration to the product.

g. *Process*

Alterations in the process that substantially change the manner in which process parameters are achieved and controlled.

h. *Product Loading or Density*

Any significant change in loading configuration.

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Conclusions

Ethylene oxide sterilization is indeed complex, but when based upon sound engineering and microbiological principles, cycle development and validation need not be complicated. The proof lies in the fact that ethylene oxide is the major sterilant used in the international disposable medical device industry.

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Ethylene Oxide – The Work Environment A Preliminary Report of a Pilot Chromosome Study in Workers at Sites Where Ethylene Oxide is Used as a Sterilant

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Introduction

For the past one and one-half years, Johnson & Johnson has been conducting a pilot research chromosome study in workers potentially exposed to the widely used sterilant gas, ethylene oxide (EO). EO is used extensively in hospitals and industry as a sterilant and it is a 'starter' chemical for a number of chemical products. At this time, there is no satisfactory substitute for EO as a sterilizing agent. EO is a known mutagen in microbial, plant and animal test systems and NIOSH recommends that it be 'regarded in the workplace as a potential occupational carcinogen'; it has been associated with chromosome changes in several human studies. This research project was undertaken in the spring of 1980, soon after information describing chromosome changes in EO-exposed workers was disclosed by the American Hospital Supply Corporation. The present study was designed to determine whether employees potentially exposed to EO showed more chromosome changes than employees thought to be unexposed.

Study Design

A cross-sectional somatic-cell chromosome study on human lymphocytes using coded specimens was performed in workers potentially exposed to EO, as compared with other workers thought to be unexposed at three different facility locations. Facilities were chosen according to the relative degree of potential exposure to EO at levels that were estimated to have existed prior to September 1980. Estimates were based on environmental sampling and monitoring as follows:

Facility	Relative exposure*	Estimated range of 8h TWA** of sterilizer operators
Plant I	Low (LRE)	< 1 ppm
Plant II	Moderate (MRE)	1-10 ppm
Plant III	High (HRE)	5-200 ppm***

LRE = Low Relative Exposure; MRE = Moderate Relative Exposure; and HRE = High Relative Exposure.

**TWA = Time Weighted Average in ppm (parts per million) of ethylene oxide.

*** An estimate of 50-200 ppm was made for samples collected on 10.3.80. Estimates for samples collected before and after that date fell within the range of 5-20 ppm.

The potentially exposed subjects included in the study were employees at each plant, who were working in the sterilizing areas and, therefore, were thought to be potentially exposed on a day-to-day basis. Employees at each plant were further categorized as to high-potential and low-potential exposure to EO, based on job descriptions, location near the sterilizer, amount of time thought to be exposed to the gas and other factors. For each potentially exposed subject in each plant, a randomly selected control, matched by age and sex, was identified in the plant and invited to participate in the study. Confidentiality of data, informed consent, and other ethical considerations were carefully observed.

After an employee agreed to participate, a work history and health questionnaire was completed and blood samples were obtained for chromosome studies. These studies included observations of the frequency of sister-chomatid exchange (SCE), and of the frequency and type of chromosome

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aberrations in lymphocytes. All blood samples were coded and sent to Litton Bionetics where lymphocyte culturing and cell scoring were performed. Referee readers from New York University Medical School and the Institute for Medical Research were appointed to monitor cell scoring regularly. The Clinical Epidemiology Unit of the University of Pennsylvania Medical School was engaged to advise on the design of the study and to uncode and analyse the data. A Scientific Advisory Board of experts in the fields of cytogenetics, epidemiology, pathology, and statistics was assembled from leading academic and research institutions to review and guide the study.

In each plant, the levels of SCE and chromosome aberrations in the potentially exposed employees were compared with those of the control employees, and findings at the three plants were compared with one another. During the course of this study, it became apparent that the SCE levels in the control group of presumably unexposed employees at Plant III were higher than the results for the other control groups available for comparison at the time. This led to a modification and expansion of the study design in that an additional set of controls, consisting of nonemployees, was selected from the community where Plant III is located. This outside control group was selected and matched by age and sex to the potentially exposed Plant III employees. These community controls were nominated by the potentially exposed employees. The outside controls employed at the time had never worked in Plant III and had no known exposure to EO.

The appendices contain brief descriptions of 1) The criteria used to categorise employees by exposure status (Appendix 1), 2) How the blood samples were managed, 3) How SCEs and chromosome aberrations were determined (Appendix 2). Tables 1 and 2 summarize the study design and the distribution and testing frequency of subjects at each plant site.

The cytogenic data described in this report are preliminary and are the subject of continuing analysis, as recommended by the Scientific Advisory Board. The preliminary analysis reports on two cell-scoring components: SCE and chromosomal aberrations of several different kinds. It is important to note that the results discussed here have been observed in relatively small study populations and must be confirmed by additional studies before any general conclusions can be reached.

Employees at Plant III (HRE) and Plant II (MRE) now have been tested three times, i.e. at the beginning of the study and twice at 6-month intervals. Employees at Plant I (LRE) have been tested only twice (baseline and 12 months later) because the results of the first test revealed no significant difference between potentially exposed and control populations. Blood samples for the 12-month follow-up at all locations were obtained during the fall of 1981. Only the baseline and 6-month follow-up data are presented here.

Table 1

Classification of sites by groups studied and measures obtained

Site studied	Groups studied		Measures obtained
	Exposed	Controls	
	High potential	In plant	SCE
	Low potential		Aberrations
Plant I (LRE)			Medical examinations
			Work history
			Exposure measurement

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Plant II (MRE)	same	same	same
Plant III (HRE)	same	In plant	same
		In outside community	

Table 2

Persons studied by worksite

Worksite exposure		Number of persons	
		Baseline	6 month retest
Plant I (LRE)			
Exposed	High potential	8	0
	Low potential	5	0
Controls	None (plant)	11	0
Plant II (MRE)			
Exposed	High potential	4	4
	Low potential	18	16
Controls	None (plant)	19	18
Plant III (HRE)			
Exposed	High potential	2	2
	Low potential	24	24
Controls	None (plant)	21	20
	None (outside)	0	24
	Total	112	108

Results

The SCE data show a consistent dose-response trend at Plant III (HRE) for both the original test and 6-month follow-up (Figure 1). Both the potentially high- and low-exposed groups had statistically significantly higher SCE scores than the control group at baseline. The SCE data for Plant I (LRE) showed no significant difference between potentially exposed and control groups.

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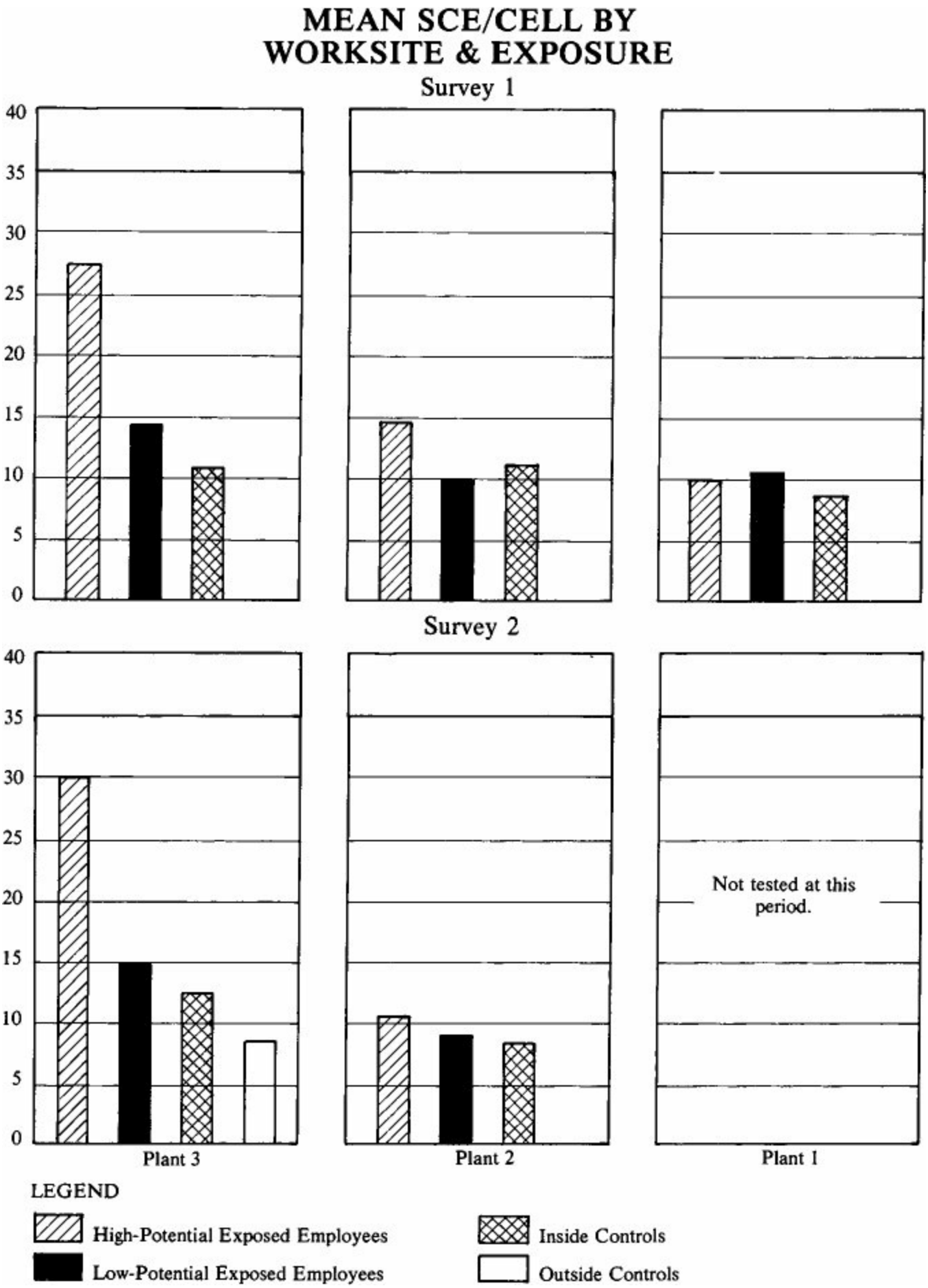

Figure 1.

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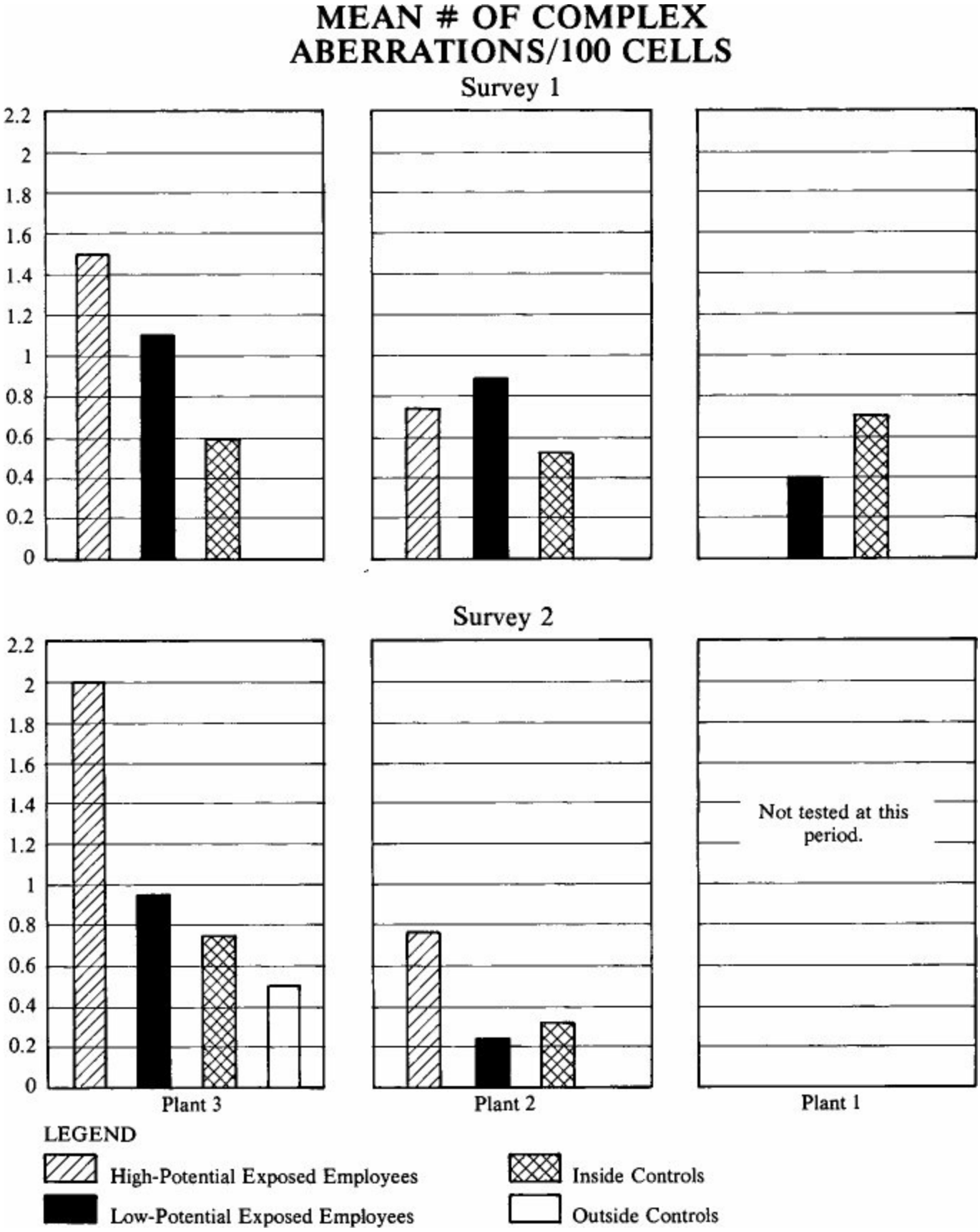

Figure 2.

There was no significant average reduction in SCE between the original test and the 6-month follow-up among all employee groups tested at Plant III. Moreover, the employee controls at this location, who were believed not to have been exposed, had significantly higher SCE than the outside controls not employed at the plant. All use of EO was discontinued at Plant III immediately after the

first survey and there has been no exposure to EO at that location for more than a year. Three employees at Plant III, including the two sterilizer operators (who comprise the high potential exposure group at that location) and one other employee, had SCE scores higher than any other person in the study (ranging from 23 to 43 SCE/cell). Even with these persons excluded from the data, there was a small but reasonably consistent dose-response trend at Plant III (HRE) for both the original test and 6-month follow-up. The chromosome aberration data do not show results as consistent as the SCE findings. Trends in complex chromosome aberrations are similar to the SCE results, but analyses of these trends are limited because complex aberrations are relatively rare (Figure 2). The complex aberration results suggest a dose-response relationship, but the magnitude of the differences between groups is not great.

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Summary

This study suggests a dose-response association between exposure to ethylene oxide and SCE in humans. It is not believed that a dose-response association between environmental chemical exposure and human SCE levels has previously been described.

An unexpected finding is that employees at Plant III (HRE), not originally considered directly exposed, may have incurred EO exposures, unknown to them as well as to the company. This is suggested by the somewhat elevated group SCE levels in what originally was thought to be an unexposed group. Transient exposure may have occurred during periods of use of certain centralized facilities that were located in close proximity to the sterilizer. Another explanation may be low-level exposure at regular work stations although this seems less likely. It is estimated that these exposures would have been considerably less than 50 ppm 8 h time weighted average (TWA).

There does not appear to be a downward trend in group SCE levels for all employee groups tested at Plant III (HRE) following six months without exposure. This stability of group SCE levels was unexpected.

No statistically significant changes in SCE or chromosome aberrations were noted between the potentially exposed and control groups at Plant I (LRE) where exposures to EO have been at levels below 1 ppm (8 h TWA) for the last several years.

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Appendix I

Selection of Study Participants

Definitions of the two categories of participants are described below:

1) Potentially Exposed Employees

For each plant location, the potentially exposed employee category includes all those employees who worked in the immediate sterilizer area for 30 minutes or more, daily, and those other individuals who, by the nature of their job assignments, were likely to have been exposed to high peak levels, but who do not meet the 'daily 30 minutes or more' definition.

a) High-Potential Exposed Employees

The sterilizer operators are called 'high-potential exposed employees'. They are categorized as a special subset of the exposed group because they are regularly assigned to the operation of the sterilizer units. For this reason, they are subject to higher risk of exposure occurring in conjunction with the operation of the sterilizer, as well as with the ambient levels in the immediate sterilizer area.

b) Low-Potential Exposed Employees

This subcategory includes all other potentially exposed employees.

2) Unexposed Controls

For each plant location, an age and sex matched group of controls was selected from employees thought to be unexposed to EO.

a) Inside Employee Controls

Inside employee controls were those employees thought to be unexposed and randomly selected to match by age and sex each potentially exposed employee studied in that location.

b) Outside Community Controls

At Plant III an additional control group was chosen from nonemployees resident in the community. Each potentially exposed individual 'self selected' his or her own community control, matching in age and sex. The following restrictions were required: All outside community controls had to be currently employed in a nonhospital environment and were to have had no known exposure to EO.

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Appendix 2

Protocol for Human Chromosome Studies

An essential feature of the study is that samples are handled and scored 'blind', without knowledge of the status (control vs. at risk) of the subjects.

i. Blood is collected in sterile heparinized 'Vacutainers' and samples are coded. Samples from groups of individuals thought to be at risk and from control individuals are handled concurrently to ensure equivalent treatment.

ii. Replicate 10-ml cultures are set up in RPMI 1640 medium supplemented with 15% foetal calf serum, 1% phytohaemagglutinin M, L-glutamine, and antibiotics. Incubation is at 37°C in humidified incubators in an atmosphere of 5% CO_2 in air.

iii. Chromosome aberrations: Three cultures are fixed between 48 and 51 hours of incubation. One hundred good quality metaphase cells are selected for each individual and analysed at the microscope. Slide location co-ordinates are recorded for all abnormal cells and all aberrations are verified by a second observer – the laboratory supervisor or the study director.

To detect unstable aberrations such as dicentrics and quadriradials, cells must be examined when they enter mitosis for the first time, before they can be lost due to cell death during the division process. To ensure that as many as possible of the cells scored are in their first mitosis (M1 cells), fixation times were based on results of experiments on twelve individuals. The staining pattern was used to distinguish cells that had cycled once, twice, or three times (M1, M2, and M3 cells) in cultures containing a low concentration (25μM) of 5'-bromodeoxyuridine (BrdUrd), known not to inhibit the cell cycle and fixed at a series of times between 45 and 60 hours. The fixation times were selected to give a good mitotic index, and the data from the test individuals give an estimate of the small error introduced by scoring a low frequency of M2 cells. BrdUrd is not used in cultures for aberration scoring.

iv. Sister Chromatid Exchange (SCE)

Three cultures per person are used, fixed between 68 and 72 hours of incubation to ensure a good yield of M2 cells. Eighty cells are scored per person.

To detect SCE cells are treated with BrdUrd for two cell cycles. To minimize variability, we: a) inoculate the same number of lymphocytes per culture for all individuals and b) use a concentration of BrdUrd (100μM) thought to reduce the variation caused by individual difference in cell growth (Carrano, *et al*, 1980) (1).

v. Cell fixation and slide preparation

During the final two to two-and-half hours of incubation, cultures are treated with colcemid (0.1 μg/mL). Cells are collected, treated with hypotonic 75mM KCl solution at room temperature for 5-10 minutes, fixed in methanol-glacial acetic acid, 3:1 v/v, washed twice in fresh fixative, and dropped onto glass slides to air dry. For aberration studies, slides are stained with Giemsa's stain. For SCE analysis, we use a modification of the FPG technique (Perry and Wolff, 1974, as modified by Goto, *et al*, 1978) (2, 3).

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DISCUSSION SESSION V – Part 3

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Q. by J. Nisbet – Australia

A question to Dr Morrissey. In a system that is at all times under vacuum, what is your view on the practice of topping up with gas to maintain a steady vacuum?

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A. by R.F. Morrissey – USA

This is a good question and it comes up frequently. There are differences of opinion on how to approach the subject. It gets back to validation. If you validate a process and you know what the biological kill is at a particular gas concentration and particular conditions, you probably do not have to add gas. I think, an additional question is why has there been a drop in chamber pressure or why was it necessary to add extra gas during the exposure phase. Quite often it is caused by packaging materials; in many cases they are corrugated cellulocics which absorb ethylene oxide (EO). You might say that if those materials are absorbing EO and the microorganisms are contained on the materials that the microorganisms also would be absorbing EO and be effectively killed. Therefore, why add additional gas? I think, it really goes back to validation. If you have shown that you can have an effective process without gas addition, fine. If one firm, for instance, does one thing in a particular situation and another firm does something that appears to be radically different and works, validation is required. I do not believe that you have to make up gas, but may be there are cases where you may have to.

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Comment by R.J. DeRisio – USA

For those persons who are not familiar with the EO process, I think what is really different with the question is that it relates to subatmospheric processing, whereas for the types of processes operated under pressure the thought of adding make-up gas immediately brings to mind the possibility of a leak in the sterilizer in contrast to absorption within the load.

———————

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Q. from the floor

Dr Herrmann, in the evacuation cycle, the EO is discharged into an open vent and then goes into drainage. We installed a system based on the solubility of EO in water. The question is, is it soluble and is there a reliable type of humidity sensor on the market today for EO sterilization?

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A. by A.A. Herrmann – USA

I will refer the questions to Dr Morrissey. On the question of sensors, I am not familiar with that type of technology. However, we are very careful how we manage all EO exhaust, as well as liquid material, so as not to put it in community air and community water.

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A. by R.F. Morrissey – USA

The whole subject of sensors for relative humidity for EO sterilization came up quite often during the development of the AAMI document. Let me just give you a minor commercial. There are two documents: one is an actual formal recommended practice guideline, the other is the proceedings of a meeting that Dick DeRisio mentioned. This was a joint meeting of the FDA, industry, and academic people who put together the guidelines. Humidity sensors, historically, are very inaccurate. In the AAMI document, there are a number of cases of caution. There are cases where it is shown that alternative procedures are quite effective and that one does not have to employ a humidity sensor. There are, for instance, pressure and temperature differentials. In many cases, sterilization cycles start off by pulling a very deep vacuum, and when one pulls a deep vacuum, one adds a quantity of steam from which the relative humidity can be determined by pressure differential. Humidity is important, but the use of a relative humidity sensor may not be critical, as there are alternative ways. If you take a look at the AAMI document, I think it might expound the subject.

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Comment by R.J. DeRisio – USA

Our experiences at the regulatory Agency in coming to grips with this subject have provided that firms measure relative humidity at the beginning of the exposure, before the gas is added, and reliably record the information. There are some firms that are using DuPont monitors. Some of these monitors tend to be very expensive, but also very accurate, and there can be conversion to relative humidity data, knowing the temperature of the chamber. We have seen firms that have to wash and clean and then dry sensors almost after every load and even then some of these sensors do not have too long a life with recurring exposure to EO.

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Comment by R.F. Morrissey – USA

Let me add to the last point. Some manufacturers have found a way to increase the life of a sensor. Those that are using sensors have derived mechanisms of removing the sensor immediately from the environment prior to EO gas addition, flushing the sensor with dry nitrogen and holding it in an antechamber configuration, so that the quality and the life of the sensor is extended.

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Q. by J. Perry – Australia

I have two questions. What does Dr Morrissey consider to be the optimal time for chamber evacuation and does he have any knowledge of a product that has been preconditioned or prehumidified for, say, 24 hours which would then still require chamber humidification?

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A. by R.F. Morrissey – USA

The simple answer is to validate it. With a particular product and a particular type of sterilizer, preconditioning may or may not be necessary. I do not think, there are any magic numbers. When you go through the AAMI document, you will find that there are not that many numbers. The EO process is so variable that if it can work in particular circumstances with a particular product in your sterilizer and you can prove it with sound scientific data, then it should be acceptable.

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Q. by R. Clemens – Australia

Dr Morrissey, with regard to the transmission of EO and water vapour through polyethylene, is there any variation rate of diffusion because of the polyethylene formulation, or doesn't it enter into it?

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A. by R.F. Morrissey – USA

I would assume so. I really cannot answer your question specifically. The reference is taken from some of the classical work of the late Bob Ernst, who, many of you may know, was a pioneer in EO sterilization and demonstrated an interaction, an interplay between moisture and EO. Understanding this relationship is really the key to success with EO. I am sure that a variety of films and compositions may affect the diffusion rate, but I do not have that answer.

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Q. by J. Perry – Australia

I have a question for Dr Herrmann. At what physical site, or where do you physically measure your ppm in the atmosphere, and could you give me an indication of the instrumentation that you use?

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A. by A.A. Herrmann – USA

The type of instrumentation has been caught up somewhat in the state of the art. It was a very difficult thing prior to 1976 to measure accurately environmental workplace EO because of lack of technique. About that time the charcoal tube surfaced. We have used a variety of techniques for personal monitoring, which is breathing zone monitoring. The charcoal tube is a unit that is attached to a pump that draws an air sample in reasonably close proximity to the breathing zone that essentially represents what a person is inhaling. We do this on gas samples as well as on 8-hour-a-day patterns that are staggered throughout the work place. We sample different jobs for different periods of time, so that we have a reasonable idea about the controls we use. In addition, because of a potential for peaks and because of a potential for the gas to be difficult to grab, and because you cannot have a charcoal tube in every place, we have developed a policy worldwide where in addition to this kind of monitoring we will be installing continuous gas Chromatograph multipoint samplers to complement area monitoring. We then can get a better feel on a continuous basis as to what is happening with exposure.

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Q. by A. Tallentire – UK

Bob, you alluded to the phenomenon of stratification, and I understand that there are cycles in which the EO and billowing gas will be added separately to the vessel. What evidence is there that in those circumstances stratification of the EO does not occur?

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A. by R.F. Morrissey – USA

I am not sure that I can answer directly, but in the AAMI guideline there is an inter-relationship between gas injection temperature and the capacity, for example, of the heat exchanger, and primarily it has to do with ethylene oxide/chlorofluorocarbon mixtures. If the gas is introduced at a low temperature, you will get fractional distillation, which is a potential problem with EO. Now, we have situations where in a number of installations there is bulk storage of EO. There is a mixer inside a large cylinder (a large chamber), and there have been no problems. However, several people have mentioned that sometimes, if you have a cylinder of an ethylene oxide mixture and it is left undisturbed for a long period, there may be differential stratification. Normally, where there is a dip tube that goes to the bottom of the cylinder, this problem is eliminated.

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Comment by A. Tallentire – UK

I was referring to the possibility of stratification in the vessel itself, not in the cylinder.

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A. by R.F. Morrissey – USA

Making the assumption that the gas has been added properly, we have not observed the phenomenon. Frank Halleck could comment on the subject.

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Comment by F.E. Halleck – USA

This is a common question that comes up many times. We have done extensive studies on this very issue. In the chamber, you never get stratification with a Freon/ethylene oxide mixture, because the vapour pressure of the Freon is so much higher than that of ethylene oxide. In most large and small chambers, using the mixture of Freon and EO, you have heat-up systems, either an expansion tank or vaporization piping, so that the liquid conversion to the gas stage is almost instantaneous. You should know, however, that when you use a carbon dioxide mixture with EO and you introduce the carbon dioxide mixture into the expansion tank, you can get stratification in the expansion tank. This may carry over into the chamber if you do not allow enough time for the equilibration of the liquefaction to take place since carbon dioxide vapour pressure is considerably lower than that of ethylene oxide. I think, we have an engineer here and perhaps he can answer the question. Would he like to comment.

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Comment from the floor

In my experiences, basically in the manufacturing and sterilization of surgical gloves, I saw gas stratification measured with a gas Chromatograph on cycles having an exposure time longer than 18 to 24 hours. Stratification is proportional to time and is calculated to be about 100 mg/L, decreasing from the top of the chamber to the bottom of the sterilizer. That is mainly due to the thermal draft currents within the chamber and also to a lack of proper ventilation blowers that could agitate the gas mixture and maintain it at a consistent gradient.

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Q. by L.F. Dodson – Australia

Dr Herrmann, have you done any other epidemiological studies on people who have been exposed to ethylene oxide in high exposure risk situations? Secondly, what is considered to be the significance of the chromosomal findings that you describe?

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A. by A.A. Herrmann – USA

We have several studies ongoing, from which the answers will probably be available two to three years from now. One study is that we will be looking at all sterilizer operators in North America, only because it is a finite group of people and we are much more likely to be able to track accurately records that date back to the start of operations. We can look at morbidity and mortality experience in that group of people. We go back to 1953. However, the group is not in the thousands, it is in the hundreds. So, as far as the sensitivity of an epidemiology study, if there are problems in three or four clusters then it would at least raise concern. The study is ongoing and has just been initiated. In the other study, we are going to track those people during a six-year period, in which a sterilizer was operating, to look at all the people who ever worked there in that facility. Essentially, other than what we did earlier in 1980, it was to take a very rough look at all of our sterilizer operators and people who had high potential exposure, as we knew it then, just to be sure that we did not have a clustering. We did not want to have the niceties of an epidemiology study interfere with just doing a very rough cut of the medical records. The rough cut of the medical records did not show us any particular clustering of problems. We had a scattering of the kind of diseases, you experience in the general population.

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Q. by L.F. Dodson – Australia

What do you think is the significance of those findings other than that EO does affect chromosomes?

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A. by A.A. Herrmann – USA

Well, there are the sister-chromatid exchanges. Sister-chromatid exchanges (SCE) are avant-garde, having been around for about five or six years, and it happens to be where a lot of the action is in terms of research, as it is a fairly easy technique to read a sister-chromatid exchange. There is not much question that virtually every carcinogen, chemical carcinogen, that has mutagenic capabilities can increase SCE. Almost every chemical that has this capability has been demonstrated to have had that capability. It is also thought from a number of different studies that sister-chromatid exchange is a fairly good evidence of past exposure to certain chemicals, and results can be used for dosimetry. It has also been looked at as an assay for mutagenic potential of a chemical, if an exposure can move sister-chromatids around, since you can compare them always with controls. It is impossible to do this kind of study without having a large number exposed and a large number of controls. However, you cannot do this study with half a dozen people and half a dozen controls. SCE do not have any well defined associated health effects, although there are in some chromosomes fragility syndromes, called Bloom's Syndrome, from an increased number of sister-chromatid exchanges. This is probably the only health exception. Concerning aberrations, there is quite a bit of documentation on the associations of aberrations and health effect, not as cause and effect, but as an association. The most significant data, of course, are the radiation data from Hiroshima and Nagasaki. This is where we have seen effects in terms of soft and hard tumours. The aberrations as they relate to health are more serious than SCE, but who knows, five years from now maybe we may be talking about the same issue with regard to sister-chromatid exchange.

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Comment by R.J. DeRisio – USA

In summarizing and closing this part of the session, I would conclude that a protocol exists for the validation of ethylene oxide cycles and that the AAMI guidelines have gained wide acceptance among regulatory bodies and manufacturers worldwide. Although strictly not within the scope of the document, the principles can be applied to hospital sterilization as well.

We have learnt that there are available practical and reliable means to monitor EO exposure in the worker environment which can be applied by users of EO.

The increasing interest in radiation notwithstanding, ethylene oxide will continue to be used as a means of sterilizing medical devices. I believe, we will see steps taken to reduce residue levels on sterilized devices with a concurrent improvement in methods for determination of residue levels

In the area of employee safety, the level of interest and co-operation among industry, hospitals, associations, and regulatory agencies should result in significant improvements in worker safety.

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DISCUSSION
SESSION V

Q. by J. Lindsay – Australia

I sat with rather rapt amusement listening to Pam Wills. She seemed to me to pour a very large bucket on some of the sterilization processes used by some of the radiation people. It would seem that it was, to coin a phrase, rather overkill and overwaste not only of resources but money and manpower and time. I wonder if somebody on the panel would care to comment.

Also, I have a question for Mr Masefield with regard to the enumeration of organisms after the sterilization processes. No mention was made of a pre-incubation or pregrowth period for those organisms that are sublethally damaged. I wonder if, in fact, the data that you have are rather tenuous in that regard.

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A. by P.A. Wills – Australia

Perhaps, you misunderstood what has been said. Somebody assumed that really there is nobody validating the use of a dose in Australia. We went through the exercise according to the *Code of Good Manufacturing Practice* to show how difficult it was. Hopefully, it will become easier when the Code is revised in accordance with the International Atomic Energy Agency recommendations. Does this answer your question?

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Comment by J. Lindsay – Australia

Partly; it just seemed that the radiation processes were too long and unnecessary. You could have got away with using less time, perhaps less effort, and still have achieved what was wanted.

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A. by P.A. Wills – Australia

Well, the work showed that you really need 2.5 Mrd, except for AAEC lyophilized products. There are probably a fair number of things in this category.

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Q. by J. Lindsay – Australia

Mr Masefield, I was concerned that when you do enumerations of organisms after radiation damage you do not have a pre-incubation period for picking up organisms that are sublethally damaged. It is common to do this in other microbiological processes, and I wonder why it is not done in radiation analysis. You made no mention of this subject.

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A. by J. Masefield – USA

I did not describe the sterility test. As far as organism recovery is concerned, most medical devices are inhospitable to nurturing growth of organisms damaged or not. In so far as allowing time to elapse between incremental dose irradiation and sterility testing, we had not noticed it having an impact on the results.

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Q. by E. Haderup – Australia

In view of what we have learnt about ethylene oxide exposure, I am concerned about the possibility of air-change rates within the CSSD. Should we increase what is a ward air-change rate of 6-10 air changes an hour to a theatre rate of 15-20 changes an hour? I would appreciate the panel's view on this.

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A. by A.A. Herrmann – USA

When you talk about the theatre, are you talking about the workplace outside the environment of the sterilizer?

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Comment by E. Haderup – Australia

I am asking whether we should increase the air-change rate, that is ventilation, within the CSSD to what we normally give the theatres, which is about 20 air changes an hour. At the moment, the CSSD in our establishment is about 6-10 air changes an hour.

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A. by A.A. Herrmann – USA

The control of ethylene oxide is submarine technology; you have to stop leaks, and to stop leaks from the tank to the jacket, from the jacket to the door, and from the jacket to the outside. Most units have adequate ventilation systems and it is surprising how you can contain ethylene oxide in the work environment using properly attached exhaust systems, if you have leak control. It is easy to get down to less than 5 ppm. I will ask someone else to answer the question on the CSSD environment, as I am unfamiliar with it.

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A. by F.L. Hebbard – Australia

The Department of Labour and Industry recommends that the safety valve orifice must be taken two metres above the roof of a building. There have been no recommendations to date to increase air change.

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Comment by A.A. Herrmann – USA

Johnson & Johnson has a variety of different operations in 24 countries. There are a dozen sterilizer operations in the United States that use ethylene oxide, and, I guess, we probably have every size, source, and variety of unit that you could imagine, from liquid in tanks where it vaporizes to vacuum cycle jacket units of laboratory size, to vacuum cycle units through which you could literally drive a railway carriage. You need to be a detective with an infrared spectrophotometer and a probe to check all of these units, the vacuum pumps, the trenches around the units, the joints that connect the exhaust pumps, etc. You have to make judgments on a basis of potential exposure. Our view is that the standard in the workplace has to be 1 ppm.

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Q. by A. Mercieca – Australia

I have a question for Richard DeRisio. Has the FDA finalized acceptable levels of ethylene oxide residues in products destined for patient use, and if so, are they available?

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A. by R.J. DeRisio – USA

There is at present no work being done. The document that I referred to was published in the *Federal Register* as a proposal in 1978. I have mentioned to Jim Whitbourne, co-chairman of the AAMI Committee working on residual determination, that maybe something will evolve that will enable a judgment on residue levels. In fact, I am not sure what extraction method is used or correlated. Granted, levels must have been set on the basis of medical opinion, and there must have been studies to look at typical levels in devices. If they were published in 1978, the data would be seven or eight years old, and, of course, since then technology has changed. Any levels would have to represent what could be determined with methodology that we will develop in the next year or so from the AAMI Committee. An appropriate residue level is based on scientific review and opinion on a particular class of device. We will look to health organizations to review the kind of levels that we are using. It appears that firms are not having a great deal of difficulty meeting the levels in that proposal, so there has not been a great deal of pressure to change those levels to something more liberal. On the medical side, they don't seem to be too high as to be hazardous.

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Q. by P.T. Doolan – France

My question is to John Masefield. John, in the AAMI B2 Method no counts of organisms are required. I am sure, I am not the first to remark on this, and I feel that this could have an impact on the audit frequency. I am interested to know if any consideration has been given to this by the Committee when developing the guidelines. It seems to me that you chose your words very carefully when you said that representative samples of the product should be chosen for the fraction-positive testing. Would you care to comment.

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A. by J. Masefield – USA

Concerning the audit frequency first. Having set the dose, we recommend that you audit your production frequently. We have discussed whether it should be a quarterly, an annual affair, or otherwise, and whether we should provide guidelines in this direction. In practice, particularly with smaller firms that have perhaps not as rigid control over their environment as some of the larger organizations, when they come to us for advice, we suggest that they sample monthly to commence with to make sure that the dose that has been set is valid. In such circumstances, we found that there were no problems. The whole dose-setting method is conservative and you are getting a lot of overkill. It is the resistant tail of the population that is driving the dose. Resistant organisms do not seem to appear very often. We get an increase inactual bioburden, but increases seem to be in less resistant organisms. We, therefore, do have frequent auditing, but it does not seem to be making a lot of difference.

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Comment by A. Tallentire – UK

I would like to comment and say that in effect you are doing a count when you do the AAMI B2 Method. As I recall, the first fraction-positive corresponds to an average three organisms per item of a resistant variety and, similarly, the first no-positive corresponds to 0.1 of an organism of a resistant variety. In reality, therefore, you are doing a count, although you are not doing a bioburden determination in the normal sense of the word.

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Comment by R.J. DeRisio – USA

We have in the Agency always required bioburden monitoring for any process that involved changes in bioburden and yet we hear from actual data that large spikes in bioburden population of more than one order of magnitude might not reflect the overall resistance of the item. What is a matter for concern is that when we see small manufacturers who have frequent changes in component suppliers, equipment maintenance and cleaning may not be at a level of those who do not see changes in a resistant population. There may be variable personnel contamination, as personnel are not as protected as they are in some other places. We should know at least what are the sources of contamination of radiation-resistant organisms. If we are to accept that these are at a low level and are unaffected by typical bioburden fluctuations, we would be more comfortable if we knew the source, e.g., if the source is common to a particular manufacturing material, or from soil, and so forth. Mr Masefield mentioned an increased audit frequency using the verification dose which may be a way to impose more control on a firm that is considering going to dosimetric release using the AAMI methods. Thus, there will still be monitoring of bioburdens to be consistent.

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Q. by E.R. Pavillard – Australia

I have a question for Dr Tallentire. In your interesting paper, you discussed wrapping and qualities of fibres in wrapping for sterilized products. You implied but did not state that trapping on fibres was associated with some type of electrostatic charge. You said, the microorganisms attach, but you did not say how, and I presume that an electrical charge may be involved. Is it reasonable to consider that a sterilizing process might alter the charge on fibres, so that the paper after sterilization did not exclude or trap particles as well as it did from the tests that were conducted prior to sterilization?

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A. by A. Tallentire – UK

What you infer is correct. The mode of capture of microbial particles as they pass through the fibre matrix is electrostatic. I think, it would be reasonable to expect the charge possibly to change, perhaps by heat. I would anticipate that it may not be affected by radiation. Our studies to date have all been done on unsterilized webbed materials, and it is our intention to do them on sterilized samples, so as to be able to make comparisons. At some future date we will answer your question.

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Comment by J. Nygard – USA

I would just like to make two comments on earlier questions. The first concerns medical problems from ethylene oxide exposure. American Hospital Supply Corporation began a programme in 1976 that included genetic studies and environmental surveys. I understand, they have detected no abnormalities in those who were exposed to ethylene oxide.

Secondly, with regard to the solubility of ethylene oxide in water, those who have done considerable survey work with ethylene oxide have found that when they used steam ejectors and water-sealed vacuum pumps ethylene oxide was solubilized in water, which is reversible in special conditions. Therefore, anyone with water-sealed vacuum pumps and steam ejectors should be cautious and suitably vent the area.

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Comment by R.F. Morrissey – USA

During the Symposium, we have talked about bioburden and overkill and comments were made about controlling the process, or refining the process, or reducing the process, whether it was ethylene oxide or radiation. I would not want our comments to be misinterpreted as decreasing the sterility assurance level What this forum has shown is that there are a variety of new methods and new technologies from which we are obtaining new information that permits us to optimize sterilization processes. The majority of the ethylene oxide processes, for instance, were designed for overkill. The cycle time was set independent of the nature of the bioburden. It must not be interpreted as cutting corners or reducing the quality. It is by advancing our knowledge of microbial resistance and process variability and control that permits process optimization.

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INDEX

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in *italics* indicate Figures and/or Tables, those in boldface indicate major discussions.

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* MANUFACTURER

Any agency involved in the conversion of raw material into parts, and parts into finished products.

For the purposes of the registration scheme major stages of manufacture are identified as:

1. Conversion of raw materials into definable components, sub-assemblies, parts.
2. Production of or assembly from components, sub-assemblies and parts of the finished unsterile, unpackaged product/device.
3. Packaging.
4. Sterilization.

Only agencies carrying out at least stages 2 and 3 for some products are considered as manufacturers.

(1) "Package" is defined as a unit consisting of one or several articles presented in the same wrapping for irradiation.

(2) The reference dosimeters are calibrated by the "Service Central de Protection contre les Rayonnements Ionisants" (Central Service for Protection from Ionizing Radiation).

(1) Biological indicators prepared with Bacillus pumilus E 601 or Bacillus sphaericus C_1A contaminated with 10^8 spores and standard inactivation curves can be obtained from the "laboratoire National de Reference Microbiologique".

1. National Bacteriological Laboratory, S-105 21 Stockholm, Sweden.
2. International Atomic Energy Agency, Wagramerstrasse 5, A-1400 Vienna, Austria.

Translation accepted by the Department of Drugs, National Board of Health and Welfare 1977-10-10.

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