STERILIZATION OF MEDICAL PRODUCTS

STERILIZATION OF MEDICAL PRODUCTS VOLUME II

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DEDICATION

Charles Artandi
1917 - 1980

During the preparation for the Second Kilmer Conference the organizing committee lost one of its most loyal supporters.

There appears from time to time an individual whose particular gifts enable him to exercise a profound and beneficient influence on his colleagues. Charles Artandi was such a man. During his long career he became a recognized international authority on industrial sterilization and occupied many positions where his understanding and willingness to help enabled him to contribute much of value. He was also the mainstay for the Johnson & Johnson high standard for sterile products.

We shall surely miss his guidance and enthusiastic support. This volume is dedicated to his memory.

PREFACE

This volume represents the proceedings of a symposium held at the Washington Hilton Hotel, Washington, D.C. on October 16 and 17, 1980. The symposium was the second in a series organized as a tribute to Fred B. Kilmer, the first Director of Research of Johnson & Johnson and a pioneer in the sterilization of medical products as well as in the microbiological control of the environment.

The Second Johnson & Johnson International Kilmer Memorial Conference was planned with several objectives in mind. The major objective of the Conference was to provide a forum for a selected group of world experts in the field of sterilization of medical products to exchange information on the forefront of this specialized technology. The divergent regulatory requirements governing sterile medical products in various countries were also examined. Approximately 150 participants from 17 nations, representing government, universities, hospitals and industry were in attendance.

Another objective was the recognition of Dr. Jocelyn C. Kelsey as the recipient of the Second Kilmer Award for his many contributions to sterlization and the control of microorganisms in the environment. Dr. Kelsey, an internationally renowned microbiologist and retired Deputy Director of the Public Health Laboratory Service of Great Britain, has published widely, but is best known perhaps for his paper entitled "The Myth of Surgical Sterility." This classical paper is reprinted in this volume by permission of the Editor of *The Lancet*.

The editors should like to express their sincere appreciation to the speakers for their efforts, cooperation and expertise in putting into final form the information presented orally at the sessions; to the chairman, especially to the Conference General Chairman, Dr. R. W. Campbell, who devoted a great deal of his time both before and during the Conference and contributed greatly to its success.

The editors are grateful also for the sponsorship of the Johnson & Johnson Corporate Office of Science and Technology, Mr. Herbert G. Stolzer of the Johnson & Johnson Executive Committee and Mr. Herbert Kramer of Johnson & Johnson International. Finally, the editors are indebted also to Mrs. Sylvia Perweiler and Ms. Janet Stavola for their assistance in the planning and the conduct of the Conference.

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1981

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FIRST SESSION

Conference General Chairman
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Validation of Sterilization Processes for Medical Devices

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Validation of the sterilization process is expensive and time consuming. The requirement to validate this process has not been clearly stated in the Medical Device Good Manufacturing Practices (GMP) (1) published by the Food and Drug Administration. However, as industry gains more knowledge about the sterilization process, I predict it will become an internal requirement by all manufacturers of sterile medical devices, irrespective of government regulations.

The medical devices industry is a non-sophisticated consumer when it comes to the sterilization process. Many manufacturers still treat the sterilizer as a "magic black box" that is absolutely infallible. Sterilization process parameters many times are established on little if any sound scientific data. These processes have evolved into pseudo-sophisticated processes. Time is the most frequently adjusted parameter to achieve an "acceptable process." For example, if an eight hour cycle has a sterility test failure, then many times the cycle is arbitrarily extended, sometimes even doubled. If the sterility tests now are acceptable, no additional data is generated to demonstrate the effectiveness on the product. Under certain conditions, time can be increased with little or no increase in sterilization efficacy. Certainly this is not the method that should be used to establish an acceptable process.

The sterilization process has been perceived by many to be an art. However, today we are attempting to approach it as a science. Sterilization has had several periods when it appeared that it was becoming a science. This first scientific approach to sterilization appeard in that classic article on "Modern Surgical Dressings" by F.B. Kilmer, published in the American Journal of Pharmacy in January 1897, (2) to whom this conference is dedicated. It took most of the scientific community about 60 years to comprehend what was revealed in that early work. It was not until the United States National Aeronautics and Space Program that a scientific approach to sterilization was really applied. The requirement was that an interplanetary spacecraft had to be sterilized to a probability of less than one chance in a million of having a single living microorganism contained on it. More recently, the pharmaceutical and medical device industry has applied these scientific approaches to process development and documentation. We have called this approach validation.

The sterilization process is the last manufacturing step applied to the product. If this process is not well understood and controlled, it can compromise the entire integrity of the product. Extreme compromises can result in sterile products that are not functional or functional produts that are not sterile. Without adequate process control, sterile products may be produced today and products produced tomorrow may be nonsterile. Validation should be a must for any sterilization process, if the manufacturer is concerned with product integrity. I believe most manufacturers certainly are.

Manufacturers typically direct their major process control efforts toward those areas that they know impact on the product quality. Manufacturers who have very little knowledge of the sterilization process and do not understand the problems that can occur to their product, pay little attention to the control of this process. There is a degree of comfort derived from naiveté. As knowledge of the sterilization process grows, no manufacturer will feel comfortable with anything less than a sterilization process based on sound scientific data, a strong validation program, and a process

appropriately controlled for routine processing. With this knowledge, manufacturers will develop cost effective processes. They will have less resterilized product, less scrapped materials, and fewer product failures in the field. All of these situations positively impact on the profits of the corporation. Validation is an investment – it takes a cash outlay before a return can be realized.

I would like to address several general questions often asked about validation before I get too deeply into the details of the process.

What is validation?

Validation is a term that has been used frequently describing a test on a sterilizer and a product to determine if the process if performing properly. Validation is much more than a single terminal test. It is an entire systems approach to the assurance thay your process performs as you expect each and every time it is performed.

Why do I have to validate?

Validation is not a specific requirement in the United States for processing medical services. However, the concept of process control permeates the entire Medical Device GMP. Validation is the means of assuring that your process is valid for its intended use and that it is controlled. Therefore, you must validate to assure that you have control of the process. Validation is also a mechanism of knowing your process and it becomes an invaluable tool in diagnosing problems when the process fails for one reason or another.

How extensively do I test?

Validation is not just a test. It has many facets. It includes documentation of the manufacturing process. It includes an installation qualification on all equipment used in the process. It includes a complete metrology program on all process sensors, indicators, recorders and controllers. It also includes performance qualification of the system. It is the last step that has been commonly and mistakenly portrayed as “validation”. Performance qualification involves the physical performance of the sterilizer as well as the biological performance of the process on the product. This final step integrates the product with the hardware.

Many aspects of validation are an ongoing manufacturing process. In certain instances, such as performance qualification specific comprehensive tests must be repeated periodically.

How often do I re-test the performance qualification?

The performance qualification must be retested at certain key times. These include:

1. After any major change has occured to the sterilizer;
2. After any modification has occurred to the product or package;
3. After any failure in the testing of the product which may be explained by the failure of the process equipment; and
4. At least annually to assure that the mechanical equipment controlling the process has not deteriorated.

What are the key elements of the validation program?

A validation program has many facets. First, the medical device must be manufactured under Good Manufacturing Practices. This will assure you that the product and the package that is being sterilized is controlled and identical each time.

Packaging in many respects is just as important as the product and it is many times overlooked. The package acts not only as a biological barrier to maintain a sterile product, but it is also a barrier to the sterilant and can retard or inhibit the sterilization process. Many times inappropriate coatings placed on normally permeable barriers may not be visibly different but can cause nonsterile products to be produced in an otherwise sound sterilization process.

Component material changes, such as different suppliers of components and slight design engineering changes may alter the device's ability to be sterilized. It is therefore, imperative to document all material changes of both the product and the package, and the manufacturing process to assure proper sterilization of the product.

Documentation

The documentation package must include: Protocols, Procedures, Specifications and Reports. All of these must have the appropriate review and approval signatures.

Protocol: The Protocol is a document that outlines in technical detail exactly the scope of a study. The study may be as simple as proving a procedure, or as complex as developing the sterilization performance qualification of a product and process. Protocols contain the following information:

1. Title of the study.
2. Organization for which this study is being performed.
3. Organization which is performing this study.
4. The person who is directing the study.
5. Scope of the study.
6. The approach used in the study, including specific detailed methods and materials.
7. Identification of all supportive documentation.
8. Definitions of specific terms that apply to the study.
9. Signatures and dates of approval for both the performing organization and the organization for whom it is to be performed.

The Protocol must be completed prior to the commencement of any testing.

Procedures: Procedures describe, in a step-by-step manner, a specific task. Each individual task necessary to perform the total validation program must be identified and documented in this manner. The procedure must be verified, using a Protocol. Following this verification, the procedure is approved with appropriate management signatures and issued. This document now needs only to be referenced by appropriate number and title designation in the protocols for the various steps in the validation process.

Specifications: Specifications describe a material or standard process in sufficient detail to assure replication each time the task is performed. Specifications are proved using the protocol process in the same manner as procedures.

Reports: The reports are the accumulated experimental data generated by performing the protocol. The reports must reference the protocol and the detailed procedures contained in them. If the data was not generated in the manner described in the protocol, the exceptional conditions must be detailed in the report. Exceptional conditions must be explained. The data format in the report may be raw data or summarized data. In cases where the raw data is too voluminous or bulky to present conveniently in a report, a summary of the data is more appropriate. If only summarized data is presented, then specific references must be supplied to indicate where the raw data is available for review. The

report should also contain the conclusions reached based on the analysis of the study data. The report should contain the appropriate signatures from performing organization as well as those from the sponsoring organization.

Validation Testing Program

The validation testing program must be documented as described in the previous section. The actual testing program is segmented into three phases: Equipment Installation Qualification, Calibration and Performance Qualification.

Equipment Installation Qualification: This phase of the validation program involves an engineering evaluation of the process equipment. Each piece of equipment involved in the process of producing a sterile product must be qualified. This qualification starts with a detailed physical description of each piece of equipment. Engineering drawings are reviewed for accuracy and completeness. Discrepancies are noted and red lined in the drawings. The manufacturer's specifications are reviewed for utility service requirements. Operating characteristics are evaluated to assure that the equipment is functioning properly. Preventative maintenance procedures are reviewed or established. Spare parts listings are also reviewed. Components are evaluated to determine which are critical to the control of the process and which are not. When a critical component is changed during a maintenance program, it may be necessary to rerun performance qualification tests.

Calibration: All sensors, indicators, controllers, and recorders must be calibrated on the equipment following the installation qualification testing. If a sensor or indicator is not critical to the performance of the process, calibration can be omitted. However, in this case, a "reference only" label should be used, thus indicating it is not important to the control of the process.

Calibration testing should be performed using instrumentation traceable to the National Bureau of Standards (NBS). The appropriate certificates indicating traceability to NBS should be included in the report.

Calibration data should reflect the precision and accuracy of the indicator, controller or recorder. Appropriate correction factors should also be included.

The frequency of recalibration is established based on past performance history and how important the system is to the process. In some cases a sensor or controller may be calibrated before each use, as well as after each use. Generally calibration frequencies range from three to six months.

Performance Qualification: Performance qualification refers to that phase of the validation test program when product and process equipment are tested together. This phase is the culmination of the validation test program and has inappropriately been referred to as validation. The equipment has been documented from an engineering perspective, all control systems have been calibrated so that they will yield meaningful data, and now the equipment will be evaluated with the product to determine its performance.

It is assumed that the sterilization cycle has already been developed for the product. All of the important process parameters must be monitored. Parameter distribution within the sterilizer as well as parameter penetration into the product must be determined. In steam and dry heat sterlization processes, only temperature distribution and penetration studies are usually performed. Ethylene oxide gas sterilization processes have ethylene oxide gas concentration, moisture and temperature as the key process parameters.

Parameter sensors are placed throughout the sterlizing chamber and inside packaged product, distributed throughout the chamber. These sensors are placed in the location that is most difficult to sterlize within both the chamber and product. The loading pattern for the product can influence the ability of the sterilant to reach the product. Therefore, if several different load configurations are used, each must be evaluated to determine the influence on the sterilant's capacity to reach the product.

The integration of parameter level and time is demonstrated by the use of a biological monitoring system. This system uses bacterial spores of known resistance and population. These spores are demonstrated to be more resistant to the sterlization process than those organisms that are naturally occurring on the product prior to sterlization.

The system which is qualified is one which that has demonstrated acceptable performance in achieving desired parameter control within the sterilizing chamber and product. Such a system has also demonstrated acceptable microbial lethality in producing a sterile product.

The completion of the above described three phases (equipment installation qualification, calibration and performance qualification), results in a validated sterilization process.

Does the medical device industry believe in validation? Certainly the major medical device manufacturers do. That belief has only come with their increased knowledge of the sterilization process. As the small medical device manufacturers gain in the same knowledge, they too will begin to see its value. Industry, in general, will proceed in the direction of validation because the knowledge gained througha proper validation program is effective in controlling manufacturing costs.

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1. Manufacture, Packing, Storage, and Installation of Medical Devices—Regulations Establishing Good Manufacturing Practices. (1978). Federal Register **43**: 31508-31532.
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Bioburden: A Rational Approach

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Historical

The man whom we are honouring at this gathering was well aware of the meaning and impact of the modern day term “bioburden”—those microorganisms found on medical products just prior to sterilization. Fred B. Kilmer went to great lengths to maintain what we refer to today as a “high degree of sterility assurance” (Figure 1).

Figure 1. Fred B. Kilmer first Director of Research for Johnson & Johnson, ca. 1930.

Without the aid of D-values of kill curves, he intuitively understood the relationship between the number of organisms on a product and the extent of sterilization processing required. Environmental control was his forté. Describing the physical layout and operation for the manufacture of the first commercial sterile surgical dressings, Dr. Kilmer stated, “The buildings set apart for this work were built for this special purpose—made plain and tight to exclude dirt. They are admirably situated away from busy and dusty streets. For miles on either side stretches river and meadow-land, securing an almost dustless atmosphere. In fitting up the rooms in which the manipulations take place, the ideas kept in view were the exclusion of bacteria, easiness of keeping clean” (10).

Within the buildings, Kilmer went on to say, “The walls and ceilings are glass-smooth. The floors are filled and polished. There are no closets or shelving, no cracks or crevices to harbor dust or dirt. The furniture consists of glass-topped tables with iron frames, allowing effectual and easy cleansing. The principal of the work is done in the ‘aseptic room,’ so called because all things within it are at all times kept surgically clean.” Kilmer developed an elaborate set of rules governing disinfection procedures for equipment and personnel who were to enter the “aseptic room.”

In his quest to reduce contamination, he went so far as to presterilize all gauze materials and glass

containers before the dressings were cut, folded and packed. After packaging, the dressings were then subjected to a terminal steam sterilization process. This same approach was followed some 78 years later when Johnson & Johnson decided to reduce the bioburden on unbleached cotton stockinet prior to component fabrication into disposable hospital gowns. Such an approach to increase the sterility assurance of Cobalt-60 sterilized disposable hospital packs was favorably received by the FDA, and helped to foster the rapid approval of "dosimetric release" within the USA (13).

Kilmer not only pioneered procedures for the control of bioburden but was the first person to use biological indicators to monitor a commercial sterilization process (5). The concepts that this first Director of Research of Johnson & Johnson followed during the late 1800's can be seen in practice today. He controlled the presterilization microbial population, then went on to use an "overkill" steam cycle which he validated using *Bacillus anthracis* inoculated product biological indicators. He was keenly aware of the impact of such important parameters as load density and geometry and took pains to insure that the biological indicators were placed in the center of the package in the middle of the trays.

Much later, other investigators referenced the importance of bioburden and environmental control. In 1940, R.M. Savage (14), while investigating various technical considerations associated with product sterility testing, pointed out that when material is heavily contaminated prior to sterilization, failure to sterilize will result in a discrete number of contaminated units. Tattersall in a 1961 report (15) entitled, "Control of Sterility in a Manufacturing Process," illustrated that fractional sterilization of a batch of plastic articles processed by ethylene oxide, was related to initial contamination. Emphasis was on "the nature and degree of contamination of production articles," rather than resistance, in the designing of "test pieces".

J.C. Kelsey discussed the importance of good factory hygiene, indicating that products should be handled as little as possible during processing. In describing an effective sterilization process Kelsey said, "For most processes both the cost of sterilizing and the damage done to the load increase with exposure, so that the smallest safe exposure must be sought" (9).

With the United States commitment to space exploration, the National Aeronautics and Space Administration (NASA) initiated a program to search for extraterrestial life. In doing so, precautions were taken to prevent external contamination of the planets. Sterilization was deemed necessary and bioburden loading became critical. Sterility testing of large spacecraft components was obviously impractical and unscientific. The emphasis shifted toward a probability approach. The official NASA policy acknowledged the possibility of extraterrestial life and recognized that microbial contamination originating from Earth would interfere in the investigation of extraterrestial life and make detection impossible (3,6,7,16).

Lawrence Hall, Planetary Quarantine Office, said that NASA standards required a "sterilization level such that the probability of a single viable organism aboard any spacecraft intended for planetary landing or atmospheric penetration would be less than 1×10^{-5}, and a probability limit for accidental planetary impact by unsterilized flyby or orbiting spacecraft of 3×10^{-5} or less." Hall discussed methods of limiting the "viable burden" on a capsule including the sterilization of raw materials. He also used the terms "microbial burden," "biological burden" and "biological loading" to describe those microorganisms found on space capsules before terminal sterilization. He concluded that if it was possible to assemble a spacecraft with sufficient assurance that the microbial population was indeed small, then an appreciable reduction in the terminal sterilization cycle was

justified (6).

Hall and Lyle summed up the essence of the NASA approach to sterilization: "A suitable low probability for the survival of microorganisms after spacecraft sterilization requires the application of sterilants in proportion to the biocontaminant load present on the spacecraft at the beginning of sterilization… It is also desirable to minimize the viable biological load in order to reduce the sterilization to be applied to the spacecraft" (7).

The concepts of environmental control and bioburden awareness enunciated in the 1890's by Fred Kilmer for the manufacture of sterile dressings, were in the 1960's refined, expanded upon and became an integral part of the space exploration effort.

Assessment of Bioburden

Figure 2 illustrates the extensive range of presterilization bioburden loading on various types of medical products. Factors contributing to the presterilization microbiological flora include the nature of the components, the type of manufacturing procedures employed, the extent of human contact, and other environmental conditions. The list (Figure 2) contains seven commercially manufactured products and one hospital prepared item. The variability between these diverse products is in the order of one million microorginisms.

As products increase in complexity frequently the microbiological load also increases. The effect of a minor component may have a major impact. Unbleached cotton was linked to 99% of the total bioburden of disposable hospital packs (13). As expected, natural products especially those of animal origin show high numbers with wide variations in count.

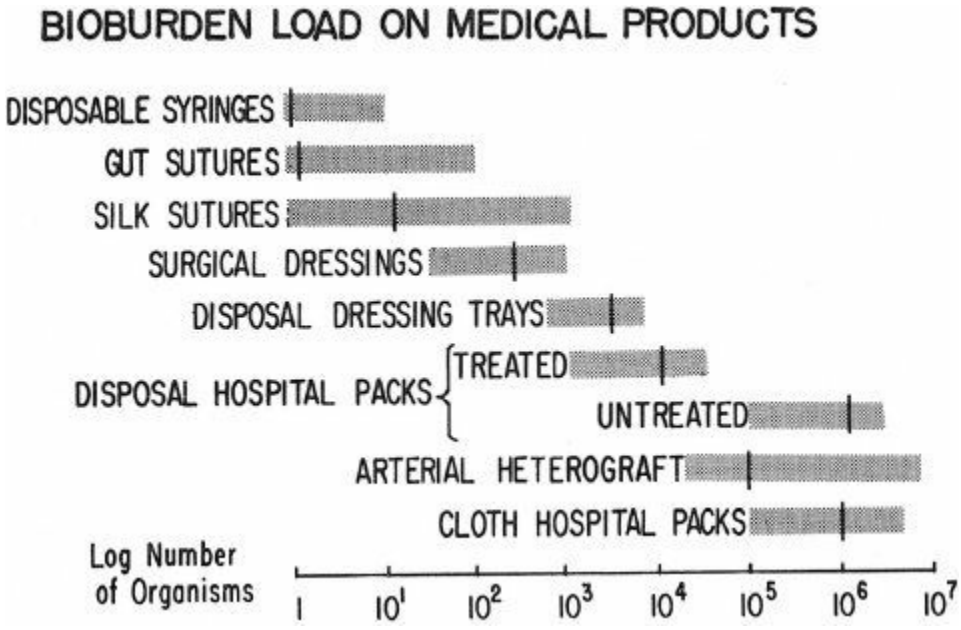

Figure 2. Bioburden load on various medical products. Vertical bar identifies the arithmetic mean (1,8,11,12,13).

Miller (12) reported that up to 60% of all plastic disposable syringes were sterile when removed from the manufacturing line prior to terminal sterilization. Since the manufacturing process included high temperature extrusion accompanied by automated assemble, it is easy to understand why the microbial population was so low.

Looking further into the relationship between product characteristics and microbial loading, it is possible to delineate several broad groups (Figure 3).

If a product fulfills certain criteria it is possible to predict, with reasonable certainty, the associated bioburden load. It has not always been recognized that the manufacturing processes for many synthetic materials have inherent sterilizing properties. High temperature is the most common, and is associated with the production of metal devices and many plastics. It is only after such products are stored or handled that the microbial population accumulates. Low counts associated with the automated production and packaging of disposable plastic syringes attests to this reality.

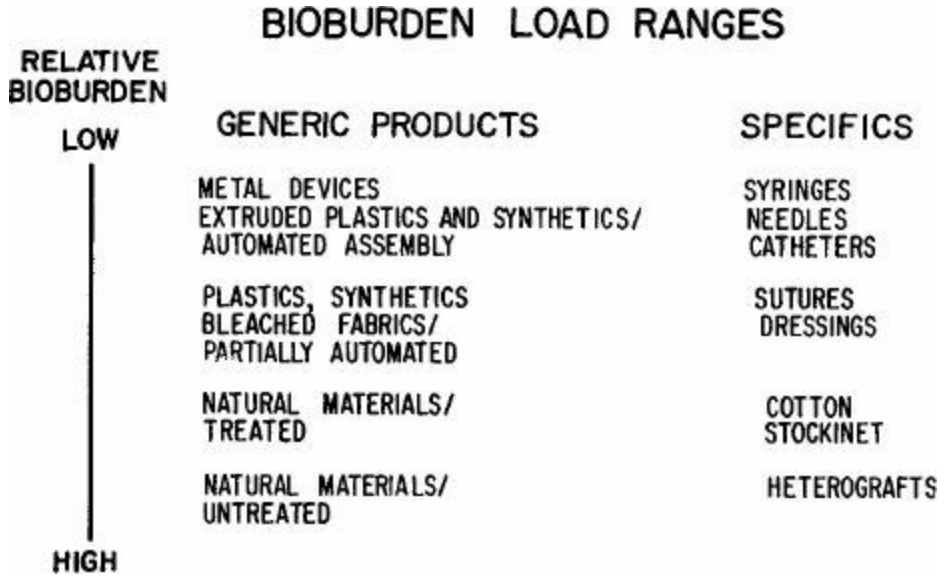

Figure 3. Classification of bioburden into four generic groups as a function of product characteristics.

An intermediate range of bioburden may result from the use of processes that only partially reduce the microbial population, along with products having high surface area and where assembly is not completely automated. At the extreme are natural materials of biological origin where the manufacturing steps introduce exacerbating factors such as water, elevated temperature, humidity, etc.

Knowledge of raw materials and manufacturing procedures is the first step in understanding and identifying bioburden loading points. An experienced environmental specialist can frequently predict the outcome of bioburden testing based on such information.

Applications of Bioburden

There are two broad applications in which bioburden information assist a medical products manufacturer:

1. Evaluation of changes that impact on the presterilization quality of a product.
2. In designing an appropriate sterilization process.

Bioburden analyses can be of great value when determining the impact of product associated changes on inherent product quality, functionability, and sterilizability. Process and equipment modifications can be evaluated, alternate raw materials qualified, and the impact of human handling versus mechanical methods assessed. Where microbiological monitoring of air, water and surfaces takes place as a part of a regulatory or in-house requirement, bioburden testing of actual product samples provides a much more simple, direct, and meaningful readout. Organisms recovered from the air, for example, may or may not come into contact with product.

There are two basic approaches in developing effective sterilization cycles: The overkill approach and the bioburden approach. A recent recommended practice document published by the Association for the Advancement of Medical Instrumentation called, "Guidelines for Industrial Ethylene Oxide Sterilization of Medical Devices" states that the overkill concept assures that the sterilization process will inactivate selected numbers of resistant spores typically *Bacillus subtilis* var *niger*, with an additional safety factor, without necessarily relating the challenge population to the presterilization bioburden. This method provides an overkill, because the cycle conditions established to kill the challenge indicator are more severe than those required to kill the presterilization bioburden (2).

The bioburden approach is to collect presterilization data and to relate the numbers and/or resistance of the bioburden to the indicator microorganism. This permits cycle selection by establishing the challenge indicator population with a safety factor added above the anticipated bioburden.

Overkill cycles are traditional for many types of sterilization processes including steam, ethylene oxide and radiation and require that the product withstand the stringent exposure and perform satisfactorilly afterwards. Bioburden cycles can be considered a refinement over the overkill approach, and require monitoring the presterilization population on a periodic basis. It is the cycle of choice for heat-labile products, such as large volume parenterals, which may not tolerate overkill conditions.

A graphical representation comparing bioburden and biological indicator resistance can be seen in Figure 4. Note the slope for the standard biological indicator organism as the surviving fraction decreases with increasing exposure time (dose). More often than not the bioburden exhibits only a fraction of the resistance of the biological indicator. Rarely do we see bioburden more resistant than the standard resistant biological indicator, and this is usually the result of an organism/substrate interaction or incomplete sterilant penetration due to the protective action of certain packaging materials.

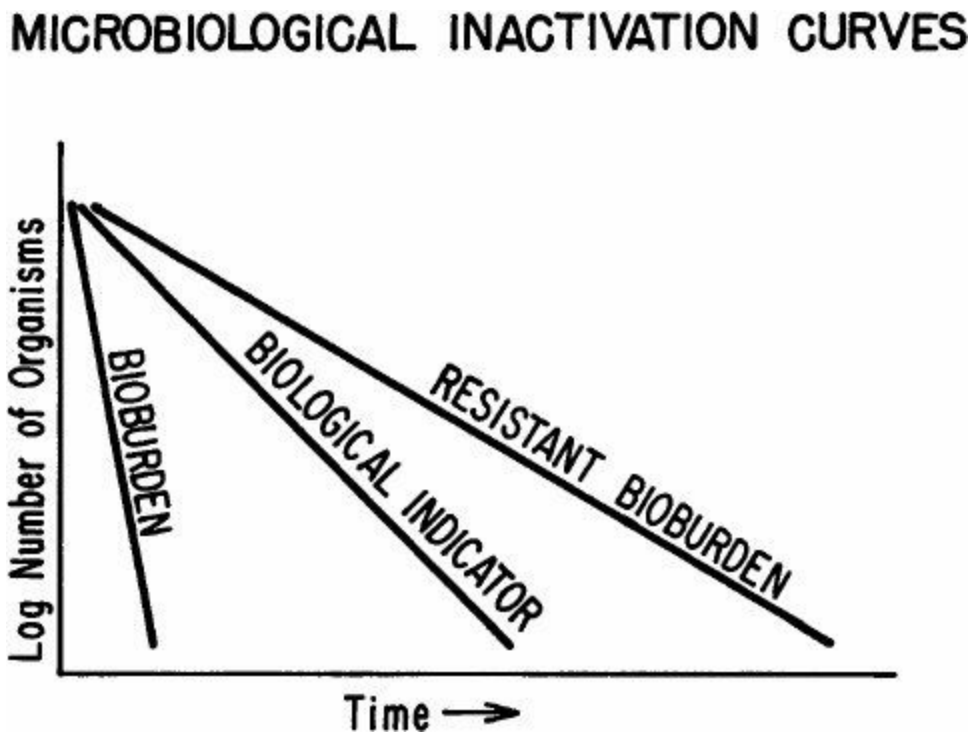

Figure 4. Microbiological inactivation curves illustrating the relative resistance of biological indicator to bioburden population.

Figure 5, adapted from actual data, shows the relationship between bioburden resistance and standard biological indicator resistance which has been extended into the probability range. There are at least four possible sterilization cycles that could be derived from these data:

Cycle 1

The classical overkill cycle requiring inactivation to 10^0 at five minutes plus an additional six log safety factor for a total exposure time of ten minutes is easily visualized. Total inactivation is 12 logs of resistant biological indicator. The D-value equals 0.83 minutes.

Cycle 2

The bioburden inactivation is extremely rapid. In two minutes approximately nine logs are reduced; the 1,000 presterilization bioburden organisms plus a six log safety factor. The D-value of 0.21 minutes if four times less resistant than the standard biological indicator, resulting in a bioburden cycle time of two minutes. The difference between the overkill exposure time and bioburden exposure time is five fold (two minutes versus ten minutes).

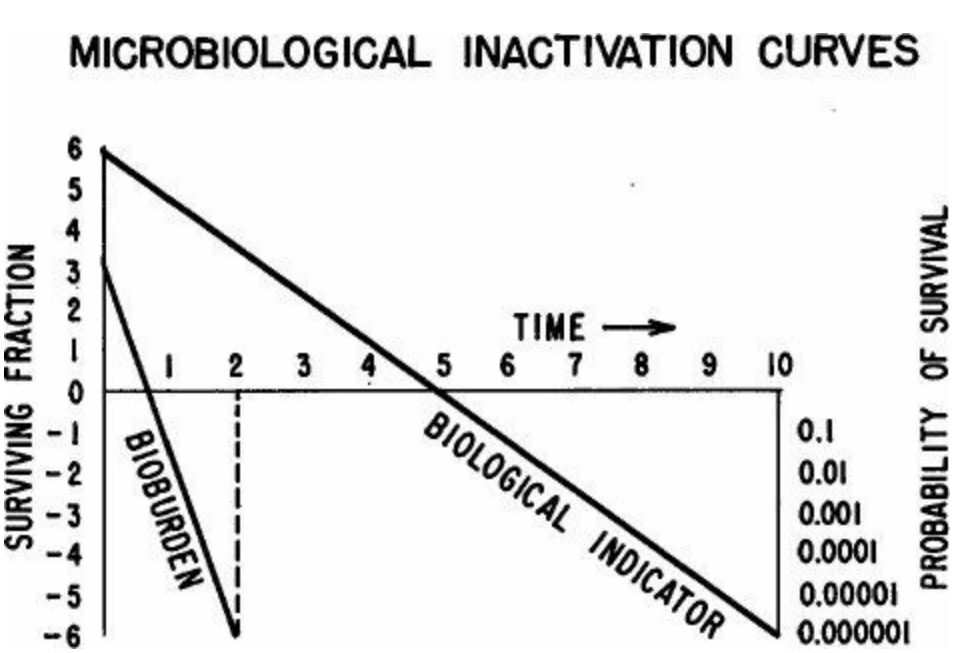

Figure 5. Derivation of sterilization cycles based on extrapolation of microbiological inactivation curves into the probability range.

Cycle 3

Another approach might consider the selection of a five minute exposure time. This would be sufficient to inactivate 1,000,000 standard resistant spores or the astronomical figure of 10^{23} bioburden organisms.

Cycle 4

A fourth, more appropriate procedure would utilize what I call a "Bioburden Calibrated Biological Indicator" (BCBI). This means that a biological indicator could be used as the routine process control monitor, but it would be calibrated against the bioburden resistance. In this case, a spore population of 10^3 would more than suffice for routine monitoring and would insure that a safety factor of at least 10^6 is maintained with an exposure time of approximately three minutes.

The point that I am trying to get across with these examples is one of *flexibility*. Flexibility in cycle design. There are a wide variety of approaches to cycle development and routine monitoring. The more knowledge you have about bioburden resistance the more you can refine the process. In the previous examples cycle *time* was refined. It is not a simple matter of classifying a set of conditions as strictly a "bioburden cycle" or an "overkill cycle." There are areas that exist between the two extremes. There are degrees of overkill. The use of biological indicators specifically calibrated against bioburden is a logical solution for everyday process monitoring.

Alternatives to Bioburden Testing

Where facilities for bioburden testing do not exist, or in those situations where such analyses are too costly or complicated, there are at least three alternatives (Figure 6).

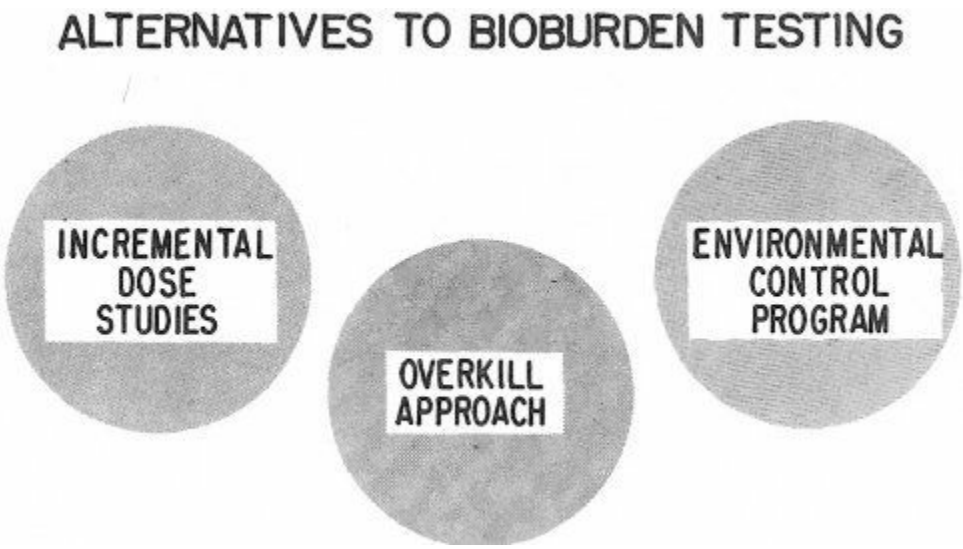

Figure 6. Some alternatives to bioburden testing. Testing being defined as the laboratory determination of microbial counts.

1. *Incremental Dose Studies*
   This procedure consists of subjecting product samples to fractional exposures and then testing the samples for sterility. Sterility tests can be conducted by the smaller laboratories or by outside contract laboratories, and are scored as positive or negative. The application of this technique is at the heart of the Dose Setting method for radiation sterilized products which will be presented elsewhere.
2. *Specialized Good Manufacturing Practices (GMP)*
   Assuming the source of bioburden loading can be identified and controlled, then procedures used to control bioburden can be institutionalized as a specialized GMP program. These GMP procedures once validated, can be monitored by Quality Assurance to insure that all parameters meet specification compliance. This can be considered an indirect method of bioburden monitoring. If the procedures to control bioburden have been validated and are reproducible, then there is no need to conduct routine bioburden tests.
3. *Use of Overkill Cycle*
   For those instances where bioburden monitoring is inappropriate, and the product can withstand excessive cycle conditions, then the use of the overkill approach may be the method of choice. Although not required, some bioburden information is useful. Knowledge of bioburden can be arrived at indirectly by an initial examination of material and process characteristics as described earlier.

The Future

In some circles bioburden testing has led to much confusion. There was a time when FDA Investigators, while inspecting medical device firms, asked for bioburden data but had little understanding as to the significance of the numerical values. Worse than that, some manufacturers started up bioburden testing programs with the sole intention of keeping the investigator happy on his next inspection. This attitude prevailed for several years but was clarified in November, 1979, in the FDA publication entitled, “Application of the Device Good Manufacturing Practice Regulation to the Manufacture of Sterile Devices” (4). The document stated that, “…bioburden testing is not required unless the firm’s own internal procedures require such testing. For medical devices FDA: (a) does not require that the sterilization process be established on the basis of bioburden and (b) does not specify that bioburden testing is essential to all sterilization processes…” The important point is that the FDA does not require bioburden data when overkill cycles are used. However, if a manufacturer utilizes a bioburden based cycle he is required to maintain bioburden at or below prescribed levels. On the other hand, FDA is not enamored with overkill processes and warned the field force that firms may grow lax in presterilization practices and cover up microbial or particulate filth problems with a severe overkill process.

The science of sterilization processing has undergone marked advances within the past ten years. The late Charles Artandi (1) characterized sterility controls as falling into three distinct periods of development:

1. The Period of Innocence
2. The Period of Doubt
3. The Period of Enlightenment

We have evolved from a period of innocence of reliance upon the statistically invalid technique of product sterility testing, advanced into the use of biological monitors, and now incorporating the common sense of bioburden with the realization that as our knowledge increases, the concept of parametric release for most forms of sterilization, including ethylene oxide, will be realized (Figure 7).

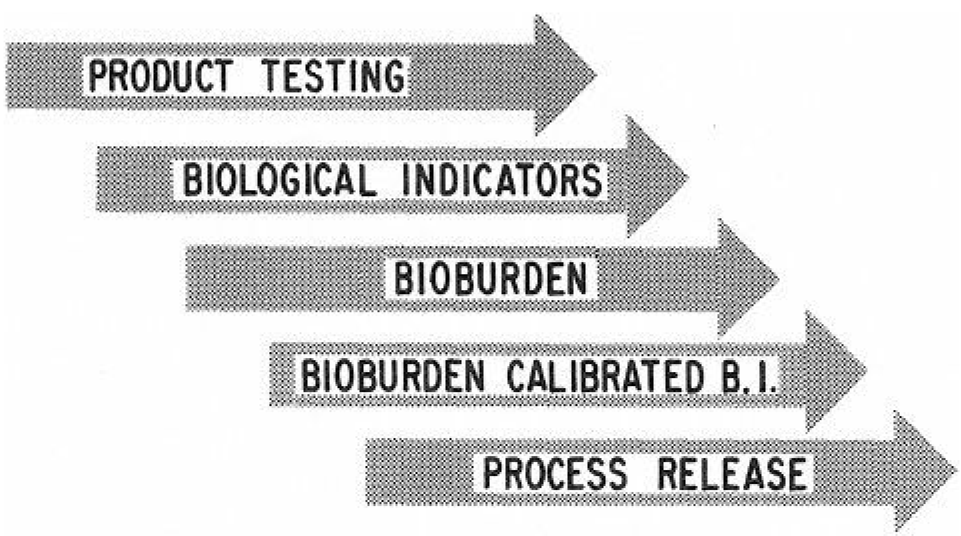

Figure 7. Chronology of sterility control.

I hope that our doubts about bioburden will diminish, and be replaced with the enlightened

realization that bioburden can be a powerful tool for optimizing our current processes.

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A Quantitative Approach to Microbiological Safety

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I had thought to use the title “How many germs?”. The present title better describes the content of this talk, but still “How many germs?” is the real problem. This is really the question which the manufacturer of medical devices must ask and it is the question which the user of those products must answer.

Germs

First of all we must know of what we speak. We are talking about germs. We are not talking about bacteria or microorganisms in general, but germs! Bacteria, or more generally microorganisms, are impersonal; they are innocuous looking and non-threatening things. There *are* microorganisms which can do nasty things when all the conditions are just right, but many are quite harmless, others are beneficial and some are essential to human well-being. They can even eat oil spills and manufacture hormones and other useful things. Now germs on the other hand are nasty, vicious creatures which cause infection and make people ill. It is germs that we are concerned with.

I think that this distinction is important to make, because what we are ultimately concerned with is clinical safety, a reduction of clinical hazard. We are looking for assurance that a particular device is safe for its particular use in a particular place. It is also important to distinguish between contamination and infection. Contamination is the presence of something which is not supposed to be there—contamination is not desirable, particularly bacterial contamination, but it does not necessarily result in infection. Infection involves the growth and multiplication of germs and undesirable reactions with living tissue.

To be a germ, a microorganism must be of the right type, in the right state, in the right quantities, in the right environment, at the right place and at the right time. If any of these conditions are not met it is *not* a germ; it does not produce infection. If we can prevent even one of these conditions from being met, we remove the threat.

We all know that general acceptance of the "germ theory" is relatively new, although such an idea had been suggested many times. It is difficult now to fully appreciate the complicated theories of disease transmission which were prevalent before the germ theory was accepted. We had fixed contagium in palpable morbid products—these were viruses; and volatile contagium in the impalpable emanations of the body—a miasm. So we ended up with infectious contagious disease, infectious miasmatic disease and miasmatic contagious disease among many other labels (1).

Environment

From the very beginning of the development of a germ theory, and even before, the environment was considered to be the most important in disease: important in its progress and in its outcome. Flint said: “The seed must have its appropriate soil and various circumstances may on the one hand promote and on the other hand prevent its germination”(1). The treatment of infections was necessarily entirely symptomatic when the cause was not known and the prevention of the spread disease was haphazard and often ineffective for the same reason.

Germ Theory

About 1540 Hieronymus Fracastorius Veronensis suggested that contagious disease may be due to invisible animal life. Then in 1640 Athanasius Kircher thought he could actually see these animals with his primitive microscope, but he thought that the various “worms”, as he called them, found in meat left outside through the night were due to “corruption contracted from the moon”(2). Perhaps they were!

Sir Henry Holland suggested in 1839 that the source of epidemic disease is animalcule life. But although support for the germ theory grew in the latter part of the nineteenth century, and Louis Pasteur’s work added supportive evidence, still in 1890 there was no universal acceptance of the theory (1).

Killing Germs

Obviously one doesn't think of killing germs until they are shown to be the cause of infection, but fumigants were used for many years as a disinfectant in contagious fevers. Dr. Carmichael Smyth received £ 5000 from the British Parliament in the early 1800's for his fumigant of nitre in heated sulphuric acid. It is interesting to note that he recommended a quantitative approach to disinfection. He instructed that: "The vessels (of disinfectant) should be arranged at a distance of 20 ft. or more from each other according to the virulence of the the contagium"(3).

Different Levels

The principle that the response should be graded according to the stimulus is a very old one. It is applied in general and it has been applied in medicine for as long as there are records. There are many catch-phrases: “Drastic cures for drastic diseases”, “You don’t amputate for ring-worm”. And in the germ area: “Some procedures require more sterility than others”(4,12).

Dr. A. Auvard in his *Traite Pratique de Gynecologie* of 1892 suggests that: “Pour une simple exploration, un savonnage ordinaire des mains (suivi au besoin de l’immersion pendant deux ou trois minutes dans une solution de sublime a 1/2000) est suffisante.” But on the other hand: “Quand on veut pratiquer une opération et surtout une laparotomie, un lavage plus complet est nécessaire”(5)—another clear statement of the need to grade cleanliness, the need for different processes to produce hands which are acceptable for different procedures.

This principle is something all physicians and nurses realize and utilize in designing procedures. Sewing up a thigh wound requires less microbiological care than aspirating a knee joint, syringing an ear less than draining a hydrocoele and removing a sebaceous cyst less than performing a spinal tap. We all know this; we all accept this; and we also know that the microbiological state of the equipment used in these procedures could vary and could be graded in such a way that the clinical hazard in each procedure would remain acceptable. This “gradation of cleanliness”, or whatever term one wishes to use, was accepted without having really been discussed. It was an intuitive thing and the result of experience in many fields. No one has really explained or codified it, though the hierarchy of required cleanliness has been developing since before Lister.

The Word "Sterile"

Perhaps everything would have continued to develop very nicely and quietly if the word "sterile" had not been pressed into use in the context of surgical cleanliness and clinical safety. People began to use the words "sterile," "sterility," "sterilization", etc. in this sense only quite recently. It is interesting to look at three dictionary definitions of the word "sterilize":

- Noah Webster's American Dictionary of the English Language 1892; to make barren, to impoverish, to exhaust of fertility. To deprive of fecundity.
- British Empire Universities Modern English Dictionary 1914; to make sterile; deprive of the power of reproduction, as bacteria.—(germs are now being recognised).
- The Oxford Universal Dictionary 1955; to cause to be unfruitful. To deprive of fecundity. To render free from microorganisms.

So here we finally have what everyone now *knows* to be the meaning! But do they?

The word "sterile" began to be used in medicine and biology without anyone really troubling to define it properly. It replaced the range of surgical cleanlinesses that the health professions had understood without defining. It became used to denote a general condition and it came to be thought desirable in itself. So now instruments, devices, products, people and places were designated either sterile, or not sterile and the use to which they were put, what happened there, was considered of very secondary importance in determining their suitability.

The use of the word "sterile" in many ways stultified thought and progress in the area of surgical cleanliness and clinical safety. Sterile was good; non-sterile was bad. Under these circumstances how can anyone begin to ask or answer the question, "How many germs?"

But luckily many people did—though not out loud at first. "Sterile"—the magic word, meant many different things to many different people. It sill kept its proper meaning of inability to reproduce, but for the surgeon's hands it meant 10 minutes of scrubbing with some specially concocted fluid (4,5), for a patient's abdomen it meant being shaved, washed with disinfectant and draped with a sheet from an autoclave, and for instruments and devices it meant coming out of a packet marked "sterile". There had been many mutterings, but doubts of the value of the word were first raised formally by Kelsey in 1972 (6).

How Sterile?

Postoperative infections did not cease following the introduction of the word, so it was obvious that some part of the system (one of the steriles) was not sterile, or at least not as sterile as one would like.

The whole process must be considered and checked to ensure an acceptable end result or product. In 1940 Dr. John Drew said: "The plentiful and regular use of soap and water is far more efficacious than the occasional application of the most powerful disinfectant"(13). This sounds very much like a plea for process control and GMP.

Medical products which require to be sterile are subjected to various processes designed to achieve this state. Testing of samples has been done by various methods to verify the sterile state, but end product testing has serious limitations when one is looking for a very low probability of contamination in products which are produced in small quantities. Unfortunately, in the case of medical devices this situation is quite common.

Arriving at a figure for the probability of contamination of a product can be done in several ways. One must take into account many factors: what potential germs are present, how these behave when subjected to the process designed to kill them, how well all the variables of the process are controlled and how stable and reproducible are the conditions in the system, from raw material to finished transported product. This is the area which is difficult, subject to much discussion and an area which requires very specialized technical expertise.

I can appreciate the many problems which have been cited as causing difficulty in determining a number for the probability of contamination (7), but the point is that manufacturers need a number (from testing, or monitoring and control of their process). This is how they decide whether or not they can call their product sterile. They still use the word "sterile" because it is comforting to everyone concerned, but they have in their head a number which indicates the acceptable probability of any one product being contaminated and this number is their cut off point. If their calculations from testing, process control, bioburden estimates, etc. give a probability of contamination less than their magic number, then they feel justified in using the magical, comforting word "sterile" on the product.

The argument that a number for probability of contamination cannot be properly calculated is specious. Despite the difficulties, it is being done, and being used to decide whether the word "sterile" is or is not used on a label.

The degree of confidence with which a probability number can be quoted will also vary with many factors, but this is so with almost any assertion that one wishes to make. Surely one gets a better, more useful, idea about the size of a table if told that it will seat about twelve people, rather than if told that it is big!

Again, to suggest that a number indicating probability of contamination would merely confuse health professionals (8) seems vaguely insulting. I have never heard anyone argue that automobile speedometers should consist of two lights: red meaning fast and green meaning slow, on the basis that marking it in k.p.h. would be too complicated and confusing for the motorist!

The idea that the word "sterile" should be used only in its proper absolute sense is by no means new. It has been proposed many times and of course we all know of the Munson case (9). If this idea were followed, then the word "sterile" would drop out of use in the field of medical products and something must replace it. If on the other hand the word is to be qualified, then the qualification must be quantified. There really is no other way; it is one or the other. In either case we will end up with a

grading of surgical cleanliness, the levels of which will have to be designated in some way—by letter, number or other symbol. We must in other words return to the orderly evolution which was taking place before this upstart word "sterile" was thrust upon us.

I think it is fair to say that if the probability of contamination cannot be calculated and stated, then one has no right to pretend that one can call the product sterile.

Clinical Safety

To try and approach this whole subject rationally it would seem that the best point of departure is the destination.

Our objective is clinical safety or lack of clinical hazard. Now this in itself is an absolute which is unattainable, but it is a target to aim at and a target which has been aimed at with varying degrees of persistence and varying degrees of success (10).

The advances of medical science, advances in available drugs and very largely the advances in medical devices and their related technology have meant that procedures previously quite impossible because of the clinical hazard involved are now acceptable. However, these advances often utilizing and relying on the many invasive probes, pumps, monitors and implants, generate their own new hazards. Someone, somewhere has always to make a judgement consciously or unconsciously as to whether the expected benefits are sufficient to warrant the risks involved.

Would it be possible to measure the factors affecting clinical safety and feed them into a formula which would generate a clinical hazard index (CHI)? And if it were possible, would it be useful?

To be able to measure something is always an advantage when comparisons must be made and, therefore, it should be useful as long as an idea of the expected accuracy of this measurement is available. So, what are the factors of which account would need to be taken in determining such an index? They can be listed under four headings:

Patient

- General condition - (resistance to insult).
- Tissue involved and the region of the body where the procedure takes place.
- Duration of contact.

Attendant

- Level of general medical competence (ability to deal with complications).
- Expertise in the procedure.

Place

Where the procedure takes place (O.R.; hospital or clinic environment; other).

Device

- Expected material/tissue interaction.
- Expected functional reliability.
- Probability of microbiological contamination(10).

It certainly would not be an easy task to decide how to measure these factors and how to weigh them in such a way as to arrive at a clinical hazard index which could be used in the comparison of alternatives and as an aid in planning treatment. It would *not* be easy, but it is something which is worth attempting. The end again seems to be a good place to start, and so in this case we can start with the probability of microbiological contamination.

Microbiological Survival Index, MSI

This is something to which a number can be given, a number which would be useful in itself and could be used eventually in the calculation of a clinical hazard index. The microbiological survival index, MSI, has been explained in several presentations. It is the absolute value of the logarithm of the probability that any one device is contaminated with a viable organism (10).

There are many problems to be solved and many agreements to be reached before different products processed in different ways can all be assigned a microbiological survival index, an index which can be compared one with the other and with an assurance that similar numbers mean the same thing. But in many cases a number could already be used, and in fact one is to be used in the near future by at least one manufacturer. It would certainly give more information than the label “sterile”.

Cooperation between users and manufacturers could quickly result in a concensus as to what is attainable, what is desirable and what is presently acceptable as an MSI rating for any particular device use. These acceptable MSI ratings could then be changed by consultation as other factors in the CHI equation changed and as processes and their control improve (11).

Setting acceptable MSI ratings would be an evolving process resulting entirely from discussions between manufacturer and user. The regulatory agencies who are charged with ensuring that manufacturers claims are supportable with adequate test data and that devices are safe and effective would be able to monitor the processes and controls and discussions with both manufacturers and users could now be quantitative rather than qualitative. A very desirable state of affairs.

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DS Gamma Radiation Dose Setting and Auditing Strategies for Sterilizing Medical Devices

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Introduction

Cobalt 60 Gamma radiation is recognized as a preferred method for sterilizing medical devices. Consequently, between 1972 and 1980 installed Cobalt 60 capacity world wide has increased from 15,000,000 to at least 55,000,000 curies. In 1976, at the instigation of C. Artandi and C.W. Bruch, a North American Working Group, under the auspices of the Association for the Advancement of Medical Instrumentation (AAMI) was created to develop guidelines for controlling the sterilization of medical devices by radiation. This included the development of satisfactory methods for determining the approximate dose of radiation required to sterilize devices. The methodologies presented here are an outgrowth of the committee's activity.

Three classes of guidelines can be recognized for determining sterilization doses (1). Class One relies on confirmation that the dose was effective using end product sterility testing. Class Two would specify a minimum dose, such as 2.5 Mrad, with possible additional limits based on the presterilization microbial challenge. This guideline was considered to be both unduly restrictive and permissive since certain North American products already had an extensive history of being sterilized at doses of less than 2.5 Mrad with no evidence of hazard, and product with a large or unduly resistant bioburden might require higher doses than 2.5 Mrad. In the Third Class, no fixed dose is specified, but sterilization dose is determined by the presterilization irradiation resistance of the product microbial bioburden. Initially we believed that the Class Three guideline, which was supported by the USP XIX and IAEA Code of Practice, was the most valid approach to determining sterilization doses.

It has now become clear that existing dose setting methodologies which satisfy the Class Three approach are seriously limited because few medical device industries have the microbiological expertise, laboratories or funds to carry out the required resistance determinations. Given this, we began the task of developing alternative class III strategies for determining sterilization dose on a product-by-product basis that could be readily performed by small as well as large health care companies.

We here present five methods or strategies for determining gamma radiation sterilization dose for medical devices. Four of the methods are included in the AAMI proposed Process Control Guidelines for Radiation Sterilization of Medical Devices, (RS-P 1/81) (2). Also included here and in the AAMI guideline is an audit strategy for a periodic Quality Control verification of established sterilization doses. Information on the rationale and the testing performed to verify the strategies is provided. It is our hope that these dose setting methods will lead to the development of an international standard for determining sterilization doses.

Desirability of Lowering Dose

A sterilizing dose of 2.5 Mrad was the standard dose in many countries, including Canada and the United States until a few years ago. Typically, deviations from the 2.5 Mrad sterilization dose have been to lower doses. However, if the presterilization microbial challenge to sterilization is significant, our methods can be expected to provide estimates of sterilization dose greater than 2.5 Mrad. Lowering dose is desirable for several reasons. Some materials (e.g., certain plastics) can undergo a dose lower than 2.5 Mrad without essential harm but will exhibit undesirable physical changes at 2.5 Mrad, precluding the use of radiation sterilization process. From an economic

viewpoint, of course, a lower dose results in greater throughput and hence improved productivity for gamma sterilization processes (3,4).

Lower doses should lead to broadened applicability. Radiation sterilization is generally recognized as superior to most other sterilizing methods because of the known assurance of dose delivery. It is this assurance which makes applicability desirable. Another factor relevant in the argument for reducing dose has been the realization that certain products (for example, those which will not come in direct contact with a patient or will not penetrate or violate the natural defense barrier of a patient), do not require the same degree of sterility assurance as other products (1,2,5). The probability of infection will not be increased by the use of a lower Sterility Assurance Level under these circumstances.

Definition of Sterility

Sterility of an item is defined as the absence of viable organisms on that item. In a sense, this definition is nearly useless in that it is practically impossible to decide for sure whether a given item is sterile. However, we can decidedly determine that certain items are *not* sterile. For practical purposes the definition should be replaced by a statement wherein "absence of viable organisms" becomes "absence of viable organisms as measured by standard microbiological procedures" or some other suitable qualifying phrase. It has become standard to define sterility assurance level in terms of the probability that a randomly chosen item will be nonsterile. In this paper we will refer to the desired probability of a nonsterile item as the Sterility Assurance Level (SAL) and our dose setting method aims to set dose to achieve a given SAL. Typical values for SAL are in the 10^{-6} to 10^{-3} range.

Difficulties in Setting Dose

Assume that a SAL of 10^{-6} has been selected. Ignoring the difficulty of determining if an item is truly sterile, it is apparent that much experimentation must be done in order to set a dose to achieve the desired SAL without further assumptions. For example, using a Class 1 approach, if one had a dose in mind (say 2.5 Mrad), then almost 3,000,000 items must be irradiated at that dose with no nonsterile results to have 95% confidence that the given dose is adequate. If even one nonsterile item appears after dosing, the assumed dose could not be verified as achieving the desired SAL. With no particular dose in mind, the potential amount of experimentation would conceivably be much greater. Note that the above example ignores both the possibility of false positives (and negatives) as well as the possibility that the product entering the sterilizer changes from time to time and hence the experimentation would have to be repeated periodically for audit purposes.

Assumptions

Some assumptions must be made to bring the experimentation down to practical levels. But it is desirable that the assumption be kept to the minimum and that they be scientifically sound.

We have made three assumptions:

1. A randomly chosen organism has a probability of $10^{-D/D10}$ of surviving a dose of D Mrad where D10 is a characteristic of the particular randomly chosen organism (6).
2. Organisms survive independently of one another.
3. There is a distribution of G(.) of D10 values independent of the number of organisms, i.e.,

probability that a randomly chosen organism has a D10 value less than or equal to t is given by G(t).

The first assumption is reasonably standard and corresponds to a log linear inactivation curve for homogeneous populations when combined with assumption 2. Certain homogeneous populations have exhibited a "shoulder effect" in that the inactivation curve is somewhat flat in the low dose range and becomes steeper and linear for higher doses (7-10). The methodology proposed is conservative with deviations from assumptions of this sort. The second assumption of independence of organisms inactivation is somewhat adversely affected when significant "clumping" occurs in the population. Most inactivation models make implicit or explicit use of this assumption and it is hard to avoid. Unfortunately clumping also tends to make such methods less, rather than more, conservative. The third assumption is reasonable and allows for a heterogeneous bioburden.

Probability that an Item is Nonsterile at a Given Dose

The assumptions allow us to calculate the probability that an item with a bioburden of N_o organisms will be nonsterile.

The probability that a randomly chosen organism will survive a dose of D is given by $\int_0^\infty 10^{-D/t} dG(t)$. This integral is a Stieltjes integral and can be interpreted for a discrete set of possible D10 values as follows:

If the possible D10 values are denoted by $t_1, t_2, \ldots, t_k$ and the proportion of organisms with a D10 value of t_i is denoted by P_i, then $\int_0^\infty 10^{-D/t} dG(t) = \sum_{i=1}^{k} P_i \, 10^{-D/t_i}$

The assumptions allow the probability of product nonsterility at a given dose to be related to the probability of nonsterility at another dose using the frequencies of product microbial flora and the distribution of resistances, G(.). This makes it possible to calculate the probability of nonsterility at a selected dose without actually experimenting at that dose. Unfortunately, except for end product sterility testing, dose setting methods, including ours, must perform the bulk (if not all) of the experimentation at doses lower than the dose required to achieve the desired SAL (particularly if the desired SAL is 10^{-6}) (9-11). Hence the sterilizing dose will be set by extrapolation rather than interpolation, even though extrapolation is undesirable. This is primarily because there is no practical way to check whether the assumptions are reasonable beyond the experimental domain.

There seems no way around this undesirable feature. Any alternative approach must deal with the problem of false positives (in the 1/100 to 1/1000 range typically) and the extremely large number of observations required to establish with confidence a probability of nonsterility in the 10^{-6} range. Extensive computer simulation has given strong indication that our methods have satisfactorily dealt with this extrapolation problem.

For ease of reference the dose setting methods, rationale and auditing procedures are presented in sections. The eight sections which follow are:

Section 1. Dose Setting Using Microbial Resistance from Unirradiated Natural Product

Section 2. Dose Setting Using Microbial Resistances from Screened Natural Product

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SECTION 1. DOSE SETTING USING MICROBIAL RESISTANCES FROM UNIRRADIATED NATURAL PRODUCT

This section contains the underlying model of microbial inactivation as a function of radiation dose. The model is applied to calculate the probability that an irradiated item with a given microbial load will be nonsterile. Therefore, it can be used to find the dose that will produce a specified probability of a nonsterile item.

The microbial load on an item is characterized by the number of organisms on the item and by the resistance of the individual organisms. The probability model postulates that the bioburden is a mixture of homogenous populations, each of which behaves in a D10 fashion, thus the probability that a given item will be sterile after exposure to a known dose of radiation is a function of the initial number of organisms on the item and of the distribution of D10 values of the homogeneous components of the bioburden.

The direct application of this model for setting dose is not recommended because of the large number of laboratory experiments that would be required to determine the exact presterilization microbial challenge. The dose setting method contained in Section 4 is designed to avoid the need for direct measurement by inferring the bioburden and its overall microbial resistance by indirect observations. This and other methods presented here have been designed to broaden the application

of information that has become available in a few centers. Since this model only assumes D10 microbial inactivation, it will most likely provide more conservative sterilization doses than methods using beginning shoulder, multiple hit theory.

Calculations

Given that radiated microorganisms are sterilized in a D10 manner and the microbial D10 resistances (D_j) and their probabilities of occurrence (P_j) are known, the probability or a random microbe surviving (P(RS)) a dose D Mrad can be expressed as:

$$\text{Equation 1.1} \qquad P(RS) = \sum_{j=1}^{k} P_j(.1)^{D/D_j}$$

Now the amount (N_i) of initial bioburden per item and the frequency of N_i occuring (f_i) is known, the probability of a sterile item can be expressed as:

$$\text{Equation 1.2} \qquad \sum_{i=1}^{n} f_i\,[1 - P(RS)]^{N_i}$$

Therefore, the probability of a nonsterile item is:

$$\text{Equation 1.3} \qquad 1 - \sum_{i=1}^{n} f_i\,[1 - P(RS)]^{N_i}$$

Armed with the microbial resistances, their probabilities of occurrence, and the frequency distribution of the bioburden it is possible to determine sterilization dose for medical devices. Dose is determined by identifying the value of D in Equation 1.1 which will make Equation 1.3 equal to the desired probability of producing a nonsterile item.

Example 1.1

This example provides a geometrical intepretation of a heterogeneous microbial challenge to gamma sterilization. Let the distribution in Table 1.1 represent the D10 resistance distribution (D_j, P_j) of 1,000 microbes per item. Here i = 1, with N_1 = 1000 and f_1 = 1.00. This distribution (W/D) is derived from Whitby (11,12) by Davis as discussed in Section 3.

Table 1.1 —A Representative W/D Microbial Distribution (D10 in Mrad).

D10 (D_j)	0.10	0.15	0.20	0.25	0.28	0.31	0.34	0.37	0.40	0.42
PROB (P_j)	0.655	0.225	0.063	0.032	0.012	0.0078	0.0035	0.001	0.0007	0.00007

The following eleven figures depict the challenge of this representative W/D distribution to radiation inactivation. Each of the figures utilize a standard D10 logarithmic inactivation graph to show stepwise the creation of the cumulative W/D heterogenous microbial challenge. Figure 1 depicts the first homogeneous population, D_1 = 0.10 Mrad, P_1 = 0.655, Figure 2 depicts the first and the second population, etc.

Figure 11 depicts, using a broken line, the overall challenge to microbial inactivation that the composite (heterogeneous) W/D distribution presents for this example. The inactivation levels by dose in Mrad were derived using the probability equations of this section.

FIGURE 1

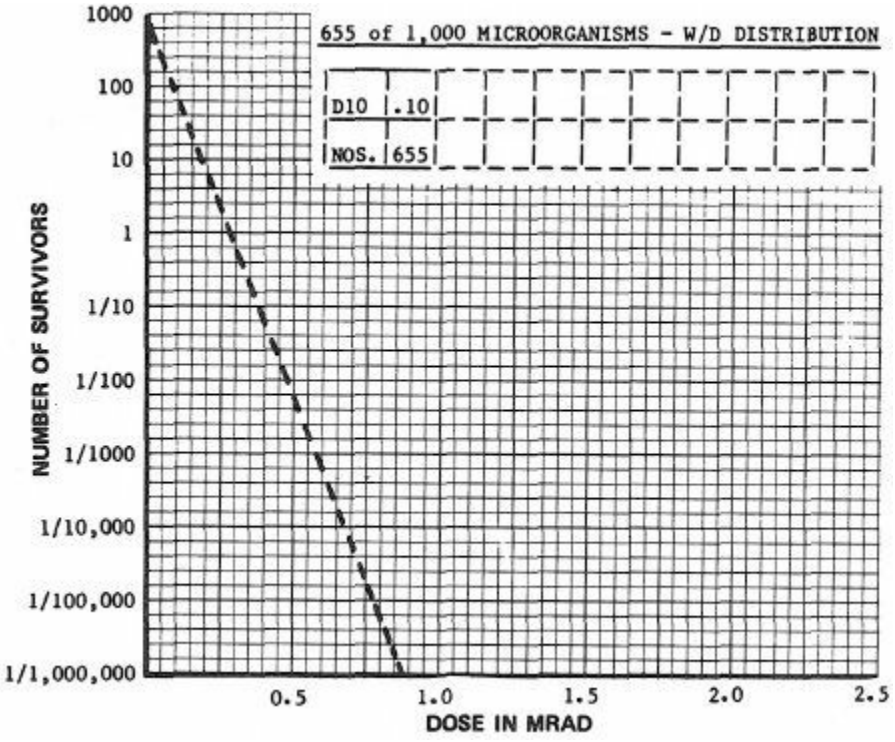


FIGURE 2

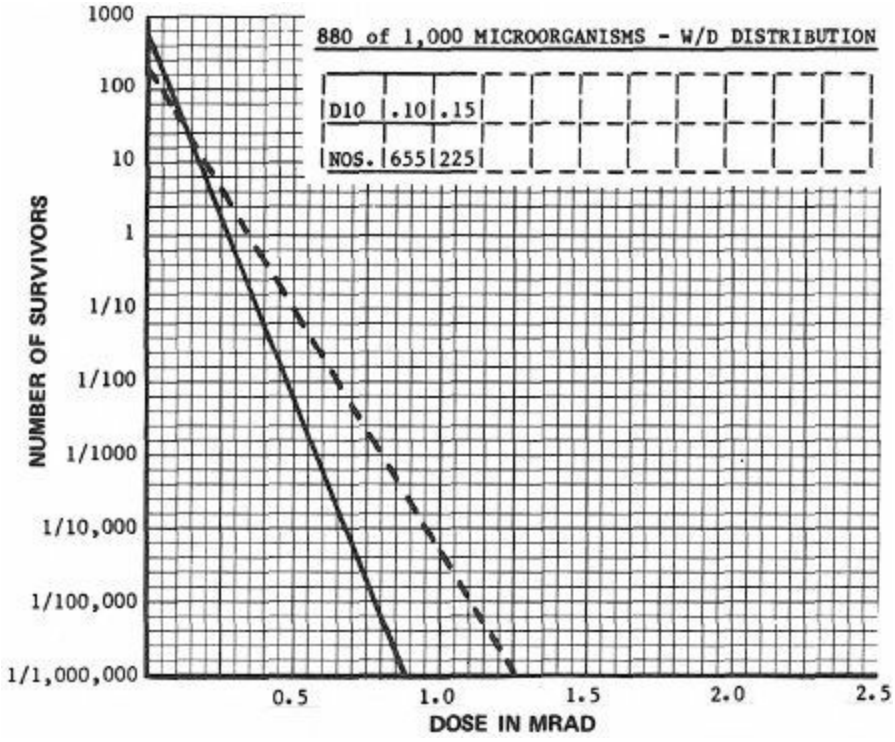


FIGURE 3

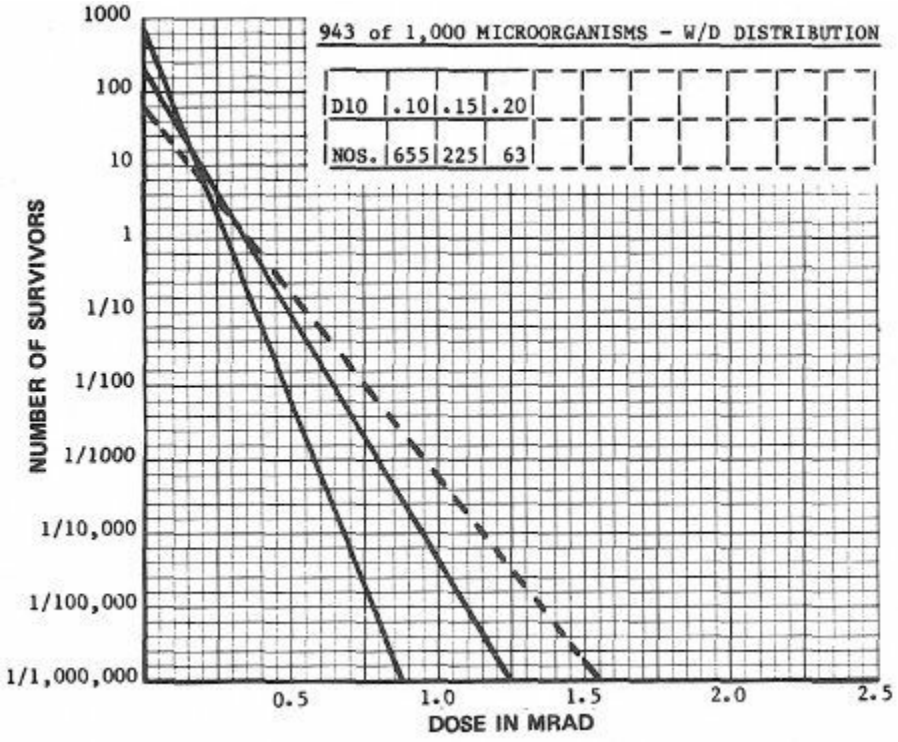


FIGURE 4

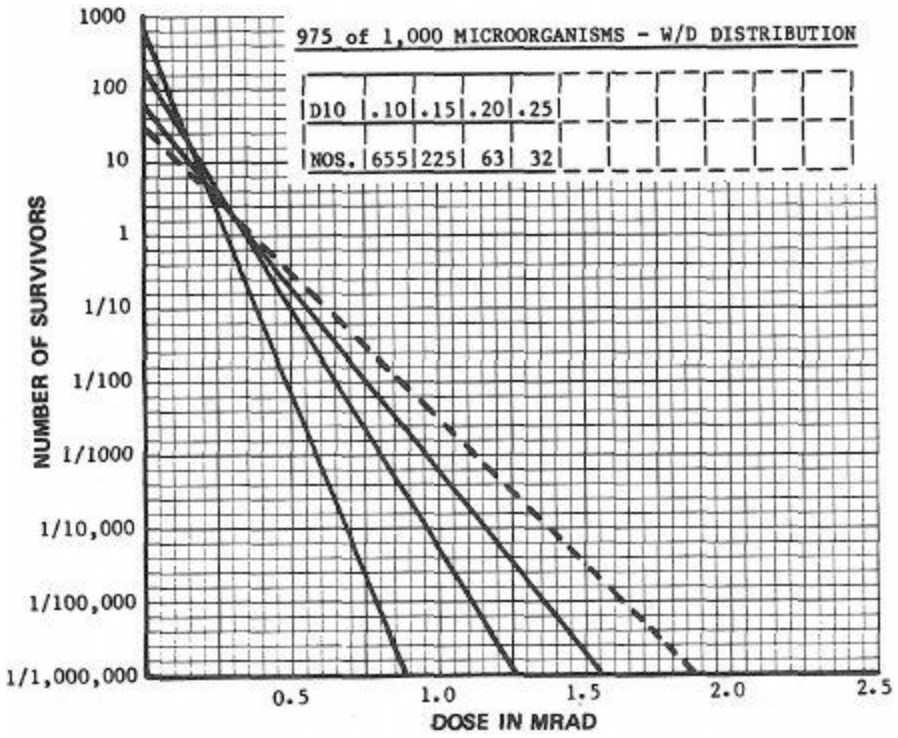


FIGURE 5

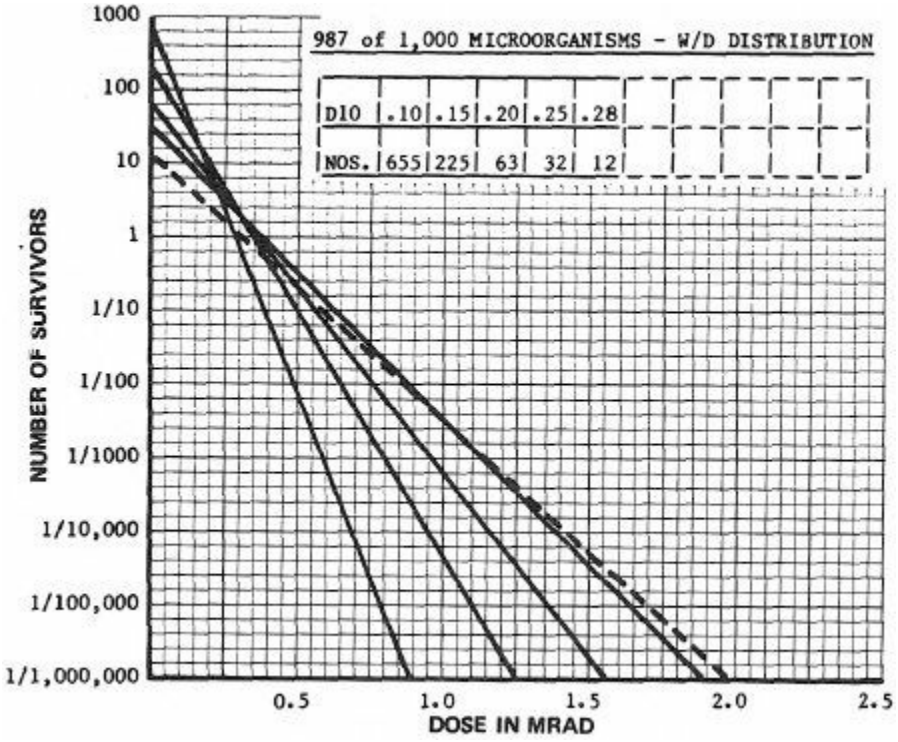


FIGURE 6

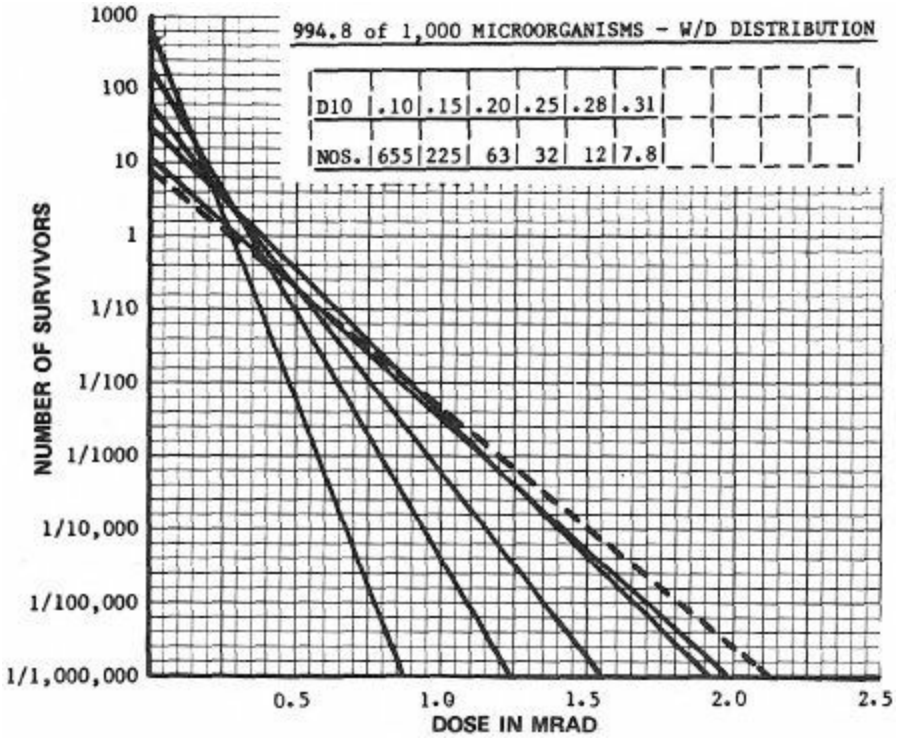


FIGURE 7

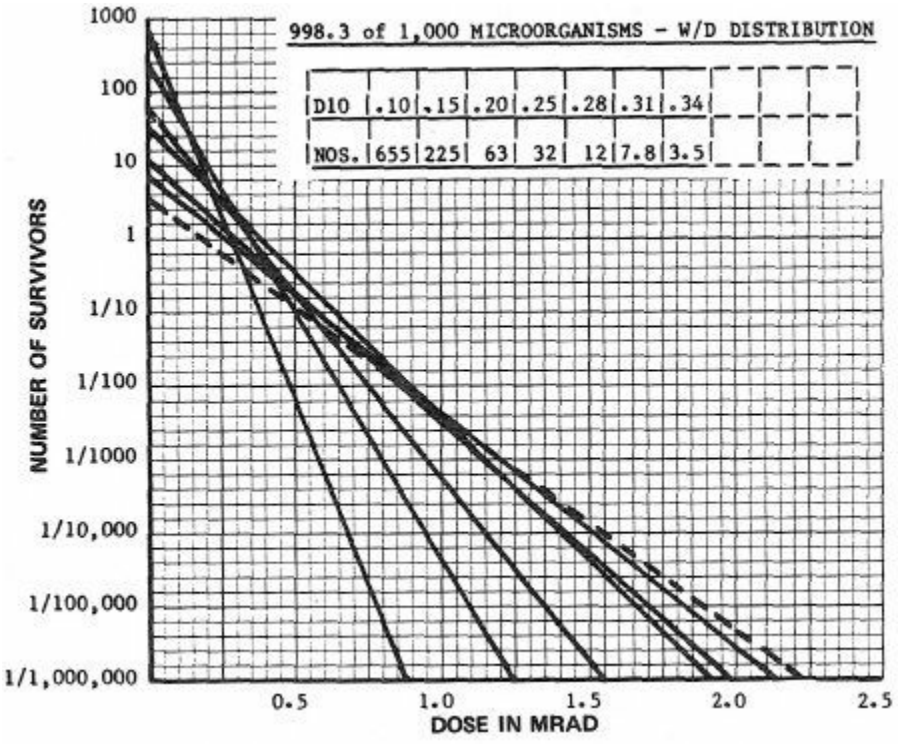


FIGURE 8

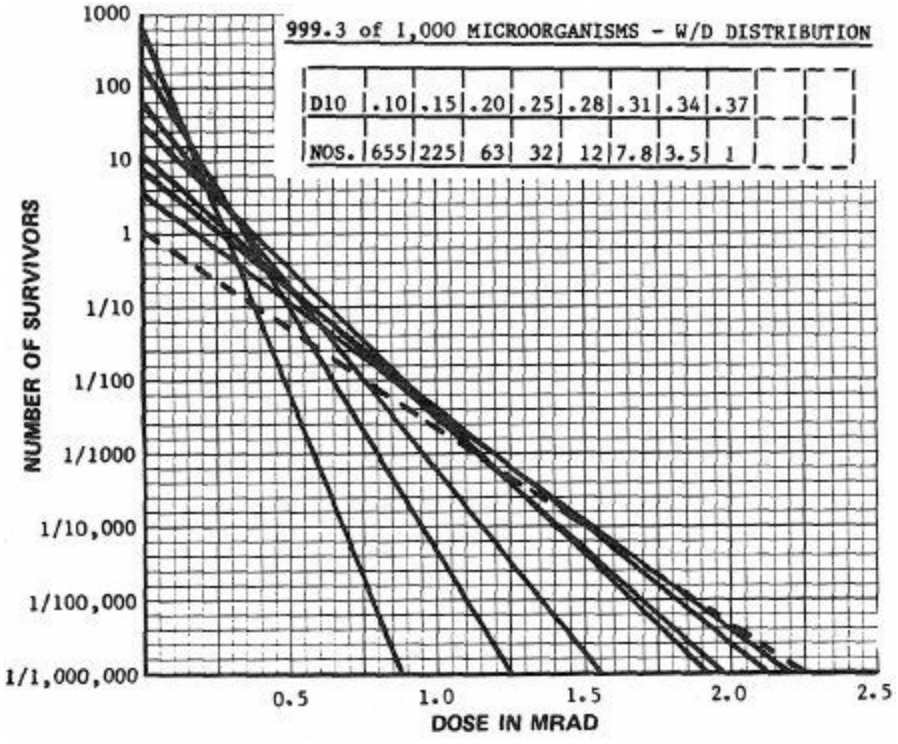


FIGURE 9

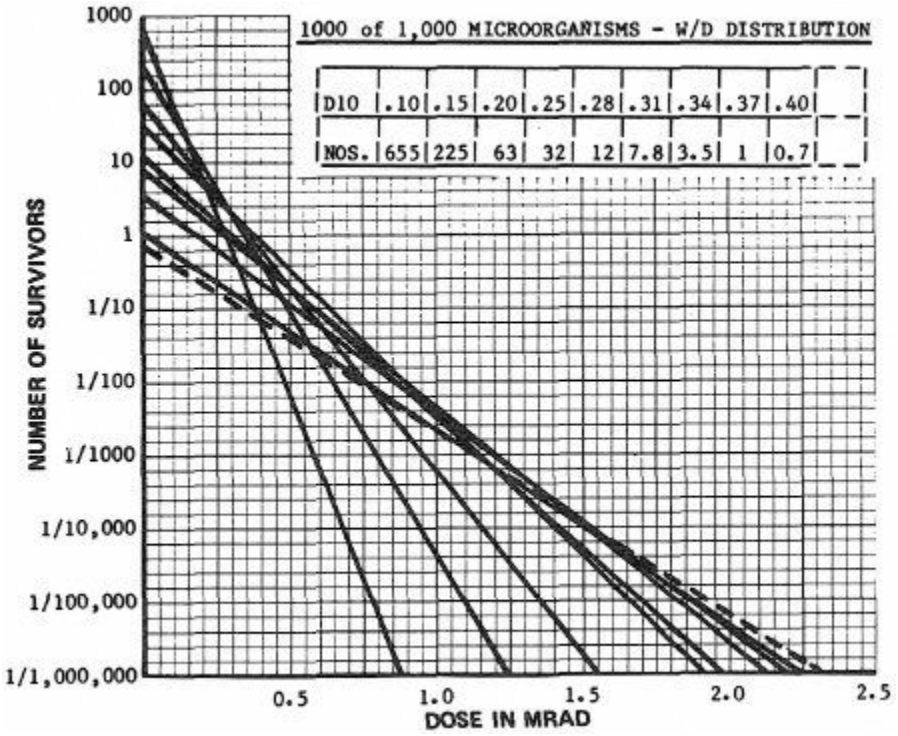


FIGURE 10

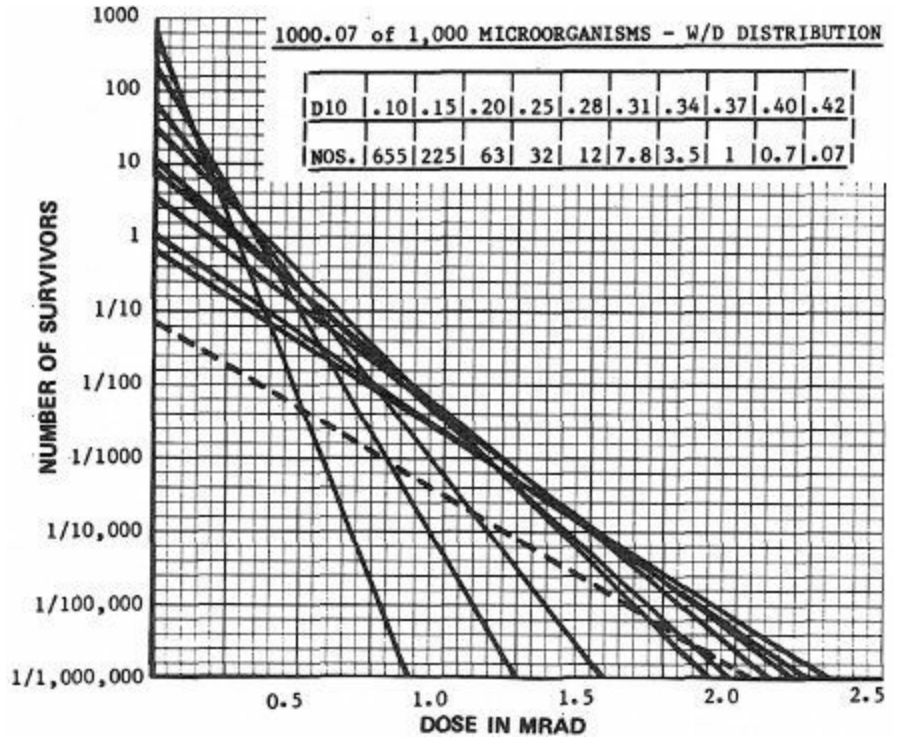
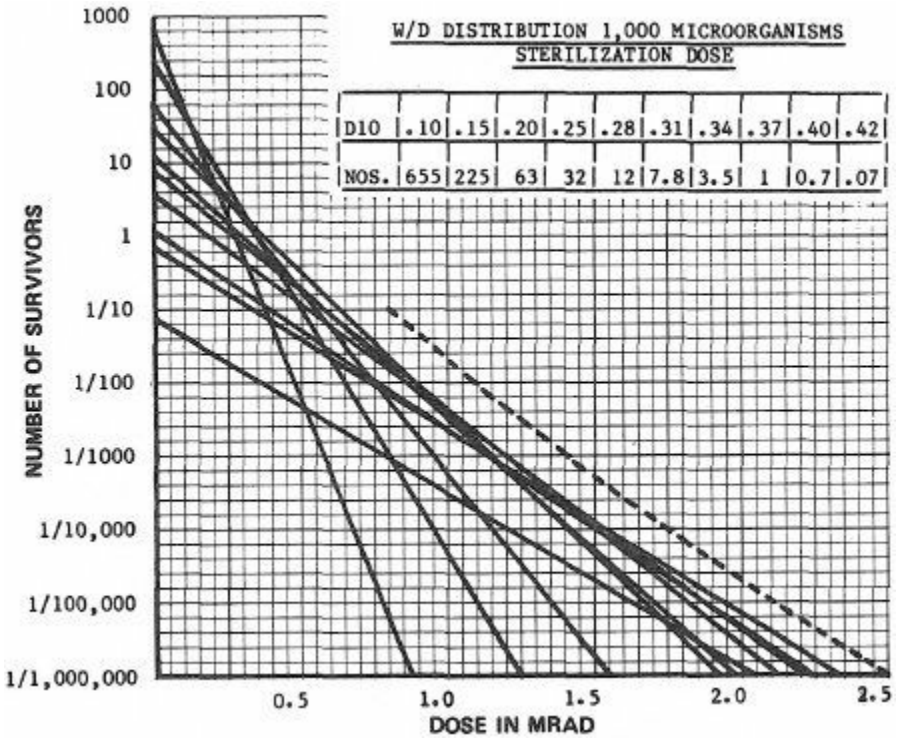


FIGURE 11



It can be observed from Figure 11 that the overall inactivation curve for the W/D distribution example is not log linear. Nor is it totally dependent on any one of the homogeneous populations. This has been shown to be generally true in practice. It is also apparent from Figure 11 that a D dose of 2.55 Mrad will provide a sterility assurance level of 10^{-6}.

Since some microbiologists prefer to determine sterilization dose using resistance information, a screening dose procedure has been applied in practice to significantly reduce the amount of laboratory resistance determinations. This approach requires that only the microbes surviving a screening dose be evaluated for resistance or sensitivity to radiation. Since the screening dose is set up to reduce the bioburden to an average of less than one microbe per item, Equation 1.1 can be applied to determine desired sterility assurance levels. Section 2 contains experimental procedures for a screening dose method. Nevertheless, such a method, even though not as experimentally demanding as the method of this section, is also expensive and can be performed by relatively few laboratories. Section 2 is included both here and in the AAMI standard for continuity with past approaches to sterilization dose setting. Section 5 provides a simpler, more cost effective and valid alternative to the method of Section 2.

SECTION 2. DOSE SETTING USING MICROBIAL RESISTANCE FROM SCREENED NATURAL PRODUCT BIOBURDEN

Introduction

To apply this method, an incremental dose experiment is performed to determine a screening dose of radiation where not more than 10 out of 20 samples tested will be positive in a sterility test. Sufficient samples are then dosed at this "50 percent sterility" dose to obtain approximately 200 microbial isolates. The radiation resistance of each of these isolates is then determined. The sterilization dose required to reduce a population containing an average of one microbe per sample with the observed distribution of radiation resistances to a desired level of sterility assurance is then calculated. This dose is added to the initial 50% sterility screening dose to give the total sterilization dose that the product will require. The rationale here is that at the 50% sterility level, the remaining bioburden typically averages approximately one microbe per sample.

Procedures

Dose setting using natural product bioburden resistance requires four stages of activity to determine a sterilization dose (2).

Stage 1: From each of three lots randomly sample complete or proportional parts of 360 medical devices which will contain a representative microbial challenge to sterilization.

Stage 2: Perform the incremental dose experimentation described in Table 2.1 using 0.1 Mrad incremental doses. From the sterility test results of this experiment, determine the screening dose, X Mrad. X Mrad is defined as the lowest incremental dose which yielded no more than 10/20 fraction positives for each of the three lots.

Table 2.1 —Number of samples for evaluations at various incremental ^{60}Co Mrad doses.

	Incremental Mrad Doses								Samples for Stage 3 Experiment	Total Samples Required
	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8		
Lot 1	20	20	20	20	20	20	20	20	200	360
Lot 2	20	20	20	20	20	20	20	20	200	360
Lot 3	20	20	20	20	20	20	20	20	200	360

Stage 3: At X Mrad, dose a sufficient number of the retained samples to obtain approximately 200 sterility test positives for resistance determination. The X Mrad screening dose is verified if there are no more than 60 percent positives. If the frequency of positives exceeds 60 percent, the screening dose must be reestimated.

The D10 resistances should be determined by using counts and linear regression or by using quantal sterility test data and the maximum D10 value determined from the Stumbo, Murphy and Cochran procedure (13):

$$D10 = \frac{\text{Dose}}{\log(\text{Inoculum count}) - \log \ln(\#\text{samples}/\#\text{negative})}$$

It is also important to use product as the substrate and assure the inoculum represents as near as possible natural microbial contamination states when developing the resistance determination data.

The sterilization dose is X Mrad + D Mrad.

Where; X Mrad is the screening dose, determined in Stage 2 and verified in Stage 3, and D Mrad is the Dose (D) which makes

$$\text{Equation 2 . 1} \qquad \sum_{j=1}^{k} P_j \, (.1)^{D/D_j}$$

Stage 4: equal to the desired Sterility Assurance Level (SAL) times the Sample Item Proportion (SIP). D_j and P_j are the determined isolate resistances and their relative

frequency of occurrence respectively.

To assure the determined dose is applicable over time, the D** sterility dose audit procedure of Section 7 should be applied. The audit dose D** Mrad for this method is determined as follows: D** = X Mrad + D* Mrad, rounded up to the nearest 0.1 Mrad, where X Mrad is as defined above and D* Mrad is the dose (D), which makes Equation 2.1 equal to a Sterility Assurance Level of 10^{-2}. The audit samples should be of the same SIP as that used for determining dose.

Example 2.1

Dose Setting Using Screened Isolate Resistances:

The 200 isolate resistances were determined and were distributed as shown in Table 2.2.

Table 2.2 —Isolate Resistance Distribution.

Numbers	90	43	30	18	8	3	5	2	1
D10 (D_j)	0.1	0.15	0.20	0.26	0.28	0.30	0.32	0.34	0.36
Relative Frequencies (P_j)	0.45	0.215	0.15	0.09	0.04	0.015	0.025	0.01	0.005

In this example, sterilization dose is calculated for a SAL of 10^{-3} and a SIP of 10^{-2}. The screening data indicated that the screening dose X was equal to 0.3 Mrad. Dose is calculated by finding the dose D Mrad at which

$$\text{Equation 2.2} \quad \sum_{j=1}^{k} P_j \, (.1)^{D/D_j} = (10^{-3})\,(10^{-2}) = 10^{-5}$$

The D that satisfies the above equation is 1.26 Mrad.

Therefore, Dose = 0.3 Mrad + 1.26 Mrad or 1.56 Mrad.

For purposes of audit dosing, D** in this sample is 0.7 Mrad, i.e., D** = 0.3 Mrad + 0.39 Mrad, rounded up to the nearest 0.1 Mrad.

Comments

This method is an application of the Section 1 model. The screening dose X Mrad, assuming the Poisson distribution, reduces the remaining product microbial contamination to a theoretical average of less than 0.92 viable organisms per item (12). Therefore the probability of a nonsterile item at (X + D) Mrad is less than the product of SAL and SIP.

The experimentation required for this method will be significantly greater than the DS + A Maximum D10 resistance method of Section 5, which can be expected to yield accurate estimates of sterilization dose with approximately 90% less laboratory resistance determinations.

SECTION 3. A STERILIZATION DOSE SETTING METHOD USING BIOBURDEN INFORMATION AND A STANDARD REFERENCE DISTRIBUTION

Introduction

This dose setting method depends upon experimental verification that the product microbial flora is more sensitive to gamma radiation than a given standard reference distribution. It requires bioburden determinations and the use of sterility testing of product samples that have received a lower dose of radiation than that which would constitute the ultimate sterilization dose (2).

As with all the methods of this report, this method is based on the probability model of Section 1. The dose setting values of Table 3.2 were derived using the probability calculations discussed therein.

This method utilizes a microbial population of standard but arbitrary radiation resistance. The distribution of resistances is defined in Table 3.1.

Table 3.1 —A Standard Microbial Resistance Distribution.

D10	0.10	0.15	0.20	0.25	0.28	0.31	0.34	0.37	0.40	0.42
Freq	0.65487	0.22493	0.06302	0.03179	0.01213	0.00786	0.00350	0.00111	0.00072	0.00007

The rationale for the selection of this microbial population as a reference standard is provided at the end of this section. The assumption is made that the distribution of D10 values in Table 3.1 represents a more stringent challenge than the natural bioburden on the item to be sterilized. This assumption is put to a test by a verification dose experiment.

In this method, the average bioburden on an item is determined. Table 3.2 is then used to determine a verification dose at which 100 items will be irradiated. To accept the sterilization dose indicated in Table 3.2, sterility tests of the samples irradiated at the verification dose must yield no more than one positive sterility test for the 100 samples tested. Table 3.2 does not provide doses higher than 2.5 Mrad because the application of this method beyond 2.5 Mrad is not recommended. If using this method does not provide a sterilization dose, an alternative dose setting method should be used. A sterilization dose audit procedure is recommended to confirm that the adopted sterilization dose will continue to apply. The audit procedures are given in Section 7.

Procedures

Determination of Bioburden.

To determine product bioburden, valid samples of at least ten are taken from each of several product lots. A sufficient number of samples should be taken to adequately represent and quantify the bioburden on the item to be sterilized. A sample can be the whole item or a proportional part of the item. The average bioburden for each lot is determined, as well as the overall average bioburden. The bioburden count used for this method is the overall average, unless one of the lot bioburden averages is two or more times as large as the overall average, in which case the largest lot average should be used.

Use of Table 3.2

The table is entered using the desired Sterility Assurance Level (SAL), the Sample Item Proportion (SIP), and the average bioburden per Sample Item Proportion. To assure that the indicated sterilization dose applies, a dose verification experiment is *required*. The verification dose is indicated in Table 3.2, according to the product's average bioburden. The verification requires dosing 100 samples (using the same SIP as used for bioburden determination) at the verification dose. The table sterilization dose may be accepted as valid if there is not more than one positive sterility test in the 100 samples. If there are two or more positives, this method is contraindicated and sterilization dose should be determined by another method. Only if it can be clearly demonstrated, that the bioburden determinations were incorrectly derived, or the sterility tests were compromised, can this method be reapplied.

Example 3.1

The following illustrates the use of the bioburden method in the case where the objective is to provide a sterility assurance level (SAL) of 10^{-3}.

1. In this example, the bioburden was determined from a sample item proportion of 1/70 of the item or unit. Lot 1 had an average bioburden of 1,000, Lot 2 an average bioburden of 500, and Lot 3 an average bioburden of 600, for an overall average of 700 per sample.
2. Dose was determined from Table 3.2, "Gamma Sterilization Dose Setting Table for Medical Devices", using parameters log(SAL) = –3, SIP = 0.01, and bioburden = 1,000. Interpolation is not allowed in the table; hence, the SIP was taken as the next lowest sample fraction and bioburden as the next highest. The table SAL dose was determined to be 2.12 Mrad.
3. The required verification dosing of 100 independent samples (SIP = 1/70) at the table verification dose of 1.10 Mrad had zero (0) positives; therefore, the 2.12 Mrad dose is verified.

Table 3.2 —Gamma Sterilization Dose Setting Table for Medical Devices (Dose Given in Mrad).

		Average Number of Bioburden per Proportion of Item Sampled												
log (SAL)	SIP	2	5	10	50	100	500	1000	5000	10,000	50,000	100,000	500,000	1,000,000
-3	1	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	1	0.88	1.02	1.10	1.32	1.42	1.66	1.76	2.01	2.12	2.37	2.49	**	**
-5	1	1.19	1.33	1.42	1.66	1.76	2.01	2.12	2.37	2.49	**	**	**	**
-6	1	1.52	1.67	1.76	2.01	2.12	2.37	2.49	**	**	**	**	**	**
-3	0.1	0.88	1.02	1.10	1.32	1.42	1.66	1.76	2.01	2.12	2.37	2.49	**	**
-4	0.1	1.19	1.33	1.42	1.66	1.76	2.01	2.12	2.37	2.49	**	**	**	**
-5	0.1	1.52	1.67	1.76	2.01	2.12	2.37	2.49	**	**	**	**	**	**
-6	0.1	1.87	2.02	2.12	2.37	2.49	**	**	**	**	**	**	**	**
-3	0.01	1.19	1.33	1.42	1.66	1.76	2.01	2.12	2.37	2.49	**	**	**	**
-4	0.01	1.52	1.67	1.76	2.01	2.12	2.37	2.49	**	**	**	**	**	**
-5	0.01	1.87	2.02	2.12	2.37	2.49	**	**	**	**	**	**	**	**
-6	0.01	2.23	2.39	2.49	**	**	**	**	**	**	**	**	**	**
-3	0.001	1.52	1.67	1.76	2.01	2.12	2.37	2.49	**	**	**	**	**	**
-4	0.001	1.87	2.02	2.12	2.37	2.49	**	**	**	**	**	**	**	**
-5	0.001	2.23	2.39	2.49	**	**	**	**	**	**	**	**	**	**
-6	0.001	**	**	**	**	**	**	**	**	**	**	**	**	**
Verification Dose		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

**If this table does not provide dose, then an alternative strategy must be used.

1. Determine bioburden, Sterility Assurance Level (SAL), and Sample Item Proportion (SIP) used for bioburden determinations.
2. Use this table to determine verification dose according to values in 1.
3. Perform the verification experiment, dosing 100 samples (use same SIP as for bioburden determinations) at the verification dose to certify that the tabled sterilization dose is acceptable. Accept, if number of positives is 0 or 1. If more than 1 positive is observed, an alternative strategy should be used.

Sterilization Dose Audit for Quality Control

To assure that the sterilization dose remains applicable through time, the audit procedure specified in Section 7 should be performed quarterly unless seasonal and/or manufacturing area bioburden conditions indicate otherwise.

Table 3.3 —Bioburden Distribution for an Average of 2 Microbes per Item.

Counts	1	3	7
Freq	0.70	0.20	0.10

Table 3.4 —Bioburden Distribution for an Average of 5 Microbes per Item.

Counts	3	5	10	25
Freq	0.50	0.35	0.10	0.05

Table 3.5 —Bioburden Distribution for Averages of 10 Through 1,000,000 per Item.

Frequencies	0.10	0.45	0.39	0.04	0.02
Bioburden Averages	Bioburden Counts by Frequency				
10	5	8	10	25	50
50	25	40	50	125	250
100	50	80	100	250	500
500	250	400	500	1,250	2,500
1,000	500	800	1,000	2,500	5,000
5,000	2,500	4,000	5,000	12,500	25.000
10,000	5,000	8,000	10,000	25,000	50,000
50,000	25,000	40,000	50,000	125,000	250,000
100,000	50,000	80,000	100,000	250,000	500,000
500,000	250,000	400,000	500,000	1,250,000	2,500,000
1,000,000	500,000	800,000	1,000,000	2,500,000	5,000,000

The sterility assurance level doses of Table 3.2 were derived using the resistances shown in Table 3.1, the bioburden distributions shown in Tables 3.3, 3.4 and 3.5 and a range of Mrad doses of 0.36 to 2.5 Mrad.

The sterilization dose values for given Sterility Assurance Levels (SAL) and Sample Item Proportion (SIP) were determined using Equation 1.3 to identify the D Mrad dose which makes the

probability of a nonsterile item equal to the product of SAL and SIP.

Since some manufacturers choose to use 2.5 Mrad as a sterilization dose, two simplified methods that can be used for verifying this dose are included here. It should be noted that these methods are not as rigorous as the preceding method because verification is being performed at the 10^{-1} SAL level rather than the 10^{-2} SAL level. The major reason for their inclusion is that some batches may have too few devices to provide 100 test samples, or the devices may be too expensive to test 100 destructively for sterility assurance purposes. The first of these additional methods verifies 2.5 Mrad as a sterilization dose on a batch by batch basis whereas the second method can be used to verify dose on a production process basis.

A Simplified Approach to Verify the Validity of 2.5 Mrad as a 10^{-6} Sterility Assurance Level Dose on a Batch by Batch Basis

Introduction

This method offers a simplified approach for verifying the validity of a 2.5 Mrad sterilization dose on a batch by batch basis. Occasionally, medical device firms are required to prepare a batch of devices on a special order basis or for clinical evaluation. This method can be used to verify, with minimal product destruction, that the batch can be effectively sterilized to a 10^{-6} sterility assurance level when exposed to a 2.5 Mrad dose of gamma radiation. This method does not require bioburden data.

Procedures

1. Randomly sample 10 units of product from the batch to be sterilized using a SIP of 1.0, 0.1, or 0.01 and irradiate the samples with ≤ 1.01, $\leq 0.75 \leq 0.49$ Mrad, respectively, depending upon the chosen SIP. The substerilization verification doses must be delivered within $\pm$ 0.05 Mrad.
2. Perform product sterility tests on the substerilization dosed samples.
3. Accept 2.5 Mrad as a viable 10^{-6} sterility dose for the batch if there are 0/10 positives. If there are 2 positives, reject 2.5 Mrad as a viable sterilizing dose. If there was 1 positive in 10, irradiate a second sample of 10 at the prescribed verification dose and accept 2.5 Mrad as a viable sterilizing dose if no further positives are observed in sterility testing.
4. If a 2.5 Mrad sterilization dose can not be verified using this method, it can be concluded the distribution of microbial resistances of Table 3.6 is not an applicable upper bound for the microbial challenge of the batch. Hence, another strategy should be used to determine a dose which will yield a 10^{-6} sterility assurance level dose.

Rationale

The verification doses were calculated to represent 10^{-1} SAL using a modification of the microbial resistance distribution discussed in this Section. The reference distribution used here is shown in Table 3.6

This distribution was arrived at by accumulating all Table 1.1 resistances less than or equal to 0.25 Mrad and assigning them a 0.25 Mrad D10 value.

The probability methods of Section 1 were applied to the above distribution and bioburdens having an average of 1000, 100 and 10, respectively, to derive the verification doses.

Table 3.6

D10	0.25	0.28	0.31	0.34	0.37	0.40	0.42
Frequency	0.97461	0.01213	0.00786	0.0035	0.00111	0.00072	0.00007

A Simplified Approach to Verify the Validity of 2.5 Mrad as a 10^{-6} Sterility Assurance Level Dose on a Product by Product Basis

Introduction

This method offers a simplified approach for verifying the validity of a 2.5 Mrad sterilization dose on a product by product basis. This method can be used to verify, with minimal product destruction, that the product can be effectively sterilized to a 10^{-6} sterility assurance level when exposed to a 2.5 Mrad dose of gamma radiation. Auditing for the continued applicability of the 2.5 Mrad dose can be carried out by repeating the verification steps. This method does not require bioburden data.

Procedures

1. Randomly samply 10 units of product from each of three batches. Use a SIP of 1.0, 0.1, or 0.01 and irradiate the samples with less than or equal to 1.01, 0.75 or 0.49 Mrad respectively, depending upon the chosen SIP. The sterilization verification dose must be delivered within ± 0.05 Mrad.
2. Perform product sterility tests on the substerilization dosed samples.
3. Accept 2.5 Mrad as a viable 10^{-6} sterility dose for the product if there are 0/10 positives for each batch. If there are 2 positives, for any of the three batches, reject 2.5 Mrad as a viable sterilizing dose. If any of the three batches had 1 positive in 10, irradiate a second sample of 10, from the batch, at the prescribed verification dose and accept 2.5 Mrad as a viable sterilizing dose if no further sterilization test positives are observed.
4. If a 2.5 Mrad sterilization dose cannot be verified using this method, it can be concluded the distribution of microbial resistance of Table 3.6 is not an applicable upper bound for the microbial challenge of the product. Hence, another strategy should be used to determine a dose which will yield a 10^{-6} sterility assurance level sterilization dose.

Sterilization Dose Audit for Quality Control

The procedures used here to verify the sterilization dose should be reapplied in sufficient frequency to assure that the 2.5 Mrad dose remains applicable through time.

A Standard Reference Distribution for Presterilization Frequencies of Microbial Resistances

Introduction

To identify a standard reference distribution of resistances which could be used to represent a reasonably significant challenge to radiation sterilization, microbial resistance distributions from the literature and industrial sources were reviewed. Tables 3.7 through 3.11 represent our interpretation of these distributions.

Discussion

Whitby (1978) presented, at the Annual Meeting of the Parenteral Drug Association, resistances for bioburden contained in a typical hospital pack (11). Whitby (1979) extended the testing to reflect 673 isolate resistances from some 70,000 microbes (12). His earlier work was based on 397 isolates.

The above data was reviewed and reassessed. It was concluded that the resistance estimates could be biased to the high side by from 0.03 to 0.05 Mrad. This is due to the conservative approach used to estimate the resistances, i.e., D10 values were calculated using the equation D10 is less than D Mrad/log (inoculum) where D Mrad was chosen as the first incremental dose at which there were zero survivors. A more appropriate calculation method would be to use

$$D10 = \frac{\text{Dose (last fraction positive)}}{\log(\text{Inoculum count}) - \log \ln(\#\text{tests}/\ \#\text{negative})}$$

It was further pointed out in both papers that the work reported measured resistances of inoculum dried in glass tubes, this was the same procedure used by Czerniawski and Stolarzyk (14). When using this type methodology, it can be concluded that organisms are more occuluded than they would be on cellulosic or absorbent substrate. Whitby also reported that the resistance data showed the D10's using a glass substrate were likely to be at least 0.1 Mrad higher than would be expected for microbes inoculated onto cellulosic substrate. This, together with the D10 overestimate, indicates the reported resistances of Whitby and Czerniawski might be 0.13 to 0.15 Mrad too high for organisms in the natural state on many medical devices.

Given this information, it was concluded that we could safely use the Whitby resistances as reported by Whitby to provide a conservative presterilization microbial resistance reference profile. Using this distribution which was derived from a screen dose of 0.4 Mrad, a calculation was made to provide a model resistance profile for unirradiated bioburden which is given in Table 3.7.

Table 3.7 —A Standard Table of Microbial D10 Resistances.

D10	0.10	0.15	0.20	0.25	0.28	0.31	0.34	0.37	0.40	0.42
Freq	0.65487	0.22493	0.06302	0.03179	0.01213	0.00786	0.00350	0.00111	0.00072	0.00007

Although these resistances were chosen somewhat arbitrarily for universal use, it is our belief that this distribution will, in most cases, represent a more severe challenge to radiation microbial inactivation than the natural bioburden on items to be sterilized.

Distributions that were available for our assessment are summarized in Tables 3.8 through 3.11. The tabled information reflects our interpretations of the data furnished by the referenced sources.

For the reference distributions which represent frequencies of microbial resistances after a subprocess screening dose, a transformation of the frequencies was required to estimate the preirradiation microbial population. This involved using the expression N = [number of survivors]/[$0.1^{(D/D10)}$] iteratively until a reasonable overall preirradiation bioburden number was

obtained. In the preceding expression N is the starting bioburden for a given D10 Mrad resistance and D Mrad represents the nominal screening dose. This procedure is conservative to shoulder deactivation phenomena because it yields larger incidences of the higher resistance microbes.

Table 3.8 —Microbial Resistance Distribution V.V. Bochkarev et.al.

D10	0.1	0.165	0.2	0.3	0.35
Freq	0.9805	0.0178	0.0015	0.0001	0.0001

This table was derived from approximately 8,000 isolates obtained after a 0.5 Mrad screening dose (15).

Table 3.9 —Microbial Resistance Distribution from Company A

D10	0.05	0.08	0.10	0.12	0.14	0.17	0.24	0.31	0.34
Freq	0.1121	0.0176	0.2436	0.0927	0.1046	0.3214	0.0090	0.0075	0.0015

This table was derived from 671 isolates representing 4,000 randomly selected microorganisms (16).

Table 3.10 —Estimated Microbial Resistance Distribution from Company B

D10	0.04	0.06	0.13	0.15	0.17	0.20
Freq	070	0.20	0.04	0.03	0.02	0.01

This table is based on 2427 isolates (16).

Table 3.11 —Microbial Resistance Distribution Due to Czerniawski and Stolarczyk

D10	0.10	0.15	0.20	0.22	0.28	0.33	0.39	0.46	0.52
Freq	0.6195	0.2128	0.0596	0.0300	0.045	0.0195	0.0094	0.0037	0.0005

This table is an interpretation of information provided by Czerniawski and Stolarczyk on resistances derived from 8576 isolates taken from needle factory halls or from within irradiation facilities (14).

The distribution in Table 3.7, when compared with the Bochkarev distribution (Table 3.8) and the industrial source distributions (Tables 3.9 and 3.10), represents an upper bound of microbial resistance. When compared to the Czerniawski distribution (Table 3.11), it appears similar except that it has smaller frequencies of higher resistances.

For purposes of standardization we believe the distribution resulting from our interpretation of the Whitby data represents a realistic upper bound for microbial resistance. Furthermore it is in line with the successful North American experience of using 2.5 Mrad as a generally accepted sterilizing dose. For instance, the 10^{-6} sterilization dose for 100 or 1000 microbes with resistances from our recommended standard distribution would be 2.12 Mrad and 2.49 Mrad respectively, whereas Czerniawski recommends 3.0 and 3.5 Mrad doses. The authors will continue to evaluate this choice of reference distribution as more data becomes available.

THE DS + A STERILIZATION DOSE SETTING METHOD – AN OVERVIEW

SECTION 4. THE DS + A STERILIZATION DOSE SETTING METHOD USING FRACTION POSITIVE INFORMATION FROM INCREMENTAL DOSING AND A TABLED DOSE SETTING FACTOR (DS + A) MRAD

Introduction – an Overview

This method provides a sterilization dose setting strategy for gamma sterilization of medical devices that a) is computationally straightforward, b) requires microbiological experimentation well within the capabilities of microbiological units of most producers and independent laboratories, c) supplies answers that are reasonably reproducible (i.e., have small variability) (2).

Method Abstract

The DS + A dose setting method requires four stages of activity to determine a dose which can be expected to provide a specified Sterility Assurance Level (SAL). The SAL dose can be expressed as:

Dose = D* Mrad + (extrapolation factor)(DS + A) Mrad
where:
D* Mrad is a measurable estimate of dose which will provide approximately 1 percent (10^{-2}) nonsterile samples; and
(DS + A) Mrad is an estimate of the tail population D10 Mrad for microorganisms surviving the D* Mrad dose.

The four stages of activity are:

1. Product sampling of three independent production lots.
2. An incremental dose experiment to identify the minimum dose which will provide some sterile samples. This dose is referred to as the First Fraction Positive (FFP Mrad) dose. Also, this experiment initially estimates the dose D* Mrad.
3. A second dose experiment, using 100 samples, is conducted at D* Mrad to refine the estimate of expected nonsterile samples at D* Mrad. This experiment also estimates the minimum dose, First No Positive (FNP Mrad), at which less than 1 percent of the samples will be nonsterile.
4. A series of calculations, based on data from Stages 2 and 3, are used to determine the desired sterilization dose.

For complete details of how to conduct this method, refer to the DS + A Sterilization Dose Setting Method – Experimental Procedures contained at the end of this section.

Main Ideas of the Dose Setting Method

This method involves two stages of experimentation. The first stage seeks to estimate a dose, D* Mrad, at which approximately 1% of the product samples will be nonsterile. In the second stage, 100 samples are dosed at D* Mrad. A dose to achieve the desired SAL is then calculated based on the results of both stages of experimentation. The estimated dose is given by Dose = D* Mrad + [log(CD*/100) – log(SAL) – log(SIP)] (DS + A) Mrad. CD* is the number of positives out of 100 tests at the second stage and (DS + A) Mrad are factors calculated from the data indicating the "tail resistance" of the bioburden. SAL is the desired sterility assurance level. SIP is the sample item proportion.

The intuition behind the formula is roughly as follows: the dose D* which results in CD*/100 positives (when CD* is not too much larger than 1) will result in a relatively homogeneous bioburden resistance on the samples. Therefore, the log of the fraction positives as a function of dose should be nearly linear for doses larger than D* Mrad. Hence, one must estimate the number of additional log reductions to achieve the desired SAL which is given by [log(CD*/100) – log(SAL) – log(SIP)] and the "tail resistance" or "tail D10" which is given by (DS + A) Mrad. The relationships are indicated schematically in Graph 4.1.

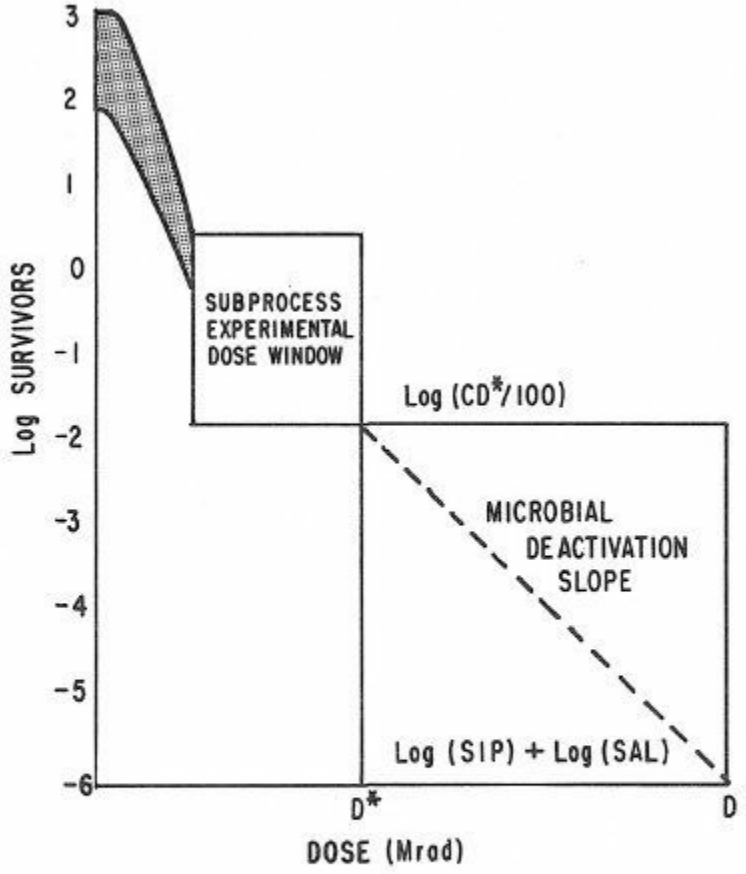
Two noteworthy aspects of the strategy are: 1) by establishing a dose which achieves sterility in approximately 99% of the product samples, some measureable level of protection is guaranteed and 2) all sterilizers will be establishing a standard value

D** Mrad = D* Mrad + [2 + log(CD*/100](DS + A) Mrad which itself should provide valuable information to industrial and regulatory bodies after substantial experience is built up using the method to set dose on product. D** is an estimate of the data required to produce an SAL of 10^{-2}. A further important feature is that there is no reliance on counts of initial bioburden or conventional resistance determinations.

We now give a more detailed description of the method. Complete details can be found at the end of this section.

1. *Determine SAL:* The sterility assurance level (SAL) is assigned by the medical device's intended end use.
2. *Determine SIP:* The sample item proportion (SIP) is the proportion of an end use item that will be used in the dose setting experimentation. The SIP may be determined by volume, weight or surface area and is governed by practical considerations, principally physical limitations involved in sterility testing. The portion selected should be representative of the proportional microbial challenge on the end use item.
3. *Select 280 independent samples from each of three production lots:* Divide the 280 samples from each lot into nine groups of 20 for Stage 1 experimentation and one group of 100 for Stage 2 experimentation.

Graph 4.1—The D/S Window and Extrapolation (Log Survivors by Dose in Mrad).



$$
\begin{aligned}
-\text{Slope} &= [\log(CD^*/100)-(\log(SIP)+\log(SAL))]/[D-D^*] \\
&= 1/\text{“Tail D10”} \\
&= 1/[DS+A] \text{ therefore,} \\
D \text{ Mrad} &= D^* \text{ Mrad} + [\log[CD^*/100)-(\log(SIP)+\log(SIP))](DS+A) \\
&\quad \text{Mrad}
\end{aligned}
$$

DS is conservatively set in Table 4.3 according to the width of the subprocess experimental dose window sterilization test results. A is a correction factor necessary when the exact beginning of the window is not experimentally established.

4. *Perform the Stage 1 experiment:* At each of the nine doses from 0.2 to 1.8 Mrads in increments of 0.2 Mrads, dose 20 samples from each of the three lots. The incremental doses should be delivered to be within ± 0.05 Mrad of the intended dose. Perform a standard sterility test (or tests) on each sample and record for each lot and each sample of 20, the number of positive test results. A typical result is indicated in Table 4.1.

Table 4.1 —Positive Sterility by Incremental Dose

Dose	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8
Lot 1	20	8	2	0	0	1	0	0	0
Lot 2	19	8	1	0	0	0	0	0	0
Lot 3	20	12	2	1	0	0	0	0	0

5. *Determine D**: D* Mrad is the dose estimated to produce approximately 1% nonsterile samples.

(It will later be refined to produce a better estimate, D**). D* Mrad is defined as the median of the three d*'s determined for each of the three lots (except as noted below). The d* Mrad (for a lot) is the minimum dose of i, or ii, below:

i. The minimum of the first incremental doses where 2 consecutive 0/20 positives occur.

ii. The first incremental dose at which 1/20 positives occur, immediately preceeded and followed by 0/20 positives.

The exception is that if any d* Mrad exceeds the median d* Mrad by more than 0.4 Mrads, D* Mrad is taken to be the maximum of the d*'s. In Table 4.1 the three d*'s are 0.8, 0.8 and 1.0 Mrad respectively, and hence D* is 0.8 Mrad.

6. *Perform the Stage 2 experiment:* Dose the 100 samples from that lot whose d* Mrad is equal to D* Mrad and has the smallest dose resulting in at least one negative sterility test. If more than one lot qualifies under this criterion, select the lot with the minimum number of positives at that dose. If more than one lot still qualifies, select any qualifying lot at random.

   The 100 samples from the selected lot are then dosed at D* Mrad and the number of positive sterility test results are recorded. CD* is the number of positive sterility test results found in this stage of experimentation. If 0 positives are observed, set CD* = 1.

   For example, in the first stage results of Table 4.1, D* = 0.8 Mrad as indicated above and the 100 samples from lot #2 would be chosen for the second stage of experimentation.

7. *Determine DS and A to find "Tail Resistance":* (DS + A) Mrad is an estimate of the resistance of the bioburden distribution after it has been subjected to D* Mrads. DS is the larger of the two values and hence has the larger impact. "A" is a relatively minor adjustment. Both factors are functions of a "window" in the data extending essentially from the first dose at which some sterile samples are found to the first dose at which no positives are found. DS is a function of the width of the window (the width of the window is a function of the apparent tail resistance of the overall product microbial flora); the wider the window the larger DS becomes. A is a function of the height of the window. The taller the window the smaller A becomes.

   To find DS and A our procedure is as follows: first determine the FFP (First Fraction Positive) dose defined as the median of the three lot doses where a negative sterility test result is first found. Then determine, at the FFP dose, the minimum number of positive test results and find the corresponding value of A in Table 4.2.

   From the data in Table 4.1, FFP is 0.4 Mrad, the median of 0.4 Mrad, 0.2 Mrad, and 0.4 Mrad. The minimum number of positives found at 0.4 Mrad is 8 and hence A is 0.017 Mrad.

   To find DS we first determine FNP (First No Positive) which is defined as D* Mrad if there were no positive sterility test results at D* Mrad experimentation or (D* + 0.2) Mrad if there were positives at D*. Then calculate (FNP-FFP) Mrad and find DS by entering Table 4.3.

   From the data in Table 4.1, D* Mrad was determined to be 0.8 Mrad and lot 2 was the lot chosen for Stage 2 experimentation. Suppose this experimentation resulted in one positive test result. Hence, FNP = (0.8 + 0.2) Mrad = 1.0 Mrad, FNP-FFP = (1.0-0.4) Mrad = (0.6) Mrad and DS = 0.32 Mrad. It therefore follows that the apparent "D10 tail resistance" (DS + A) Mrad = (0.32 + 0.017) Mrad = 0.337 Mrad. The tabled DS Mrad values were chosen so as to represent a conservatively high D10 Mrad tail resistance.

Table 4.2 —A (Mrad): The Dose Setter Augmentor.

#Positive Tests of 20	A (Mrad)
19	0.000
18	0.003
17	0.004
16	0.006
15	0.008
14	0.009
13	0.010
12	0.012
11	0.013
10	0.014
9	0.016
8	0.017
7	0.019
6	0.021
5	0.023
4	0.026
3	0.029
2	0.033
1	0.040
0	0.040

Table 4.3 —Dose Setter Values (Mrad) Calculated Using First Fraction Positive to First No Positives.

(FNP – FFP) Mrad	DS D10 Mrad
0.0	0.20
0.2	0.24
0.4	0.28
0.6	0.32
0.8	0.36
1.0	0.40
1.2	0.48
1.4	0.56
1.6	0.64
1.8	0.72
2.0	0.80

Rounding Procedures for Table 4.3.

1. Do not interpolate within Table 4.3.

2. If the (FNP–FFP) Mrad is within ± 0.05 Mrad of the table value in Column 1 use that value; if not, use the next higher (FNP-FFP) Mrad value.

8. *Calculate the required dose:* The DS method estimates the dose required to sterilize to the given sterility assurance level (SAL) by calculating the additional dose (Dose – D*) Mrad required to sterilize a homogeneous population of D10 resistance equal to (DS + A) Mrad and average bioburden per SIP equal to CD*/100 to a level of sterility of (SAL)(SIP). The additional number of "log reductions" is therefore [log(CD*/100) – log(SAL) – log(SIP)]. The approximate "tail D10" is (DS + A) Mrad and therefore the required additional dose, (Dose–D*) Mrad = [log(CD*/100) – log(SAL) – log(SIP)] (DS + A) Mrad. Hence Dose = D* Mrad + [log(CD*/100) – log(SAL) – log(SIP)](DS + A) Mrad. For the data of the hypothetical example we have been following, assuming the whole item has been sampled, i.e., SIP = 1, and the desired SAL = 10^{-6}:

Dose = 0.8 Mrad + [log(1/100)–log(10^{-6})–log(1)](0.337) Mrad
= 0.8 Mrad + (–2 + 6 + 0)(0.337) Mrad
= 2.15 Mrad

9. *The D** Audit to insure adequacy of dose:* It is recommended an audit be conducted at least quarterly in order to detect changes in the manufacturing facility or raw material sources that have the potential of increasing the established dose. Section 7 contains the procedures for conducting the sterilization dose audit.

Summary

In summary, the rationale behind the method rests on the approximate homogeneity of the population after a dose of D* Mrad. In fact, there is a shift in the distribution of resistances towards the most resistant organisms present and the log of the number of survivors per dose becomes more nearly linear beyond D* Mrad. However, the dose D* Mrad clearly does not produce absolute homogeneity and there is still curvature in the log survivor curve. Hence, the "tail resistance", (DS + A) Mrad, is more properly viewed as an attempt to find an effective upper bound to the resistance of the population remaining after a dose of D* Mrad.

The values for DS and A were derived from computer generated simulation studies. The simulation studies covered a wide range of initial levels of contamination and of distributions of microbial resistance. The whole dose setting methodology was then tested by further computer simulation studies. Some details of these investigations are given in Section 6.

The DS method of dose setting has two major practical advantages:

1) experimentation is performed on natural product microbial flora, and

2) the method is based on sterility test results (fraction positive) rather than bioburden (microbial) counts and their respective resistances.

It is reasonably well documented that the resistance of organisms are affected by the substrate on which they are deposited and also by the extent to which they have been washed. Therefore, using fraction positive results rather than resistance determinations is advantageous in that it avoids problems associated with the representativeness of resistances derived in the laboratory.

Although it is usually assumed that false positive sterility tests occur with a rate of approximately less than 1 in 1000 tests, our methods are relatively insensitive to this. Additionally, it should be remembered that there is an unknown false negative rate since sterility test methods use growth media and conditions which might not be conducive to the growth of certain organisms. All methods will be affected by this latter possibility.

A corollary to the DS method makes use of cultures from the positive sterility tests found at the higher incremental doses. The corollary states that the maximum laboratory culture D10 resistance value can be substituted for the DS + A factor to determine an acceptable SAL dose. This corollary method is shown to be generally accurate, given valid representative resistance determinations, for a wide range of presterilization microbial flora and has variability similar to the DS + A method of this section. The DS + A maximum isolate resistance method is discussed in Section 5.

The DS + A Sterilization Dose Setting Method — Experimental Procedures

Introduction — Experimental Procedures

The rationale for this method has already been discussed in this section. However, some of the salient points are restated here. The DS + A method requires the use of sterility tests applied to product that has received lower doses of radiation than that which will constitute the ultimate sterilization dose. Tables are supplied which will allow sterility test results of samples exposed to radiation to be extrapolated to specify an appropriate sterlization dose.

This method does not require the enumeration of bioburden. A protocol for a series of incremental dose experiments is provided to allow a dose D* Mrad to be established such that approximately 1 in 100 samples dosed at D* Mrad will be nonsterile. The method provides a sterilization dose by extrapolation from the 10^{-2} level using the appropriate DS + A dose setting factors which characterize the remaining microorganism resistance.

Overview

The DS + A dose setting method requires four stages of activity to determine a dose which can be expected to provide a specified Sterility Assurance Level (SAL). The SAL dose can be expressed as:

Dose = D* Mrad + (extrapolation factor) (DS + A) Mrad

where:

D* Mrad is a measurable estimate of dose which will provide approximately 1 per cent (10^{-2}) nonsterile samples; and (DS + A) Mrad is an estimate of the tail population D10 Mrad for microorganisms surviving the D* Mrad dose.

The four stages of activity are:

1. Product Sampling of three independent production lots.
2. An incremental dose experiment to identify the minimum dose which will provide some sterile samples. This dose is referred to as the First Fraction Positive (FFP Mrad) dose. Also, this experiment initially estimates the dose D* Mrad which will provide approximately one per cent nonsterile samples.
3. A second dose experiment using 100 samples, is conducted at D* Mrad to refine the estimate of expected nonsterile samples at D* Mrad. This experiment also estimates the minimum dose, First No Positives (FNP Mrad), at which less than one per cent of 100 samples will be nonsterile.
4. A series of calculations based on data from Stages 2 and 3 are used to determine the desired sterilization dose.

Also included at the end of this section (Example 4.9) is a special application of the DS + A dose setting method for product made under Ultra Clean Good Manufacturing Practices.

A D** sterilization dose Quality Control audit procedure (Section 7) is provided to assure the determined sterilization dose continues to be applicable through time.

Note: Notation is lower case when it refers to results for product samples for single lots, and upper case when it refers to a summary of all three lots.

Procedures

Stage 1 — Product Sampling. From each of three independent manufacturing lots produced under GMP conditions, 280 samples are randomly selected for use in partial dose determinations. The samples should be selected to represent the microbial challenge to sterilization.

Stage 2 (Experiment 1) — Determination of FFP, A, D*, and CD* Lot.

1. Irradiate twenty samples of each lot at 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 Mrad, respectively. Hold 100 samples per lot for experimentation at D*. A total of 840 samples will be required (Example 4.1).

Example 4.1 — Number of samples for evaluations at various incremental ^{60}Co Mrad doses.

	Incremental Mrad Doses									Hold Samples for Stage 3 Experiment	Total Samples Required
	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8		
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

Note: Computer simulations indicate that for an average sample bioburden of 1,000 or less, fewer incremental doses should be sufficient. Recommended guidelines are: If bioburden is 10 or less, use incremental doses of 0.2 through 1.0 Mrad. If bioburden is 11 through 100, use incremental doses of 0.2 through 1.2 Mrad. If bioburden is 101 through 1,000, use incremental doses of 0.2 through 1.6 Mrad.

2. Determine the First Fraction Positive dose (FFP Mrad), i.e., the median value of three lot ffp's. A lot ffp is the first dose where at least one of the twenty samples is sterile (Example 4.2).

Note: If the three lot ffp Mrad doses are different, FFP Mrad is the median of the three. If any of the ffp Mrad doses are equivalent, select as the FFP Mrad that ffp dose common to the two lots.

Example 4.2 — Determining FFP Mrad.

	Incremental Dose (Mrad)								
	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

Sterility testing results: number of positives out of 20 tests. In this example, the values of ffp for Lots 1, 2, and 3 are 0.4, 0.4, and 0.2, respectively. Hence, FFP, the median ffp is 0.4 Mrad.

3. To determine A Mrad, take the minimum number of positive results at FFP Mrad (Example 4.3) and refer to Table 4.4.

Example 4.3 — Determining A.

	Incremental Dose (Mrad)								
	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8
Lot 1	20	8	2	0	0	1	0	0	0
Lot 2	19	8	1	0	0	0	0	0	0
Lot 3	20	12	2	1	0	0	0	0	0

Sterility testing results: number of positives out of 20 tests. Since ffp 1 is 0.4, ffp 2 is 0.2 and ffp 3 is 0.4, FFP = median ffp = 0.4 Mrad. Therefore, A is determined by the minimum number of positives at 0.4 Mrad. Using 8 positives and Table 4.4, A = 0.017 Mrad.

Table 4.4 — A (Mrad) — The Dose Setter Augmentor.

# Positive Tests of 20	A (Mrad)
19	0.000
18	0.003
17	0.004
16	0.006
15	0.008
14	0.009
13	0.010
12	0.012
11	0.013
10	0.014
9	0.016
8	0.017
7	0.019
6	0.021
5	0.023
4	0.026
3	0.029
2	0.033
1	0.040
0	0.040

4. Determine the D* Mrad dose, i.e., the median of the three lot d*'s (Example 4.4).
   Note: d* Mrad for a lot is equal to the minimum dose of (a) or (b) below:
   a) The first incremental dose at which 0/20 positives occur immediately followed by 0/20 positives.
   b) The first incremental dose at which 1/20 positives occur, immediately preceded and followed by 0/20 positives.
   If any d* is greater than the D* determined above by more than 0.4 Mrad, then D* must take the value of the greatest d*.
5. The CD* Lot is the lot that will be used to characterize D* Mrad by dosing 100 samples from

that lot at D* Mrad. The lot used to characterize D* is the d* lot which is equal to D* Mrad and which has either the smallest ffp or, if there is a tie in ffp, the lot which has a minimum number of positives at the tied ffp's.

Note: When all d*'s are not the same, the CD* lot is the lot with the median d*. If two of the d*'s are equal, then the CD* lot is chosen from the equal d* lots, such that either the ffp is the minimum or the ffp has the least number of positives (Example 4.5).

If all d*'s are equal, the CD* lot chosen is that which has the minimum ffp (Example 4.6).

Example 4.4 — Determining D*.

	Incremental Dose (Mrad)								
	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

Sterility testing results: number of positives out of 20 tests. Lot 1 d* Mrad is 0.8, which represents the minimum of the first incremental dose where 2 consecutive 0/20 positives occurred. Lot 2 d* Mrad is also 0.8 Mrad. Lot 3 d* Mrad is 0.8 Mrad, which is the first incremental dose at which 1/20 positives occurred, immediately preceded and followed by 0/20 positives. Hence, D* is 0.8 Mrad.

Example 4.5 — Determining CD* Lot (case 1).

	Incremental Dose (Mrad)								
	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8
Lot 1	20	7	1	1	0	0	0	0	0
Lot 2	20	9	1	0	0	0	0	0	0
Lot 3	20	12	0	0	0	0	0	0	0

Sterility testing results: number of positives out of 20 tests. In this example, CD* Lot is Lot 2, which has the median d* of Lot 1 d* (1.0 Mrad), Lot 2 d* (0.8 Mrad) and Lot 3 d* (0.6 Mrad).

Example 4.6 — Determining CD* Lot (case 2).

	Incremental Dose (Mrad)								
	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8
Lot 1	19	9	2	0	0	0	0	0	0
Lot 2	20	8	1	0	0	0	0	0	0
Lot 3	20	1	1	0	0	0	0	0	0

Sterility testing results: number of positives out of 20 tests. In this example, the CD* Lot is Lot 1. The d* from each lot was equal (0.8 Mrad), so Lot 1 is chosen because it has an ffp dose (0.2 Mrad) which is smaller than the the ffp doses from the other two lots.

If this choice is not unique, i.e., a tie exists in ffp doses, the CD* lot chosen is that with the minimum number of positives at the first ffp dose (Example 4.7).

Example 4.7 — Determining CD* Lot (case 3).

	Incremental Dose (Mrad)								
	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8
Lot 1	20	8	4	0	0	0	0	0	0
Lot 2	20	12	2	0	0	0	0	0	0
Lot 3	20	8	3	0	0	0	0	0	0

Sterility testing results: number of positives out of 20 tests. In this example, the CD* Lot is either Lot 1 or Lot 3. The d*'s from each lot are equal (0.8 Mrad); the ffp dose from each lot is also equal (0.4 Mrad). Therefore, either Lot 1 or Lot 3 is randomly chosen as the CD* Lot, because both have a minimum number of positives at ffp.

Stage 3 (Experiment 2) — Determination of CD, FNP, and DS.*

1. Perform the characterization of D* (CD*). Irradiate the 100 samples retained from the lot determined in Stage 2 (Experiment 1) at D* Mrad. CD* is the number of positives resulting from the 100 samples dosed at D* Mrad. If 0 positives in 100 samples are observed, set CD* = 1.
2. To determine FNP Mrad, i.e., the dose at which First No Positives is predicted to occur:
   a) Set FNP Mrad = D* Mrad, if CD* was equal to zero positives; or

   b) Set FNP Mrad = D* Mrad + 0.2 Mrad, if CD* was less than 10 positives; or
   c) Set FNP Mrad = D* Mrad + 0.4 Mrad, if CD* was 10 to 15 positives; or
   d) If CD* is greater than 15 positives, D* Mrad should be redetermined.
3. To find the value of DS (D10 Mrad), refer to Table 4.5, using FNP minus FFP Mrad.
   Example: If FNP = 1.4 Mrad and FFP = 0.4 Mrad, then DS = 0.4 Mrad.

Table 4.5 — Dose Setter Values (Mrad) Calculated Using First Fraction Positive to First No Positives.

(FNP – FFP) Mrad	DS D10 Mrad
0.0	0.20
0.2	0.24
0.4	0.28
0.6	0.32
0.8	0.36
1.0	0.40
1.2	0.48
1.4	0.56
1.6	0.64
1.8	0.72
2.0	0.80

Rounding Procedures for Table 4.5

1. Do not interpolate within Table 4.5
2. If the (FNP-FFP) Mrad is within ± 0.05 Mrad of the table value in Column 1 use that value; if not,

use the next higher (FNP-FFP) Mrad value.

Stage 4 — DS Gamma Radiation Dose Calculations

The dose setting method can be expressed as follows:

Dose = D* Mrad + [log (CD* 100) – log (SAL) – log (SIP)] (DS + A) Mrad. See Example 4.8.

Example 4.8 — DS + A Dose Calculations.

	Incremental Dose (Mrad)									
	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	D* = 0.8
Lot 1	20	8	2	0	0	1	0	0	0	**
Lot 2	19	8	1	0	0	0	0	0	0	$\frac{1+}{100}$
Lot 3	20	12	2	1	0	0	0	0	0	**

Poststerility testing results: number of positives out of 20 tests.

(**Since the 100 samples from Lots 1 and 3 were not used to characterize D* Mrad, they may be discarded).

Dose = D* Mrad + [log (CD*/100) – log (SAL) – log (SIP)](DS + A) Mrad
Let SAL = 10^{-6} and SIP = 1
From the data:
D* = 0.8 Mrad and CD* = 1 +
FFP dose = 0.4 Mrad
FNP dose = D* + 0.2 Mrad = 1.0 Mrad
A = 0.017 Mrad (the value in Table 4.4 corresponding to 8 positive = tests)
DS = 0.32 Mrad (the value in Table 4.5 corresponding to 0.6 Mrad)
Then the Dose is:
Dose = 0.8 Mrad + [log (1/100) – log (10^{-6}) – log (1)] [0.32 + 0.017] Mrad
= 0.8 Mrad + [–2 + 6 + 0] [0.337] Mrad
= 2.15 Mrad

Dose Setting for Ultra Clean Good Manufacturing Practices — a Special Application of the DS + A Method

"Ultra Clean" implies that the organisms that make up this bioburden are of minimal challenge to the sterilization process. This method may be used when: 1) The bioburden is not more than an average of 10 per item, (SIP = 100%) and, 2) No incremental dose has more than 5 out of 20 positives.

The procedures for setting dose for Ultra Clean GMP product are the same as those for GMP product, except that Table 4.6 is used to determine UDS Mrad. UDS is used in place of DS for Ultra Clean product dose setting. An immediate verification of dose is performed, on the 100 samples retained from the lot with maximum d* Mrad, before accepting the determined dose. The verification audit should be conducted according to the audit procedure of Section 7. Also, future sterilization

dose audits should be conducted according to the procedures of Section 7.

Table 4.6 — UDS Dose Setter Values Calculated Using First Fraction Positive to First No Positive.

(FNP – FFP) Mrad	UDS D10 Mrad
0.0	0.16
0.2	0.16
0.4	0.24
0.6	0.28
≥0.8	**

**Switch to Table 4.5

Rounding procedures for Table 4.6

1) Do not interpolate within Table 4.6
2) If the (FNP – FFP) Mrad is within ± 0.05 Mrad of the table value in Column 1, use that value; if not, use the next higher (FNP – FFP) Mrad value.

Example 4.9 — UDS + A Ultra Clean Dose Calculations.

	Incremental Dose (Mrad)									
	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	D* = 0.4
Lot 1	1	0	0	0	0	0	0	0	0	$\frac{0}{100}$
Lot 2	2	1	0	0	0	0	0	0	0	** ***
Lot 3	3	0	0	0	0	0	0	0	0	**

Poststerility testing results: number of positives out of 20 tests.

(**The 100 samples from Lots 2 and 3 were not used to characterize D*. Lot 2 was used to confirm the predicted dose, because it has the maximum d*. Lot 3 may be discarded.

***Audit of Lot 2 showed zero positives in 100 tests. Hence the established 1.20 Mrad sterilization dose is verified.)

Note: Clearly in this case all incremental doses were not necessary. Judgement from historical information may have dictated that the higher doses would not be useful. An experimental design using incremental doses of 0.2, 0.4, 0.6, 0.8 and 1.0 should be sufficient for Ultra Clean product dose setting.

Let SAL = 10^{-6} and SIP = 1

From the data:

D* = 0.4 Mrad and CD* = 0

(Note: Since CD* = 0, substitute CD* = 1)

FFP dose = 0.2 Mrad

FNP dose = 0.4 Mrad (Note: Since there were no positives in experiment 2 FNP = D* = 0.4 Mrad)

A = 0.04 Mrad (the value in Table 4.4 corresponding to 1 positive test)

UDS = 0.16 Mrad (the value in Table 4.6 corresponding to FNP – FFP Mrad)

Then the Dose is:

Dose = 0.4 Mrad + [log (1/100) – log (10^{-6}) – log (1)] [0.16 + 0.04] Mrad

= 0.4 Mrad + [–2 + 6 + 0] [0.20] Mrad
= 1.20 Mrad

SECTION 5. THE DS + A MAXIMUM ISOLATE RESISTANCE STERILIZATION DOSE SETTING METHOD

STERILIZATION DOSE SETTING USING THE MOST RESISTANT ISOLATE AND THE EXPERIMENTAL DESIGN OF THE DS + A METHOD

Introduction

This method requires, as in the DS + A Method, that D* Mrad be determined. However, the extrapolation from D* to the required sterility assurance level is made by substituting for DS + A the maximum D10 value of the microorganisms surviving the substerilization doses (2).

Procedures

Perform the experimental procedures of Section 4. Dose is set according to the dose setting formula of the DS + A method, using the most resistant isolate, D10 Mrad, in lieu of (DS + A) Mrad. To determine the most resistant microorganism, it is sufficient to isolate, culture and determine a D10 value for each positive sterility test growth where the fraction positive is less than or equal to 10/20. The D10 determinations should be made with product as the substrate and the inoculum representing as nearly as possible the natural microbial contamination states.

To assure that the determined sterlization dose is applicable through time, D** sterilization dose quality control auditing should be applied as specified in Section 7.

Example 5.1

In this example the objective is to provide a sterility assurance level (SAL) of 10^{-6} for product produced under GMP conditions. Also, the Sample Item Proportion (SIP) is 0.1, D* was determined to be 0.8 Mrad, CD* was 1. A typical result from the DS + A experimentation of Section 4 is shown in Example 5.1.

Example 5.1 — Number of Positives Observed out of 20 Tests.

	Incremental Dose (Mrad)									
	0.2	0.4	0.6	0.8	1.0	1.2	1.4	1.6	1.8	D* = 0.8
Lot 1	20	8**	2**	0	0	1**	0	0	0	–
Lot 2	19	8**	1**	0	0	0	0	0	0	$\frac{1+**}{100}$
Lot 3	20	12	2**	1**	0	0	0	0	0	–

**These positives were cultured for D10 resistance determinations.

The most resistant of the 24 isolates had a D10 = 0.28 Mrad. On the basis of this data, sterilization dose may be calculated as follows:

Dose = D* Mrad + [log (CD*/100) – log (SAL) – log (SIP)] (Max D10 Mrad)

= 0.8 Mrad + (–2 + 6 + 1) (0.28 Mrad)

= 2.2 Mrad.

Comments

If culturing to determine the maximum isolate resistance can be performed accurately to represent the natural microbial resistance on product, this method can be expected to give sterilization doses equal to or slightly more valid, over a large range of microbial resistances, than those provided by the DS + A method. This method should not be used if the product presterilization bioburden is less than 10. See discussion in Section 6.

SECTION 6. COMPUTER SIMULATION VERIFICATION OF THE DS + A DOSE SETTING METHODS

Using the Ethicon computer sterilization simulator described at the end of this Section, many sterilization dose estimation strategies were tested. The final methods evaluated are described as follows:

(Method M1)

A method approximating to Bruch's proposal to use Stumbo procedures to calculate overall microbial D10 resistance for use in sterilization dose estimation (17).
The dose setting formula used was:

$$\text{Dose} = 1/3 \sum_{i=1}^{3} (\log (A) - \log (SAL) - \log (SIP))\ D10_i$$

where:
A is the average bioburden,
SAL is the Sterility Assurance Level,
SIP is the Sample Item Proportion.

$$D10_i = 1/n_i \sum_{j=1}^{n_i} D_j/(\log A - \log \ln(B_j))$$

where:
n_i is the number of D10 estimates from lot i,
D_j is the substerilization does in Mrad,
B_j is the number of tests/number of nonsterile tests.
Theoretical considerations and our simulation results indicate that this method leads consistently to doses which are too low.

(Method M2)

The UDS + A method described in Section 4.
Dose = D* Mrad + [log (CD*/100) – log (SAL) – log (SIP)] [UDS + A] Mrad

(Method M3)

The DS + A method described in Section 4.
Dose = D* Mrad + [log (CD*/100) – log (SAL) – log (SIP)] [DS + A] Mrad

(Method M4) The DS + A most resistant isolate method described in Section 5.
Dose = D* Mrad + [log (CD*/100) – log (SAL) – log (SIP)] [Max D10]

(Method M5) An average of the M3 and M4 estimates.

We now discuss the results of five sets of simulations (50 for each of five microbial distributions). These five characteristic simulations are representative of results from some 60 sets of simulations performed on various microbial distributions.

Graph 6.1 represents 50 sterilization dose estimates each for Methods M1, M3, M4 and M5. The estimates are the results of 50 simulations for items having an average of 1000 microorganisms with resistances varying from 0.10 to 0.42 Mrad. The distribution of resistances is given in Table 6.1. This distribution is described in Section 3 and is considered a stringent microbial challenge to gamma sterilization.

Table 6.1 — Whitby/Davis Microorganism Distribution Resistances in D10 Mrad.

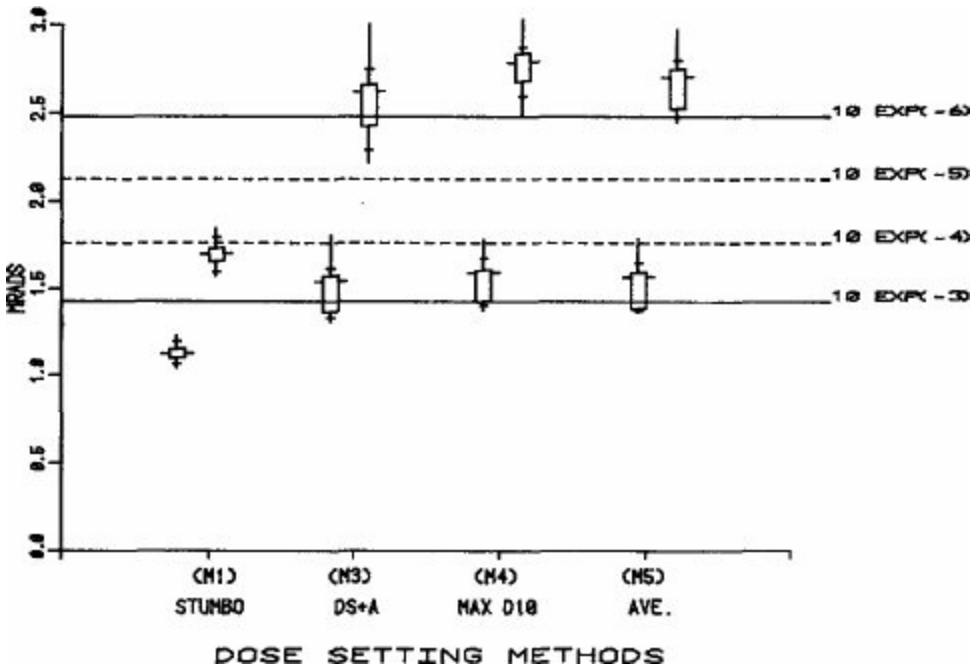
D10 Mrad	0.10	0.15	0.20	0.25	0.28	0.31	0.34	0.37	0.40	0.42
Frequency	0.65487	0.22493	0.06302	0.03179	0.01213	0.00786	0.00350	0.00111	0.00072	0.00007

Bioburden Distribution Numbers and Frequencies

Number	500	800	1000	2500	5000
Frequency	0.10	0.45	0.39	0.04	0.02

Graph 6.1 is interpreted as follows: Each method was used to estimate the required 10^{-3} and 10^{-6} sterilization doses. The required doses were calculated by the model of Section 1. The dose estimate pictures graphically depict the minimum and maximum estimate as well as the 10th, 25th, 50th, 75th and 90th percentiles. The 10^{-4} and 10^{-5} sterilization doses are depicted as broken lines to allow judgements about the accuracy of the dose estimates.

From Graph 6.1 it can be seen that application of Method M1 using Stumbo procedures, to estimate a SAL of 10^{-3} or a SAL of 10^{-6} dose is not supported. However, the DS + A Method M3 and the DS + A Max D10 Method M4 provide acceptable estimates. Method M5, an average of the DS + A and the DS + A Max D10 estimates, also provides conservative sterilization doses.



Graph 6.1 — Whitby/Davis Distribution Sterilization Dose Estimates 1000 Microorganisms.

Graph 6.2 depicts the results of 50 sterilization dose setting simulations using all five dose setting methods for items having an average bioburden of 500 with resistances varying from 0.08 to 0.33 Mrad. See Table 6.2. This distribution was derived from the Whitby/Davis distribution discussed in Section 3 by calibration of resistance modified for cellulosic substrates and is considered to represent an expected microbial challenge to gamma sterilization.

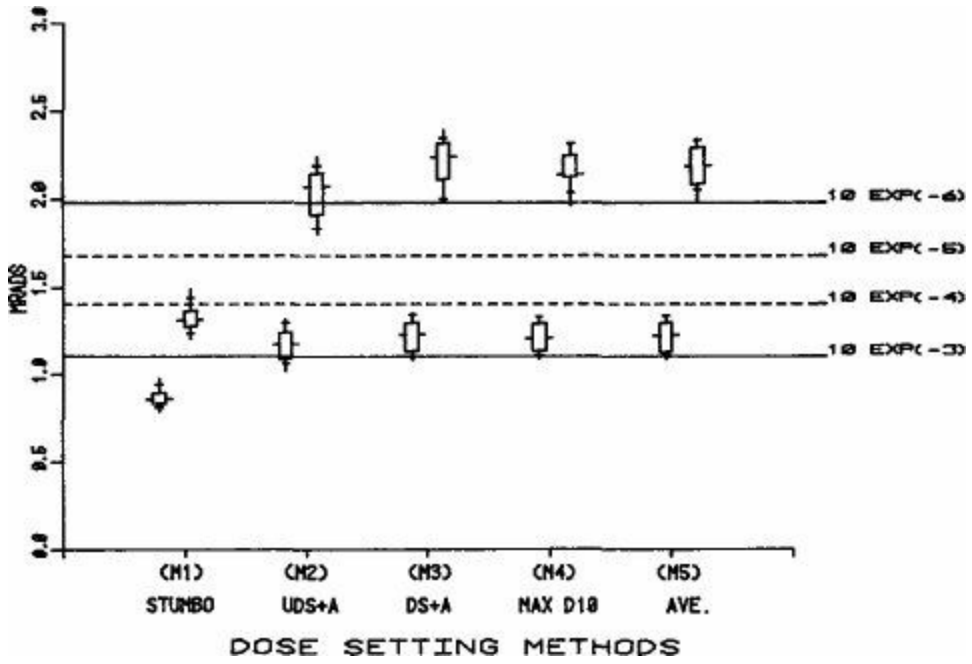
In Graph 6.2, it can be observed that all methods except Method M1 give acceptably conservative dose estimates. The Ultra Clean UDS + A Method M2 of Section 4 was included here for reference purposes only, because the bioburden count would not allow its use in practice.

Table 6.2 — Whitby/Davis Microorganism Distribution Resistances in D10 Mrad.

D10 Mrad	0.08	0.10	0.15	0.20	0.25	0.27	0.29	0.31	0.33
Frequency	0.5509	0.2626	0.1316	0.0351	0.011	0.0039	0.0025	0.0016	0.0008

Bioburden Distribution Numbers and Frequencies.

Number	250	400	500	1250	2500
Frequency	0.10	0.45	0.39	0.04	0.02



Graph 6.2 — Expected Whitby/Davis Distribution Sterilization Dose Estimates 500 Microorganisms.

Graph 6.3 provides the results of 50 simulations using five dose setting methods for items having an average bioburden of two microorganisms with resistances varying from 0.05 to 0.25 Megarads. See Table 6.3. This distribution of microbes is considered to represent a minimal challenge for gamma radiation sterilization.

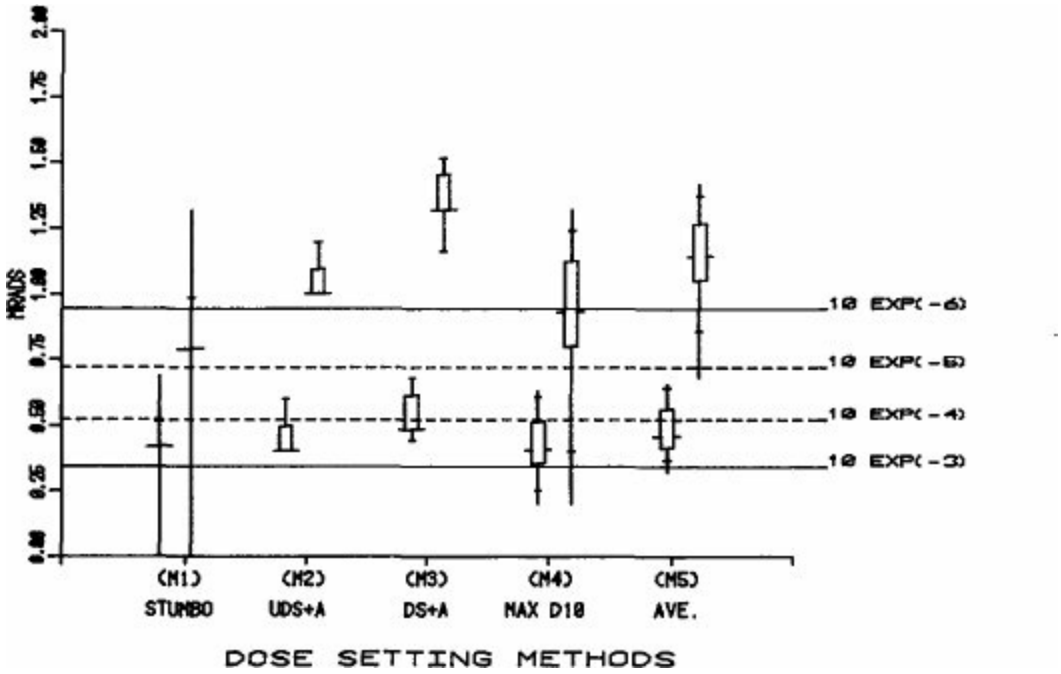
The UDS + A Method M2 of Section 4 for Ultra Clean product provides acceptable sterilization dose estimates whereas Method M1 and Method M4 lack accuracy or are too variable. The unacceptably excessive variability for these two methods is due largely to the small numbers of microbes available on the items for evaluation. A method of reducing the variability in the DS + A resistance Method M4 would be to increase significantly the sample size of the experimental design.

Table 6.3 — Minimum Whitby/Davis Microorganism Distribution Resistances in D10 Mrad.

D10 Mrad	0.05	0.07	0.10	0.15	0.17	0.19	0.21	0.23	0.25
Frequency	0.5509	0.2626	0.1316	0.0351	0.011	0.0039	0.0025	0.0016	0.0008

Bioburden Distribution Numbers and Frequencies.

Number	1	3	7
Frequency	0.70	0.20	0.10



Graph 6.3 — Minimum Whitby/Davis Distribution Sterilization Dose Estimates 2 Microorganisms.

The simulation results shown in Graphs 6.1, 6.2 and 6.3, observed generally in some 50 additional sets of simulations, indicate that the dose setting procedures of Methods M2 through M5 are adequate for gamma sterilization dose setting.

Czerniawski-type distributions were also used to evaluate the consequences of applying our methods to populations which might have microbial resistances significantly higher than the ones used in generating the information of Graphs 6.1, 6.2 and 6.3 (16).

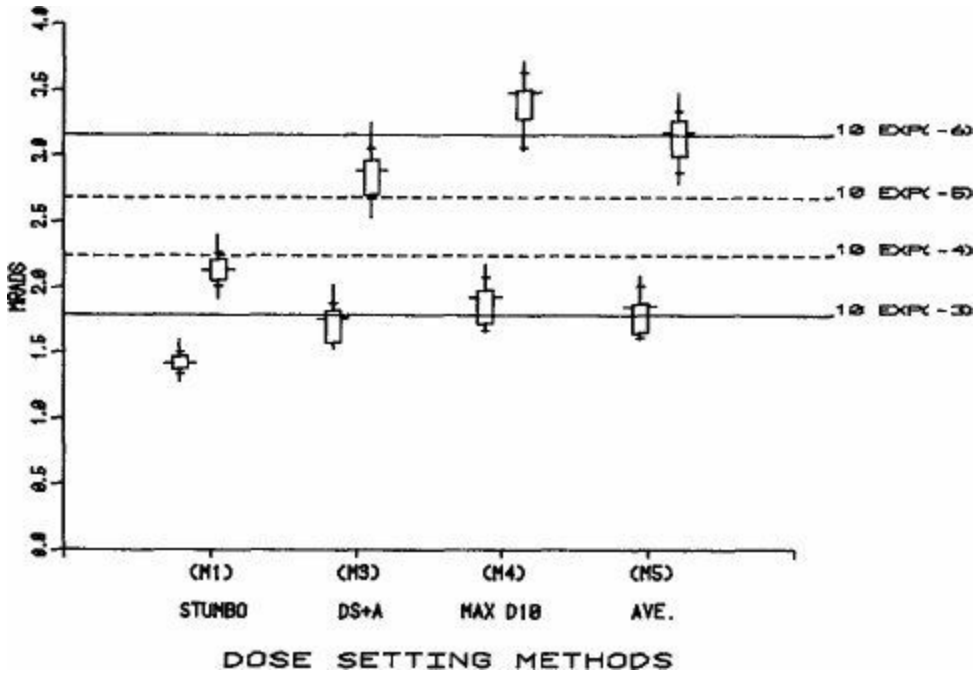
Graph 6.4 contains the results of 50 simulations for items having an average bioburden of 1000 with resistances varying from 0.1 to 0.52 Mrad. See Table 6.4.

Table 6.4 — Czerniawski Microorganism Distribution Resistances in D10 Mrad.

D10 Mrad	0.10	0.15	0.20	0.22	0.28	0.33	0.39	0.46	0.52
Frequency	0.6037	0.2074	0.0581	0.0527	0.045	0.0195	0.0094	0.0037	0.0005

Bioburden Distribution Numbers and Frequencies.

Number	500	800	1000	2500	5000
Frequency	0.10	0.45	0.39	0.04	0.02



Graph 6.4 — Czerniawski Distribution Sterilization Dose Estimates 1000 Microorganisms.

The results of Graph 6.4 as might be expected, show the DS + A Max D10 Method M4 gives acceptable sterilization dose estimates, whereas the DS + A Method M3 does not. However, it should be noted that 90% of the DS + A method estimates are above the 10^{-5} SAL. The DS + A method most likely would give satisfactory estimates if more samples, perhaps 100, were used in each experimental cell and 1000 samples were used for defining CD*.

To further challenge the methods, another Czerniawski-type distribution was evaluated. See Table 6.5. This distribution was formulated to have an even higher frequency of radiation resistant microbes. The results of 50 simulations are given in Graph 6.5.

Table 6.5 — Czerniawski Plus Microorganism Distribution Resistances in D10 Mrad.

D10 Mrad	0.10	0.15	0.20	0.22	0.28	0.33	0.39	0.46	0.52
Frequency	0.5032	0.2074	0.0581	0.0527	0.0450	0.0195	0.0094	0.0037	0.1000

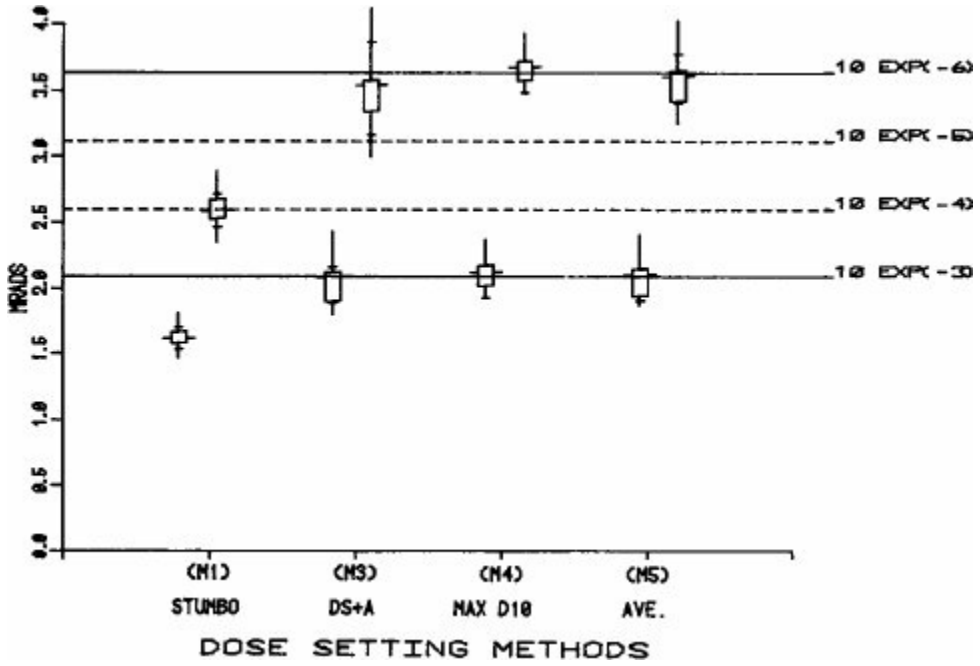
Bioburden Distribution Numbers and Frequencies.

Number	50	80	100	250	500
Frequency	0.10	0.45	0.39	0.04	0.02

Results for this higher resistance distribution are similar to the simulations using the Czerniawski distribution.

Simulations based on homogeneous D10 microbial populations of resistances, 0.2, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50 and 0.60 Mrad were also run. As expected, the results of these simulations showed that the M1 and M4 methods gave accurate estimates with minimal variability. The DS + A method M3 provided estimates which were usually 1 to 2 logs of sterility assurance too high. This was expected because the DS + A Mrad values were chosen to be conservatively high for reasonable populations of heterogeneous microbial resistance.

Tables 6.6 through 6.11 contain representative coefficient of variability information for the five methods as determined from the simulations. The coefficient of variability is calculated by dividing the standard deviation of the estimates by the average of the estimates times 100% and represents the variability of the estimates relative to their average. This gives information about precision but not about the accuracy of the dose specified. For instance, the variability of the Bruch/Stumbo method is low but the dose set is usually unacceptably low.



Graph 6.5 — Czerniawski Plus Distribution Sterilization Dose Estimates 100 Microorganisms.

Table 6.6 — Coefficients of Variation for 10^{-3} SAL Dose Estimates from Simulations on the W/D (Table 6.1) Distribution.

Dose Setting Methods	Average Number of Microbes per Item						
	10	100	500	1000	5000	10,000	100,000
(1) Bruch/Stumbo (M1)	5.6	8.4	5.3	4.6	4.2	3.9	3.1
(2) UDS + A (M2)	9.5	11.1	7.6	—	—	—	—
(3) DS + A (M3)	9.2	7.8	6.1	7.3	5.1	7.2	3.9
(4) DS + A Max D10 (M4)	10.0	7.1	6.2	7.0	5.0	5.8	3.8

Table 6.7 — Coefficients of Variation for 10^{-6} SAL Dose Estimates from Simulations on the W/D (Table 6.1) Distribution.

Dose Setting Methods	Average Number of Microbes per Item						
	10	100	500	1000	5000	10,000	100,000
(1) Bruch/Stumbo (M1)	9.5	8.4	5.7	4.9	4.7	4.3	3.7
(2) UDS + A (M2)	16.0	10.6	9.1	—	—	—	—
(3) DS + A (M3)	7.8	6.6	5.4	5.8	4.2	7.2	3.6
(4) DS + A Max D10 (M4)	9.0	6.5	5.6	4.5	3.5	3.9	2.7

Table 6.8 — Coefficients of Variation for 10^{-3} SAL Dose Estimates from Simulations on the Expected W/D (Table 6.2) Distribution.

Dose Setting Methods	Average Number of Microbes per Item						
	10	100	500	1000	10,000	100,000	
(1) Bruch/Stumbo (M1)	11.2	7.9	5.8	5.0	4.7	3.6	3.2
(2) UDS + A (M2)	14.7	10.2	7.3	—	—	—	—
(3) DS + A (M3)	12.3	9.9	6.9	7.5	6.4	4.9	5.7
(4) DS + A Max D10 (M4)	12.6	9.5	6.7	6.5	5.8	4.0	4.8
(5) Averages (M5)	12.1	9.7	6.6	6.9	6.1	4.4	5.2

Table 6.9 — Coefficients of Variation for 10^{-6} SAL Dose Estimates from Simulations on the Expected W/D (Table 6.2) Distribution.

Dose Setting Methods	Average Number of Microbes per Item						
	10	100	500	1000	5000	10,000	100,000
(1) Bruch/Stumbo (M1)	11.2	7.9	6.4	5.5	5.4	4.2	3.8
(2) UDS + A (M2)	14.1	7.7	7.11	—	—	—	—
(3) DS + A (M3)	8.6	6.7	5.6	6.5	4.9	4.6	5.6
(4) DS + A Max D10 (M4)	11.1	6.6	4.5	4.0	3.7	2.6	3.2
(5) Averages (M5)	8.5	6.4	4.5	5.1	4.2	3.4	4.3

Table 6.10 — Coefficients of Variation for 10^{-3} SAL Dose Estimates from Simulations on the Czerniawski (Table 6.4) Distribution.

Dose Setting Method	Average Number of Microbes per Item				
	10	100	1000	10,000	100,000
(1) Bruch/Stumbo (M1)	13.3	6.1	4.3	3.1	2.6

(2) UDS + A (M2)	11.4	8.8	–	–	–
(3) DS + A (M3)	10.4	7.6	6.1	5.8	4.4
(4) DS + A Max D10 (M4)	11.7	8.9	5.9	4.9	3.7
(5) Averages (M5)	10.3	7.9	5.6	5.1	3.9

Table 6.11 — Coefficients of Variation for 10^{-6} SAL Dose Estimates from Simulations on the Czerniawski (Table 6.4) Distribution.

Dose Setting Methods	Average Number of Microbes per Item				
	10	100	1000	10,000	100,000
(1) Bruch/Stumbo (M1)	13.3	6.1	4.3	3.4	3.0
(2) UDS + A (M2)	10.1	9.5	–	–	–
(3) DS + A (M3)	8.1	6.4	5.2	6.9	5.3
(4) DS + A Max D10 (M4)	9.7	7.2	4.6	3.3	2.4
(5) Averages (M5)	7.3	5.9	3.9	4.6	3.5

The following provides a description of the Ethicon Sterilization Computer Simulator which was developed to evaluate the methods set forth in Sections 4 and 5 of this document.

A Description of the Ethicon Sterilization Computer Simulator

In order to evaluate and refine our dose setting strategies a computer simulation system was developed. To have confidence in the information from the computer simulations and to simulate as near as possible actual sterility testing conditions, we minimized assumptions about the distributions of microbes on product and their resistance to gamma radiation.

The computer simulator makes use of a random number generator to choose microbes from given discrete distributions for assignment to items on a microbe by microbe and an item by item basis. Random numbers are then used to determine if the item will be sterile after receiving a given gamma radiation dose. These procedures are repeated until a complete experimental design of sterility results is available. To simulate the design of the DS + A method (Section 4) for product having an average bioburden of 1000 requires more than 840,000 uses of the random number generator. Since we repeat these designs 50 to 100 times to get estimates of variability and accuracy for the sterilization dose estimates, there are some 40 to 80 million random number accesses required per simulation.

An overview of the computer simulator program follows:

Input Data Contains:

a. The number of simulations desired.
b. The numbers (N_i) of possible microbes per item and their probabilities (f_i) of occurrence (N_1, f_1; N_2, f_2; … N_k,f_k).
c. The classes of resistance (D_j) and their probabilities of occurrence (p_j): (D_1,p_1; D_2, p_2; …; D_n, P_n).

d. The incremental radiation doses to be used in the experimental design, were usually 0.2, 0.4, … 1.8 Mrad.

In order to use the random number generator to determine the amount of microbes (bioburden) on a given item the data from input (b) are arranged in a 2 × k array. The array is represented as:

Bioburden Number	N_1	N_2	N_3	…	N_k
Random Number intervals*	$[0, f_1]$	$\left(f_1, \sum_{i=1}^{2} f_i\right]$,	$\left(\sum_{i=1}^{2} f_i, \sum_{j=1}^{3} f_i\right]$	…	$\left(\sum_{i=1}^{k-1} f_i, 1\right]$

*These intervals are used in conjunction with random number generators to determine the bioburden on an item.

The random number generator is then accessed to determine a random value between 0 and 1. The number of microbes on the item is then the N_i corresponding to the interval containing the random number value.

Once the order of microbes (N_i) for the item is determined, the program returns to the random generator to determine the kinds of microbial resistances that the N_i organisms have.

In order to use the random number generator to determine the microbial resistance the data from input c) is arranged in a 2 × n array. The array is represented as:

D10 Value	D_1	D_2	…	D_n
Random Number Values*	$[0, p_1]$,	$\left(p_1, \sum_{j=1}^{2} p_j\right]$	…	$\left(\sum_{j=1}^{n-1} p_j, 1\right]$

*The intervals are used in conjunction with the random number generator to determine the D10 of each microorganism of the bioburden.

Once all (N_i) of the resistances have been selected they are put into classes according to their D_j value. Given M_j equals the number of items a particular D_j was selected, the probability A that the item will be sterile after an incremental dose D Mrad can be calculated using:

$$A = \sum_{j=1}^{n} (M_j/N_i)\ (1 - P(RS))^{M_j}$$

where P (RS) is the probability of a random microbe survivor, and is represented as:

$$P(RS) = \sum_{j=1}^{n} (M_i/N_i)\ (0.1)^{D/D_j}$$

Since A equals the probability that an item is sterile, (1 – A) equals the probability an item is nonsterile.

The random number generator is then accessed again to determine a number in the interval from 0 to 1. If the random number chosen is contained in the interval [0, A] the item is judged sterile, if not, then the item is judged to be nonsterile.

The above procedures are repeated until a full set of sterility tests are obtained to fill out the experimental design. Given a complete design of sterility test results, sterilization dose estimates can be calculated.

In addition to using the simulator to assess item sterility, the random number generator is invoked to simulate culturing so as to determine D10's for the higher incremental dose nonsterile items. This information was used to evaluate the DS + A Max D10 M4 dose setting method of Section 5.

Since the computer simulator provides accurate sterility results without the affect of laboratory testing variability and false positives, it is reasonable to conclude that methods that prove to be better under simulation will be similarly better in practice. The computer simulator has provided much insight into the deactivation of heterogeneous microbial populations and the resultant "sterility tests".

SECTION 7. THE D** STERILIZATION DOSE AUDIT FOR QUALITY CONTROL

Introduction

The sterilization quality audit is conducted to detect changes in the manufacturing facility or raw material source that have the potential of increasing the established sterilization dose. The established dose is based either on the most recent sterilization dose setting experiment or on an augmented dose action indicated by a previous quality audit. To determine the continued validity of a sterilization dose for specific sterility assurance levels, the audit should be performed at least quarterly, unless seasonal and/or manufucaturing bioburden indicate otherwise.

Procedures

1. From a randomly selected production lot, randomly sample 100 units using the same SIP on which sterilization dose was determined.
2. Dose these 100 samples at an audit dose of D** Mrad. The delivered audit dose must be within ± 0.05 Mrads. D** Mrad is the standard estimated dose that is expected to provide a SAL of less than or equal to 10^{-2}. The determination of D** Mrad for each dose setting method is:

   Screened Resistances (Section 2): D** Mrad is equal to the sum of the screen (X Mrad) and D* Mrad, rounded up to the nearest 0.1 Mrad, where D* Mrad is the dose, D, which makes

$$\sum_{j=1}^{k} P_j \, (0.1)^{D/D_j}$$

   equal to a sterility assurance level of 10^{-2}. See the example of Section 2.

   Bioburden Method (Section 3): D** Mrad is equal to the verification dose given in Table 2 rounded up to the nearest 0.1 Mrad.

   DS + A Method (Section 4): D** Mrad is the larger of the two doses, D* Mrad + [2 + log (CD*/100)] (DS + A) Mrad rounded up to the nearest 0.1 Mrad or (D* + 0.2) Mrad.

   DS+A Max D10 Method (Section 5): D** Mrad is determined as described for the DS + A method above.
3. Perform sterility testing on the 100 audit samples and determine the number of nonsterile samples.

D Audit Actions**

Refer to Table 7.1 to determine the action required as indicated by the audit results. Four types of actions are possible:

1. A = Acceptable. The sterlization dose is acceptable.
2. C = Caution. The sterilization dose may be borderline. A review of Good Manufacturing Practices as they relate to product sterility may be indicated.
3. C + = Caution +. There is an indication that the sterilization dose may be too low. Hence, it should be augmented by the Mrad value shown in Table 7.1. A review of Good Manufacturing Practices, as they relate to product sterility is indicated.

Table 7.1 — Action Criteria for the Established Dose Audit Criteria Based on 100 Tests at D** Mrad.

Degree of Extrapolation		Action Criteria for Quality Audit at D** Mrad				
(1) lg (SAL)	(2) SIP	(3) 0 pos	(4) 1 pos	(5) 2 pos	(6) 3 pos	(7) ≥ 4 pos
3	1	A	A	A	C	R + (0.28)
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 + (0.33)	R + (0.44)	R + (0.52)	R + (0.52)

Legend for Table 7.1:

pos	=	Number of positive sterility tests observed.
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. Check GMPs.
R +	=	Reestablish dose—increase dose immediately by the value given in parentheses and then reestablish dose. Check GMPs.
log(SAL)	=	log of Sterility Assurance Level.
SIP	=	The Sample Item Proportion.
D**	=	The audit D** is the larger of D* + .2 Mrad, or D* + [2 + log (CD*/100)] (DS + A) rounded up to the nearest 0.1 Mrad, or an audit augmented D**. When sterilization dose is augmented, augment D** by the same Mrad value.

Columns 3, 4, 5, 6 and 7 give the number of positive tests of 100 samples at D** Mrad dose.

4. R + = Reset. The sterilization dose should be immediately augmented by the Mrad value shown in Table 7.1, and a new sterilization dose should be determined. A review of Good Manufacturing Practices, as they relate to product sterility is indicated.

Table 7.1 is entered by determining log (SAL) and SIP. The action (and potential dose augmentation) is determined by consulting column 3, 4, 5, 6 or 7, according to whether there are 0, 1, 2, 3 or 4 or more positives in the audit results.

Note 1: If the dose is augmented by Y Mrad on the basis of audit results, then subsequent audits should be performed at D** + Y Mrad rounded up to the nearest 0.1 Mrad.

Note 2: A repeat of the quality audit to overrule action criteria is not permitted, unless there is good evidence that the quality audit was compromised by unacceptable procedure.

Note 3: If sterilization dose is augmented twice, then sterilization dose should be redetermined.

SECTION 8: GLOSSARY OF TERMS FOR DS GAMMA RADIATION DOSE SETTING AND AUDITING STRATEGIES FOR STERILIZING MEDICAL DEVICES

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 of all three lots.

Bioburden: the total of all viable microbes on a packaged item or unit immediately prior to radiation sterilization process.

CD:* The number of positive sterility tests from 100 samples irradiated at D* Mrad.

D10: The amount of radiation required to kill 90% of the organisms of a homogeneous microbial population. It is defined on the basis that the death of microbes follows first order kinetics. The use of D10 values allows a theoretical calculation of the probability of a survivor from a radiation sterilization process.

d Mrad:* 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 Mrad:* An initial estimate of that dose of irradiation which 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 D10 value assigned to the population of microorganisms surviving a dose of D* Mrad. 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 the 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 in the twenty samples is sterile.

First Fraction Positive (FFP): The median value of three lot ffp's.

First No Positives (FNP): The lowest dose in an incremental sterilization dose series where 100 samples of a lot are expected to be sterile.

Incremental Dose: Irradiation doses ranging from 0.2 Mrad to 1.8 Mrad delivered in increments of 0.2 Mrad.

Sample: The experimental unit which is either the whole item or unit or a proportional part as determined by weight, volume or surface area so as to validly represent the bioburden.

Sample Item Proportion (SIP): The proportion of the item that was sampled for dose setting procedures. When sampling small items such as sutures, an entire item (SIP = 1) should be taken for testing, whereas when sampling large items such as surgeons' gowns, it may be necessary to select only a portion of the item, e.g., 1% (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. SAL's range from 10^{-3} to 10^{-6} depending on product use.

Ultra Clean GMP: Items or units from manufacturing processes where the bioburden on items or units is minimal and of minimal challenge to the sterilization process.

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Process-Control Release of Terminally Sterilized Medical Devices

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Introduction

Sterility is classically defined as an absolute condition, i.e., the complete destruction or removal of all forms of life. It is my view that the sterility of medical devices be assured to the extent that the probability of microbial contamination or survival is one in a thousand (10^{-3}) or less for each topical medical device and one in a million (10^{-6}) or less for each implantable device. To estimate the state of sterility by the United States Pharmacopeia (USP) finished product testing (1) is an absolute condition for all items in a lot, is extremely difficult.

The following discussion will analyze a more direct mechanism for how sterility assurance on a batch-by-batch basis can be derived. It is recognized that several satisfactory approaches may exist and there is no intent to discourage manufacturers from using or developing different types of methodologies. The following discussion should be used, therefore, as a general guideline to the types of necessary data. A key thrust is that the manufacturer provide D-values for the resistance of microbial contamination on medical devices exposed to a described sterilization process. These data are then used to calculate sterilization cycles which assure kill of the natural flora (bioburden) on the device and allow estimates of the probability of a survivor for each item so sterilized.

In this analysis terminal sterilization of medical devices will be approached as a probability function, which is susceptible to measurement in physical-chemical terms. A D-value (time to kill 90% of the organisms) is defined on the basis that the death of microbes usually follows first order reaction kinetics (2,3,4). The use of the D-value allows a theoretical calculation of the probability of a survivor from a terminal sterilization process.

It is my opinion that the term "sterile" has been compromised, in that the term covers a too broad of a range of probabilities of survivors on products, i.e., from 10^{-2} to 10^{-9} probability of a survivor per-item. Table I gives estimates of the probabilities of survivors for several terminally sterilized medical items. R. W. Campbell of the Canadian Health Protection Branch (5) has suggested that the word "sterile" be dropped in favor of a microbiological survivor index (MSI). The MSI is a positive term derived by taking the reciprocal of the logarithm for the probability of a survivor from a sterilization process (a further discussion of the MSI concept is given in the paper by Smith (6).

Table I. — Estimates of Probability of Survivors for Sterlized Items.*

Item	Probability of Survivor/Unit**
Canned chicken soup	10^{-11}
Large-volume parenteral fluid	10^{-9}
Intravenous catheter and delivery set	10^{-6}
Syringe and needle	10^{-6}
Urinary catheters	10^{-3}
Surgical drape kit	10^{-3}
Small-volume parenteral drug (sterile fill)	10^{-3}
Laparpscopic instruments (processed with liquid chemical sterilants)	10^{-2}

**The USP 20-item sterility test will detect probability of survivor/unit of $10^{-1.3}$ with 95% confidence.

**Minimal estimates based on bioburden and its D-value to sterilizing agent being used.

Sources and Control of Bioburden during Manufacture

Medicine was one of the first disciplines to recognize the need for control of microbial contamination, particularly in surgical suites. A most dramatic push to limit the numbers of microorganisms in the industrial environment came from the activities of the space program to restrict the microbial contamination of planets during extra-terrestrial exploration (exobiology). Studies sponsored by the National Aeronautics and Space Administration (NASA) since the 1960's have shown that significant numbers of microorganisms can exist on surfaces and in the air of intramural industrial environments. However, if proper control measures are continually employed the level of microbial contamination can be kept low, i.e., from 0.1 to 3 viable particles per ft^3 of air and less than 500 microorganisms per ft^2 of surface area in the work environment. Where contamination control programs have been estabilished and monitored, it has been shown that people are the primary source of microbial contamination on the product being produced.

Microbiological control procedures should be instituted in any facility manufacturing sterile medical devices. These control procedures are directed towards the limiting of the presterilization microbial load (bioburden) on the products to a level compatible with the sterilization cycle to be employed. Many sterilization cycles are established on the basis that the presence of a given number of microorganisms on each device can be used to predict the probability of a survivor per device. Estimates of survivors can be accomplished by a carefully planned and executed program of microbial contamination control combined with qualification and vigorous monitoring of the terminal sterilization cycle. Such programs constitute the basis of process control release of sterilized product, i.e., no finished product sterility testing.

For estimations of the effectiveness of biological contamination control systems, criteria or standards should be established. The usual goal will be to limit, control or reduce the number and the types of microorganisms occurring on specific components and subassemblies during the assembly of the finished device. For example, one specific criterion might specify the number of bacterial spores allowable per finished device immediately prior to sterilization. If the device is too large to be sampled, then a specification for the number of spores per unit area could be established. Sometimes, the criteria will also specify allowable number of airborne contaminants in the assembly area and frequently other particulates as well. It is usually necessary to specify the assay techniques and other tests and procedures to be used in these monitoring activities.

In any microbial contamination control system one or more of the following techniques is usually employed to assess whether the proper level of microbiological control has been established:

1. Microbial Air Sampling: air impaction samplers, liquid impingers, and settling plates are used most frequently.
2. Particle Size Sampling: liquid impinger sampler with preimpingers offer some particle-size selectivity; the Anderson cascade sieve sampler is frequently used to descriminate the airborne viable particles in a microbiological aerosol into six particle-size ranges.
3. Surface Sampling: cotton swabs or Rodac plates are usually used.
4. Surface Contamination Accumulation Test: small sterile strips of stainless steel, glass or plastic are placed in the environment; after various exposure periods the strips are collected and assayed for viable microoganisms.

5. Component Surface Testing: small components and systems under microbiological contamination control may be tested by complete immersion in an appropriate bacteriological culture broth or by washing the component in a sterile rinse solution that is then quantitatively assayed either by plate counting or membrane filtration.

The bioburden control problem on medical devices in various stages of manufacture is primarily a personnel problem, either through inattention to basic control procedures or as an ecological source of microorganisms. Of the great spectrum of bacteria, fungi, yeast and viruses which comprise the natural microflora, a few find the inner and outer surfaces of the human body to be hospitable for growth. As greater attention is paid to the cleanliness of a manufacturing area, a greater proportion of the environmental microbes constituting bioburden will be contributed by these organisms originating from humans.

Particular attention should be paid to the cleaning of exposed skin area since contact with the human skin or shedding from human hair is frequently the largest source of bioburden on medical devices. Since sterilization of the skin is practically impossible, it is recommended that various types of antiseptic (skin-compatible disinfectant) cleaning solutions be used to allow a significant reduction in the number of organisms carried on exposed skin areas. Other procedures that provide a significant means of control for bioburden shedding from personnel are the following:

1. Mask and hair caps can provide a reasonable barrier for the shedding of microorganisms from the nose and mouth area, the lower extremities of the face and neck, and the top and back of the head. Frequent changes of masks are recommended to prevent overloading with microorganisms.

2. Gowns should be prepared in laundering facilities that prevent the growth of microorganisms during various stages of the laundering process. Following laundering the gowns should be wrapped in various types of protective packages to prevent an accumulation of microorganisms on the laundering clothing.

3. Footwear and gloves. In many sterile device manufacturing areas protective garments for the feet and hands are worn. Various forms of disposable plastic coverings are available to limit the introduction of microorganisms into device processing areas.

Clean rooms, clean enclosures, and clean work stations are installations that reduce the bioburden as well as particulate contamination of the air around devices under manufacture. By themselves these physical facilities are not sufficient to provide hardware or devices with low bioburden levels. The goal of low bioburdens is accomplished through the use of this type of physical facility by trained people who are properly garbed and are following prescribed procedures that prevent unnecessary accumulation of organisms on the medical devices.

Laminar flow clean rooms represent the ultimate approach to cleanliness based on the isotropic flow of filtered air. The exhaust air is circulated through a high efficiency particulate-removing (HEPA) filter for greater efficiency. The direction of flow may be vertical (downflow rooms) or horizontal (crossflow rooms).

Clean work stations or benches are similar to laminar flow facilities in the degree of microbiological contamination control that can be achieved. They are ideally suited for critical operations on small assemblies or small finished devices. The principles of laminar air flow are

utilized to prevent and even remove particulate contamination on devices. Comercially available laminar flow benches provide environments having fewer than 0.3 organisms per ft^3 at normal working distance from the HEPA filter face.

Characterization of Bioburden (Types and Numbers)

Ideally, the kinetics of a sterilization process and the degree of microbiological loading (bioburden) determine the probability of a survivor per finished packaged device. Industrial microbiologists interested in determining the bioburden on finished medical devices immediately prior to sterilization have necessarily borrowed and adapted techniques developed for other applications. During the past 50 years microbiologists in other fields, particularly in the food and dairy industries and some medical facilities, have developed suitable methods for microbiological sampling of various materials and/or items. These techniques can be grouped basically into 4 categories: swabbing, agar contact (Rodac plates), direct surface agar plating, and fluid rinses. Most microbiologists working with medical devices are employing variations of rinse techniques to determine the bioburden on the devices immediately prior to terminal sterilization.

It should be noted that the Spacecraft Sterilization Technology effort of the NASA has investigated and adapted techniques from other microbiological applications for use with the quantitation of microorganisms on/in aerospace hardware. As part of the NASA policy of standardizing microbiological procedures it was decided to utilize a single broad spectrum recovery agar medium in a single incubation sequence for all bioburden determinations. It was recognized that no single growth medium or single incubation sequence could recover all viable microorganisms occuring on a spacecraft. It was decided therefore that maximal counts from one broad recovery agar medium would be preferrable to multiple media and varied incubation sequences which could well make the entire bioburden assessment effort unmanageable. Trypticase Soy Agar (TSA) was chosen by NASA scientists as the optimal recovery medium and 32°C for 72 hours as the incubation sequence to be employed. The emphasis of this approach was the recovery of sporeforming species.

The first opportunity for the Bureau of Medical Devices of the FDA to use the characterization of bioburden in the definition of sterilization cycles came in 1976 with the document entitled “Guidelines for Sterilization of Intraocular Lenses by Manufacturer” (7). Since the medical device industry has in the past looked to the general information contained in the USP as a guide for its own activities, it was decided to use the procedures listed for the USP microbial limits test as a way to define bioburden on intraocular lenses. The main difficulty with the USP procedure is that it was designed to test a small subportion of lots of dry powders or liquid medications and is not fully applicable to the testing of all available surfaces on a device to be sterilized. Another limitation is that the estimate of total aerobic microbial count through serial dilution with growth/no growth endpoints in multiple broth tubes at each dilution is more difficult to manipulate and to extrapolate to a true bioburden determination on the device. In some situations such most probable number (MPN) estimates can be satisfactory.

This paper provides the opportunity to give more visibility and applicability to the procedures developed by NASA for microbial counts on spacecraft surfaces to the bioburden analysis for medical devices (8). The NASA method, which is excellent for bioburden determination on small implantable devices, or in the fluid path of larger devices, is outlined in Figure 1. It is an adaptation of the rinse technique employed in the dairy and food industry. The finished devices (intact or broken-up) are placed in individual bottles containing 50 ml of sterile 0.1% peptone water. The bottles are mechanically shaken to dislodge contaminants. Duplicate 5 ml aliquots of the peptone water (or serial dilutions thereof) are then plated in 20 ml of TSA agar (identical with the Soybean-Casein Digest Agar Medium of the USP method) and incubated aerobically, while two other 5 ml aliquots are

incubated anaerobically. The remaining 30 ml of the diluent are transferred to a large test tube and heat-shocked at 80°C for 15 minutes. The purpose of this step is to destroy all non-spore forming species so that spore-formers can be separately estimated. After heat-shocking, duplicate 5 ml aliquots are plated in 20 ml of TSA agar and incubated both aerobically and anaerobically. In addition, the item itself after shaking can be removed from the diluent and plated directly in melted TSA agar. After the agar hardens this plate is incubated aerobically at 32°C for 72 hours.

Under FDA contract 73-241 an adaptation of the NASA procedure was investigated and reported by West (9). The modifications included the following: longer contact with diluent on a shaker followed by brief insonation; diluent filtration through membrane filter; membrane filter plated directly or blended for serial dilution and plated in TSA medium. West used these modified procedures with syringes, drape material, and catheters.

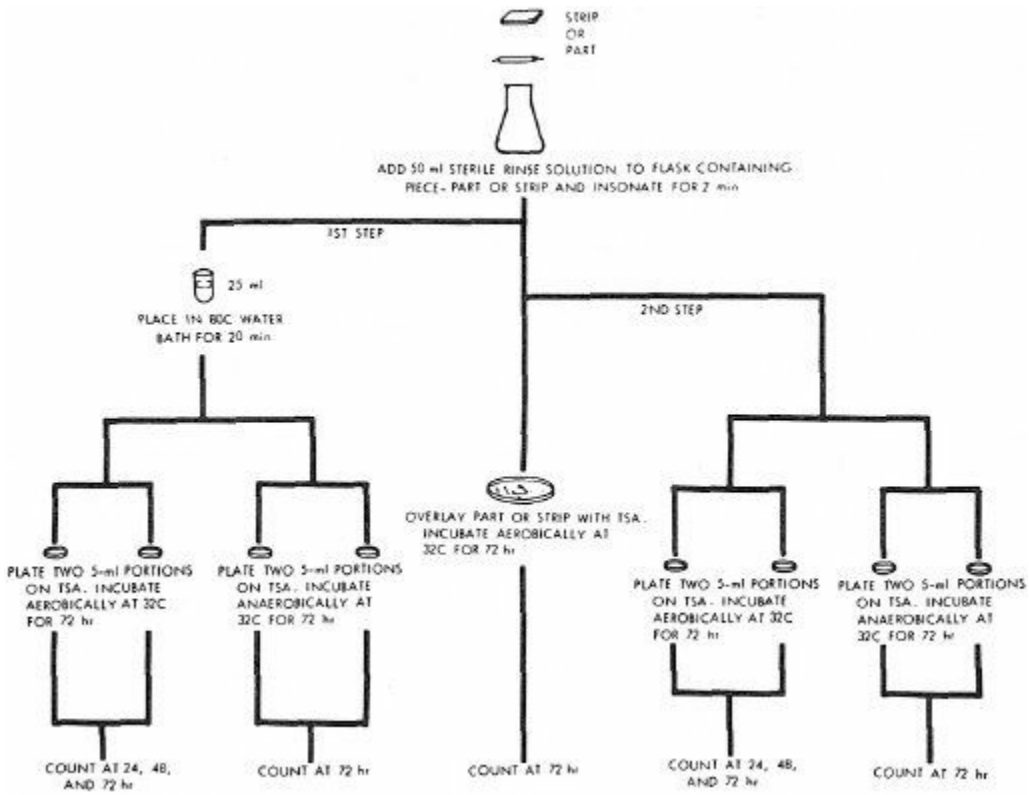

Figure 1. Schematic outline of NASA standard procedure for microbiological examination of spacecraft components (from Reference 8).

Development of Sterilization Cycle

The procedure that has been most in vogue to assure the sterility of pharmaceutical or medical items is a sterility test of a small sample of the treated products. In the past, the official drug compendia (USP and National Formulary, now combined) relied extensively on such tests as the way to judge the adequacy of a sterilization cycle. I have characterized the USP finished product sterility test as legally acceptable but scientifically inadequate. Over the years many articles have been published that show the biological as well as statistical inadequacy of finished product sterility testing as the basis by which to assess terminal sterilization of medical products. Conventional sterility tests can not provide sufficient confirmation of the sterility of a lot of treated items unless extremely large sample numbers are taken, and even then the analyst is confronted by the dilemna of extrinsic contamination from his own body or from the testing environment.

Recent experience has shown that two other procedures can be used either separately or together to bring about a much greater degree of assurance of sterility in processed items. The first approach centers on studies that establish the rates of kill (kinetics) which take place during the destruction of various types and numbers of microorganisms expected to be present on the product during a particular terminal sterilization process. Determination of the kinetics of microbial destruction by a given sterilization treatment yields D-values from which the probabilities of survivors per item can be calculated. The basic mechanics of this approach have been presented by the authors of other papers in this symposium.

The second approach that has come into recent use is that of biological indicators placed in each batch of the product to be sterilized. Usually, the biological indicator is a bacterial spore suspension which has been dried on a suitable carrier or, more ideally, placed directly on the sample items to be sterilized. The destruction of these positive microbial controls provides excellent support (validation) that sterilization of a given batch of material has been achieved.

Ideally, the organisms in these biological indicators should have their resistance characterized in terms of D-values. The destruction of a known or graded series of populations of organisms of defined resistance (D-value) when correlated with the resistance (D-value) of the natural bioburden can allow a reasonable estimate of the probability of a survivor per item from a given sterilization process. The basic philosophy behind the use of biological indicators is that this procedure provides a more rigorous control of a sterilization cycle than a sterility test of treated products that usually have only a low level of random contamination before entering the sterilizer.

When the physical variables of a given sterilization procedure can be rigorously controlled and monitored by physical means, then the concept of dosimetric or process-control release, i.e., no finished product sterility test, can be utilized. Process-control release assumes that the kinetics of microbial destruction for the bioburden has been defined and the associated probabilities of a survivor for a particular sterilization process have been calculated based on that bioburden.

Process-Control (Dosimetric) Release for Products Sterilized by Ionizing Radiation or Steam Under Pressure

An examination of the kinetics of microbial kill by radiation sterilization will show that this sterilizing method is as efficient as moist or dry heat sterilization and under usual operating conditions is much easier to control than ethylene oxide gaseous sterilization. Because of the simplicity of this sterilization process, the number of variables that must be regulated is at a minimum. Only a brief analysis of several key factors associated with radiation sterilization of medical supplies will be discussed here. The two chief factors determining the level of sterility (probability of survivors) attained form radiation processing under ususal manufacturing conditions are the average bioburden on the items and the radiation resistance of that bioburden.

The first step in developing a dosimetric release program for radiation sterilized devices should start with a program of environmental microbial monitoring. Through the use of various aerosol samplers, swab techniques, and Rodac contact plates the sources of microbial shedding and build-up in the processing environment can be determined and suitable restrictive measures taken to limit the accumulation of microorganisms on the items to be sterilized. The average bioburden on each type of product as manufactured should be determined. Through the use of subprocess (incremental) doses, a calculation of the D-value for the bioburden can be obtained through the use of the Stumbo equation or through refinements of this equation discussed by other authors in this symposium. Once the D-value has been estimated, then a calculation of the process dose for either a 10^{-6} or a 10^{-3} probability of a survivor per item can be undertaken.

Many manufacturers going into radiation dosimeteric release initially employ a biological indicator, usually spores of *Bacillus pumilus*, to serve as an integrator of all factors that could be involved in the sterilization process. The sterilization of products inoculated with spores of *B. pumilus* can be correlated with the destruction of the natural bioburden on the product. Based on the published D-values for *B. pumilus*, it has been found that the inoculum level of 10^6 spores of this organism on an item will not survive a dose beyond 1.2 Mrads. The manufacturer then places greater reliance on the use of primary and secondary chemical dosimeters to assure that the load has achieved the necessary amount of energy (dose) to achieve the desired level of probability of a survivor.

The purpose of dosimetry is to measure the energy transferred by radiation to the treated material. Physical and/or chemical dosimeters are available which are easy to use, relatively precise, and reliable (10). The qualities that a good physical and/or chemical dosimetric system should have are the following: it should be independent of the dose rate and only record the total dose received; it should be small in size; and it should be reasonably precise, reliable, and reproducible.

In order to achieve these later objectives, the sterile disposable device industry has utilized the concept of primary and secondary dosimeters. The primary dosimeters can be considered “the gold bar” type of standard and are employed during the initial phases of commissioning of a facility or the introduction of a new product line to be sterilized in a particular sterilization facility. Calorimeters, ionizing chambers, and chemical dosimeters such as ferrous sulfate solution, and ceric-sulfate solution are considered the techniques for primary standard reference dosimetry.

In the day-to-day monitoring of radiation sterilized medical products secondary dosimeters which can be related back to the primary dosimeters are routinely employed. Liquid solutions of radiochromic dyes, ceric-plus cerous sulfate, undyed plastics such as clear Perspex (polymethyl methacrylate) and polyvinyl chloride, and dyed plastics such as red Perspex which can be read

spectrophotometrically or potentiometrically are the materials of choice. The absorbed dose levels currently being used in radiation dosimetric release programs range from approximately 1 Mrad for products desiring a probability of a survivor of 10^{-3} or less per item to 1.6 - 2.0 Mrads for products having a probability of survivor of 10^{-6} or less per item.

The approach to have steam sterilized products released process-wise is similar to that described above for radiation dosimetric release. The bioburden on a sample of items from the load to be sterilized should be determined. However, it is not necessary to go through subprocess (incremental) dosing to obtain a D-value for the steam resistance of the bioburden. Instead, the bioburden should be exposed to a heat shock for approximately 10 minutes at 80°C to determine the spore load in the bioburden. If the number of spores per item is less than 100 then the use of an F_0 of 10 or greater will provide a probability of a survivor of 10^{-6} or less. The F_0 is the amount of time that the most difficult to penetrate portion of a load is at the temperature of 250°F. Thus, if an item can be shown through various techniques involving the integration of lethality (11) to be at a temperature of 250°F for 10 minutes, then it has an F_0 of 10. Similarly, if the rates of microbial destruction at a lower temperature can be related to the rates of microbial destruction at 250°F, i.e., the use of the "z" function (11), then a longer processing time at a lower temperature can have an F_0 of 10.

Unlike radiation dosimetric release, steam process release does not usually employ a steam dosimeter as such. If the use of thermocouples shows that the center of load is at a temperature of 250°F for 10 minutes or more or the integration of lethal rates through the come-up, holding, and cool-down times will summate to an F_0 of 10, then the product can be released on a process basis alone. The routine use of thermocouples or thermistors is not required after the initial validation of an F_0 of 10 has been established for a specific sterilizer with a particular load configuration. Some manufacturers do utilize heat and steam integrating chemical indicators as a form of steam sterilization dosimeter for lot-by-lot release of their products. These steam sterilization dosimeters are widely used by hospitals to monitor their steam autoclave cycles.

As an adjunct to the steam process-control release program, some manufacturers during the initial development phases of the program will use a biological indicator challenge of spores of *B. stearothermophilus*. It is not necessary to have these spores carried on the product since there is an equivalence of resistance between spores carried on adventitious carriers such as paper strips and those carried directly on product for this sterilization method. Frequently, it is found that the number of spores on the biological indicator must be decreased from the 10^6 level since the D-value for many lots of spores of *B. stearothermophilus* will exceed 2 minutes at 250°F. Ideally, the destruction time for the biological indicator should not come closer than two D-values to the total time desired for the steam sterilization cycle. The instrumentation used to follow the temperature, pressure, and time of sterilization cycle should be properly calibrated and rechecked at periodic interval (at least annually) to assure that the steam process release variables are still within control.

Summary

The United States Pharmacopeia XX (1980) accepts the limitation of finished product sterility tests as a batch release mechanism. The use of alternative procedures as batch release mechanisms for terminally sterilized products, while no longer described as such in U.S.P. XX, are allowed under the general notices section of the USP. The Bureau of Medical Devices of the FDA began accepting in 1975 voluntary data submissions for process-control or dosimetric release of terminally sterilized products. The BMD has now determined that a pre-market notification (510k) submission is a suitable mechanism by which to inform the Agency of a shift to dosimetric or process-control release of terminally sterilized products other than those which require a premarket approval application (or supplement).

Some of the common elements in a program of process-control release are the following: environmental monitoring programs; choice of sterilant; bioburden and its resistance (D-values) to sterilant; sterilization equipment; control of the sterilant during product processing; and overall control of the process, including audits of various parameters for both product and process.

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Q. by M. Duncan – England

How did you decide on a two-day release for ethylene oxide? Is this two-day release taken from the moment the batch is removed from the sterilizer or after a period to allow gas dissipation?

A. by C.W. Bruch – USA

The issue of gas dissipation limits the amount of interest in a two-day release procedure, which is calculated from the moment the product leaves the sterilizer. Companies are not attracted by this option because frequently it takes longer than five days to degas at room temperature the product down to the residue levels that have been proposed by FDA. These residue levels are still in the proposal stage; they are not final.

The two-day release procedure is approved on the basis of company-supplied information. The companies make data submissions under the 510 (k) mechanism. They provide data from half-cycle times of exposure. If the product is inoculated with 10^6 spores and the inoculated product is inactivated with a half cycle, the manufacturer has high assurance that a full cycle will provide a 10^{-6} probability (or even lower probability) of a survivor from that cycle. The use of the two-day biological indicator merely confirms that the basic processing was followed and that the product was indeed processed in a previously validated ethylene oxide cycle.

Q. by W.A. Staub – USA

Who is to determine what is an acceptable MSI number? Under what conditions? Determined by what procedure?

A. by M.T. Cooper – Canada

There is no question that our approach is that the determination of a number should be something between the manufacturer and the users, and the users' organizations—professional organizations. This is a very definite decision on our part as to how it should be done. How it should be determined is another question which is very difficult to answer because it is agreed that there are many ways of doing this. Many ways which are not comparable one with another. So we do not know exactly how it is going to be done and this is the whole point. This is what the discssion is hopefully about. How do you come out with something which everybody can agree is a way of determining the number. I think that answers the question.

Q. by T.W. Gorski – USA

On the substrate effect on spore resistance. If we start with a uniform standard spore suspension and contaminate paper strips and other substrates such as plastics, cloth, metals with the same number of spores, then subject all inoculated carriers to an identical threshold sterilization cycle:

1. Is there any variation in performance between these products?
2. If so, why?

A. by J.R. Gillis – USA

In answer to your first question, yes there is a difference in the performance of a "uniform" preparation of spores placed on different carrier substrates. For example, spores placed on paper will respond differently to identical sterilization cycles than the same spores placed on metal or glass surfaces.

As to why this happens, the physical characteristics of the carrier must be considered and how that carrier responds to the physical/chemical conditions of the sterilization process. Such characteristics as thermal conductivity, mass, affinity for moisture or absorption of sterilant, all affect the response of the spores contained therein to the sterilization process.

There are two other considerations. One is the suspending liquid and the other is how the suspension is dried on the carrier surface. Solvent suspensions may tend to wick the spores and dry more evenly with less clumping on the surface. Such carriers will respond differently than carriers having the spores clustered and clumped on the surfaces.

The real significance to the traditional use of paper as the substrate for characterizing the performance of a spore suspension is that a reference standard must be selected so that one spore suspension can be compared to another. Spore suspensions qualified in this manner as acceptable for monitoring a sterilization process may then be inoculated if desired onto other carriers more appropriate to simulate actual product characteristics.

A. by R.F. Morrissey – USA

I agree. There definitely is an organism-substrate interaction. Similar numbers of organisms on different substrates will exhibit different resistances. Spores certified on a standard paper carrier may show greater or lesser resistance when deposited on plastics. There is another point that you should be aware of: whatever inoculum challenge you use should be related to the microbial load on the product. It should not be our goal to produce biological indicators of such resistance that the sterilization cycle will never kill them; this is very easy to do! Our goal is to have a realistic biological indicator that is representative of the microbial load on the product. Also, be aware that artifacts can be produced during the preparation of biological indicators. People should be aware of this. In addition to substrate-spore interactions, the type of suspending medium can impact resistance and sometimes result in tremendous tails on resistance curves. You really need to understand what you are trying to accomplish before setting up a biological program.

Q. by A. Tallentire – England

I was somewhat surprised when you produced your survival curve for bioburden against treatment. You show in fact a linear response with increasing treatment. Do you really believe this? This is very fundamental, of course, in relation to what has been said about the modeling.

A. by R.F. Morrissey – USA

I was waiting for you to comment on that. As I mentioned, some of the idealized curves presented were based on actual experimental data. Although it is sometimes thought that bioburden populations are primarily heterogenous, and that this heterogenity is exhibited by nonlinear kinetics, we have encountered instances where the population is homogenous or at least responds to treatment in a linear (homogeneous) manner. I believe the situation with the radiation resistant unbleached cuff material would support this. Enumeration technique can also play a role. Sometimes tailing effects can be seen when performing a viable plate count of survivors at low population levels. We have found that fraction negative end point techniques should be used to confirm whether or not a tail actually exists; this will eliminate potential artifacts. I believe that both linear and nonlinear situations exist with respect to inactivation of bioburden.

Q. by K.H. Morgenstern – USA

I agree with your conclusion on the growth of gamma sterilization, but why limit it to gamma? Why not radiation, which includes electrons and X-rays?

A. by K.W. Davis – USA

All of the work we have done to date to develop the DS + A methodology is based on data derived from gamma processes. The reason for emphasizing this is so that people do not go out and apply this methodology outside the gamma data domain. That is the only reason. However, we believe it is possible to develop similar methods for other modes of sterilization.

Q. by R.L. Kronenthal and V. Ross – USA

Has the Whitby distribution been verified? Are there exceptions? What is the consequence on DS if it is incorrect? Can you comment on the confidence that the bioburden proposed is of more than adequate resistance?

A. by J.L. Whitby – Canada

No one can be sure that anything is universal, but I think the great thing about the DS method is that it does require that the bioburden be exposed to a specified does of radiation and if the expected result is not obtained it must be assumed that the bioburden is more resistant and further tests carried out.

What we really want to know is the frequency of occurrence of radiation resistant microbes. What Mr. Davis said was very important in respect to ongoing confidence in the DS method. That is, in the audit procedure, we are constantly shooting at a 10^{-2} target.

We should also attempt to discover if there are a significant number of radiation resistant microbes, by determining the radiation resistance of isolates obtained in positive samples irradiated within D* or with the audit dose. We could then compare the frequency with which isolates with a given D10 were found with that expected if the distribution was reasonably correct. I think this is an important corroboration.

Q. by R. Garrison – USA

What reasons exist for not using the DS method for other methods of sterilization than gamma?

A. by K.W. Davis – USA

I can give the same answer I gave earlier in response to Dr. Morganstern. The point that Dr. Tallentire raised, in the discussion of Dr. Morrissey's paper, about knowing the behaviour of the microbial tail resistance in other forms of sterilization makes us very reluctant to generalize our methods to other sterilization processes. The ETO sterilization process, for example, is more difficult to model than the gamma processes.

Q. by A. Skopek – Australia

Could you please elaborate on the acceptability of the proposed labeling of commercial products with MSI?

A. by C.W. Bruch – USA

I feel that manufacturers here in the United States will have to keep the word “sterile” on their products. The MSI value would be placed in apposition to the word “sterile”. In the labeling or in a brochure that accompanies the product, the Canadian definition of what MSI means would be provided. This approach envisions a voluntary mechanism for the use of MSI. I just do not see that the word “sterile” will ever be dropped from the labeling of sterile products in the United States.

Comment by R.W. Cambell – Canada

I might say something about the Canadian situation on MSI. This whole question of numbers on the labels has gone out of proportion altogether. This is not the important thing. The important thing is that we should be thinking MSI and that we should be process-oriented rather than end product testing-oriented. I think that if we are all thinking the numbers involved in MSI, then eventuallly, if the thing meets the criteria which everybody agrees upon, it could be called sterile, and may or may not have a number on it. But there are a large number of other situations where a product does not have to be sterile in the present sense of the word, but it has to have some degree of microbiological protection. These are the labels of which we will be thinking, e.g., MSI-1 and 2. The word "clean" does not mean a thing; it is just as bad as "sterile". There is no such thing as a clean product. But we could say, in our discussion with industry, that is, between the regulatory agency and the industry, there could well be agreement that in the manufacturing of a particular product, a level of microbiological safety must be achieved, which between ourselves, the industry and ourselves, we agree to call MSI-1 or MSI-2. Then the product must be manufactured to this level, but the level does not have to appear on the label anywhere. There are large numbers of products where, at present, we are talking about "clean" or "free from pathogens" or things of that sort, which mean nothing at all. It seems to me that by using the term MSI-1 or 2, we could deal with situations like this. Another example is the continuing argument about scopes of all kinds and what should be done with them. Should they be disinfected or should they be sterilized? We cannot seem to come to any agreement as to what is desirable in such cases. Why do we not all agree that they should be processed to MSI-2? Then we can get around to working out some kind of process which will achieve that level. Then we do not have to say whether it is disinfection or sterilization or what it is is. It is processing to MSI-2.

Comment by: Chairman R.W. Cambell – Canada

I think we are all agreed now that we are not going to have MSI numbers on labels for years to come, but it does not stop us thinking them.

Q. by Mr. D. Duncan – England

You stated that the three lots and the sample size from these lots used to determine bioburden resistance were chosen by computer calculations. Could you elaborate on the basis for the computer calculations for this sample selection?

A. by K.W. Davis – USA

We used the computer to simulate as near as possible the destruction of microbial flora and the laboratory sterility testing of subprocess dosed samples. We evaluated our methods using several different subprocess dose sample sizes as well as different numbers of lots. Our decision to go with a sample size of 20 and three lots was dictated both by the results of the computer simulations and ecnonomic considerations. If more precision for sterilization dose estimation is required there is no problem on my part if you wish to increase the 20 to 100 and make the number of samples tested at D* Mrad equal to 1000. This will give more precision if the false positives inherent in sterility testing can be controlled to less than 1 in 10000. It should be noted, however, that the sample size and number of lots we finally chose to adopt for our strategies give acceptable and for the most part conservative sterilization dose estimates.

Q. by R.A. Fuller – USA

You remarked that hospitals release products everyday based on dosimetry. What percentage of hospitals meet the standards you discussed for dosimetry release? Do not too many hospitals release more on faith than dosimetry?

A. by C.W. Bruch – USA

Many hospitals release on faith rather than dosimetry. In terms of steam cycles, I think they do a very adequate job. With ethylene oxide they have been very dependent on the sterilizer companies to provide adequate cycles for their use. I am not sure that they process to the same sterility assurance level that industry does. Neal Danielson of the Wesley Medical Center has done a tremendous job in trying to bring more knowledge to the hospital people who sterilize supplies for central service. So even if they are not achieving high sterility assurance levels now, they will in the future.

Comment by J.L. Whitby – Canada

In hospitals while we do not have complete control, we do rely on a lot of other things, many of which have already been mentioned. We use log books and we do, of course, use biological indicators. We make up test packs for gas sterilization in particular, which pose a challenge to the system and we have an ongoing record over the past that these test packs have been satisfactorily processed. We occasionally get a failure and that tells me that we are not quite meeting the standards that we had before. Our MSI is down. It does not tell us that a product is not sterile and therefore should all be recalled. But, of course, if we do not act at once when we find that there has been a failure in the sterilization testing system, we are being irresponsible. Thus, on the basis of log book records, etc., we are developing a record of experience, and ultimately, we have a good case to release our products on the basis of dosimetry.

Q. by Mr. T. Hudson – USA

Is your sample size that rigid? How would one approach expensive, dense products (e.g. oxygenators which could cost $300 each)?

A. by K.W. Davis – USA

Yes the sample size is rigid. If you can assure your self that ten independent samples from each oxygenator would adequately represent ten independent oxygenators I have no problem with you doing the experimentation using two oxygenators where we call for 20. If you chose to follow the strategy of using partial oxygenators in your experiments, then you should randomly distribute these ten samples to different subprocess doses and not use all ten with the same dose.

Comment by R.F. Morrissey – USA

I have one comment that will take only a second. Dr. Bruch alluded earlier to the Scandinavian situation with biological indicators, and we had a question on the effects of oranism-substrate interaction. I would like to read a quotation from a paper by Dr. Charles Artandi that is very appropriate here: "There is no particular merit in using microorganisms of extremely high resistance in biological indicators unless they are ordinarily present in the products, or in arbitrarily creating conditions which produce excessive resistance to the process unless there is evidence that similar conditions may occur with the product itself."

SECOND SESSION

Session Chairman Martin Stephenson

Ethicon Sutures Ltd.
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Ethylene Oxide Toxicity: Review and Update

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Introduction

About 10 years ago, I presented a paper entitled "Ethylene Oxide Sterilant Residues" at a meeting of the Parental Drug Association (1). The paper was primarily a review of the toxicity of ethylene oxide and its major reaction products, and included a brief outline of some studies that had recently been initiated by the Sterile Disposable Device Committee (SDDC) of the Health Industries Association (HIA). A recent rereading of the paper disclosed that no mention whatsoever was made of "mutagenesis" or "mutagenic effects"! Although reports of ethylene oxide induced mutations in *Drosophila* (2) and *Neurospora* (3) had been available since at least the early 1950's, toxicologists were not talking to geneticists back then or, perhaps, we thought that events that happened in flies and fungi were somehow irrelevant for man. Things have certainly changed dramatically in the last decade!

Review of Published Information

It would be redundant and unprofitable for me to undertake a comprehensive review of the toxicity of ethylene oxide (ETO), ethylene chlorohydrin or 2-chloreothanol (ETCH) and ethylene glycol (ETG) considering the many in-depth literature reviews that have become available since 1975. I refer specifically to the 1975 report of the Ethylene Oxide Review Committee of the Food and Drug Administration (FDA) (4), the 1977 report of the Subcommittee on the Benefits & Risks from the Use of Ethylene Oxide for Sterilization of the Department of Health, Education, & Welfare (DHEW) (5) and the 1977 National Institute for Occupational Safety and Health (NIOSH) report entitled "Special Occupational Hazard Review and Control Recommendations for the Use of Ethylene Oxide as a Sterilant in Medical Facilities" (6). In addition, the 1978 "Notice of Rebuttable Presumption Against Registration (RPAR) and Continued Registration of Pesticide Products Containing Ethylene Oxide" of the Environmental Protection Agency (EPA) (7), the 1978 FDA's Proposed Maximum Residue Limits and Maximum Levels of Exposure for ETO, ETCH and ETG (8), and the Health Industry Manufacturers Association's (HIMA) lengthy response to the former document (9) provide additional valuable review information.

Instead of going over much of the old material, I shall limit my remarks to selected topics only, or to articles that have appeared in the literature since the EPA (7) and FDA (8) notices.

Woodard Studies

In November 1971, a report of toxicity and irritation studies of ETO, ETCH and ETG was published by the Sterile Disposable Device Committee of the Health Industries Association (10). These studies, conducted by the Woodward Research Corporation, were cited in both government notices, particularly the FDA document. In some instances, I believe the information was misinterpreted and misused. Consequently, it may be useful to review some of these data again and point out some of the areas of disagreement. Table I summarizes the acute studies of ETO, conducted in three species by four routes of administration. Tables II and III contain similar information for ETCH and ETG. In general, these data are consistent with other published acute toxicity information. For each chemical, there was a remarkable absence of major differences due to sex or species. On the basis of the acute oral toxicity studies in rats, ETO was approximately 4 times, and ETG 200 times, less toxic than ETCH.

Table I. – Acute Toxicity – Ethylene Oxide* (LD_{50} g/kg)

		P.O.	I.V.	I.P.	S.C.
MICE	male	.37	.26	.18	.19
	female	.28	.26	.18	.26
RATS	male	.24	.36	.18	.14
	female	.28	.38	.15	.13
RABBITS	male	.63	.18	.25	.20
	female	.63	.18	.25	.20

*Aqueous Solution (25 mg/ml)

(Data from reference 10)

Table II. – Acute Toxicity – Ethylene Chlorohydrin* (LD_{50} g/kg)

		P.O.	I.V.	I.P.	S.C.
MICE	male	.15	.12	.12	.12
	female	.18	.12	.13	.15
RATS	male	.07	.11	.06	.07
	female	.05	.10	.07	.07
RABBITS	male	.06	.08	.09	.10
	female	.06	.08	.09	.10

*Aqueous Solutions (4.8 to 19.2 mg/ml)
(Data from reference 10)

Table III. – Acute Toxicity – Ethylene Glycol* (LD_{50} g/kg)

		P.O.	I.V.	I.P.	S.C.
MICE	male	17.0	4.5	2.6	6.6
	female	17.0	5.2	2.4	6.6
RATS	male	10.4	5.2	6.1	7.7
	female	15.3	5.2	4.5	7.7
RABBITS	male	6.3	7.9	6.3	10.0
	female	6.3	7.9	6.3	10.0

* Undiluted
(Data from reference 10)

As a point of interest, it should be noted that ETCH is reported to be more toxic by cutaneous application than by oral administration (11).

A number of standard tissue irritation tests were also part of the Woodard program. These included intramuscular, intracutaneous, eye, skin, and subcutaneous irritation studies. Tables IV, V and VI summarize the results of these studies.

ETO, ETCH and ETG were also tested for sensitization potential in guinea pigs by two different methods; topical application, and intracutaneous injection. Negative results were obtained in both instances.

Table IV. – Irritation Studies - Ethylene Oxide (% – Aqueous Solutions)

Test System	Maximum Concentration Tested	Highest "No Effect" Concentration
I.M.*	2	2
I.C.*	2	0.1-0.2

Eye*	10	2
Skin*	5	1
S.C.**	1	0.1

*Rabbits
**Guinea Pigs
(Data from reference 10)

Table V. – Irritation Studies – Ethylene Chlorohydrin (% – Aqueous Solutions)

Test System	Maximum Concentration Tested	Highest "No Effect" Concentration
I.M.*	2	2
I.C.*	5	1-5
Eye*	100	10
Skin*	5	0.5-5 ?
S.C.*	1	0.5

*Rabbits
**Guinea Pigs
(Data from reference 10)

Table VI. – Irritation Studies — Ethylene Glycol (% – Aqueous Solutions)

Test System	Maximum Concentration Tested	Highest "No Effect" Concentration
I.M.*	2	2
I.C.*	5	2
Eye*	100	10
Skin*	5	2
S.C.**	1	1

*Rabbits
**Guinea Pigs
(Data from reference 10)

Table VII. – Subchronic Toxicity Studies
– Dosage Levels (mg/kg/day - 28 days)

Ethylene Oxide	54*, 18, 6
Ethylene Chlorohydrin	27, 9, 3
Ethylene Glycol	450, 150, 50

*Reduced to 36 mg/kg/day after 7 days - dog study
(Data from reference 10)

The Woodard program also included subchronic studies of all three compounds in both rats and dogs. The subcutaneous route of administration was a compromise choice, reflecting the primary product interest of the sponsors, i.e., sterile disposable devices. Daily injections were given for 28 consecutive days. Dosage levels were as shown in Table VII. All doses were given in a constant volume of 3 ml/kg, and distilled water was used to prepare the respective dilutions. Distilled water was chosen so as to avoid complications resulting from the possible degradation of ETO and ETCH. In order to administer the dosages chosen in the volume that was stipulated, it was necessary to exceed the threshold irritant concentration for each material. Unfortunately, this resulted in varying degrees of inflammatory change at the injection sites which complicated the study and made interpretation of the findings difficult. As a result, some of those who have reviewed the studies have taken the easy course, which is obviously to consider all changes as being compound related. This has resulted in frequent judgments that "no effect" levels were not established and that, consequently, further downward extrapolation was required. Had the reviewers studied the full report in detail, they would have seen that many of the observations, particularly at the lower dosage levels, were undoubtedly related to the inflammatory reactions and not to the compounds, per se. Commenting on this very point, Dr. G. Woodard, one of the study's authors, criticized the setting of arbitrary "no effect" levels based on the appearances of increased ectopic hematopoiesis, and increased sedimentation rates and leukocyte counts in the dog. "In the report," he noted, "we pointed out that these changes were attributed to the inflammatory reactions and in some cases local infections at the site of the repeated subcutaneous injections." In addition, he emphasized that "these changes should not be attributed to the direct toxic effect of ethylene oxide, ethylene glycol and 2-chloroethanol" (12).

EPA RPAR Notice

As mentioned previously, EPA published a "Notice of Rebuttable Presumptions Against Registration and Continued Registration of Pesticide Products Containing Ethylene Oxide" (RPAR) on January 27, 1978 (7). This action was based on the authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) which requires that EPA render a determination regarding the continued use of a pesticide as part of the re-registration process for all presently registered pesticides (13). Benefit/risk considerations must be taken into account in rendering such a determination. The ETO RPAR was based on two alleged risk presumptions: mutagenic effects by multitest evidence, and reproductive effects in animals. The scientific evidence cited in support of the mutagenic effects allegation included studies with ETO involving gene mutations in microorganisms, plants, and invertebrates, as well as chromosomal mutations as determined by cytogenetic and dominant lethal studies in rodents. Evidence for ETCH induced gene and chromosomal mutations were also cited. Support for the reproductive effects allegation included, primarily, some evidence of testicular atrophy in ETO exposed guinea pigs and rats. Although not pivotal to the RPAR position, EPA also cited evidence for other possible chronic or delayed adverse effects of ETO, ETCH or ETG.

Apart from questions of legal authority and benefit/risk considerations, many believed that the studies relied upon to show mutagenic and reproductive risks for man were of insufficient quantity and quality to warrant firm regulatory conclusions (9). Dr. S.R. Wolman of the N.Y.U. School of Medicine has recently published a very thorough review of the data base used for the mutagenic effects allegation (14).

The EPA has not yet made a final decision of the registrability of ETO. A recent government publication (15) points out that "EPA is continuing to review the comments and rebuttals and is awaiting the results of several long term studies sponsored by FDA and Union Carbide." The notice further states that EPA intends to complete its review by January 1981.

FDA Proposal

FDA published its Proposed Maximum Residue Limits and Maximum Levels of Exposure for ETO, ETCH and ETG on June 23, 1978. The *proposed* rule would establish maximum residue limits for ETO and its two major reaction products, ETCH and ETG, in drug products for human and veterinary use, and in medical devices for human use. In addition, it would establish maximum daily levels of exposure for drug products for ETO, ETCH and ETG. The FDA stated that the "action was being taken because residues of ethylene oxide and its two major reaction products in drug products and devices for which ethylene oxide is used as a sterilant may produce reactions in patients, and because of the potential risk of mutagenicity from exposure to these residues" (8). Table VIII illustrates the maximum residue limits proposed for devices. Table IX contains the proposed maximum residue limits for drugs, and Table X contains the proposed maximum daily levels of exposure to residues in drug products.

Table VIII. – Maximum Residue Limits* – Devices

Device	ETO	ETCH	ETG
Implant			
Small (<10 g)	250	250	5000
Medium (10-100 g)	100	100	2000
Large (> 100 g)	25	25	500
Intrauterine Device	5	10	10
Intraocular Lens	25	25	500
Devices Contacting Mucosa	250	250	5000
Devices Contacting Blood (ex vivo)	25	25	250
Devices Contacting Skin	250	250	5000
Surgical Scrub Sponges	25	250	500

*parts per million
(from reference 8)

Table IX. – Maximum Residue Limits* – Drugs

Drug	ETO	ETCH	ETG
Ophthalmics	10	20	60
Injectables	10	10	20
Intrauterine Devices (cont. drug)	5	10	10
Surgical Scrub Sponges (cont. drug)	25	250	500
Hard Gelatin Capsule Shells	35	10	35

*parts per million

(from reference 8)

For a number of reasons, many individuals and groups have taken issue with these residue limits and levels proposed by the FDA. I intend to comment only on the proposed maximum daily levels of exposure for residues in drug products, although I find many other aspects of the proposal confusing and inconsistent. As indicated in Table X, according to the proposed rule, the maximum daily levels of exposure to ETO, ETCH and ETG should not exceed 30 μg/kg/day/30 days, 15 μg/kg/day/30 days and 2.5 mg/kg/day/30 days, respectively. These values were derived, as shown in Table XI, from the aforementioned Woodard study. The additional 10-fold safety factors that were utilized for ETO & ETCH were applied, according to the Commissioner of Food & Drugs, because of mutagenic potential "for which sufficient dosage data are not available" (8). As mentioned previously, inflammatory reactions at the injection sites undoubtedly accounted for many, if not all, of the changes seen at the lower dosage levels in the Woodard study. To further reduce these doses by 50 percent, and then to apply a 100-fold (10×10) safety factor, in the case of ETO and ETCH, seems like regulatory overkill. It also seems strange to use data from routine subchronic animal toxicity studies as the basis for setting maximum levels of exposure when the cited risk is mutagenicity!

Table X. – Maximum Daily Levels of Exposure – Drugs

ETO	30 μg/kg/day/30 days
ETCH	15 μg/kg/day/30 days
ETG	2.5 mg/kg/day/30 days

(from reference 8)

The FDA has not yet issued a final regulation and, according to a recent document (15), continues its review of the comments received in response to the proposed rule.

AAMI Studies

In 1973, the Ethylene Oxide Subcommittee of the Association for the Advancement of Medical Instrumentation (AAMI), in collaboration with the FDA, instituted a series of studies designed to determine safe limits for ETO, ETCH and ETG in medical products. A preliminary, summary report of these studies was presented at the first Kilmer Conference in 1976 (16). Since that time, the studies have been completed and results have become available.

1. Subcutaneous implantation studies
   These studies were conducted by F.F. Becker at the New York University School of Medicine. Small sections (1.5 cm long) of polyvinyl chloride tubing containing different amounts of ETO residue were implanted subcutaneously in mice. After 24 and 48 hrs, the mice were sacrificed and the tissues in the region of the implants were examined microscopically. Direct comparisons were made with tissues associated with control implants containing no ETO residue. It was concluded that concentrations of 0 through ca. 850 ppm produced no significant damage, whereas doses of ca. 2000 ppm or above caused significant tissue damage. Between 850 and 2000 ppm was a "grey zone" where the results were equivocal.

Table XI. – Derivation of Maximum Daily Levels of Exposure – Drugs (mg/kg/day/30 days).

	ETO	ETCH	ETG
Lowest Dosage Level-Woodward Study	6	3	50
Estimated "No effect" Level	3	1.5	25
10-Fold Safety Factor	0.3	0.15	2.5*
Additional 10-Fold Safety Factor	0.03*	0.015*	

*Maximum levels proposed by FDA

2. Hemolysis studies
These studies were conducted by A.B. Jones at the University of Mississippi, and the results have been published (17). The hemolytic potential of pure ETO in solution was evaluated in a number of test systems. Concentrations of 2 mg/ml (2000 ppm) were necessary before cell lysis was observed in a system using diluted whole blood in isotonic saline, or a system using erythrocytes washed and resuspended in isotonic saline. Very erratic data were obtained when attempts were made to correlate hemolysis and cell culture toxicity with residual ETO in some selected medical materials.
3. Human skin irritation studies
These studies were conducted by J.L. Shupack at the New York University School of Medicine. While a summary of the results was presented in 1977 (18), the entire study has not yet been published. Patches retaining ETO were applied to the skin of human volunteers. The patch materials (nonwoven fabric or brown-milled rubber), from which ETO eluted rapidly, produced little or no reaction even at levels as high as 3000 – 5000 ppm. Polyvinyl chloride (PVC) film patches, which retain ETO longer, produced mild reactions above 1700 ppm. Exceptionally slow-airing materials (2 mm thick PVC patches) produced mild to moderate reactions at 1000 ppm and above. One subject, responding in a peculiar delayed manner to ETO, developed a spontaneous flare of the skin reaction approximately three weeks after exposure. Although the FDA was quick to label this as delayed sensitization (8), Dr. Shupack, in a letter to the Commissioner of Food and Drugs (19), stated that "although we cannot fully explain this unusual reaction, it certainly should not be considered an example of delayed sensitization."

Another phase of the human skin irritation studies involved patch testing with different aqueous concentrations of ETCH, ETG, or combinations of the two. The appropriate solutions were applied to patches of nonwoven gauze held in place with semiocclusive tape. One of twelve volunteers showed a reaction to all solutions containing ETCH at 550 ppm and above.

Recent Reports or Abstracts

I shall now review briefly some of the more important recent references pertaining to ETO toxicity.

Tissue dosimetry, using the degree of alkylation of histidine in hemoglobin as the marker, was recently described as a means of evaluating exposures and assessing risks in individuals occupationally exposed to ETO (20). The authors claim to have corroborated previous work from the same laboratory where tissue doses in mice were used to make risk estimates for man using the "rad-equivalence" concept (the number of rads of acute α-radiation that gives the same effect as a unit dose of a chemical). This concept, of expressing genetic risks of chemicals in the frame of reference of radiation hazards, has drawn criticism for many reasons, not the least of which is the uncertainty of a constant quantitative relationship between the two factors over a wide dose range.

Hogstedt and coworkers (21,22) have published two recent epidemiological reports in which they associate carcinogenicity with ETO exposure. The first report concerns three cases of leukemia that occurred in a small technical factory in Sweden where 50% ETO and 50% methyl formate had been used for sterilization purposes. Based on national statistics, they assert that only 0.2 cases would have been expected. The time-weighted average ETO concentration was reported to be 20 ± 10 ppm, with exposure durations of ca. 4 to 8 years. The second report concerns a cohort study conducted in a Swedish chemical factory which produced or used ETO since the early 1940's. In the full-time exposed cohort, there was an excess mortality and cancer incidence. However, the majority of these workers had been exposed to other chemicals in addition to ETO, including ETCH, ethylene dichloride, bis-(2-chloroethyl) ether and chloroform. Thus, as the authors point out, "the excess mortality and cancer incidence cannot be attributed to any particular chemical."

Garry et al (23) reported increased sister chromatid exchange (SCE) frequencies in the cultured lymphocytes of a group of hospital worlers exposed to ETO. Although a one time ETO measurement of only 36 ppm was reported for the exposure area, the incidence and severity of the symptoms reported by the exposed individuals suggest that the ETO level may have been considerably higher at other times. It has also been suggested that a respiratory virus infection may have affected personnel in the sterilization area prior to or at the time blood samples were initially obtained.

Four cases of apparent ETO-induced neurotoxicity were described by Gross et al (24). Acute encephalopathy occurred in one individual who had been exposed to ETO from a leaking sterilizer for only 3 weeks. A peripheral neuropathy, associated with abnormal nerve conduction studies, was noted in the other three individuals who had a more chronic ETO exposure history. Unfortunately, ETO levels were not monitored, but they were probably in excess of 700 ppm during the 2-8 week period that preceeded the onset of symptoms.

Dolovich and Bell (25) described follow-up studies of a previously reported case involving a patient on chronic hemodialysis who experienced systemic allergic reactions following exposure to plastic tubing and hemodialysis supplies that had undergone ETO sterilization. They demonstrated the presence of ETO specific IgG and IgE antibodies in the patient's serum.

In two separate papers, Appelgren et al (26,27) reported mutagenicity tests of ETO in rodents. Dominant lethal tests in mice, following intravenous injection of ETO at doses of up to and including 100 mg/kg were negative. However, positive results were obtained with a micronucleus test in both rats and mice using high doses of ETO intravenously.

Tyler and McKelvey (28) have studied the disposition and distribution of ^{14}C labeled ethylene

oxide vapor in rats at concentrations of 10, 100 and 1000 ppm. The animals were exposed for 6 hours and then held in metabolism cages for an additional 18 hours. At all exposure levels, urine was the primary route of excretion. The results indicated that the absorption of ETO was not linearly related to exposure levels for concentrations above 100 ppm. In addition, dose-dependent shifts in the quantity of ETO metabolites in urine were noted.

Update of Scheduled or Ongoing Studies

Carnegie-Mellon Studies

Most of the ethylene oxide producers worldwide are cosponsoring a series of studies of ETO at Carnegie-Mellon Institute of Research in Pittsburgh. These studies include chronic (2 year) inhalation toxicity, dominant lethal, one-generation reproduction, and teratology studies in rats.

Reports of the latter two studies have been made public (29). In both, exposure concentrations of 0, 10, 33 and 100 ppm of ETO were employed. In the reproduction study, male and female weanling rats were exposed 6 hrs/day, 5 days/week for 12 weeks. They were then mated and exposure was continued through day 19 of gestation. Beginning again on day 5 of lactation, the dams were exposed until day 21 postpartum. A reduced fertility index, longer gestation periods, and fewer offspring per litter were noted only in the females exposed to 100 ppm. ETO was judged not to be teratogenic in the teratology study.

Reports of the other studies have not yet been made public, but some preliminary announcements have appeared. Results of the dominant lethal study were said to be inconclusive at 100 ppm and negative at 10 and 33 ppm. In the chronic inhalation toxicity study, a moderate depression of weight gain at 100 ppm, a slight depression at 33 ppm, and negative bone marrow cytogenetic test results were reported after 1 year. Earlier this year, a number of cosponsoring companies announced that exposure at the 100 ppm level for 2 years had led to an increase in the incidence of mononuclear cell leukemia, and an increase in the incidence of suspect tumors in certain organs where such tumors normally develop. It is my understanding that the complete report of this study will be made public later this year.

American Hospital Supply Corporation (AHSC) Studies

In April of this year, AHSC made available a summary report of an investigation of the possible health effects of ETO on employees (30). The study was conducted by Occupational Medicine Associates, Inc. and involved nine manufacturing facilities. Seventy-five exposed employees were given physical and laboratory examinations, as well as cytogenetic testing. The cytogenetic testing involved karyotyping of cultured lymphocytes, enumerating the cells with chromosomal abnormalities, and determining sister chromatid exchanges. Chromosomal abnormalities were classified as breaks, exchanges, unstable forms or stable forms. A randomly selected group of forty-one unexposed individuals served as the control group for the cytogenetic evaluation.

According to the summary report, physical and laboratory examinations revealed no significant unusual findings. However, cytogenetic testing disclosed a statistically significant difference in the number of chromosomal aberrations in the lymphocytes obtained from the blood of employees exposed to ETO as compared to those not exposed. Chromosomal aberrations included increases in breaks, unstable forms, exchanges and total aberrations. The number of quadriradial exchanges, in particular, was judged to be a most unusual finding. In addition, increased numbers of sister chromatid exchanges were present in the blood of some, but not all, exposed individuals. ETO levels in the work environment were measured and were all reported to be below 50 ppm TWA. However, there were instances when short-term exposure levels exceeded 75 ppm.

Based on the cytogenetic results, AHSC removed twelve of the most “seriously affected” employees from further exposure to ETO. The twelve individuals removed had been exposed for periods of 2½ to 10 years. It is my understanding that some of these workers have reverted back to

normal on subsequent testing.

Some criticism has been directed at this study because of the lack of simultaneous test and control sample harvesting.

National Institute for Occupational Safety & Health (NIOSH) Epidemiology Study

When this study was first announced (31), many felt that it might povide definitive evidence as to whether or not ETO represents a significant occupational hazard. However, as now constituted the study is focused on ca. 44,000 chemical workers in the Kanawha River Valley of West Virginia, rather than on ETO workers, exclusively. Even though the records are apparently coded to enable identification of an ETO-exposed subgroup, the mixed chemical exposure work patterns make interpretation of the study difficult. Basically, the plan is to concentrate on mortality rates, with particular emphasis on cancer specific deaths.

Union Carbide Corp. Ethlyene Glycol Studies

A series of studies with ETG are being conducted, or have recently been conducted, at the Carnegie-Mellon Institute of Research by Union Carbide Corp. These studies include three-generation reproduction, dominant lethal, teratology and chronic (2 yr.) oral toxicity studies. Reports of the first two listed have already been made public (32). In the first, ETG was included in the diet of rats at dosage levels of 1.0, 0.2 and 0.04 g/kg/day during three generations of reproduction. With the exception of a marginally lower body weight gain in F3 pups at the 1.0 g/kg/day dosage level, all parameters were unaffected by treatment. In the dominant lethal study, F2 males from the three-generation reproduction study were bred to three consecutive groups of untreated females at weekly intervals. No dominant lethal effects were noted. To my knowledge, the results of the other studies have not yet been published.

Government Studies

A number of important studies are currently underway or are scheduled within various governmental agencies. I am aware of the following:

National Institute of Occupational Safety & Health

1. *Chronic (2 yr) inhalation toxicity studies* with ETO are being conducted in monkeys and rats at levels of 0, 50 and 100 ppm. Neurophysiology and pulmonary function experiments are part of the monkey protocol.
2. *Teratology studies* are to be conducted with ETO in rats and rabbits at levels of 0 and 250 ppm.
3. *Sperm morphology studies* have been conducted in mice following ETO exposure for up to nine consecutive weeks.

National Center for Toxicological Research

1. *Teratology studies* with ETO have been conducted in mice following 3-day intravenous injections at 4 separate periods during gestation: days 4–6 (a), 6–8 (b), 8–10 (c) and 10–12 (d). Dosage levels were high enough (75 and 105 mg/kg) to cause toxic effects in the maternal animals. A significant increase in per cent resorptions per litter was noted in both dosage groups treated during periods a. and d. In contrast, significant increases in malformations (fused vertebral arches, fused and branched ribs, scrambled sternabrae) were noted in the

150 mg/kg dosage groups treated during periods b. and c. (33).

Additional teratology studies are now being conducted under contract. They involve studies with intravenously administered ETO in rabbits, and intravenously administered ETCH in mice and rabbits.

Oak Ridge National Laboratory

1. *Heritable translocation studies* in mice have been conducted with both ETO and ETCH using the intraperitoneal route of administration.
2. *Unscheduled DNA synthesis studies* have been conducted following inhalation exposure of mice to ETO.
3. *Tissue dosimetry studies* are being conducted using tritium labeled ETO, inhalation exposures, and DNA alkylation measurements in various vital tissues, including testes.

Carcinogenesis Testing Program

A program for the carcinogenesis testing of ETO, ETCH and ETG has been developed under the National Toxicology Program (34). The bioassay of ETO has begun; mice are being exposed by inhalation in a prechronic test. The bioassay of ETCH is more advanced, with the actual chronic test in progress; rats and mice are being exposed by skin painting. ETG has been tentatively selected for testing.

Summary and Conclusions

Concern over the potential adverse health effects of ETO exposure is reflected in the prominent position that the compound usually occupies on lists of suspect chemicals. In 1977, the first official report of the Toxic Substances Control Act (TSCA) Interagency Testing Committee listed alkyl epoxides (alkylene epoxides or epoxy alkanes) in the initial group of ten substances identified as requiring testing to determine their hazard to human health or the environment (35). In 1978, ETO and its residues was included among a group of twenty-four materials identified as hazardous substances by the Interagency Regulatory Liason Group (36). And, in 1980, ETO appeared on the Environmental Protection Agency's (EPA) Carcinogen Assessment Group (CAG) "List of Carcinogens" (37), as well as the Occupational Safety and Health Administration's (OSHA) List of Potential Occupational Carcinogens (38).

From this review, it can be seen that much is already known about the biological properties of ETO, ETCH and ETG. It is also evident that a considerable amount of additional valuable information is currently being developed. Hopefully, in the near future sufficient information will be available to enable the regulatory agencies to make truly informed risk/benefit decisions.

It is estimated that less than 0.5% of the total U.S. annual production of ETO is used for sterilization purposes (9). This figure is deceiving, however, when one considers the enormous benefits provided by this method of sterilization. Unfortunately, there are no readily available, safe and practical substitutes for the ethylene oxide sterilization of many health care items, particularly for certain of the more important life-sustaining and life-supporting medical devices.

Based on current information, it would seem essential that all reasonable steps be taken to reduce ETO exposure to a minimum. This applies equally to both industrial and hospital uses. It has been amply demonstrated that much can be done to accomplish this reduction. While beyond the scope of this review, an adequate control program should include environmental surveillance, medical surveillance, engineering controls, education, improved work practices, etc. Table XII lists some threshold limit values (TLV's) for occupational exposure to ETO. Included are some recently announced voluntary limits that have been adopted by a number of U.S. corporations, demonstrating that there appears to be a real commitment to reducing ETO exposure. I have included ETCH in the table to emphasize the extremely low level of exposure that is suggested for this chemical.

There has been an ever increasing demand for ETO for the manufacture or synthesis of other chemicals, for the fumigation of agricultural products, and for industrial and hospital sterilization. Hopefully, we can learn to live safely with this extremely versatile and highly reactive chemical.

Table XII. – Occupational Exposure Limits (ppm)

ETHYLENE OXIDE	TWA*	STEL**
United States - OSHA (6)	50	
American Conference of Governmental Industrial Hygienists (ACGIH)–		
Current (39)	50	75
Future (39)	10	
Sweden (21)	10	
U.S.S.R. (40)	0.5	
Union Carbide (41)	5	

PPG Industries (42)	5	10
American Hospital Supply Corp. (30)	10	
Medtronic (43)	5	15
ETHYLENE CHLOROHYDRIN		
United States - OSHA (6)	5	
ACGIH (39)	**1*** **	
U.S.S.R. (40)	0.2	

*The time-weighted average concentration for a normal 8-hour workday.
**The maximal concentration to which workers can be exposed for up to 15 minutes.
***The concentration that should not be exceeded, even instantaneously.

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Expiration Dating of Single-Use Sterile Medical Devices

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To most of you, I suspect that the dullest question of the century may be, "What's new in expiration dating?" The answer, obviously, is "Not much."

But, as it relates to the maintenance of sterility of packaged single-use medical devices, it is perhaps useful to review the status of expiration dating.

What I would like to discuss this afternoon is the status of what is known about the maintenance of sterility as it relates to any suggested requirement for expiration dating. I would like to share with you some data provided me by several Health Industry Manufacturers Association (HIMA) member firms and review briefly the situation in the U.S. with regard to the accreditation of hospitals using single-use sterile items.

Manufacturers of sterile medical devices are often asked why a product does not have an expiration date on its label and how long the product will remain sterile. Hospital personnel are sometimes concerned about the sterility of items and compliance with the "Infection Control" section of the *Accreditation Manual for Hospitals* established by the Joint Commission on Accreditation of Hospitals (JCAH).

Expiration dates appear on the labels of many hospital products for reasons other than to indicate loss of sterility. Products containing short-lived radioisotopes, *in vitro* diagnostic products, and medical products that contain a component known to degrade over a period of time will usually specify an expiration date or shelf life. This paper does not discuss these products which have an expiration date unrelated to maintenance of sterility. Instead, we are discussing packaged, sterile medical devices purchased by the hospital from industry. The label on each device states that it is sterile, but no expiration date is given.

To print a date showing when the device is no longer sterile, manufacturers would have to do testing that provides data substantiating the shelf life for eacy type of device. Despite efforts by industry over many years, using a variety of packaging materials, the estimation of shelf life for sterility based on elapsed time has proved impossible to do. Data covering long time periods have clearly shown that contamination of a sterile device is not time related.

Kereluk (1) has indicated that presterilized industrial products are so packaged that it is almost impossible for normal biological contaminants to gain access to the interior of the packages. Evans (2) reported that articles properly sealed and sterilized in plastic film were still sterile after a 10-year storage period. Additional endorsements of sterility retention are provided in articles by Autian (3), Walter (4), and Prickett (5).

One of the most striking examples of data relating to the maintenance of sterility is the report by Johnson & Johnson in which samples of absorbent gauze sterilized in 1917 were tested in January 1962, 45 years later, and were found still to be sterile.

Some other examples of studies on maintenance of sterility and expiration dating being carried out by some HIMA member companies will serve to illustrate the fact that expiration dates that suggest cessation of sterility are impossible to determine.

One company conducts sterility tests on stored samples of its sterile, single-use devices as part of its audit program. Recent results show:

- 294 hypodermic needles from 11 lots with an average age of 9.3 years were tested; all were sterile.
- 132 hypodermic syringes from 4 lots with an average age of 10.9 years were tested; all were sterile.
- 8 catheters from 2 lots with an age of 5.4 years were tested; all were sterile.

Another company carries out two types of testing programs related to the maintenance of sterility of its sterile single-use items.

One program consists of the testing of products from distribution centers. When inventories are conducted, random samples are selected and shipped back to the laboratory for testing. The tested samples, therefore, have been subjected to typical transportation and storage conditions. The following is a list of the products and package type that were tested and found sterile after the storage time indicated:

Surgeons gloves in heat sealed paper packages.	- 5 years
Surgical drapes in heat sealed paper packages.	- 4 years
Urethane foam devices in heat sealed paper packages.	- 4 years
Cotton in paper packages.	- 4 years
Plastic devices in poly bags.	- 3 years

The second program is a part of a product stability investigation. Products are stored in three separate stability storage rooms.

1. A hot room at 90°F and 75% RH.
2. An ambient room at 68°F and 70% RH.
3. A cold room at 37°F and 35% RH.

This testing program has been underway for two years and the various rubber and cotton products submitted to sterility testing have remained sterile.

A third company is conducting a testing program on sterile, single-use devices packaged in blister packages. The five-year program thus far relates to products that have been stored for three years and tested at six-month intervals. At each testing interval, tests were done on both "stressed" and "non-stressed" products. The conditions of stress applied prior to each testing period, are:

1. Expose product to 150°F for 8 hours followed by −40°F for 8 hours.
2. Expose product to five rapid alternating cycles of 150°F to −40°F.
3. Expose product to 160°F at 96% RH for 21 days.

All stressed and non-stressed products have remained sterile for the three year period.

Another company has conducted a testing program with stored reference samples of hypodermic syringes, hypodermic needles, connecting tubes, suction kits, suction trays, catheter needles, and surgical blades over a period of almost 20 years. Literally thousands of warehouse stored products have been tested after having been stored for periods of up to 15 years. There has been no evidence that sterility is lost over time.

It is clear from this data that items properly packaged and sterilized by industry do not loose sterility through the passage of time. Therefore, it makes little scientific sense to have an expiration date on a product because of cessation of sterility. Rather than being time related, sterility maintenance is event related. Events that may compromise sterility of a device include:

1. Abrasion or contact with a sharp object that mechanically tears or penetrates the package. When this occurs, contamination is almost certain. This is a gross defect and is usually evident to the user.
2. Breathable packages that become wet and subsequently dry. This may occur during shipping, on the loading dock of the purchaser, or by improper handling in the hospital. Packages known or suspected of having become wet should not be assumed sterile unless there is assurance that water did not penetrate the package.
3. Items stored improperly. Abnormal temperatures, extreme humidity, and exposure to unusual amounts of radiant energy are examples. Such stresses can affect the package seals.

The above events can compromise the sterility of an item but have nothing to do with the age of the item, and, therefore, no basis for expiration dating is established.

With regard to expiration dating, the regulations on expriation dating vary from country to country. In the U.S., no expiration date on the label is required on most sterile medical devices. One caution for those who would prefer to put an expiration date on a product indicating the expiration of sterility is that the FDA may well ask for the data substantiating the expiration claim on the label.

In the U.S. there is often concern among hospital personnel that nonconformance to JCAH accreditation requirements may result if commercially sterilized products stored in a hospital do not contain an expiration date for sterility.

This need not be a concern, but it is easy to understand the confusion.

The *Accreditation Manual for Hospitals* says: "There shall be written policies and procedures for the decontamination and sterilization activities performed in central services and elsewhere in the hospital, and for related requirements. These policies and procedures should relate, but are not limited to the following:

> "Designation of the shelf life for each hospital-wrapped and hospital-sterilized medical item and, to the maximum degree possible, for each commercially prepared item, by a specific expiration date that sets a limit on the number of days an item will be considered safe for use. Where possible, load control numbers should be used to designate the hospital sterilization equipment used for each item, including the sterilization date and cycle" (6).
>
> The Health Industry Manufacturers Association (HIMA) requested a clarification from JCAH because of frequent questions from hospitals. The JCAH reply was clear: "There is no problem when either a specific date or the words 'sterile unless the integrity of the package is compromised' (or words equivalent in intent) are present on the commercial packaging. The words 'sterile unless' … constitute a day-to-day expirtion date in our opinion.
>
> However, when there is no such statement or date on the commercial package, then someone (hospital, manufacturer, or both) must decide on the acceptable shelf life." (7)

HIMA was assured that this interpretation would be transmitted to all Hospital Accreditation Program Surveyors.

Summary

Commercially sterilized items in the U.S. usually do not carry a label indicating a date when sterility is no longer guaranteed. This is because testing by industry has failed to show that sterility ceases over time. The maintenance of sterility is event, not time, related.

If a commercially sterilized item purchased by the hospital displays the words “sterile unless the integrity of the package has been compromised” (or words equivalent in intent), this serves as a day-to-day expiration date. No further labeling related to sterility is needed and the user’s only obligation is to store and handle the items properly. Of course, it is always best to practice a first-in, first-out system to reduce the possibility of package damage. This practice is stated as acceptable by JCAH for conformance with the accreditation requirements.

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Bioburden of Nondisposable Surgical Instruments and Operating Room Textiles

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Introduction

Much information is available about the bioburden of disposable medical products before sterilization, much less about nondisposable products before resterilization.

The reasons for this are obvious. It is not practicable to determine in all hospitals the bioburden of all the very many products to be resterilized. Steam sterilization is the most common procedure for resterilization in hospitals. It has a very broad margin of safety as long as it functions properly. It has thus been natural for regulations of hospital sterilization to focus more on monitoring autoclaves as to their functioning than on the bioburden of products to be sterilized.

However, the bioburden of nondisposable products to be resterilized is of practical interest for the determination of rational procedures for their treatment after use and before resterilization. Is it sufficient to clean surgical instruments after use, or should they in addition be disinfected? Procedures vary between hospitals and between countries. In Sweden official recommendations are that instruments should be disinfected and cleaned.

It is well established that a laundry procedure above 71°C in well functioning laundry machines properly disinfects hospital laundry (1). However, recontamination can occur. Hambraeus et al (2) have demonstrated bacterial contamination on clean staff clothing for use in the operating room. Is the laundry properly protected against recontamination?

These and similar questions induced us to investigate the bioburden of nondisposable surgical instruments and of operating room textiles at Huddinge Hospital in Stockholm, Sweden.

Materials and Methods

Surgical Instruments

Surgical instruments have been collected after unselected operations in the central operating department. Bacterial counts have been performed in the following manner: The instruments have been shaken in 1% peptone water for five minutes. The shaking fluid has been membrane filtered. The membrane filter has been placed on a blood agar plate and incubated aerobically for two days at 37°C.

Bacterial counts have been performed on 195 instruments after use: 105 used instruments after immersion in a liquid detergent for 60 minutes and a tap water rinse, 108 used instruments after cleaning and disinfection in an automated instrument-washer that disinfects by a final hot water rinse above 85°C (Dekospol S 126®) (Washer I), and 252 used instruments after cleaning in a similar machine (Decomat S 128™) (Washer II) but without the final hot-water rinse.

In Washer I the instruments to be investigated were run with other instruments in a fully loaded machine. In Washer II, however, they were run alone without other instruments.

Control runs were performed on instruments artifically contaminated with a known number of microorganisms suspended in blood. In other control runs the effect of adding Tween 80, with or without lecithin, to the shaking fluid was studied.

Textiles

At Huddinge hospital the operating room textiles are cleaned, inspected, folded and packed in sets at a central laundry serving most Stockholm hospitals. The packed sets are autoclaved in the hospital's central sterile supply department (CSSD). Newly delivered sets from the laundry to the CSSD were selected at random before sterilization, opened in the laboratory, and pieces of 1 cm^2 were cut from various items in the sets. Totally 44 items from nine sets were examined.

Bacterial counts were performed by a method described by Jerram (3). The textile square was cut up as finely as possible with sterile scissors into physiological saline containing 5% nutrient broth. The fluid was treated in an homogenizer. Surface viable counts were performed on the homogenizing fluid on blood agar plates.

Results

Instruments

With instruments artifically contaminated with known numbers of microorganisms suspended in blood and dried onto the instruments it was established that the method for retrieval of microorganisms from instruments resulted in a loss of approximately one log over a reasonably wide variation of contamination levels. Addition of Tween 80, with or without lecithin, to the shaking fluid did not affect the results even with instruments disinfected in a phenolic.

As is seen in Figure 1, 62% of the instruments were contaminated with less than 10^1 colony-forming units (cfu) after use, 81.5% with less than 10^2 and 91% with less than 10^3 colony-forming units. The median was 4.2 cfu, and the range 0 - more than 6000 cfu. Instruments used in bowel surgery were not more heavily contaminated than the others.

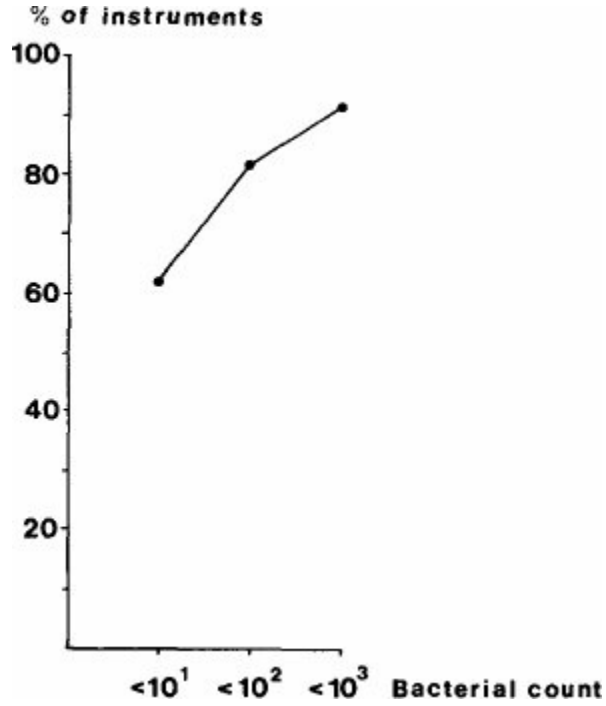

Figure 1. Bacterial counts on surgical instruments after use.

Figure 2 illustrates the corresponding figures after varying treatments. Immersion in a liquid detergent did not significantly influence the contamination level. Immersion in a phenolic diminished the contamination level significantly, as did cleaning and disinfection in the fully loaded instrument-washer I. Significantly, even more effective was the not fully loaded instrument-washer II.

After immersion in a phenolic or washing in an effective instrument-washer all instruments carry less than 10^3 microorganisms, and at least 96% carry less than 10^2. Table I gives the ranges of bacterial counts on instruments after use and after various types of treatment.

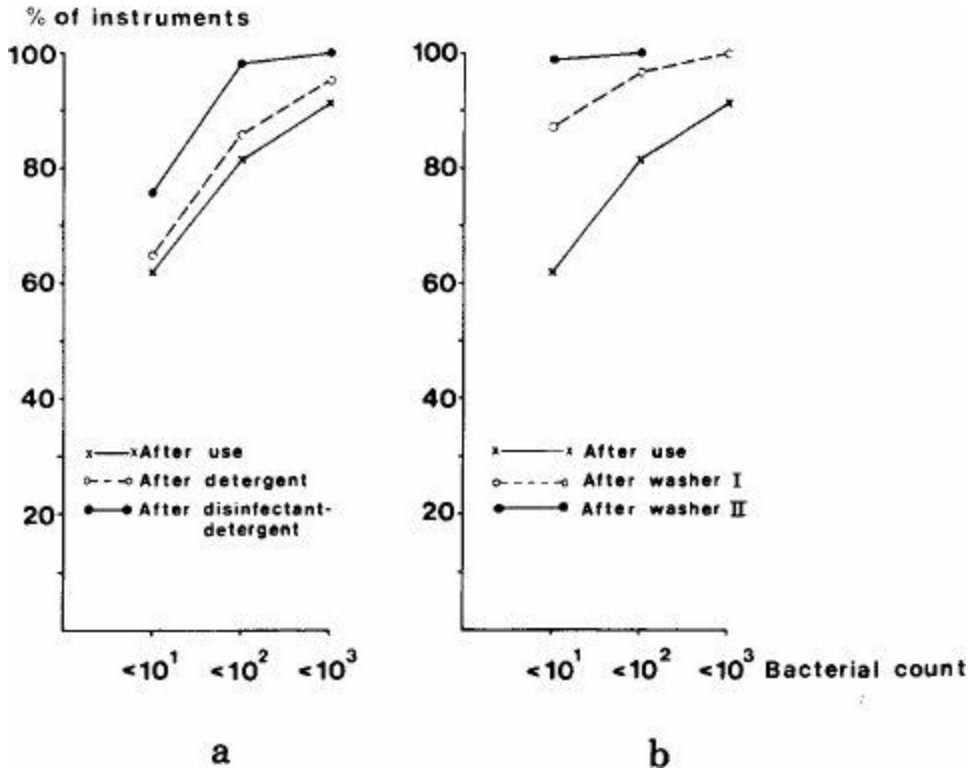

Figure 2. Bacterial counts on used surgical instruments after; (a) immersion for 60 minutes in a detergent and in a use-dilution of a phenolic disinfectant-detergent, respectively, plus a tap water rinse and (b) washing in an automated instrument washer with and without a final hot-water rinse, respectively.

Table I.—Ranges of bacterial counts on surgical instruments after use and after various types of treatment.

After use	0 –	> 6000
After detergent	< 2.5 –	> 4200
After disinfectant-detergent	< 2.5 –	760
After instrument-washer I	< 2.5 –	640
After instrument-washer II	< 2.5 –	17.4

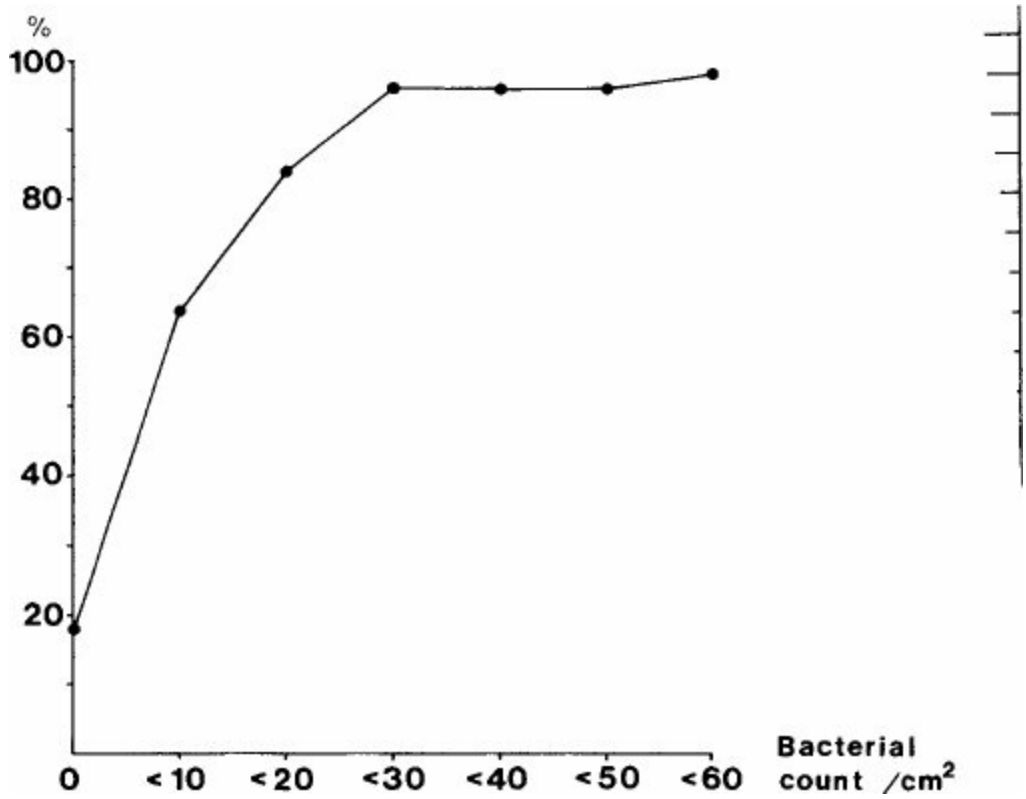

Figure 3. Bacterial counts on laundered and packed operating room textiles, per cm^2 of fabric.

Textiles

Figure 3 illustrates the bacterial counts per cm^2 of operating room textiles. Eighteen per cent of the pieces carried no microorganisms, 96% less than 30 and 98% less than 60 microorganisms. The median was five and the range 0 - 683. The next highest count was 59. No explanation could be found as to what had happened, or not happened, to the one operating towel with 683 microorganisms per cm^2.

Staphylococci, micrococci and *Bacillus* species, together with some few *Enterobacteriacae* were the species found on the textiles.

Discussion

With the surgical instruments, washer II was more effective than washer I. This may be due to inherent differences in cleaning effect between the two machines. However, it can also be explained by the fact that washer I was run fully loaded, but washer II loaded only with the instruments to be investigated. The manufacturer of the two machines states that with fewer instruments per load the cleaning is more effective.

Assuming 100 instruments in a set, a loss of one log with the method used, and instruments either cleaned in a well-functioning instrument-washer or disinfected in a phenolic disinfectant-detergent, 96 instruments would carry less than 10^3 microorganisms and four between 10^3 and 10^4. This would give a bioburden of less than 1.5×10^5 microorganisms. It would, in fact, be substantially less, as the median for all instruments in these processes is less than six with a range from zero to 760. A bioburden of approximately 10^4 is a reasonable assumption.

There are up to 30 m^2 of textile in a set of operating room textiles. Assuming a median of five microorganisms per cm^2 this gives a bioburden of 1.5×10^6 per the largest set of textiles, one to two tenfolds more than in a set of instruments.

Our main concern has not been whether the bioburdens in these sets are satisfactory or not. Nothing indicated any substantial risk in using properly sterilized surgical instruments or operating room textiles. Badly functioning hospital autoclaves are probably a much higher risk for the sterility of these items than too high a bioburden, even if this risk too is small in countries and hospitals where the monitoring of hospital autoclaves is well regulated.

For instruments we have been concerned in finding the best treatment after use and before sterilization. It appears from these experiments that cleaning in a well functioning instrument washer is the treatment of choice, and that this washer as long as it cleans well, need not disinfect the instruments. Before manual cleaning it appears prudent to disinfect instruments in a suitable disinfectant-detergent. This will not only lower the bioburden, but also, and probably more important, protect the hands of the staff from unnecessary contamination.

Few studies have been published on the bioburden of operating room textiles. However, at a symposium in Canada two years ago Goodlad (4) reported figures of 2×10^4 to 3.5×10^5 aerobes and some 10^3 anaerobes per pack in a study made with a similar technique. The difference between Goodlad's figures and those reported here are approximately one tenfold. This is an indication that it should be possible for us to raise the standard of laundry hygiene.

Summary

The bioburden has been determined on surgical instruments after use and after various types of treatment after use. After use 62% of the instruments carry less than 10^1 microorganisms and 9% more than 10^3. After treatment in a well functioning instrument-washer with or without a final hot water rinse for disinfection, or after immersion for one hour in a use dilution of a phenolic disinfectant-detergent, more than 75% of the instruments carry less than 10^1 microorganisms, more than 96% less than 10^2 and none more than 10^3 microorganisms. This gives a bioburden of large sets of instruments of approximately 10^4.

The bioburden has been determined on operating room textiles after laundering and packing. The median was five microorganisms per cm^2, and only one of 45 items carried more than 60 microorganisms/cm^2. This gives a bioburden of large textile packs of approximately 1.5×10^6.

Surgical instruments should be cleaned in a well-functioning instrument-washer after use. If cleaned by hand they should first be disinfected in an appropriate disinfectant-detergent. Efforts should be made to diminish the recontamination of operating room textiles, and other hospital textiles, in the laundry after treatment in the laundry machine.

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B.G.R.I.P. - A Hundred Year War Against The Spore

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Preface

I consider the invitation to participate in the 2nd International Kilmer Memorial Conference a real professional honor. The opportunity to speak to and listen to the recognized world leaders in the field of medical products sterilization would be a highlight of any year. Moreover after the First Kilmer Conference four years ago, by some editorial oversight, my remarks were included in the proceedings among all of the scholarly research reports and discussions. Minnesota is so far away from New Jersey I was able to convince several Deans and Department Heads at my University that the Eastern Sterilization Establishment put me in the same league as the real scientists on the program. There's no telling what this second invitation will do.

Here in Washington, however, where *truth* permeates the very atmosphere, we know better. My function is not to enlighten the conference with the results of my sterilization research. Instead between desert and coffee, I must provide some relatively harmless entertainment—a few moments of relief between the erudite philosophy of the morning and technical presentations of the afternoon—without disturbing either your thoughts or your digestion. Thus, I have chosen as my thesis another digression into the history of medical sterilization and have titled it:

"*Bacillus globigii - requiescat in pace*" - *B.G.R.I.P.*

In our hundred year war against the spore we have killed so many of these harmless little creatures their microscopic carcasses litter our labs and your factories. It behooves us to pause in momentary tribute to their selfless sacrifice for the cause of sterility. They deserve an appropriate memorial. Let them rest in peace.

A Hundred Year War Against the Spore

Nineteen eighty is considered an anniversary year for most of the people in this room. In Perkin's magnum opus *Principles and Methods of Sterilization in Health Sciences*, the date of the development of the first pressure steam sterilizer — the autoclave — is given as 1880, when Charles Chamberland provided us all — laboratory bacteriologists, hospital workers, the medical device industry — with the essential mechanism to destroy the most resistant forms of life ever created on this planet. This is not a trivial anniversary. For the first time in history we were able to *consistently* destroy all forms of life on and in inanimate materials, and were able to achieve absolute contamination control. If, indeed, infections ever resulted from the transmission of viable microbes via instruments, solution, medications, and textiles, we now had the ultimate weapon for their eradication.

(We are a little wiser now than we were a hundred years ago. We know that infectious agents find many other paths of transmission other than inanimate fomites — we can sterilize nearly everything, but we haven't eradicated infections. We have developed materials which can't be sterilized by saturated steam under pressure and we have had to develop other sterilizing weapons. Ironically, we even found that the diseases caused by sporeformers, diseases against which the autoclave should have been a specific, aren't really that important in the big picture of nosocomial infections and that the hospitals of our century have spent a lot of time attacking fleas with elephant guns.)

Still, what we like to call scientific management of environmental infections can be traced back to Chamberland's autoclave. Many of us actually owe our careers and our very livelihood to that significant development. It is appropriate, therefore, and perhaps even instructional to relive that great breakthrough year, 1880.

I was a little embarrassed to discover that 1880 was the wrong year and that the invention of the autoclave was not really marked by fireworks and champagne — even by the inventor. Considering some of the "breakthroughs" we publicize and celebrate today, one has to admit that Chamberland really hid his light under a bushel. Try as I might, I couldn't find an original publication devoted to the first autoclave. The way I reconstruct the situation, Chamberland was a 26 year old graduate student in 1877 when he came to work in Pasteur's laboratory as a media preparer. The old master was embroiled in a continual controversy about spontaneous generation with an Englishman, Bastian, and asked young Charles to discover the errors in Bastian's experiments. Tyndall, by this time, had already discovered what he called "these stubborn germs" living on old hay and many other types of vegetation and which could survive boiling for as long as five hours. Cohn saw them under the microscope and named them spores. In order to prepare media that would yield consistent spore-free results, Chamberland had to develop a device to heat his solutions to 115°. So he did. But that was not his major aim. It was just a laboratory device which would permit him to conduct his research. His real interest lay with anthrax vaccines and rabies, subjects on which he published 20 of his 30 papers between the years 1878 & 1894. (It appears that he was much more proud of his sterile filtration apparatus which he described in 1884 and which bears his name even today.) But as far as I know, the anniversary of our modern autoclave should really have been celebrated last year — a hundred years after the submission of Chamberland's doctoral thesis to the Ecole Normale Superieure in Paris — the first document which describes his adaptation to bacteriology of Papin's steam digester, invented originally in 1680.

As is the case with most inventions, their significance and historical importance depends less on the inventor's opinion than on popular acceptance and fulfillment of a need. Within 20 years, bacteriology laboratories, surgeries, and lying-in hospitals considered Chamberland's autoclave indispensible to their work. And in the 20th century Metchnikoff listed Chamberland among the Founders of Modern Medicine - along with Pasteur, Koch, and Lister - because of his contribution to sterilization. (Even Chapin, who was one of history's greatest skeptics about the role of fomites as sources of infection conceded that infant mortality from tetanus in Havana, decreased after cotton lamp wicks used for tying the umbilical cord by midwives, were sterilized.)

Today, the steam autoclave is so commonplace, bacteriology professors complain that graduate students qualify for Ph.D. degrees without ever learning how to operate one. We are equipped with such an array of sophisticated sterilizing devices and powerful germicides — gamma irradiation apparatus, high vacuum autoclaves, ethylene oxide sterilizers, activated glutaraldehyde — we forget that not too long ago our predecessors had trouble killing the spores on old hay. Whereas our professional progenitors were troubled by tetanus spores in Havana at the beginning of the century, some of you in this room were able, only a few years later, to assure the world that 1000 Viking landers would only have *one* spore *among* them when they landed on Mars. Let us honor for a moment — at this Kilmer Conference — the young graduate student who had to invent an autoclave to finish his doctoral research — and who didn't publish his discovery.

(And it wasn't even that long ago. Chamberland's papers haven't yet been placed in our library's History of Medicine repository. I get them from the open shelves of our Chemistry Library where they are catalogued as current serials.)

And a Century of Monitoring

It follows logically that if we are celebrating a century of sterilization, we are also commemorating a century of frustration experienced by those who wanted to know if the darned sterilizing devices were really working. Time and the occasion does not permit a detailed exploration of this enterprise, but it sounds like it wasn't much fun.

The original sterilizer users really didn't have much trouble. The food canners and the bacteriological media preparers just put the processed items aside for some time. There were arguments about the length of time required: some recommended three months, others fifteen days. But the essence of sterility testing in the "Golden Days" was simple empirical observation of can swelling or changes in turbidity of the medium. The logic was deceptively beautiful! If the sterilizer worked, the medium or food would remain unchanged during incubation and ergo, it could be considered sterile. Conversely, if the processed item was not rendered sterile, the fact was literally visible.

What a lovely world — no statistics, no interferences, no mathematical models, no commercial puffery. And above all, no semantic convolutions. The broth was sterile or it was not sterile — no legal decisions, no bureaucrats, no microbial indices, just perfect sterility status testing.

We started getting into trouble when we decided to sterilize products that either didn't support microbial growth or didn't let the growth manifest itself to the naked eye. Bottles of saline, for example, or pharmaceutical solutions prepared in bulk and dispensed into smaller units probably posed the first challenges to bacteriologist quality control testers. But it was a challenge that was quickly dealt with. All you needed was an adequate and representative sample; shake it up; innoculate it into a medium which does support and demonstrate growth; incubate it and you are back in the "Golden Days." (At least until USP XI in 1936).

Even this type of monitoring, however, demands a minimum of statistical sophistication, a quality which seems to be in shorter supply than political sophistication in our country. For example, this scenario, suggested by Kelsey's whimsy, is only slightly exaggerated:

> A surgery needs some sterile saline, but wants laboratory certification that the saline is sterile. Thus the central supply manager (a person for whom I am developing a particular sort of sympathy) prepares a "batch" of saline, or gets it from the pharmacy, and slides the 24 bottles into an autoclave. The lights flash, the steam hisses, the bells ring, in some models the eagle screams, and ergo, the autoclave regurgitates 24 bottles of sterilized saline. (Note: I didn't say sterile! That status could only be proved by a USP-type test. All I said was that the product had been subjected to a *process* that could be grammatically — albeit not legally or bacteriologically — called sterile.) Now we want to send some of this stuff to surgery. But they want certification before they use it. So we take two litter mates or replicates and send on to surgery and one to the lab. After three days, if the lab doesn't lose the bottle or otherwise goof it up, they inform CSS that the saline we sent them is sterile — three days ago. We hurry to tell this good news to the surgeon who has been waiting all this time in position one—gloved, masked, with his hands above his umbilical plane — sterile. Our surgeon, however, does not want to open the bottle he has; he wants the one that was tested and proved to be sterile. He didn't study statistics in medical school; instead he amused himself by reading 1978 law court records from Syracuse Federal District court.

What to do?

Ultimately we test all the bottles and find they were all sterile simply because nothing could be cultured from them on many good media, tested at many temperatures, for varying incubation times. Even Kelsey is satisfied and Bruch's inspectors are surfeited with data. But there's no saline left to use in surgery. That, of course, is the essential difficulty of status monitoring. It is inherently destructive. And even if the U.S. Pharmacopeia prescribes the size and number of samples, the medium, the incubation periods, and the standard testing techniques — criteria which have assumed awesome and legal authority with time — this testing system is still inferential. You don't test the specific device that is going to be used, implanted, injected, or infused. Statistical sophisticates and quality control managers know that you don't have to test everything. Random representative samples will do. But the closer you want to get to elusive truth about product status, the more replicates you have to test. Thus we created a fifty year long headache for those who wanted to test sterility in devices and items that were technically difficult or economically expensive to test by direct culturing. My friends at Medtronic Inc. have special things to say to me when I suggest grinding up a few dozen thousand-dollar pacemakers and plunking them into some nutrient broth. In fact they say much the same things that I heard when I made the same suggestion to the Martin-Marietta people who built the Viking lander. And anyone who has tried to grind up tape recorders, endoscopes, and surgeons' gowns will recognize their comments also.

It is quite understandable, therefore, that during the last century other monitoring attempts were being made that could bypass culturing of real and expensive products. Not at all coincidentally, F.B. Kilmer was a pioneer in this field:

> "The effectiveness of sterilization procedures can be readily confirmed,"
>
> "In the writers laboratory the practice is substantially as follows: A portion of the dressing material (for example, a piece of gauze) is impregnated with an infected nutrient fluid. The thus infected material is then dried in air, that the organisms may, as far as possible, be placed in a resistant condition. As a check experiment, a portion of this infected and dried material is placed in sterilized nutrient jelly in the culture chamber. This is done to ascertain whether the test material has surely been infected. The remaining portion of the infected material is then passed through the sterilization process, care being taken that it passes through like conditions as would the sterilized dressings."
>
> "In the case of gauze or cotton, the writer's practice is to wrap the test material in the center of the package."
>
> "After the infected material has passed through the sterilization process, it is placed in nutrient media in a culture chamber. After a suitable time (at least three days) if a growth is found in the check experiment, we are certain that our test material was infected. If no growth has taken place in the infected material that has passed through the sterilization processes, we are certain that sterilization has been complete in all the dressings. This conclusion needs no verification. If a certain portion of material purposely infected, in passing through the sterilization process with them, is rendered sterile, it is conclusive proof that the whole of the dressings cannot fail to be sterile and aseptic"…
>
> "…in surgical bacteriology, the bacillus of anthrax is used as the standard test organism; whatever will destroy the vitality of this bacillus will destroy all the known organisms of wound infection…"

The above is perhaps one of the most succinct and rational statements I have ever read in the

sterility monitoring literature. It was published in the American Journal of Pharmacy in January 1897.

Interestingly, this is not the earliest report describing the use of biological indicators to monitor sterilizing processes, though it is certainly among the first systematic attempts to develop an inferential routine sterility test. For example, as early as 1881 Koch placed packages of garden soil between layers of linens to see how well dry heat and steam penetrated through textiles. Even more remarkably, in 1831 Dr. William Henry heated suspensions of cowpox so that he could use such treatments to destroy plague on wool. Thus we have a biological indicator or analogue being used 20 years before Pasteur or Lister; fifty years before plague was shown to be caused by a visible agent; and nearly eighty years before we even knew about viruses.

These early biological indicators were either ignored or forgotten. Perhaps the quality control problems of producing consistent crops of spores were as frustrating as the quality control problems of manufacturing and operating autoclaves. But by the 1930's, sterility monitoring has become polarized into two major areas. Porudct monitoring was done by culturing with all the concomittant drawbacks mentioned above. And process monitoring was done by chemical tell-tale indicators, most prominently Diack® indicators and thermosensitive dyes printed onto paper.

A perusal of the sterilization literature of the middle 1930's reveals a lot of general dissatisfacton with the available monitoring methods. Endpoints were not accurate; it was difficult to integrate time and temperature and saturated steam detection into the same indicator; and their statistical innocence was so poignant, it's hard to believe the authors were some of the most arrogant researchers of their decade. Several investigators were on the verge of inventing the modern spore strips nearly fifty years ago. Indeed, the idea kept reappearing and such items were actually used at University of Southern California in 1933 and Western Reserve in 1937 — to calibrate and test the consistency of the chemical indicators in current use! Koch's garden soil monitors also appear and reappear — with no reference to Koch or anyone else, as if there were some magic quality of soil microbes that would detect autoclave errors passed by other tests.

And, as might be expected, the last word on sterility monitoring was provided by Carl Walter of Harvard in 1937 who tested 1000 of each of the most widely used commercial controls on the market. He used a recording potentiometer to check the accuracy of the commercial indicators, and thus introduced the plasmid of reliance on potentiometers that we find in most New England microbiologists even today. He also introduced the following dogma which follows us currently and probably does more to prevent good statistical monitoring than any other single factor:

> …"the experience of certifying sterilization by tell-tale indicators costing approximately two cents each, is not justified because of the disparity in individual performance in the commercial supply of each type of control. The large personal equation involved in the proper location and interpretation of this type of sterility indicator renders their use as a check on routine sterilization of dubious value for general hospital practice."

Don't you sometimes get the feeling of *déjà vu?* Perhaps because it *did* happen previously or in a prior life!

Epidemiology Revisited

The medical device-bacteriorlogy laboratory literature since Chamberland describes a well equipped armory of approaches and apparatus for sterilizing. It also describes a remarkably diverse array of mechanical, chemical, and biological tests and commercially available devices to monitor

sterility — both product status and operational process. What seems to be lacking in the literature is a substantial and convincing epidemiological argument that all of this is absolutely necessary. In other words, when one sums up the time, effort, cost, and concern devoted to sterilization, can we complete the equation by demonstrating how many infections we prevented from improperly sterilized or unsterilized materials and devices?

This is not to imply in any way that such unfortunate events are unknown. Far from it. They certainly did, probably do, and perhaps will keep on occurring. And some of these cases are actually documented. We have already referred to neonatal tetanus resulting from unsterilized lamp wicks used to tie the umbilicus. And there were a series of clostridial infections in New York hospitals in 1927 traced to improperly sterilized catgut sutures. Williams and his coauthors cite a number of references which implicated catgut, cellulose wadding, cotton wool, cardboard, plaster of paris bandages, dusting powders and drug injections as sources of tetanus infections — the implication being that since these are sporeformer diseases, the infections might have resulted from improper sterilization. Closer to home, we shudder when we recall a number of nosocomial epidemics in the 1970's traced to contaminated IV products; and we have become concerned about such diverse infection sources as plants, stethoscopes and laparoscopes. Mallison, as usual, did a thorough job in several publications gathering the evidence for fomite-borne infections. His lists become so extensive and encompassing, I decided to make an A-B-C list of them to teach my graduate students. Now they will both learn epidemiology as well as the alphabet:

A is for aspiration equipment and aerators too,
B is for bed pans in lieu of a loo
C is for cystoscopes so smooth and so clean
D is for dialyzers removing toxins unseen
E is for enemas
F is for fabrics
G is for gloves
H is for hydrotherapy equipment
I is for implants
J is for joint prostheses
K is for knife blades
L is for laundry
M is for medicines
N is for needles
O is for oxygenators
P is for pressure transducers
Q is for quilts
R is for rectal thermometers
S is for suction equipment
T is for tubing
U is for urinary catheter
V is for venous catheter
W is for wadding
X is for x-ray equipment

Y is for yolks (from eggs)

Z is for zinnias and other flowers

The nice thing about Mallison's lists is that he doesn't imply that faulty sterilization or slovenly monitoring is to blame, if he hasn't any evidence to back him up. To his credit, he doesn't imply more than what can be shown — that some associations exist between clinical infections and demonstrated contaminations on fomites, medications, blood, food, water and air.

He is nearly alone in his honesty. It is much more dramatic and economically rewarding to jump from clinical associations to epidemiological causality — even if the latter cannot be supported by statistics and case controls.

The whole field of sterilization and sterility monitoring must sooner or later come to grips with the following epidemiological reality:

a. The pathogenic spore formers — anthrax, tetanus, and gas gangrene — which need sterilizers for their control, are of very little consequence in today's nosocomial infection picture and in any event can be best controlled by vaccination, surgical technique, and/or antibiotics. Indeed, in the whole U.S. in 1978 there were only 86 cases of tetanus altogether (24 fatal cases), not one of them transmitted nosocomially.

b. The nosocomial infections that are being transmitted today can be controlled by decontamination techniques considerably less drastic than autoclaves. Even autoclaves that permit *Bacillus* spores to emerge unscathed are probably good enough to kill the common staphylococci, pseudomonads, coliforms, and enterobacters involved in the most serious outbreaks and experience.

c. Contaminated fomites, medications, and parenteral solutions do not automatically imply sterilization failures. More often than not, careful investigation points to poststerilization recontamination. (Unpublished data from my own laboratory show clearly that 30 minutes after a sterile pack is opened 20% of the sterile instruments on the back table are contaminated; within 3 hours, this number rises to 35%.)

This should not be construed as an attack on sterilizers or sterility monitoring. Rather it is a plea to put these enterprises in perspective. Hospital acquired infections are so serious, sterilization failures must always be considered seriously. But we must do something to demystify sterilizers and to pay as much attention to the handling of sterile items after sterilization as we do to the process of sterilization itself.

The Sterility Mystique

Several years ago, Kelsey published his "Myth of Surgical Sterility" and tried to demythologize some of the terminology in our field. It was a thankless task. I don't think we want to demythologize sterility. It's such a comfortable concept. It satisfies both our desire to be scientific and our primordial urge to believe in demons. Indeed, as early as 1906 Chapin referred to his contemporary sanitarians as "a cult of purification deriving psychologically from the demonic theory of disease." This may have been too radical a description and it may have been unkind. But it is true that many of our current concepts about sterilization were developed when the world worried about catching scarlet fever from infected books; gonorrhea from doorknobs; and yellow fever from mattresses. Moreover, beer glasses become cleaner, sterilizers become more frequently used, and infectious diseases did diminish. It's hard to contradict such facts.

In particular, the hospital world became absolutely obsessed with sterilizers. The hospital licensing laws of every one of the fifty states insist that an autoclave be installed. It's one of the few consistent features of such regulations. What can you say to demythologize the following contentions by Dr. Walter Dandy of Johns Hopkins who wrote in 1932:

> "That wound infections occur from time to time in every hospital is a well known fact. Every one has a pet explanation which satisfies for a time but gradually new reasons must be invented as the infections continue to occur. For the past 20 years I have carefully studied this problem from every angle and now believe it is possible to assure you, without fear of contradiction, that *every wound infection* can be prevented… The practical proof of this statement is an entire year's freedom from the slightest trace of infection. Moreover, the formula I have to offer is a very simple one — namely longer — much longer — better and more directly controlled sterilization of towels, gowns, and other supplies that pass through the sterilizer…"

There it is, all the elements of mystification: perfect success, simple solutions, shifting responsibility to a machine and its nonmedical operators, no data, and complete ignorance of statistics.

During the same decade surgeons were communicating clinical impressions about the magical qualities of gloves, gowns, masks, ultraviolet lights, and their own infallibility. Each could be a classical study model in the philosophy of science: a few verifiable facts plus a hodge podge of speculation presented by a twentieth century god — the surgeon. Superimpose onto their testimony the salesmanship skills by those who profited from the sale of soaps, sterilizers, and sterility indicators; the evolution of bacteriological consultants from Universities who chased bugs for a living; the creation of a new cadre of professionals who were hired to staff hospital departments of sterilization; and the emergence of government regulators who could satisfy their psychological need to be policemen while doing something useful to control disease — and you get the picture of a multimillion dollar industry that resembles a symbiotic ecosystem which supports itself and feeds on itself. And every time we try to dislodge this multicellular creature, it is pulled together by lawyers who survive by engulfing any element which deviates ever so slightly from the zoogleal mass.

Maybe this is why we do research on such trivia like "how many patches can you put on a surgical wrapper?" or "how does a 2-day Biological Indicator System compare to a 7-day system?" These are legitimate questions within the established community. Questions about the value of the system itself are not tolerated and are not asked.

Far be it from me to shake this boat. But it's becoming terribly expensive to ride in it. And it's really not as tight a boat as we would like to think. How can we explain away our glaring inconsistencies in sterilization? How can we justify the use of non-sterile laparoscope in the same procedure where we insist on a sterile knife to make the incision, sterile sutures to close the wound and sterile dressings to cover it? How can we insist that manufacturers of sterile devices certify freedom of contamination to the 10^{-6} level when we know that the operating room team which handles these things will probably contaminate them within minutes of their removal from the package?

My plea, therefore, is not for a curtailment of sterilizing or sterility monitoring. Far from it. I just suggest that the time is appropriate to devote just a little of the thought and money and enthusiasm which usually is devoted to sterility control to some consistency in the whole business. I don't want to rock the boat. But can't we trim the sails a bit and get it on an even keel?

Is the sterilization of a medical product still necessary today? Which products? How much

sterilization and disinfection and monitoring is essential? How much is trivial? How much is a waste of time? How much of the two hundred billion dollars spent for health products and services should be devoted to this enterprise? How diligently should we look at the product to see if it is sterile? How much money should we devote to monitoring the process? And can we get answers based on the bottom line of infection control instead of evangelistic testimonials? Or is it all—like several other hospital activities—another chapter in a book I intend to write when I get fired by my University for skipping classes to come to these meetings and get ostracized by the sterilization establishment for being irreverent. The title for the book will be: "Sterility Control for Fun and Profit - B.G.R.I.P."

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Technology Update

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Thermal Sterilization Update

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The use of elevated temperatures for sanitation, disinfection and sterilization of products and equipment is the classical and time-proven method for microbial destruction and control dating back to the time of the discovery of microbial agents as the causative agents of spoilage and disease. Thermal energy derived heated air or gases and wet vapors, including free flowing steam, air/steam mixtures and saturated steam under pressure, have been shown to meet most of the basic criteria for ideal disinfection and sterilization processes, as shown in Table I.

Table I.— Important Microbicidal Factors as Applied to Thermal Energy.

1. Sterilant Type and Source	1. Ease of Thermal Energy Generation and Transfer
2. Sterilant Equipment	2. Hot Air Ovens, Nonpressurized and Pressurized Vessels, Thermal Energy Exchange Devices and Systems
3. Sterilant Penetrability	3. Thermal Energy Transfer by Conduction, Convection and Direct Permeation
4. Product Compatibility	4. Stability to Elevated Temperature or Pressurization Required
5. Packaging or Sterility Containment Barrier	5. Hermetically Sealed or Permeable Packaging Sytems
6. Thermal Exposure Time	6. Variable Times Dependent Upon Thermal Energy Sources— Minutes to Hours
7. Sterilant Removal or Release and Residuals	7. Easily Removed by Cooling Systems, Including Vacuum and Nonvacuum Drying Systems
8. Economics	8. Lowest Cost of any Microbicidal Control Process

Thermal energy as a destructive force on microorganisms affords a close to ideal system and generally meets the physicochemical law of first order reactions wherein the rate of microbial destruction is a logarithmic function with respect to time. The only major divergencies of thermal microbial destruction in logarithmic kill curves occur during the initial thermal lag phase of the process due to thermal equilibration and permeation and the asymptote at terminal phase of the cooling cycles due to thermal energy dissipation vagaries and potential mechanical microbial protective systems.

The history and documentation of thermal disinfection and sterilization processes has demonstrated repeatedly that the integration of thermal energy permeation, penetration and contact with time is the fundamental principle of thermal disinfection or sterilization attainment. Hot air, free flowing steam, pressurized steam with saturated steam or air/steam mixtures afford various temperature ranges for thermal microbial destructive processes.

The most commonly employed and well known systems are pressurized steam sterilization processes that utilize 121°C (250°F) as the temperature of choice for ease of reliable sterility attainment. However, temperatures below and above 121°C (250°F) are commonly employed for

microbial destruction processes of disinfection and sterilization, but require diligence, knowledge and documented control programs to assure consistent, reliable achievement of microbial disinfection or sterilization. The classical gravity displacement steam sterilization cycle, consisting of displacement of air within a pressure vessel by steam, has not been appreciably modified from the early developed processes of steam sterilization. Sophistication of the classical gravity displacement steam sterilization cycles is mainly attributed to unique mechanical, electrical and electronic control systems that have been engineered into sterilizers to yield precise, reliable, consistent and reproducible cycles from one cycle excursion to another.

Air entrapment in chambers and packaged products employing saturated steam sterilization has been shown to be one of the prime factors for lack of reliable sterility attainment because trapped air acts as a barrier for direct steam contact and simulates conditions of dry heat sterilizers that require considerably longer exposure times to assure sterility achievement. Since entrapped air is the nemesis of saturated steam sterilization assurance, sterilization research in the past has concentrated on efficient air removal systems for steam sterilizer cycles. Thus, vacuum pumps, steam ejectors and other devices have been added to steam sterilizers to more effectively remove air from the chamber and packaged products. Controversy still exists today as to the degree of air removal that is required to assure reliable sterility attainment. Special devices have been developed to detect air removal efficacy in sterilizer chambers and packaged products and some devices are incorporated and mandated in steam sterilizer designs. The technical significance and value of these air detection devices are yet to be universally recognized because no support data has been adequately presented to technically justify these products and devices, other than as indicators of serious air leaks in a steam sterilizer system whose sterilization efficacy is dependent upon saturated steam contact of the packaged products. It is inconceivable that very small amounts of entrapped air in the chamber would affect steam sterilization assurance because the physical gas laws would prevail and the air would easily equally diffuse throughout the steam effecting steam permeation, penetration and contact. However, entrapped air in a packaged product, usually not detectable by entrapped air detection devices, is a serious problem of standardized steam sterilization cycles and it must be removed to assure consistent sterility attainment where pressurized steam contact is the cidal mechanism of sterilization. Newer devices, placed directly into the packaged product, have been developed that can be utilized in cycle development programs to guard against saturated steam sterilization failures of this type.

In contrast to saturated steam processes, other thermal energy transfer processes are commonly and routinely employed for disinfection and sterilization processes. Perhaps some of the most interesting pressurized steam processes are those which utilize both steam and air (or gases) in a pressurized chamber. Generally these unique processes are employed by industry because greater sophistication of sterilization control is required for sterility attainment and assurance. These steam cycles will be generically classified as air/steam cycles, but there are many variations of these cycles depending upon the stability of packaged product to the selected air/steam mixture cycle. A simple air/steam cycle employs equal barometric pressures of air and steam in the chamber, but the steam and air are uniformly diffused throughout the sterilizer chamber by a fan. The pressurization of the chamber is usually double that of saturated steam sterilization cycles. With these higher chamber pressurizations, stability of the package and the product must be assured not only during the sterilization exposure phase, but also during the pressure changes of temperature come up and cool

down of the cycle. Sterilization temperatures employed for these air/steam cycles are 110°C to 143°C. Many industrial air/steam cycles utilize 115°C for flexible film packaged parenteral solutions, provided that other quality assurance manufacturing procedures are employed to control the indigenous contamination or bioburden level of the packaged product before sterilization. Examples of other types of packaged products that utilize air/steam mixture sterilization cycles are crimp sealed vialed liquid drugs, liquid vitamin and nutritional concentrates, soft contact lenses, impregnated dressings and sutures. Newer applications of the air/steam mixture cycles will be forthcoming as familiarity of their cycles become better known to international sterilization scientists.

During the past decade or less, quantum changes have taken place in development of different saturated steam sterilizer cycles for special hospital and industrial applications. These newer cycles employ vacuum and other unique systems for air removal to yield shorter sterilization cycles. Although the basic steam sterilization principles are employed in these unique processes, greater sophistication of cycle control is achieved by specialized cycle sensing devices and newly developed microcomputer units to control and monitor all phases of the steam sterilization cycle. The employment of microprocessor controls and integration of cycle sensors data into master computer systems is also relatively new and more utilization of this type of sterilization control will be seen in industrial and hospital sterilization processes. Human errors of manual sterilization cycle control will be nonexistent in the not too distant future due to the entry of electronics into sterilization technology. In the future computer controlled thermal energy input into packaged products by the established or set and controlled F_0 values will serve to achieve sterilization requirements and concomitantly monitor the entire process, store data and afford hard copy data for cycle records.

Since recordkeeping of a controlled sterilization process is mandatory in some countries to comply with regulatory statutes, more use of centralized computers tied in with the operating sterilizers will be seen in industrial and possibly hospital operations. The options for manual sterilization operations will be minimized or nonexistent, to further guard against human errors in sterilization procedures.

Although there are advocates that believe the sophistication of many steam sterilization cycles today is adequate to guard against human error in sterilization processes, it is not the time to abandon sterilizer independent control systems for sterility assurance, such as biological indicators, chemical indicators and other analytical instrumentation. Based upon experiences of the author encompassing many industrial and hospital sterilization operations, independent control systems are still a basic safeguard and practical procedure for sterility assurance monitoring. Newer biological, chemical and analytical devices independent of the control system of the sterilizer can and do detect sterilization malfunctions not detectable by sophisticated electronic controls and computers because they are utilized in the packaged product and are highly sensitive to unrecognized sterilant steam penetration vagaries of the packaged product itself. A good example of a new biological chemical indicator is one that was developed for monitoring solution sterilization processes employing temperatures of 115-143°C. The chemical indicators show through presence or absence of pellet fusion whether or not the time-temperature conditions were achieved in the packaged solution by visual examination of the vial after cycle completion. Microbial kill is subsequently verified by incubation of the vial and observation for the absence or presence of growth in the vial ampule. This unique monitoring device is also applicable to steam cycle development and sterilization validation systems because it is not totally dependent upon the sterilizer control system and aseptic laboratory culturing techniques. Other

indicator systems of this type will become available as different steam processes are employed in industrial and hospital operations.

In summary, although thermal disinfection and sterilization processes have been the mainstay of standard international sterilization processes, continued research will be carried out on novel thermal processes, often coupled with newer energy permeation systems, to further the value of thermal sterilization for industrial and hospital operations.

Advances in Ethylene Oxide Sterilization

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Little was it realized in 1859, when ethylene oxide was discovered, how important a role this chemical would play in the health care of the world populations. This chemical has made the medical device industry as it is known today possible through the ability of manufacturers to use low cost, thermoplastic materials for sterile disposable medical devices. It took some 64 years, however, after its discovery before the biocidal activity of ethylene oxide was first reported. These initial reports were of its activity as an insecticide (1). It was not long after that the first patents were applied for the use of ethylene oxide for the destruction of microorganisms in spices and gums. Griffith and Hall (2,3) in these patents were the first to describe an industrial gaseous ethylene oxide sterilization process. The process that they described employed vacuum chambers and pure (100%) ethylene oxide gas. Considerable activity took place in the 1940's and 1950's with experimentation that demonstrated that laboratory plastics, medical and biological preparations, hospital bedding, plastic bandages, medical instrumentation, surgical transplants and many other items could be successfully sterilized using this chemical agent. (4).

A diversity of equipment has evolved over the years for ethylene oxide treatments. The equipment has ranged from the chambers of Griffith and Hall (2,3) to the simple atmosphere pressure metal fumigation chambers and gas tight plastic bags reported by Phillips (4). Since most early sterilization equipment was of such simple design, the sterilization process and its control was also believed to be simple and uncomplicated. As the use of this chemical sterilant grew in popularity, scientific studies demonstrated that effective reproducible sterilization was not as uncomplicated as it was believed, and that it required the controlled interaction of the parameters of heat, moisture and gas concentration.

As the complexities of ethlyene oxide sterilization became understood, it was soon recognized that more sophisticated control of the parameters would be necessary. Figures 1, 2 and 3 show a progression of ethylene oxide sterilizer control systems through the 1950's, 1960's and 1970's. These typical sterilization control monitoring systems, although increasing in sophistication, are basically similar in principle and are comprised of several sequenced electro-mechanical devices, such as pressure switches, timers, and temperature controllers.

Of major concern in sterilization processing is that there is a consistent assurance that multiple parameters (pressures, temperatures, times, rates, and sterilant concentration) are adequately controlled, monitored and documented. Table I from Burrell et al (5) shows that a most simple ethylene oxide sterilization cycle typically requires at least twelve different hardware components. As multiple cycles and additional constraints are placed upon the various parameters, the need for increased individual components increases accordingly.

Figure 1. Ethylene Oxide Control System Circa 1950's.

It is in the area of sterilizer control systems that the most noted changes and improvements have been made in the past few years. With the advent of microprocessor technology, the availability of low cost hardware and the application to process control systems, it was only a matter of time before this technology was applied to sterilizer control systems. The microprocessor approach dramatically minimizes the number of individual hardware components that are required in electromechanical systems. Microprocessor systems operate from set points and control algorithms incorporated into the system's software. The result is that the multiple pressure switches of the electro-mechanical type systems can be replaced with a single pressure transducer, several timers, and an electronic clock. Such component minimization increases reliability, minimizes maintenance and simplifies calibration.

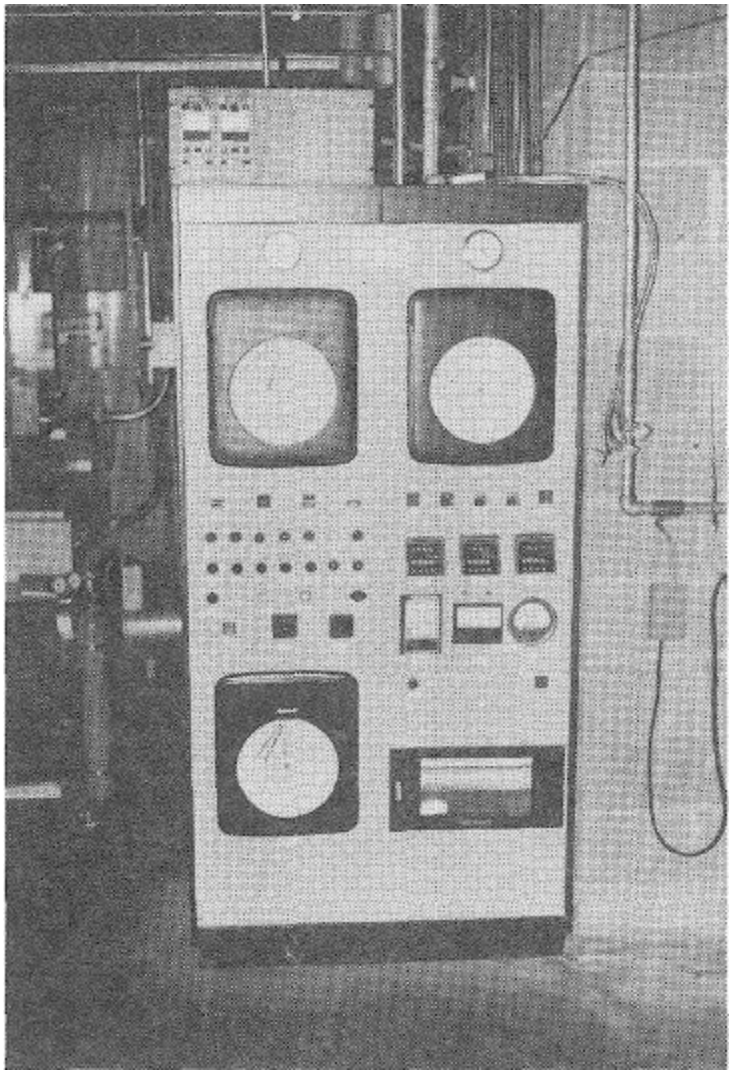

Figure 2. Ethylene Oxide Control System Circa 1960's.

Table I.— Electromechanical Components Required for a Simplified Ethylene Oxide Process.

	Minimal Controller Constraints for Sterilization Control		
Cycle Step	Pressure	Time	Temperature
Initial vacuum	PS1		
Vacuum dwell	PS2	TM1	
Steam injection	PS3		
Steam dwell		TM2	
Gas charge	PS4		TC1
Exposure period	PS5	TM3	TC2
Final vacuum	PS6		
Air inbleed	PS7		

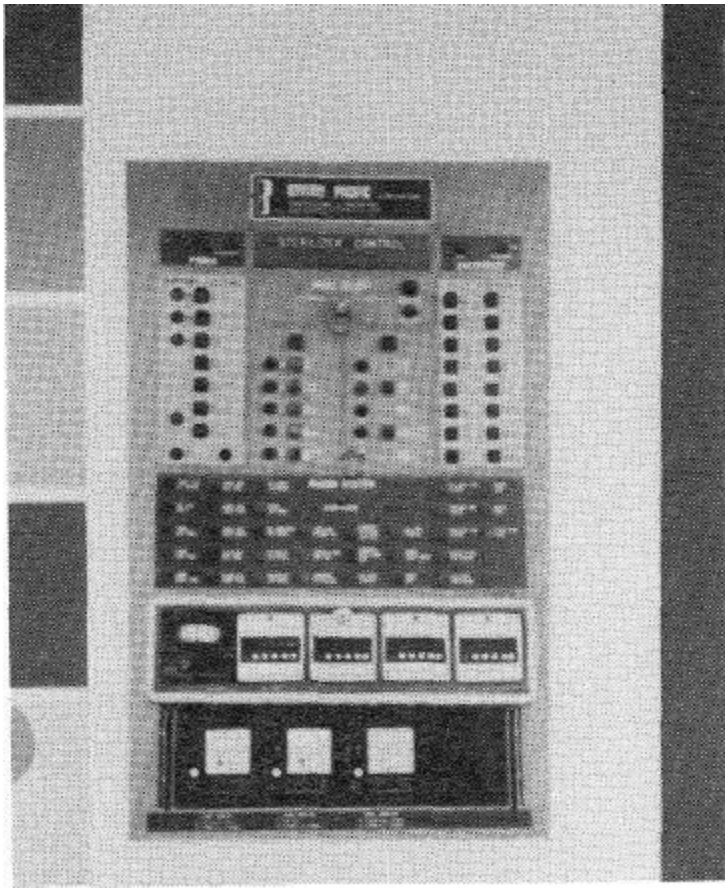

Figure 3. Ethylene Oxide Control System Circa 1970's.

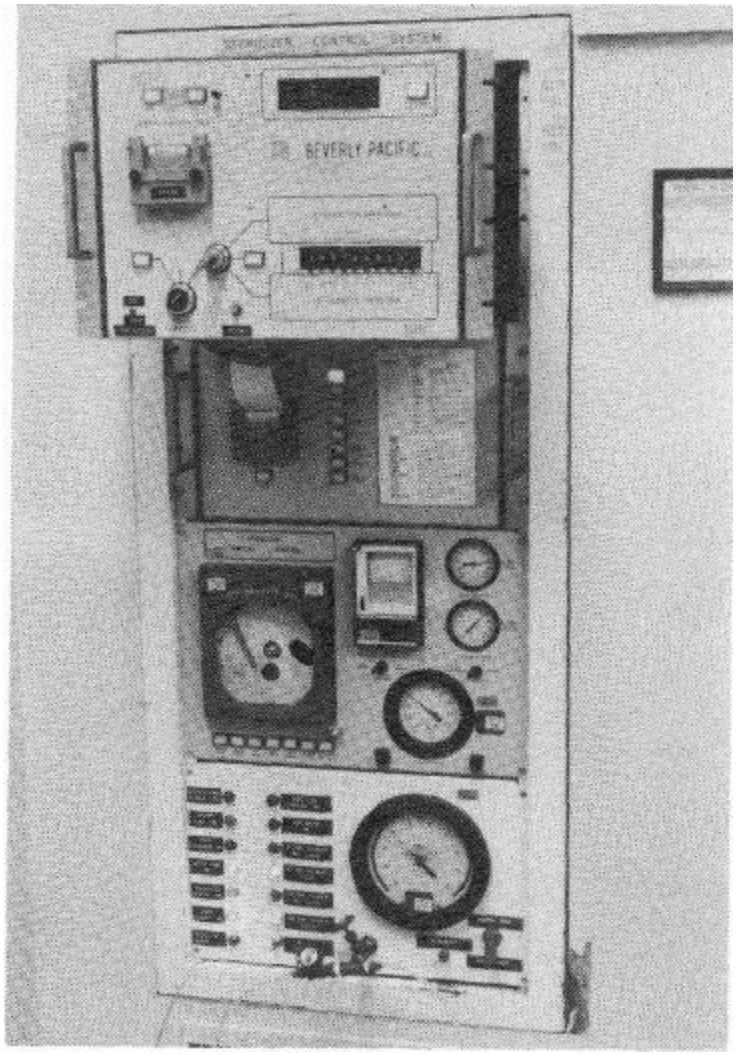

Figure 4. Beverly Pacific Co. Microprocessor Control System, American Edwards Laboratories, Irvine, California.

To the best of my knowledge the first commercially built microprocessor control system was fabricated for American Edwards Laboratories by the Beverly Pacific Company (Burbank, California) and Digital Dynamics (Palo Alto, California). This control system is shown in Figure 4. In this system, cycle process information from the Basic Functions and the Support Functions is printed out on the two strip recorders. Capabilities of this system are limited to a single preprogrammed cycle. Additionally, there is operator-limited keyed access to a variable or developmental cycle. Cycle parameter changes to the master program are less simple than with the next generation systems, since machine language, not control basic language, is utilized. A manual backup system is also provided in the event of computer problems.

The next generation of equipment, and one that we at the American Pharmaseal Company have had immense success with, is the “SCOT” system - Sterilizer Computer Operating Terminal (Figure 5). The first two of these systems were put into production operation in 1978. One system operates and controls a large production sterilizer and the second operates and controls our experimental or cycle development unit. Three additional systems have been fabricated and are presently being installed on production vessels. The main components of the SCOT system are:

Figure 5. SCOT – Sterilizer Computer Operating Terminal – American Pharmaseal – Glendale, California.

Microcomputer

The Cromenco Microcomputer includes a central processing unit, an electronic clock, and a memory (both as PROM, programmable read only memory, and magnetic disk). The Microcomputer processes inputs, makes decisions and provides inputs.

Video Monitor Tube

The Video System provides an ongoing display of the critical cycle process parameters as well as the system support variables. It is also used during troubleshooting and calibration. Satellite video display systems can be incorporated in the system if desired.

Teleprinter

A periodic hard copy printout of process parameters being monitored and controlled by the system during cycle operation is on the teleprinter. The teleprinter is the sterilizer operator's interface with the process. A keyed lock is utilized to limit access to the teleprinter and thereby access to the system.

Manual Backup System

This system is completely independent of the Microcomputer System and is used primarily for troubleshooting. It can, however, be used for cycle operation in the event of computer problems. A keyed lock is provided to limit access to this system.

Interface System

This system accepts signals from the sterilizer, directs them to the computer for analysis and redirects outputs to control sterilizer operation.

Audio Alarm System

The Audio Alarm System is coupled with a warning printout on the teletype of situations out of normal but not critical, and an audio, video and printed alarm of critical problems which causes an emergency shutdown of the system, and which requires subsequent corrective action before continuation of cycle operation.

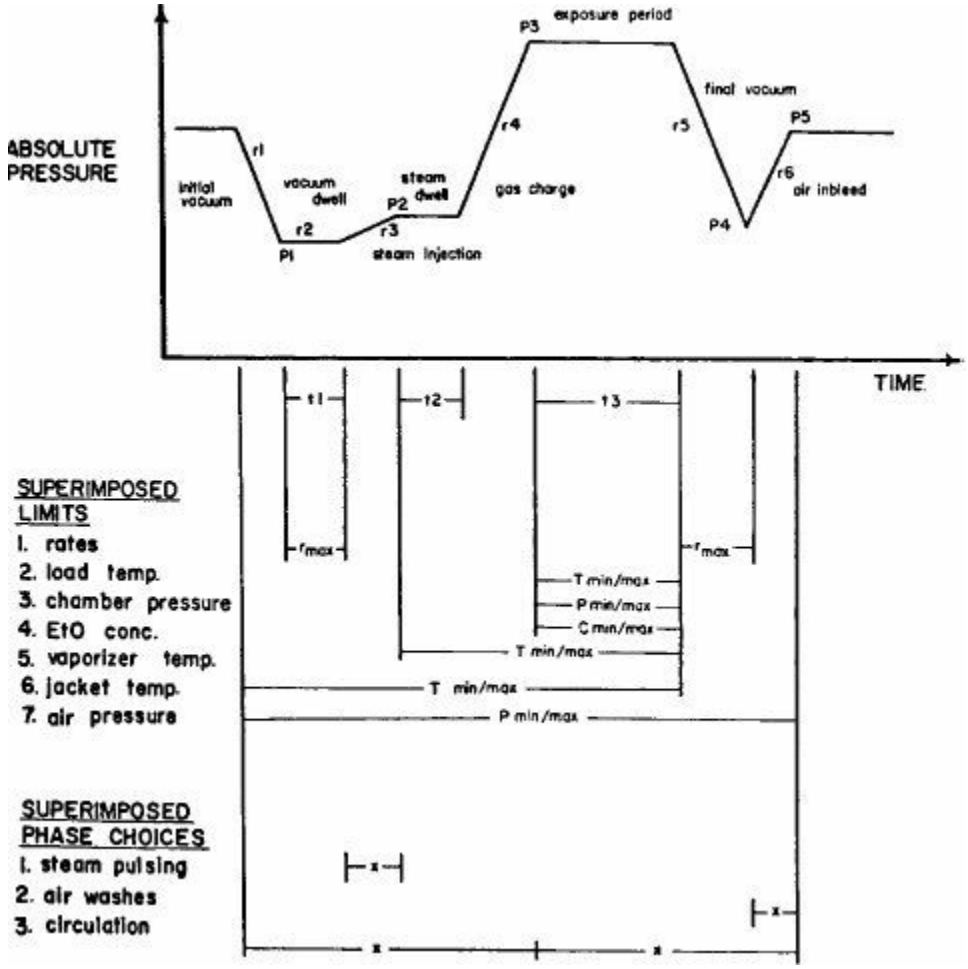

Figure 6. Simplified Example of Process Model Showing Superimposed Cycle Limits and Phase Choices.

With the SCOT system, sterilizer operations are directed by a preprogrammed process model which provides the user with the flexibility of selecting process options such as set points and alarm system constraints. A simplified model of the SCOT process model is found in Figure 6. In this example, eight process steps (initial vacuum, vacuum dwell, steam injection, steam dwell, gas charge, exposure period, final vacuum and air inbleed) are detailed with variable set points for five pressures (P1 – P5), six rates (R1 – R6) and three times (T1 – T3). Limits for rates, low temperature, chamber pressure, gas concentration, vaporizer temperature, jacket temperature and air pressure are superimposed for discreet periods of the process model. Phase choices such as the number of additional air washes, the action of steam pulsing and internal circulation also are superimposed on the model for the appropriate time period. The process model is permanently stored as software in the computer memory and when programmed with the desired variables provides the sterilization cycle profile. If it is necessary the process model can be modified by software changes.

Advantages of sterilizer control systems such as SCOT when compared to traditional sterilizer control systems include the capability to meet varied and changing production demands, usually with

no major changes to control system hardware.

The SCOT system is designed to permanently store up to eight production cycles. The process parameters for these permanent cycles are stored on a single PROM chip. Modifications to one or more of these permanent cycles can be accomplished by the substitution of a reprogrammed PROM chip. Reprogramming does not have to be performed at each control system location. Process changes are reprogrammed at a central facility and sent by mail to the remote sterilization facility if needed. This allows the absolute control of process operating parameters by the central facility.

In addition to the "permanent" cycles, SCOT is further equipped to conduct "development" cycles. The use of the development mode allows a facility to evaluate a new process or small modifications to an existing process without the need for changing the PROM chip. Access to the development mode is by a password. During the development mode the process set points, the operation constraints and the phase choices are all set by the process control operator. SCOT prints on the teleprinter a request for each variable to which the operator responds with the desired value. When all developmental cycle variables have been entered the cycle plan is complete and the cycle can be initiated.

Variations to the basic program can be performed by personnel having basic software expertise. The SCOT system was designed to utilize Control Basic, the conversational computer language which simplifies reprogramming. The reprogramming option is limited and controlled by the requirement for an additional plug-in solid state card.

The routine operation of a sterilization cycle using the SCOT system typically is conducted as follows. The sterilizer control operator specifies a cycle by designating the cycle identity code by typing it on the teleprinter. The teleprinter then prints a series of questions concerning the materials to be processed. Operator identification is requested. Although not presently utilized, unauthorized sterilizer operation can be restricted by placing a constraint on operator identification. When the operation is ready to start the operator initiates operation by answering the question "Ready?". All information previously entered in response to the computer-generated questions is collated in a printed format which then becomes part of the hard copy run record. This run record is a valued part of the device history record.

Next, processing begins. Cycle step, time, and the critical process parameters are all printed by the teleprinter as the cycle progresses through its various steps. Concurrently the video display presents a continuous update of the critical process parameters and support variables. The recorder of the manual backup system provides a circular chart recording of temperature and pressure during the entire process. If, during cycle operation, a situation arises which could have an impact on operations but is not yet affecting operations, there is a printout of a warning condition and an audio alarm. If a situation is sensed which must be resolved to prevent a product or personnel safety problem, an "emergency stop" situation results with a printout of the problem condition. At this time there is also an audio alarm, a cessation of the cycle, and a reverse video on the video display highlighting the alarm condition. The option of aborting a cycle or continuing a cycle is then presented to the operator by a printout on the teletype. In the event that the operator signals the SCOT system to continue the cycle without resolving the emergency condition, the "emergency stop" will automatically be reinitiated. Only when the proper corrective action has been taken will the computer allow the cycle to continue. Once all cycle steps have been satisfactorily completed a hard copy record of the sterilization run is available, presented complete with space designated for operator signature, Q.A. approval, and comments. Although not presently used, the magnetic disk is a parallel

option for data logging. This can be used to accumulate and later analyze trends and/or transmit run conditions to a central data logging library.

At the Johnson & Johnson Products Company the microprocessor based control system shown in Figure 7 was installed at the North Brunswick, New Jersey plant in 1979. This system operates two sterilizers, one steam and one ethylene oxide. The heart of the system is the microprocessor supplied by Eagle Signal Company (Davenport, Iowa) Model EPTAK. This completely solid state system has a 16K memory, and is capable of operating ten distinct cycles. As with the SCOT system, a cycle is initiated by the operator supplying the necessary code. Other features include digital printout of cycle parameters with concurrent visual display. It is flexible to accommodate changes, has set point protection, and has the capability for data analysis. This system does not employ video tube display. As with the other microprocessor systems, there is a manual backup mode.

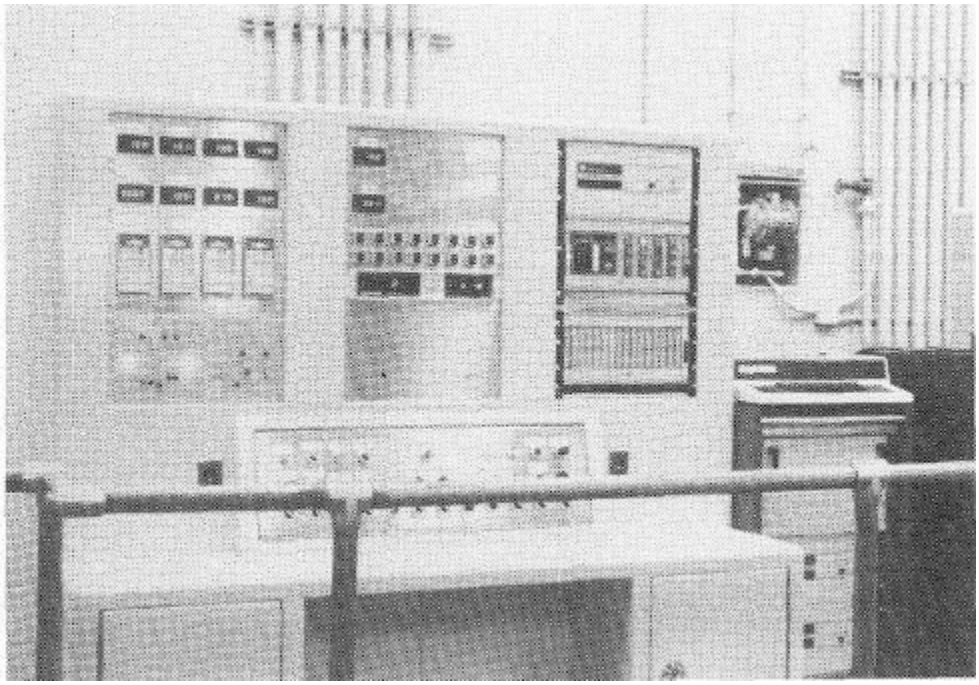

Figure 7. Eagle Signal Co. Microprocessor Control System, Johnson & Johnson Products Inc., North Brunswick, New Jersey.

In addition to the Beverly Pacific Company mentioned earlier, other sterilizer equipment manufacturers now offer various types of microprocessor based control systems. These may be purchased as a part of a complete new system or retrofitted to an existing vessel. The microcomputer based system built and sold by Vacudyne Altair (Chicago Heights, Ill.) and shown in Figure 8 is quite similar to the SCOT system previously described. With this system there is no limit as to the number of cycles capable since the information specific to operate each cycle is permanently stored on a magnetic card. During routine operation the magnetic card is simply inserted into the magnetic card reader which then loads the program into the random access memory (RAM). The cycle is then initiated by depressing the start button. A detailed run record printout is supplied from the teletype detailing elapsed time, cycle step, temperature-pressure-humidity values, out of specification events and, upon cycle completion, a space for operator's comments and the necessary approvals. Operational safeties are also built into the system, in that, if modifications or decisions must be made, a supervisor must be called to insert a key to gain access to the system. This system also contains a manual override mode for each operating valve or motor so that the unit can be operated manually or

the system can be used for troubleshooting mechanical problems.

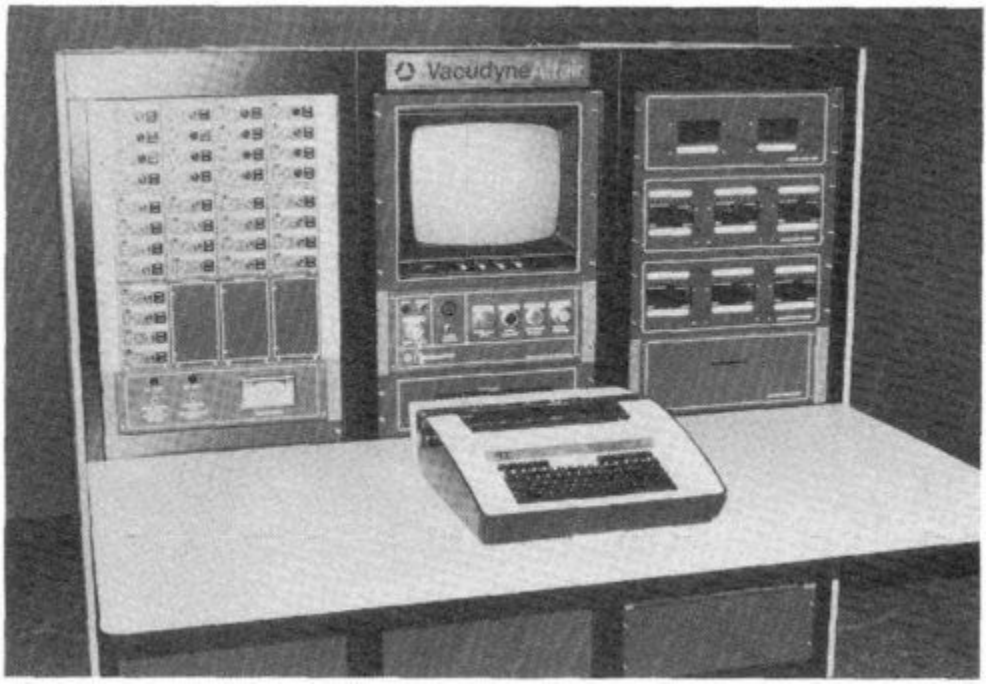

Figure 8. Vacudyne Altair Microcomputer Control System, Chicago Heights, Illinois.

The American Sterilizer Company (Erie, Pa.) offers a microprocessor based sterilizer control system called the Eagle 2200 (Figure 9). A similar system is used for the operation of steam sterilizers. As with the Vacudyne Altair system, the Eagle 2200 operates cycles from the information contained on magnetic cards. A specific card is used for each cycle. A manual backup system is also included and a cycle run record is supplied in hard copy form. This system does not offer video display of ongoing cycle parameters.

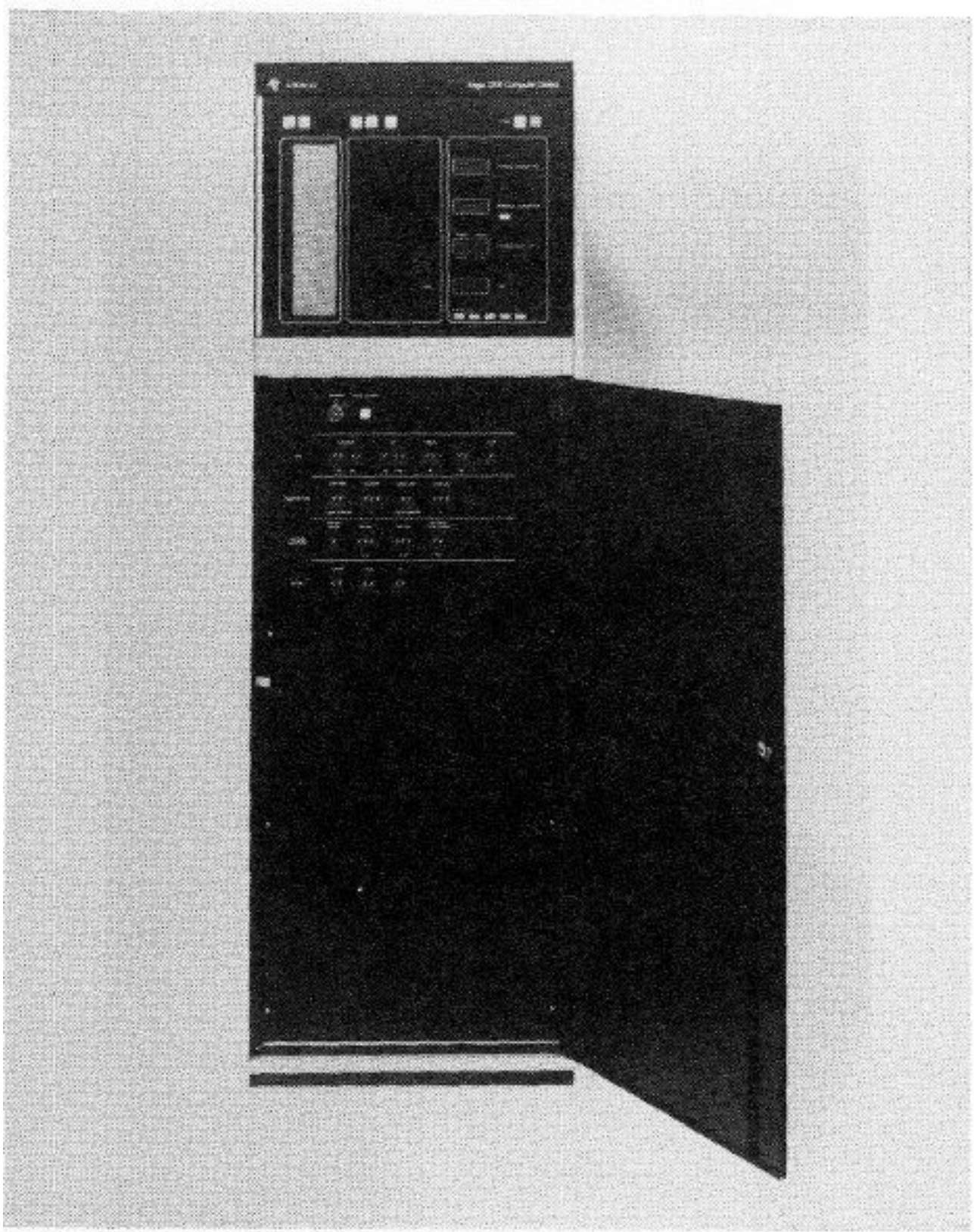

Figure 9. Eagle 2200 Computer Control System, American Sterilizer Co., Erie, Pennsylvania.

The Environmental Tectonics Corporation (Southampton, Pa.) microprocessor control system (Figure 10) is available for all small rectangular and medium size sterilizers for both steam and ethylene oxide. As with other microprocessor systems it is built on a state of the art large scale integrated silicone semiconductor "chip". To control cycle selection, digital thumbwheel switches are provided for setting the appropriate times, temperatures and pressures. Timers are not changeable once a cycle has been started and are automatically reset upon completion of a cycle allowing

simplified repetition of cycles. Digital readouts are provided for times and may be specified to indicate elapsed time or alternatively time remaining. Instrumentation includes an indicating recorder/controller that provides temperture controls to the set point programmed in the microprocessor and records process temperature on a circular chart. Standard pressure and vacuum gauges are provided on the control panel. No video display of cycle events is available, nor is a run record printout. The only hard copy information is provided by the indicating recorder controller.

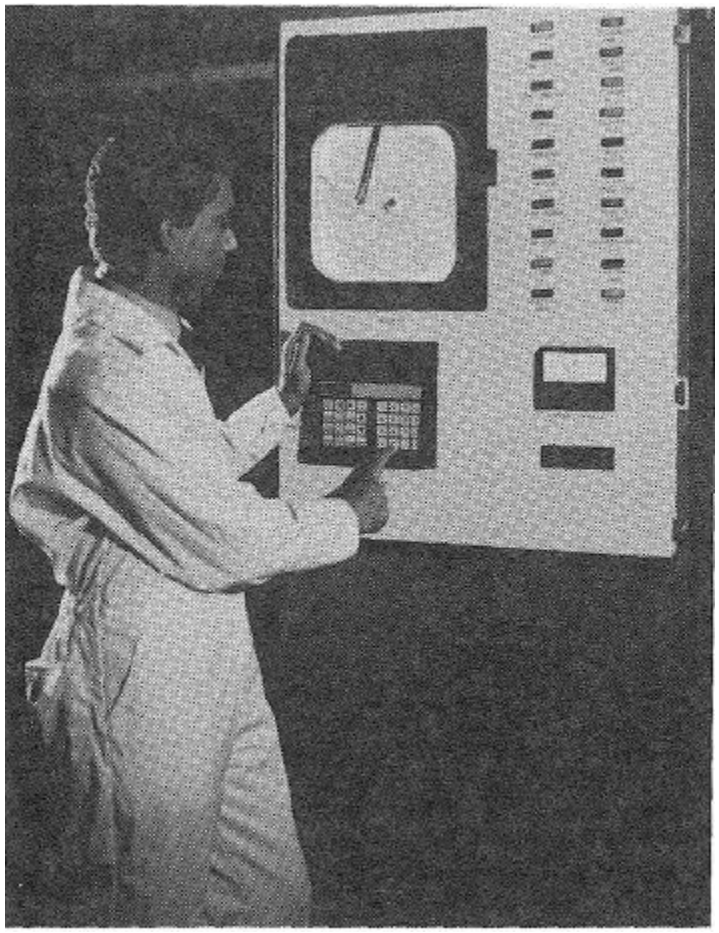

Figure 10. Ethylene Oxide Microprocessor Control System, Environmental Tectonics Corp. Southampton, Pennsylvania.

In summary, the most significant recent advance in ethylene oxide sterilization processing has been in the utilization of microprocessor technology in sterilizer control and monitoring systems. These provide significantly more control of sterilizer cycle operation than the former electro-mechanical systems by minimizing cycle variability, reducing operator decisions, easing maintenance, facilitating cycle validation, and simplifying calibration. The microprocessor systems when teamed up with a knowledge of cycle humidity and gas concentration conditions have also become the accepted tool that is the key to FDA approval for the use of parametric or integrated-variables release. It is interesting to note that it was just a few years ago when cobalt radiation sterilization gained process or dosimetric release status that it was thought that ethlyene oxide cycles were just too variable to qualify for similar status. It is now recognized, as Dr. Carl Bruch mentioned this morning, that these microprocessor-based control systems, when teamed with the well documented cycle validation programs that are now possible and the ensuing wealth of ongoing data of cycle operation parameters, provide a valid indicator of the achievement of successful sterilizing parameters.

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Advances Made in Cobalt-60 Gamma Sterilization

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Whippany, New Jersey, USA

The Historic 2.5 Mrad Dose

At the time of the first Kilmer Conference in 1976 the standard practice in North American irradiation sterilization was to expose all medical devices to a sterilizing dose of 2.5 Mrad. It was also common practice to attempt to verify product sterility by poststerilization testing.

Today, as a result of the development of scientific dose setting procedures that accurately account for heterogeneous microbial populations, that arbitrary 2.5 Mrad dose has been reduced to a variety of lower levels. Also, poststerilization testing is being replaced by a new quality assurance technique, "dosimetric release." Dosimetric release enables the immediate release of product (without quarantine) upon verification that the pre-established sterilizing dose has been delivered.

The Growth of Gamma Irradiation Sterilization in North America

The healthcare industry in North America has experienced a growing awareness of the unmatched reliability of the gamma irradiation sterilization process. Coupled with the elimination of quarantine periods and the increasing availability of irradiation compatible materials, the use of gamma sterilization techniques has been greatly expanded in North America. This expansion is perhaps best demonstrated by the wide-ranging list of products being gamma sterilized today, Table I.

As gamma sterilization embraces an increasing list of products, the number of gamma irradiation facilities has grown in similar fashion. Currently, North America has some 25 gamma irradiators, with a combined design capacity of 54 million curies, capable of processing 60 million cubic feet of product annually. This represents approximately 52% of the current world cobalt-60 irradiation capacity, Table II.

Table I. — Representative Gamma Sterilized Medical Products Dose Range and Dosimetric Release Status.

Product	Dose Range	Dosimetric Release	
Diagnostic Strips	< 0.8 Mrad	—	No
Electrodes	0.8 – 1.5 Mrad	Yes	—
Saline Lens Solution		—	No
Blood Tubes		Yes	—
Cotton Balls/Swabs		—	No
Plastic Labware		Yes	—
Surgeons Gloves		Yes	—
Specimen Containers		Yes	—
Disposable Thermometers		Yes	—
Eye Ointment		—	No
Grounding Pads		—	No
Syringes	1.5 – 2.0 Mrad	—	No
Infant Wear		Yes	—
Hospital Packs		Yes	—
Surgeons Gowns		Yes	—
Surgeons Gloves		Yes	—
Packaging Materials		—	No
Catheters		Yes	—
Empty IV Solution Bags		—	No
Culture Collection Systems		—	No
Lap Sponges		Yes	—
Scrub Brushes		—	No
Bovine Serum		—	No
Bandages	> 2.0 Mrad	Yes	—
Orthopedic Prosthesis		Yes	—
Glove Powder		—	No
Stockinette		Yes	—

Orthopedic Mixing Bowls	—	No
Water-filled Syringes	—	No
Needles	—	No
Surgical Blades	—	No
Vascular Grafts	Yes	—
Surgical Marking Pens	Yes	—
Needle Counting Systems	—	No

The Growth of Gamma Irradiation Sterilization Worldwide

Gamma irradiators have appeared in 35 countries besides the United States, stretching across the globe from England to Australia, Sweden to South Africa.

As the process grows on a worldwide scale, it becomes doubly important to arrive at an international agreement on dose setting, so countries can exchange products and communicate meaningfully on future research, and the development of irradiation sterilization technology.

Table II. — Irradiators in North America

Company	Location	(Mega Curies Capacity)
American Convertors	El Paso, Texas	4.0
American Convertors	El Paso, Texas	4.0
The Applied Radiant Energy Corp.	Lynchburg, Virginia	.55
Becton Dickinson	Broken Bow, Nebraska	3.0
Becton Dickinson	Canaan, Connecticut	1.0
Becton Dickinson	Oxnard, California	3.0
Ethicon	San Angelo, Texas	2.2
Ethicon	Peterborough, Ontario	1.0
Ethicon	Somerville, New Jersey	2.2
International Nutronics	Palo Alto, California	.5
Isomedix	Morton Grove, Illinois	.5
Isomedix	Parsippany, New Jersey	2.0
Isomedix	Columbus, Mississippi	2.0
Isomedix	Spartanburg, S. Carolina	4.0
Johnson & Johnson	Sherman, Texas	3.0
Neutron Products	Dickerson, Maryland	1.15
Neutron Products	Dickerson, Maryland	.2
RSA Corporation	Dover, New Jersey	.2
Radiation Sterilizers	Tustin, California	6.25
Radiation Technology	Rockaway, New Jersey	1.5
Radiation Technology	Rockaway, New Jersey	2.0
Sherwood Medical	Norfolk, Nebraska	3.0
Surgikos	Arlington, Texas	3.0
3M	Brookings, S. Dakota	3.0
Toronto Sterilized Products Limited	Markham, Ontario	.5

Today gamma irradiation doses of less than 2.5 Mrad are not yet accepted in many countries outside of the United States, and American sterlization products for export often have to be “topped off,” or resterilized at the 2.5 Mrad level, in order to meet these earlier standards. Since many of the long list of products listed in Table I are for export, we must recognize that a failure to approach new dose setting methodologies with an open mind unnecessarily increases the cost of European healthcare.

Recent Irradiation Sterilization Studies by the Association for the Advancement of Medical Instrumentation (AAMI)

Against a background of lack of agreement on dose setting and on dosimetric release methodology, AAMI established a gamma irradiation sterilization sub-committee to develop guidelines for the establishment of gamma irradiation sterilizing doses.

An exhaustive examination of the irradiation process concluded:

1. In many instances the gamma irradiation sterilization process was more effective, even at doses significantly lower than 2.5 Mrad, than alternative sterilizing techniques.
2. A range of sterility assurance levels should be adopted which takes into account the end use of the products to be sterilized. Thus, a single level of sterilization can be replaced by a range of levels, which depends on an understanding of a product's end use.
3. Dose setting procedures developed by the committee should accurately account for the naturally occurring heterogeneous microbial populations (bioburden) in the various products, and for microbial resistance to the sterilizing process. (Four such dose setting procedures were presented during this conference and are embodied in the AAMI document entitled "Process Control Guidelines for the Radiation Sterilization of Medical Devices (Proposed)."
4. It was further observed that general contamination levels on nonsterile products are also being reduced as a result of the implementation of Good Manufacturing Practices.

The New Incremental Dose Irradiator

Establishment of individual sterilizing dosage requirements (on the basis of end use, bioburden, and organism resistance) has led to the design of commercial sterilization facilities capable of handling the range of doses required.

The new type of irradiator handles a larger range of products and eliminates the more complex dual-track/vertical indexing conveying systems. Most important, it 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 in the incremental dose irradiator receives a multiple of any preselected incremental dose. For example, if one product carrier requires a 0.5 Mrad dose, and the second carrier 1.0 Mrad, each receives 0.5 Mrad during the first cycle; the second carrier passes through the chamber a second time to receive another 0.5 Mrad dose.

The first computerized incremental dose irradiator control system has been designed by Isomedix, Inc. The Isomedix irradiator features a computerized controlling device for each product carrier, to ensure that each carrier receives the required number of increments. The Isomedix Computer Control system, furthermore, offers total process monitoring and final hard copy documentation.

The Isomedix Computer Control system traces the progress of each carrier through the irradiator until the carrier arrives at the unloading station. To accomplish this, each carrier is assigned a binary identification number, read by light sensors strategically located at the product loading station, the irradiator entrance and exit, and the product unloading station, see Figure 1.

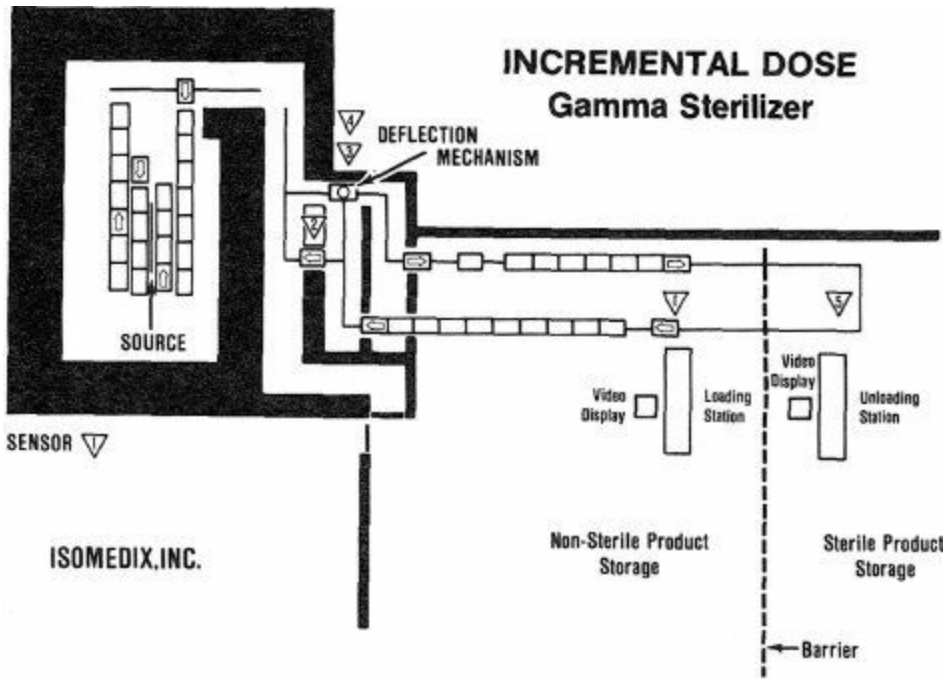

Figure 1. Incremental dose gamma sterilizer.

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, see Figure 2.

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, see Figure 3.

At the exit to the irradiation chamber, Sensors 3 and 4 read the carrier number. The computer subtracts the cycle just completed from the number of cycles required for the total process, and indicates the number of cycles remaining. If the result is zero, the Carrier Return Control discharges the carrier into the unloading station on the sterile side of the warehouse. If the result is other than zero, the Control returns the carrier to the chamber for another incremental dose.

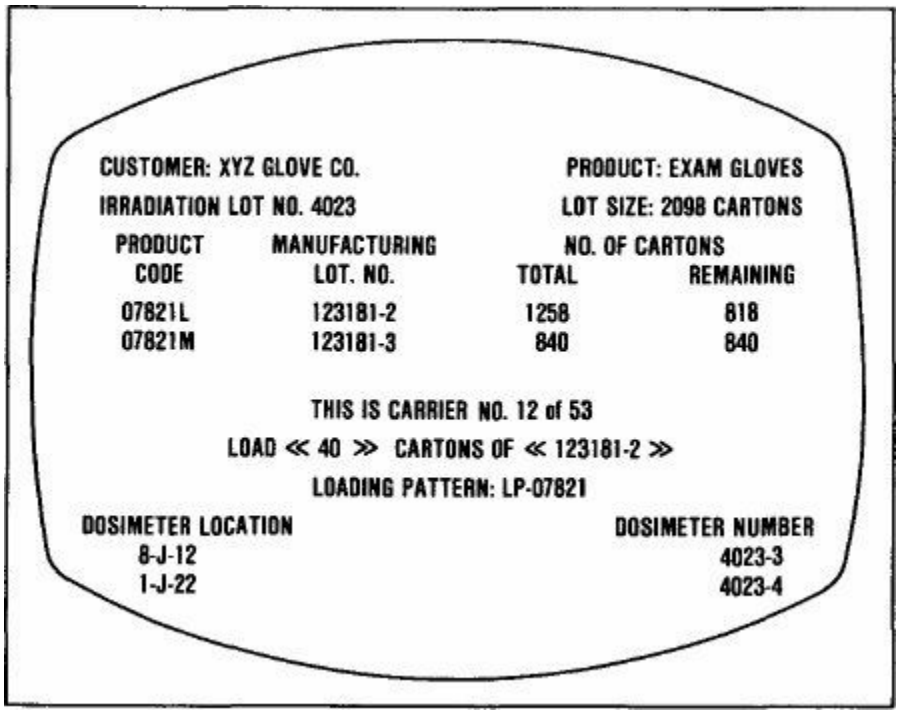

Figure 2. Video monitor display Sensor No. 1.

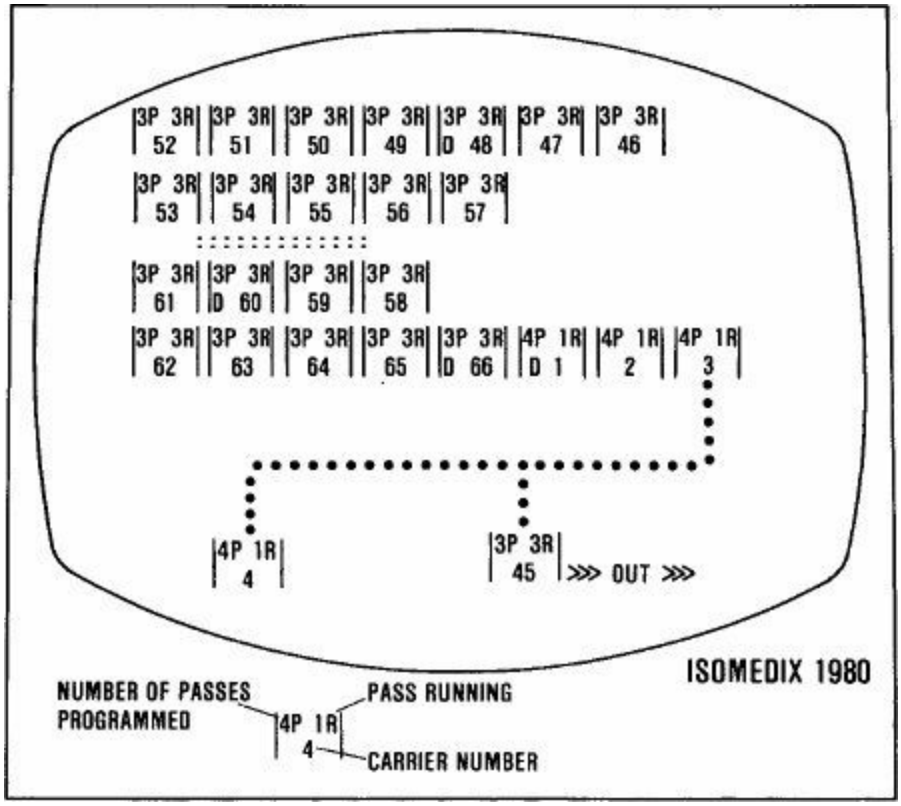

Figure 3. Video monitor display Sensor No. 2.

At the unloading station a video monitor displays the same information as was displayed at the loading station for that carrier, see Figure 2. 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.

As a final measure, the computer prints out hard copy documentation of all the information relating to the product.

Conclusion

The 1970's have provided the healthcare industry with a better understanding of the effectiveness of gamma radiation against the natural heterogeneous microbial populations occurring on nonsterile medical products.

As a result, the guidelines, the methodology, and the technology for varying sterilizing doses have been established that will give any medical device its desired margin of sterility assurance.

We confidently predict the 1980's will continue to reflect *a* significant growth in gamma sterilization around the world.

Progress Toward Practical Electron Beam Sterilization

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Introduction

The efficacy of energetic electrons as a sterilizing agent has been well understood for several decades. Three major obstacles have precluded the widespread use of on-line electron sterilization in commercial product treatment/packaging applications: concern over equipment reliability, size and safety, for a line dedicated to the process; the difficulty of sterilization assurance for both the in-line and terminal applications; and finally, the inertia of the sterile products industry to any departure from the widely practiced and proven techniques of batch terminal sterilization.

This paper presents a brief review of recent progress in the application of compact electron process machinery for industrial use. A discussion then follows in a review of real time monitoring techniques for the acquistion of archival data for process monitoring and control. In short, the paper presents progress in the first two areas which will assist in the industrial acceptability of the process.

Equipment Developments

Since the first impact of the oil embargo in 1973, there has been an increasing effort on the part of energy intensive industries in the United States to seek alternate technology. One of these "new" technologies has been that of electron processing (1) where electron beam machinery has been used to replace gas or oil fired thermal ovens. The reader will be familiar with the successes in the wire and cable (vulcanization) and film (crosslinking) fields (2) where relatively high power, high energy equipment has been successfully used for some time. In the past decade, the much broader application of electron curing has emerged in the addition polymerization of surface coatings and adhesives (3). These uses are now commercialized in the packaging, textile, wood products, and paper-finishing industries, and are beginning to impact the graphics, magnetic tape, and semiconductor industries as well. This dramatic change has been motivated at least as much by pollution reduction as by energy conservation thanks to the "solvent-less" nature of the coatings used with these processes. Much of the groundwork for the chemistry of these 100% solids systems has been laid by ultraviolet curing, which has enjoyed an even broader application in the metal decoration and graphic arts fields (4).

The significance of these developments over the past decade to the field of sterilization lies in the improvements in, and field testing of, the electron beam equipment employed in industrial processing. One of the most important of these developments has been the emergence of selfshielded equipment which can be utilized in unrestricted areas (5), that is, in areas of the plant where *no* restriction of employee access is required in those regions in the immediate vicinity of the working equipment.

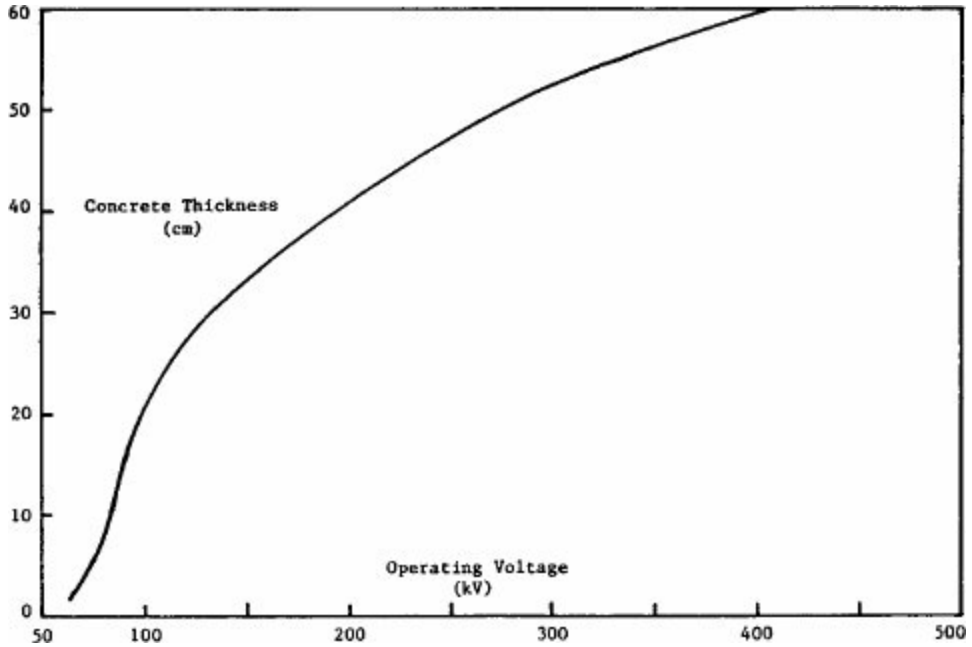

Figure 1. Typical shielding thickness for OSHA compliance for "unrestricted" use.

Figures 1 and 2 present data relating to the thicknesses of concrete and lead required in general, if the environmental radiation requirements for "unrestricted use" as specified by OSHA (5) are to be exceeded using electron beam machines in the voltage range and at power levels of approximately 10 kW of up to 500 kV. These curves are taken from our own experience (6) and extrapolated from the NCRP data (7). Since the large volume industrial curing operations referred to earlier here involved penetration requirements of less than 250 gm/m^2 (i.e. 250 microns of unit density matter), much of the machinery developed for these uses has involved operating voltages of 250 kilovolts or less, hence

relatively modest lead shield thickness (6 mm) are adequate.

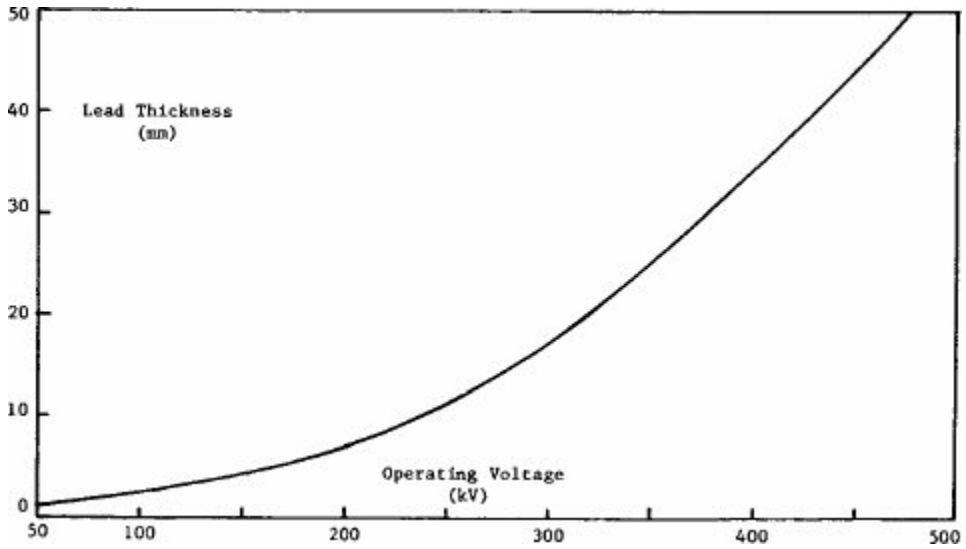

Figure 2. Typical shielding thickness for OSHA compliance for "unrestricted" use.

A schematic of an in-line, self-shielded machine is shown in Figure 3. These machines (8), developed for web and sheet surface curing applications, and for web lamination, typically operate at levels of 1 mA of current per cm of length. At the voltages indicated, this level of operation will deliver 1 Megarad at product speeds of up to 300 m/minute. This level of performance is adequate for the dose to cure requirements of most coatings, inks and adhesives currently available, while the maintainability and reliability of these units has been documented (9).

A view of large (1.7 m × 20 kW) processor installed on a coater-laminator is shown in Figure 4, and demonstrates the compactness of the energy source. The edge: edge uniformity of the beam delivered from such a unit is shown in Figure 5. Such machinery has been used successfully for many years in demanding industrial applications, occasionally requiring 20 shift (160 hr) use per week; i.e. 95% utilization. As a result, the concern about the reliability of such compact electron equipment has diminished, as precedent setting examples of its use in a number of industries continue to be publicized.

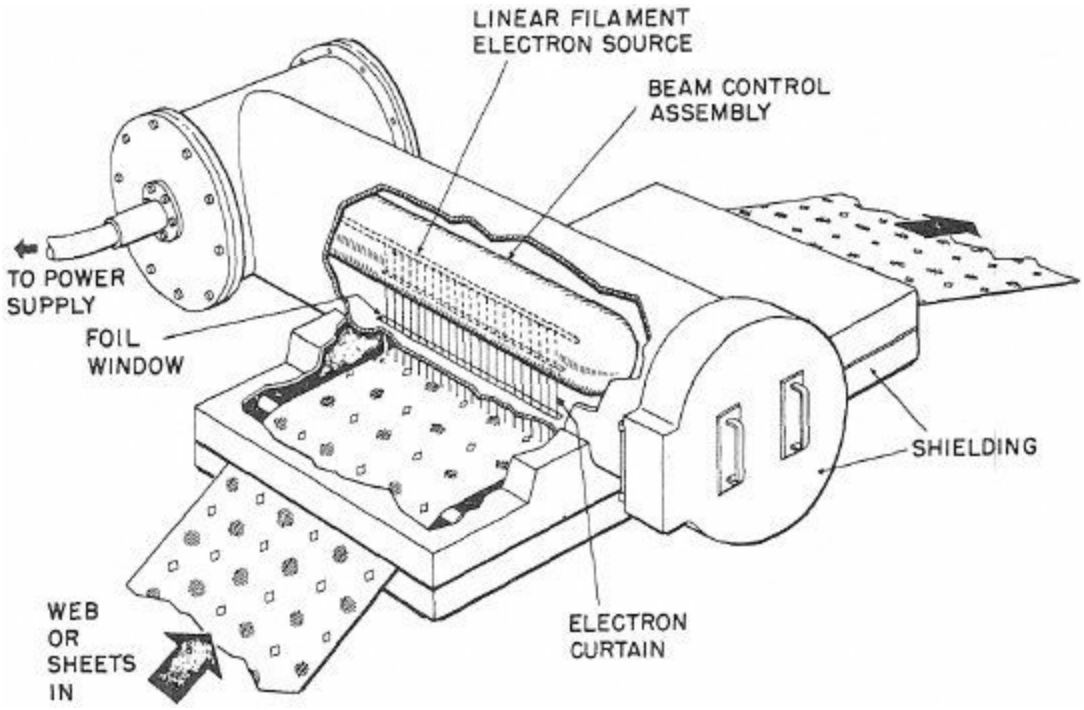

Figure 3. Schematic of the Electrocurtain® Processor.

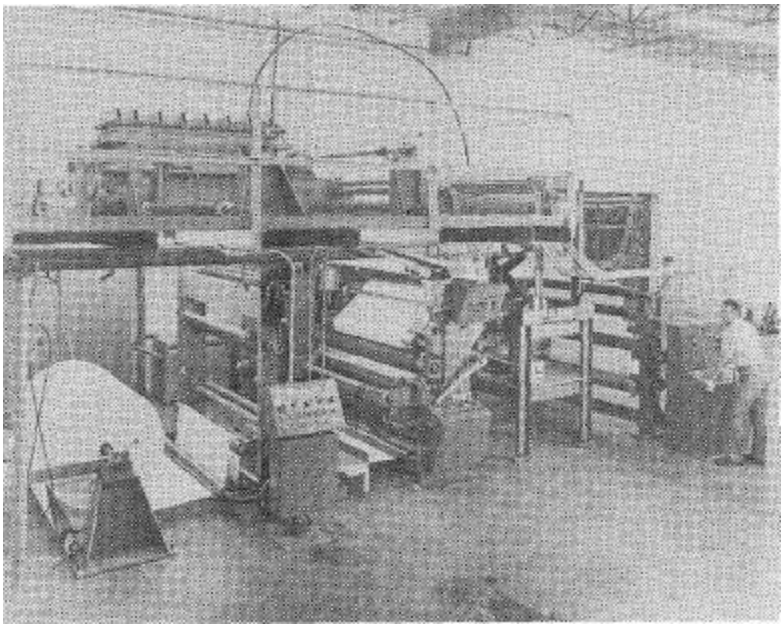

Figure 4. CB 175/170/110 Electrocurtain® installation on a 66 inch coater-laminator.

Due to the nature of these commercial finishing applications, automated control of the processor is mandatory. A schematic of the control philosophy used with these self-shielded curtain type machines is shown in Figure 6. In general, the control signal is provided by a tachometer driven by the product handling machinery. The "dose to cure" (sic: to sterilize) can then be set so that the operational amplifier, acting as a comparator, maintains the machine current (dose rate) so that the pre-set dose is delivered regardless of product line speed. This feature is particularly important where expensive substrates are being handled in short runs at high speed — waste is nearly eliminated and uniform product quality assured.

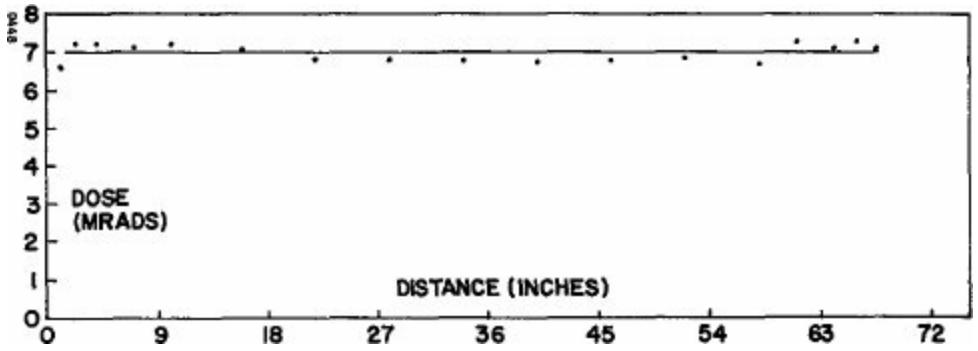

Figure 5. 1.7 meter Electrocurtain® uniformity map. January 10, 1978. (100 ma × 101 fpm × 165 kV)

In the foregoing applications, "transverse" motion of the product to the beam is typically employed. However, for certain uses involving cylindrically symmetric products such as yarn, filaments, tubing and wire, "longitudinal" motion of the product is used to achieve higher product speeds. Such a system is shown in Figure 7(a) in which a 30 cm long × 20 ma × 250 kV system is used to illuminate a product treatment zone some 5 cm in width. The 10 pass unit shown in greater detail in Figure 7(b) will deliver one Megarad at 4570 m/min (15,000 fpm) utilizing the backscattered albedo from the water cooled plate over which the product moves. Depth: dose and uniformity measurements have shown this system to provide adequate uniformity for shrink tubing applications (which is considerably more demanding than the traditional extruded jacketing cross-linking) - its potential for surgical tubing sterilization is obvious.

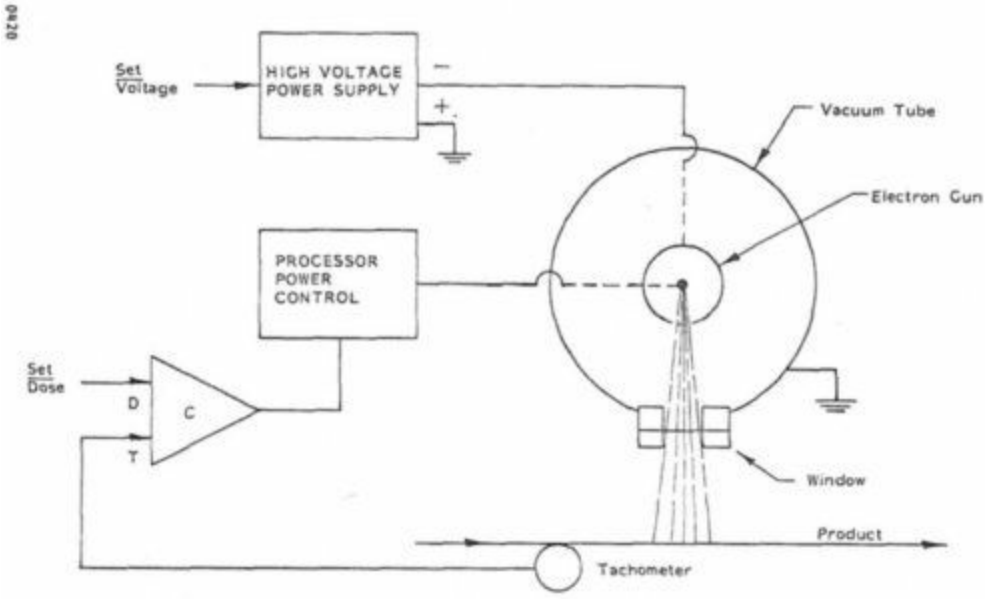

Figure 6. Typical Electrocurtain® Processor schematic.

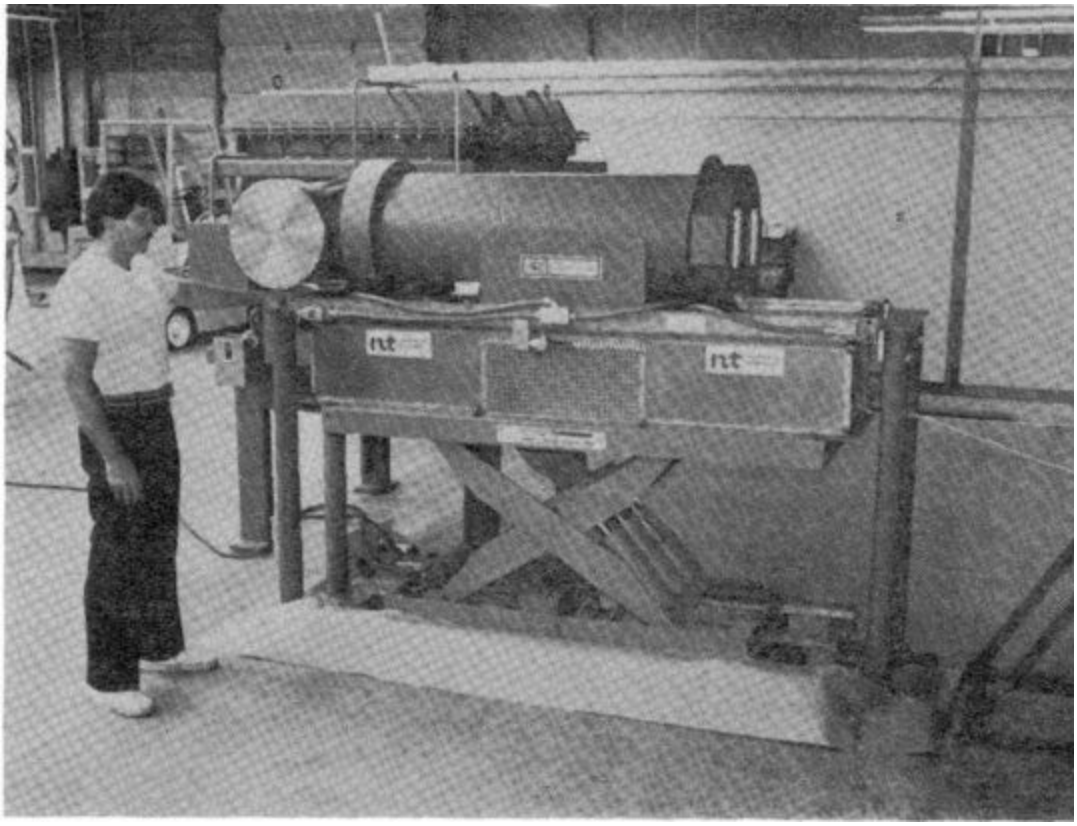

Figure 7(a). CB 258/30/20 Electrocurtain ® with Selfshield® wire handling assembly.

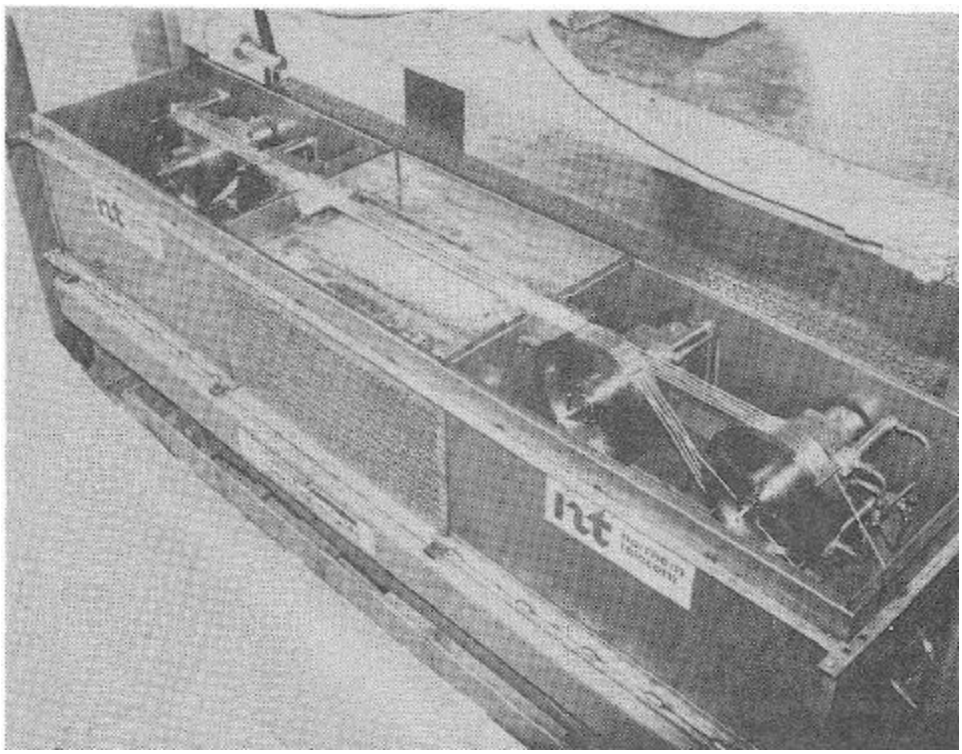

Figure 7(b). Ten-pass Selfshield® system for wire/filament processing.

Finally, one should note the development of compact pulsed electron beam machines for industrial sterilization use. A self-contained Electropulse® unit 600 mm wide × 1100 mm deep × 1200 mm high is shown schematically in Figure 8 and occupies a volume of only 1.2 m^3. This system utilizes dual

(bilateral) self-shielded electron guns which can illuminate web or sheet for surface sterilization at the relatively low speeds required. Power levels in such devices are modest (1–10 kW) and the instantaneous dose rates (10^{12} – 10^{13} rads/sec) compared with the lower dose rates (10^{7} – 10^{8} rads/sec) in the d.c. systems described earlier. It seems clear that the pulsed system offers advantages of compactness, high dose rate and simplicity of construction although there has been relatively little commercial experience with this equipment to date. This is partly due to the fact that such high dose rates are not desirable for the free-radical-initiation of addition polymerization (curing) but may, in fact, offer significant advantage for reduction of physical damage (10) in labile materials.

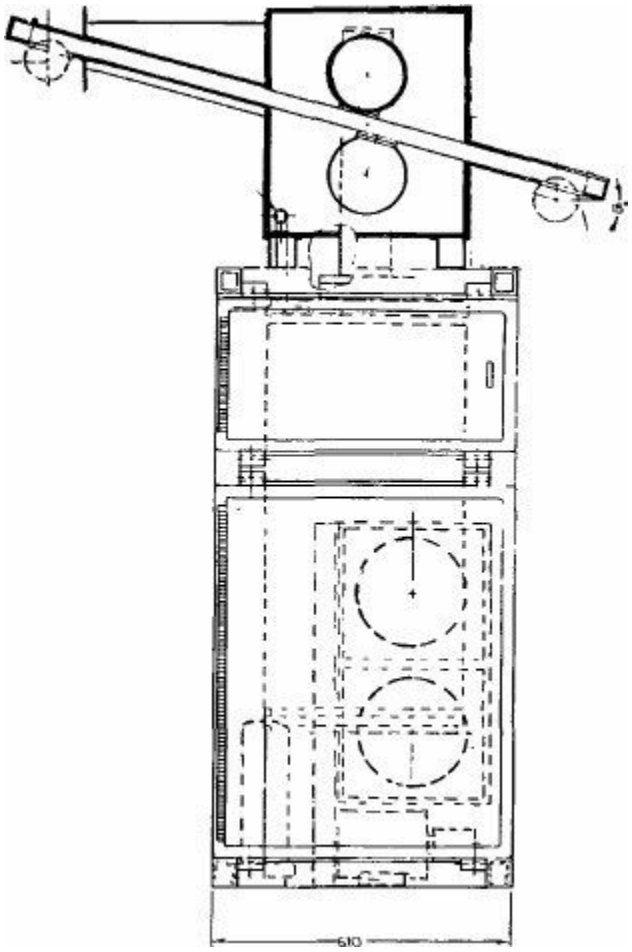

Figure 8. Schematic of the Electropulse® 306 Sterilizer.

Rather more conventional self-shielded machines at 300 kV (High Voltage Engineering Corp. and Radiation Dynamics, Inc. in the US) and at 150 kV (Polymer-Physik in West Germany) are in use commercially so that rather broad experience with this type of machinery is being accumulated within a diverse spectrum of industries.

Sterilization Assurance

It is evident that the availability of compact, in-line machinery will now permit a number of unique geometries appropriate to product sterilization. It is surprising, at first examination, that so little work has been done along these lines to date. The reason for this lies in the profound differences in the quality assurance programs associated with in-line and batch treatment. The Q.A. technology developed for terminal CO^{60} sterilization is totally inappropriate for in-line electron sterilization. Yet the fact that terminal X-ray sterilization is philosophically close to that of batch steam/heat/ETO sterilization in terms of product flow logistics (11), has made its entry into the surgical goods industry much less "disturbing".

It is somewhat fortuitous that the major market areas for inline electron *curing* are equally as demanding, in terms of treatment monitoring and control, as are most applications of it as a *sterilizing* agent. Experience has shown that most curing, in its general form, is relatively undemanding in that as long as the threshold value for "cure" is achieved, the physical properties of the coating or adhesive are unaffected by overdoses by as much as on order of magnitude! Of greater concern is the level of unreacted volatiles which remain in the product after curing (12). If such converted products are to be used in direct or indirect food contact applications, the "safety" of the cured materials will have been determined from gas chromatographic data taken on films cured under well controlled conditions. It therefore becomes desirable to monitor such critical processes with a monitoring system which can evaluate, measure and store, if necessary, data relating to electron penetration, uniformity and dose. Furthermore, it is desirable to perform these functions in "real time" so that the information can be used for instantaneous process control, as well as for archival storage and retrieval for quality control use.

One approach to this problem for a commercial scanned electron processor was utilized many years ago by the Riso group (13). The scheme is shown in Figure 9 and involves the use of fixed plates in vacuo which can monitor the albedo electron flux from the window as a measure of direct electron flux. This can be related to the electron fluence received by the product and hence offers a technique for instantaneous dose control. Such a scheme has also been used for beam "centering" to ensure uniform illumination of the window (and hence the product) by the accelerated beam.

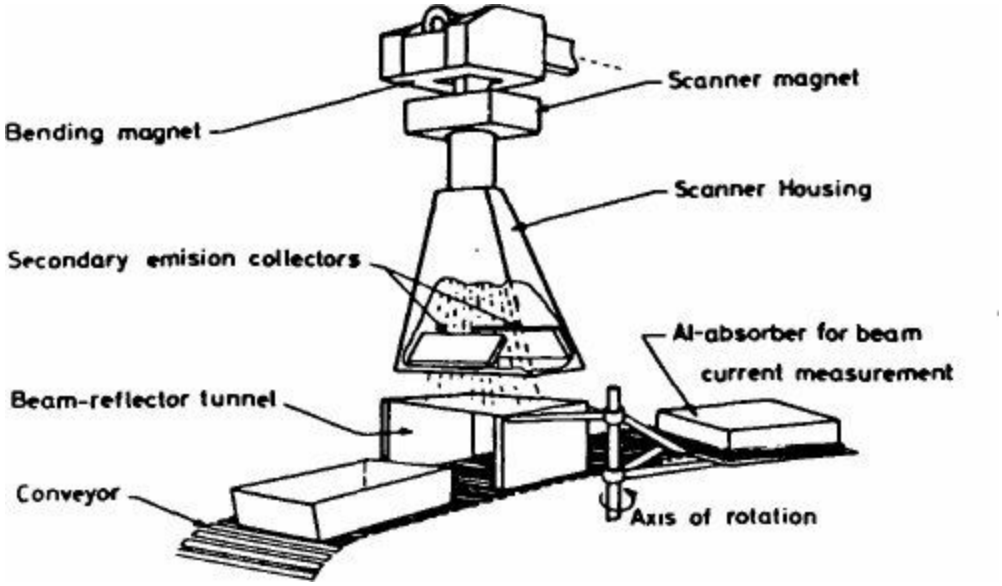

Figure 9. Sketch of beam monitoring system at the Risö accelerator plant.

In the application of the self-shielded processors/sterilizers described in the previous section, another "indirect" route to real time monitoring has been utilized. Such units normally utilize a supported electron beam window so that some conductive cooling of the window foil, in addition to mechanical support along both areas, is provided. Of necessity, these support struts or ribs are "thick" with respect to the electron beam range. As a consequence, the 20% or so of the direct beam which strikes them is fully absorbed, and serves as an intense source of penetrating bremsstrahlung. This bremsstrahlung can now be used to sample the electron flux at the window plane, and hence indirectly, at the sample plane.

Since the bremsstrahlung has good penetrating power, the sampling can be accomplished by monitoring it *through* most of the products of interest in packaging or web sterilization, or in paper/film/foil converting. One such geometry which is being applied to our Selfshield® product handling assemblies which are utilized with the Electrocurtain® and Electropulse® processors, is shown in Figure 10. It uses either a scannable narrow angle X-ray detector, or a fixed array of X-ray detectors, with critical filters. The bremsstrahlung spectrum and hence the *electron energy* can be determined by simple critical edge absorber techniques, while the electron delivered *dose rate* can be related directly to the detector current. *Uniformity* is determined in realtime as well by scanning of the single detector, or by the use of several wide angle fixed channels.

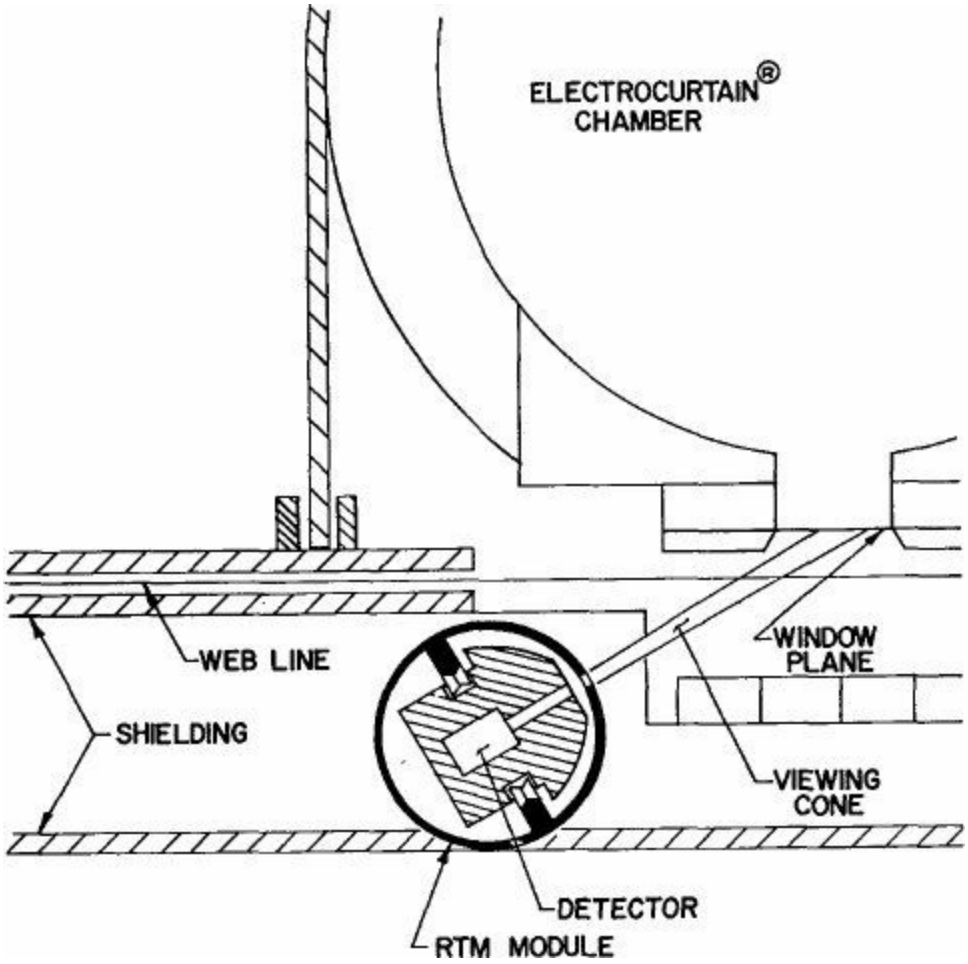

Figure 10. RTRM geometry in Selfshield® system.

It seems likely that the use of digitized data from such a monitor, for archival tape storage and real time control will ultimately eliminate the need for the laborious and time consuming techniques of electron dosimetry, which are particularly onerous in the energy region of interest here (less than 500 keV). The real time process monitor should prove to be an essential element in such critical applications of energetic electrons in the future.

Conclusion

The successful experiences with in-line electron processors over the past ten years have established a firm base for their application in many new industrial fields where “all-electric” processing can be used advantageously. The ability to monitor these systems in real time will offer a new standard of process control when compared with the thermally and chemically initiated processes which they replace. It seems certain that the sterilization of converted products and aseptic packaging are areas which can take full advantage of these benefits.

Acknowledgement

The author acknowledges the contributions of R.N. Cheever to the equipment and concepts discussed in this paper.

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Technological Innovations for Pyrogen Testing with *Limulus* Amebocyte Lysate (LAL)

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Introduction

For 37 years the rabbit pyrogen test was the only practical pyrogen test procedure described in regulatory compendia throughout the world. Although it has met its purpose relatively well over the years, it remains an elaborate procedure that is subject to the test variability inherent in all biological assays. The need for a simple, specific, and accurate pyrogen test has been recognized. Although a number of test systems have been devised for endotoxin detection, only the *Limulus* Amebocyte Lysate (LAL) test is potentially a satisfactory alternative to the USP rabbit pyrogen test.

Levin and Bang (1,2) were the first to report that thermostable endotoxin preparations isolated from *E. coli* and a marine *Vibrio* induced the extracellular coagulation of *Limulus* hemolymph. Their work demonstrated that amebocytes were required for coagulation, and that breaking up the amebocytes enhanced the clotting reaction. Levin et al. (3) later developed a sensitive LAL assay for bacterial endotoxin in human plasma using material lysed from amebocytes. As little as 0.5 ng/ml endotoxin could be detected, and the rate of the reaction was shown to be dependent on the concentration of endotoxin. Yin and co-workers (4) refined Levin's original assay so that picogram amounts of endotoxin could be detected and demonstrated that the Lipid A portion of endotoxin was responsible for lysate gelation.

With the development of commercial lysates, the sensitivity of which could be controlled from lot to lot, and growing awareness of the LAL test among regulatory agencies, a number of workers investigated its potential use in the health care industry for pyrogen testing water, drugs, biologicals, and medical devices. Today, the LAL assay is recognized as a simple, rapid, inexpensive, and accurate replacement for the more cumbersome and variable USP rabbit pyrogen test. A number of papers have been published demonstrating the superior utility of the LAL endotoxin assay relative to the official rabbit pyrogen test (5,8).

In November 1977, the Bureau of Biologics (BOB) accepted the LAL assay as a replacement for the USP rabbit pyrogen test with the provision that the manufacturer submitted parallel test data documenting that their LAL test was as sensitive as, or more sensitive than, the rabbit pyrogen test (9). Since 1977, the Bureau of Medical Devices (BMD) has also provisionally permitted the use of LAL for final release of medical devices and like the BOB, requires that parallel data be generated using rabbits and LAL to document the validity of the LAL test (10). In the near future, the BMD is expected to release final guidelines for adoption of the LAL test for pyrogenicity (11). The draft guidelines currently request manufacturers to include the following in their submissions:

1. Data demonstrating the sensitivity and reproducibility of the LAL test if the manufacturer intends to pass/fail lots based on the LAL test. A manufacturer must be able to demonstrate a test failure rate of 90% or greater at the sensitivity endpoint determined for the "average" rabbit colony, i.e., 0.1 ng/ml of *E. coli* 055:B5 endotoxin (11). Thus the BMD will permit as much as 100 pg/ml of endotoxin in medical device rinses and eluates, which is equivalent to the USP rabbit pyrogen test standard (12).
2. Checking for inhibition or activation of the LAL test by the different materials and methods of manufacture used in each product line of devices
3. Parallel testing of device production lots by both rabbit and LAL.

On January 18, 1980, the Bureau of Drugs (BOD) issued a Federal Register announcement of the availability of "Draft Guidelines for the Validation of the Limulus Amebocyte Lysate Test as an End-Product Pyrogen Test for Human and Veterinary Injectable Drugs Other Than Licensed Biologicals"

(13). Responses from industry had to be made to the Hearing Clerk by March 18, 1980. Some of the industry's requests were incorporated in a subsequent draft guideline, which should be published this year. This document requests manufacturers to comply with the following:

1. Determine the sensitivity of lysate being used. The BOD will permit no more than 50 pg/ml in a final product with the exception of intrathecals for which the limit will be even lower.
2. Before using the LAL test for end product pyrogenicity testing, it must be determined that the product does not inhibit or activate the LAL gelation reaction.
3. Attachment A of the draft guidelines provides an initial quality control procedure to assess the proficiency of a testing laboratory using LAL.
4. Attachment C of the draft guidelines provides a mechanism for determining the relationship between the control standard endotoxin (CSE) used by the given laboratory, and the national reference standard endotoxin (RSE).
5. A method of determining the maximum valid dilution of product is presented in Attachment D. This determination is predicated on the maximum human dose/kg or the USP rabbit dose, whichever is more stringent.

Methods of Endotoxin Detection with LAL

1. *Clot Endpoint*

The simplest and most widely used procedure for the detection of endotoxin in solutions is the clot end point (14,15). An equal volume of lysate and test solution (0.1 ml of each) are mixed in depyrogenated 10 × 75 mm glass test tubes. The mixture is then agitated gently and incubated in a water bath at 37°C for 1 hour. Because the LAL clot end point of some lysates is modified by handling, the reaction mixture is usually incubated uninterrupted. The end point is read easily by carefully withdrawing each tube from the water bath and inverting the tube through 180° to determine the clot end point. If a solid clot is formed and remains solid through inversion, the test solution is said to be positive for endotoxin. When used in this manner, the clot end point is primarily a pass/fail test, limited only by the sensitivity of the LAL employed. However, this sensitivity can be used to indicate quantity. Thus, if an internal manufacturing specification called for endotoxin levels to be less than 0.0625 ng/ml for a particular product, and the gel clot end point were positive with lysate of this sensitivity, the product would fail.

Because several commercial lysates are now available that have various standardized end points, the clot end point may also be used to quantify the level of endotoxin in a particular solution or production. A number of twofold serial dilutions of the test solution are made and the clot end point determined. The level of endotoxin is calculated by multiplying the reciprocal of the highest dilution of the test solution, giving a positive end point by the sensitivity of the lysate preparation. For example, if the sensitivity of the LAL employed were 0.010 ng/ml, and the dilution end point were 1:16, then the endotoxin concentration would be 16 × 0.010 = 0.16 ng/ml. This approach is particularly helpful in monitoring in-process materials and water. A positive control consisting of a product sample spiked with a known concentration of endotoxin and a negative control using nonpyrogenic water should be used in all LAL test procedures.

2. *Turbidimetric Assay*

Although the solid gel clot assay is the most widely used LAL test, it has the disadvantage of being an “early end point.” Consequently, if it is used, endotoxin can not be quantified below the level at which a solid clot is formed. The LAL turbidimetric assay, on the other hand, gives a more quantitative measurement of endotoxin over a range of concentrations (15). This assay is predicated on the fact that a proportional increase in endotoxin concentration causes a proportional increment in turbibity due to the precipitation of coagulable protein in lysate (coagulogen). Thus, the optical density of various dilutions of the substance to be tested are read against a standard curve obtained using samples of the test substance spiked with known quantities of endotoxin. It should be kept in mind that quantification of endotoxin by a turbidimetric method requires that lysate be diluted to prevent clot formation. As can be seen in Figure 1, the clot end point was reached at 400 pg/ml. However, the LAL reagent was still able to detect levels of endotoxin ranging from 6 to 200 pg/ml of endotoxin. Although a simple clot end point may be adequate for routine release testing of various pharmaceuticals, the ability to quantify endotoxin is invaluable for troubleshooting production-related pyrogen problems. Daily monitoring of plant water and in-process testing can alert production personnel to potential pyrogen problems before they become critical. Corrective action can be taken to reduce pyrogen loads and levels of endotoxin at this time.

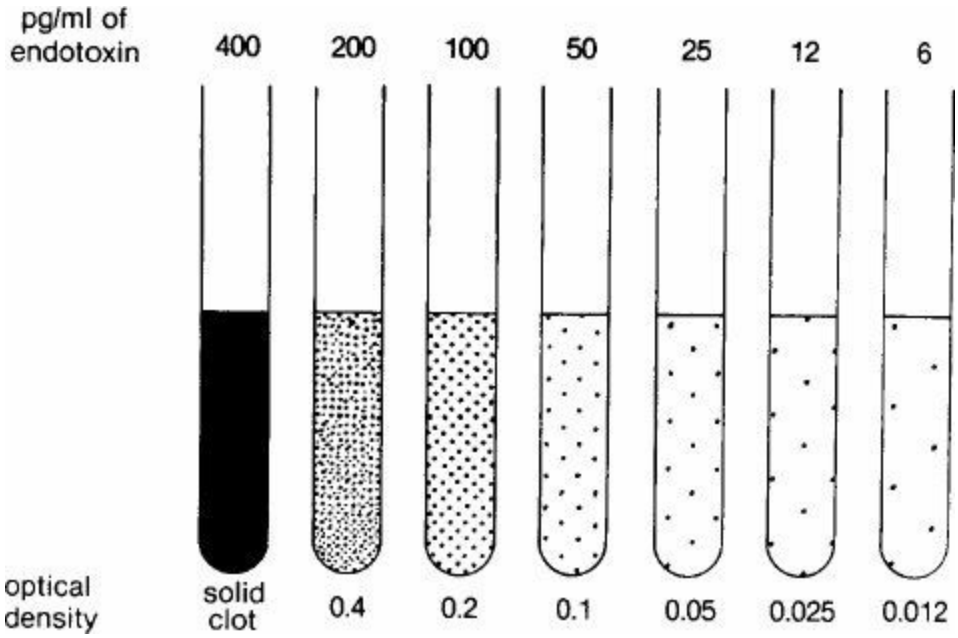

Figure 1. Quantitative LAL assay showing the increased sensitivity relative to the clot end point.

Currently, two commercial manufacturers of LAL supply lysate reagent that can be used in a quantitative, turbidimetric assay procedure (16). Lysate available from Worthington Biochemical Corporation, a subsidiary of Millipore Corporation, can detect as little as 5 pg/ml of the firm's reference endotoxin.

A typical standard curve using the turbidimetric method under plant conditions can be seen in Figure 2. Three samples of water of the day were tested, having an optical density of 0.133, 0.134, and 0.134, respectively, and measured against the standard curve. All of these samples were below the 50 pg/ml alert limit.

One of the major problems with the tubidimetric method is lotto-lot variability of lysate as can be seen in Figure 3. At the present time, the sensitivity of the lysate can be controlled relatively easily. However, when the endpoint is amplified spectrophotometrically, variability of lots is also increased.

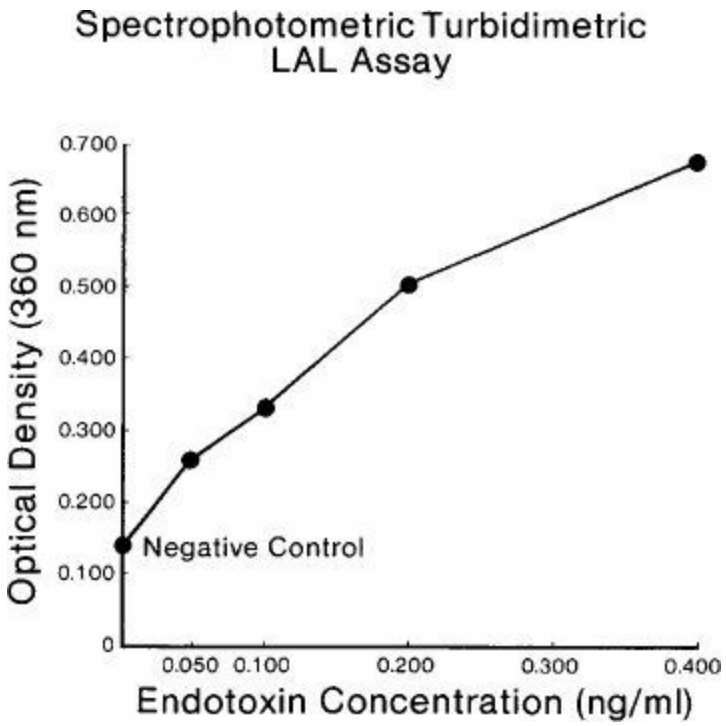

Figure 2. Sample spectrophotometer turbidimetric LAL assay curve.

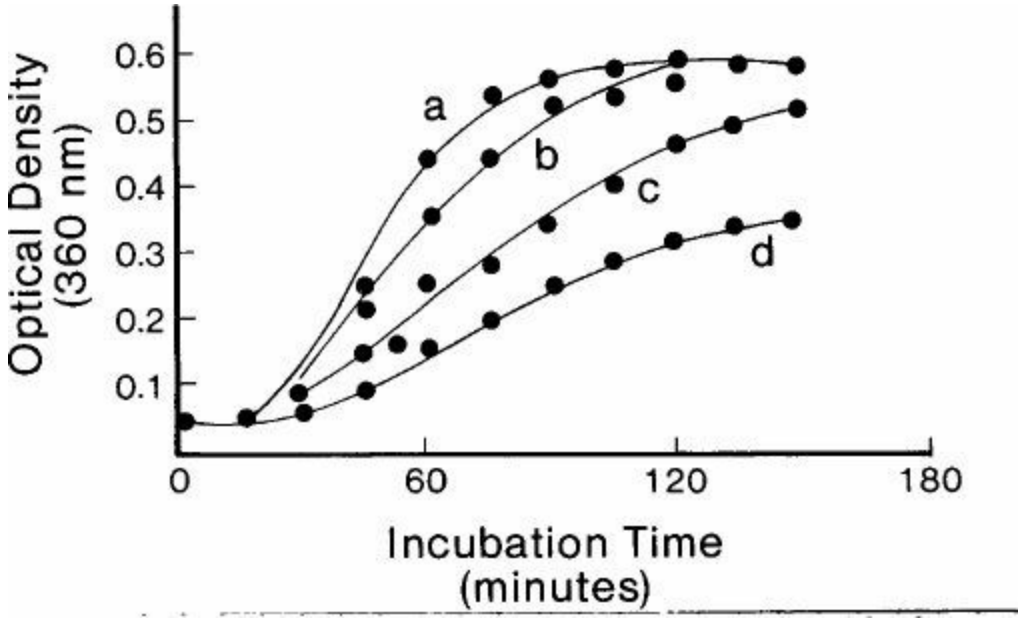

Figure 3. Lot-to-lot variability of lysate that may occur with the turbidimetric assay.*

3. *Lowry Protein—Colorimetric Method*

The Travenol Optical Density-Lowry Protein LAL method also offers all the advantages of a quantitative test for endotoxin. Like the turbidimetric method, it is based on the observation that increasing concentrations of endotoxin will precipitate proportionally increasing amounts of lysate protein. Thus, the amount of lysate-specific protein (coagulogen) that is precipitated can be quantified by peforming a simple Lowry protein determination (17).

As was done with the other methods, equal volumes of test sample and lysate (0.1 ml) are mixed in 10 × 75 mm pyrogen-free test tubes. Tubes are incubated at 37°C for one hour and then centrifuged

at approximately 1375 × g in a clinical centrifuge for ten minutes. Supernatant is then removed from precipitated protein by vacuum aspiration. The amount of lysate specific precipitated protein is determined using the Oyama and Eagle (18) modification of the Lowry protein assay (17). Using a flow-through spectrophotometer, the optical density is recorded at 660 nm. The results can be related to a standard reference curve, as described below.

It is necessary to run positive and negative controls with each test to rule out the possibility of false positives due to contamination, and false negatives due to the inhibition of the clotting reaction by the test sample. Negative controls consist of solutions identical in chemical composition to the test samples that have previously been tested and shown to be nonpyrogenic by measuring endotoxin precipitable protein. Solutions are acceptable for use as negative controls if the optical density of the precipitable protein is less than 0.2.

Positive controls consist of three concentrations of an *E. coli* endotoxin standard (055:B5; Difco Laboratories, Detroit, Michigan), prepared in the same solution used for the negative control. The three endotoxin concentrations—200 pg/ml, 50 pg/ml, and 12 pg/ml—are used to generate a standard curve of endotoxin optical density against which test samples are compared. Test samples of devices with optical density values at or above that of the 50 pg/ml endotoxin control samples are considered to have failed the lysate test.

The Travenol LAL test procedure was introduced into domestic and international plants early in 1974. It rapidly became the most important quality control tool for in-process pyrogen testing of raw materials, plant water, and parenteral solutions. For the first time, using the Travenol LAL test, a method was available for monitoring nonpyrogenic levels of endotoxin that remained undetected when the rabbit test was used. During the first year of use, the LAL test served to monitor the gradual elimination of background levels of endotoxin as applications of new technologies were introduced in manufacturing. Since that time, LAL testing facilities have been installed in 11 domestic plants and 15 international plants where thousands of LAL tests are performed (18).

4. *Nephelometric Method*

Currently, a nephelometric LAL pyrogen test is under investigation as a possible means of pyrogen detection in LVP's (19). This method employs a Hyland PDQ® Laser nephelometer which is designed to quantify specific protein concentrations. Unlike the spectrophotometric method, in which endotoxin concentration is read as optical density, the nephelometric methods reads relative light scattering (RLS) and is more sensitive and accurate (Figure 4). Sodium dodecyl sulfate (SDS) is used to terminate the enzymatic reaction at its optimal point and to ensure that the particle size of precipitated lysate-specific protein is uniform. Sodium carboxymethylcellulose (CMC) is then added as a suspension stabilizer. Like most other LAL procedures, the test is rapid and can be completed within an hour. Only 50 μl of lysate are required for the test. It can readily detect picogram amounts of endotoxin and exhibits a coefficient of variation of 8.6% at 50 pg/ml endotoxin.

Nishi and colleagues (20) reported on a semiautomated nephelometric procedure for the detection of bacterial endotoxin. The assay is conducted by mixing 5 μl of sample in the presence of 150 μl of lysate. The intensity of light scattering is recorded every five minutes on a strip chart recorder and printed on paper tape. Although the procedure uses more lysate than the conventional LAL assay, more tests can be performed simultaneously, and more precise end points can be determined than with either the clot end point or the current turbidimetric methods. The coefficient of variation of samples

spiked with 200 pg/ml of endotoxin was 1.0% within a run. Variation over trials was reported to be 2.1%. As little as 10 pg/ml of endotoxin could be detected, which is comparable to levels reached with the clot end point when the most sensitive lysate preparations are employed. However, the clot end point suffers from as much as a twofold variation on either side of the end point. This assay does not appear to be as sensitive as the chromogenic substrate assay which will be discussed later.

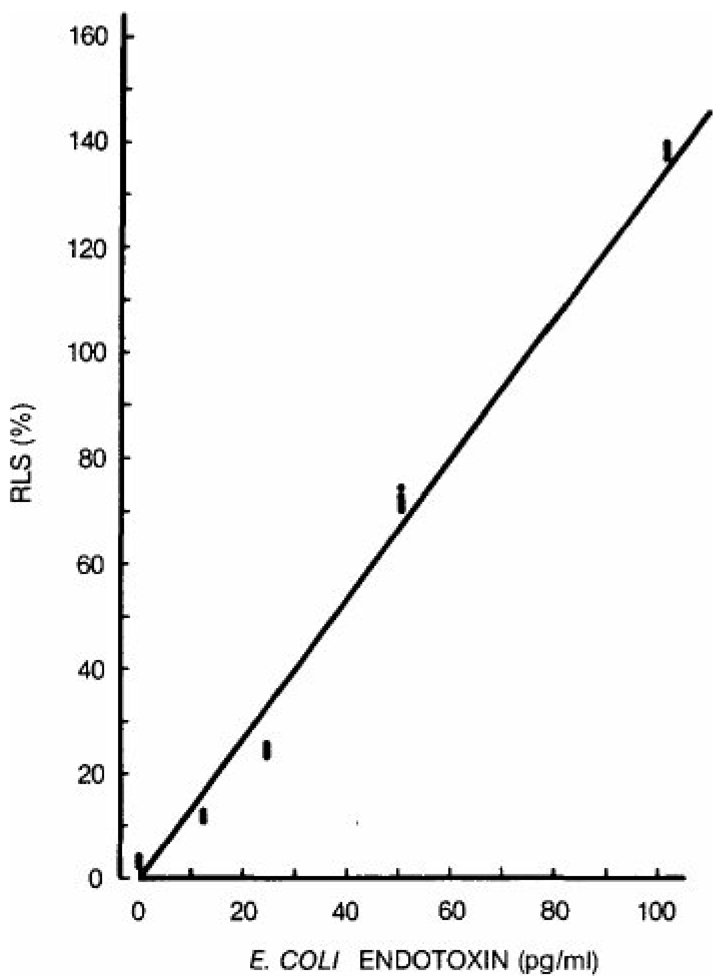

Figure 4. Standard curve for nephelometric procedure.

5. *Slide Test*

Frauch (21), was the first to suggest a simple LAL slide test. Using a calibrated capillary pipet, only 10 μl of lysate is mixed with an equal volume of sample. This preparation is incubated in a moist chamber at 37°C for 30 minutes (reducing conventional LAL incubation by a factor of two). A positive control, a negative control, and test samples are prepared on slides with a black background. They are easily differentiated on the basis of viscosity and turbidity. Little enthusiasm was generated for this method during the mid 1970s, but recent work by Japanese and British investigators is indicative of renewed interest in this simpler, more rapid, and inexpensive LAL test.

Goto and Nakamura (22) developed a slide test using only 20 μl of lysate reagent and employing a novel end point. Lysate and sample are mixed on a silicone-coated glass slide and incubated at 37°C for 30 minutes. Then the reaction mixture is "dried up" and forms a configuration on the slide that is consistent with the end point. Negative reactions exhibit a characteristic central ring. The end point achieved is 100 pg/ml, which is a function of the sensitivity of the lysate used (Pregel, Teikoku Hormone Mfg. Co., Japan). The end point of this system could be lowered by using a more sensitive

lystate or increasing the amount of Pregel lysate employed in the reaction mixture.

Another Japanese worker, Okuguchi (23), reported an improved LAL microslide method employing 10 μl of lysate. Samples are incubated in the presence of lysate on a tissue culture chamber slide for 30 minutes at 37°C. Test samples are then stained with one drop of bromphenol blue (BPB) and end points determined using an inverted phase contrast microscope. Samples forming a ring filled with cell debris are negative for endotoxin, but positive samples exhibit a cloud-like formation throughout the mixture. Typical reactions can be seen in Figure 5. While the modified slide procedure compares favorably with Frauch's microslide method and the clot end point, endotoxin can be detected only at a concentration of up to 10^{-3} μg/ml. Again, this is a function of the sensitivity and concentration of lysate used in the system. Unlike the clot end point, nephelometric, turbidimetric, and chromogenic substrate assays, the microslide method has not been subjected to statistical analyses of its accuracy and precision. The single greatest virtue of the microslide method is lysate cost savings, because the price of lysate is considerably higher elsewhere than it is in the United States.

Flowers (24), also reported a modified slide method in which only 10 μl of LAL is used. A 9 mm depyrogenated glass tube which has been dipped in paraffin is placed on a nonpyrogenic glass slide creating a well in which the reaction is placed. End points are read by placing a glass capillary tube in the center of the ring and measuring the flow of reaction mixture into the tube. Because the flow is inversely proportional to the endotoxin concentration, minimal flow constitutes a positive reaction and maximal flow indicates a negative sample. Positive tests cause a rise of only 4 mm, whereas sample mixtures containing little or no endotoxin rise 10 to 12 mm. End points between 4 and 6 mm are regarded as equivocal. Positive and negative reactions are shown in Figure 6.

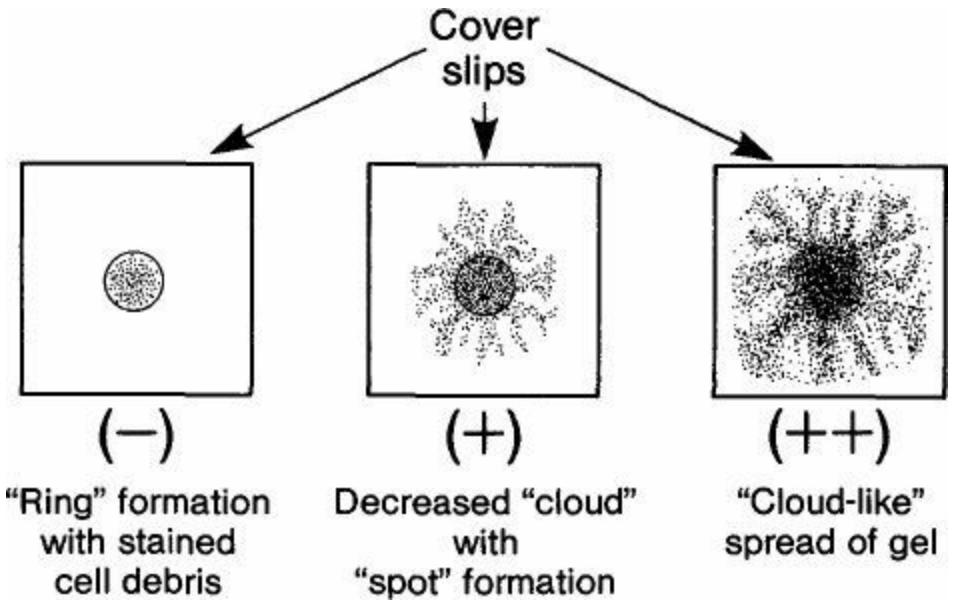

Figure 5. Typical reactions using the improved LAL microslide method.*

6. *Chromogenic Substrate*

Japanese workers have recently pioneered the use of chromogenic substrates and lysate (from *Limulus* and from *Tachypleus* the Japanese Horseshoe crab) for the detection of endotoxin (24, 25). This method takes advantage of the specificity of the endotoxin-activated proclotting enzyme, which exhibits specific amidase activity for carboxy terminal glycine-arginine residues. When such sequences are conjugated to a chromogenic substance, p-nitroanilide (PNA) is released in proportion

to increasing concentrations of endotoxin. Thus, it is possible to measure endotoxin concentration by measuring endotoxin-induced amidase activity as release of chromophore. Release of chromogenic substrate is measured by reading absorbance at 405 nm. Testing is conducted with 50 μl of lysate. The quantitative relationship between the logarithm of the endotoxin concentration and amidase activity can be observed between 5×10^{-6} and 5×10^{-2} μg/ml of endotoxin and, therefore, can be used for detection of picogram quantities of endotoxin associated with medical device eluates, immersion rinse solutions, and certain drug products.

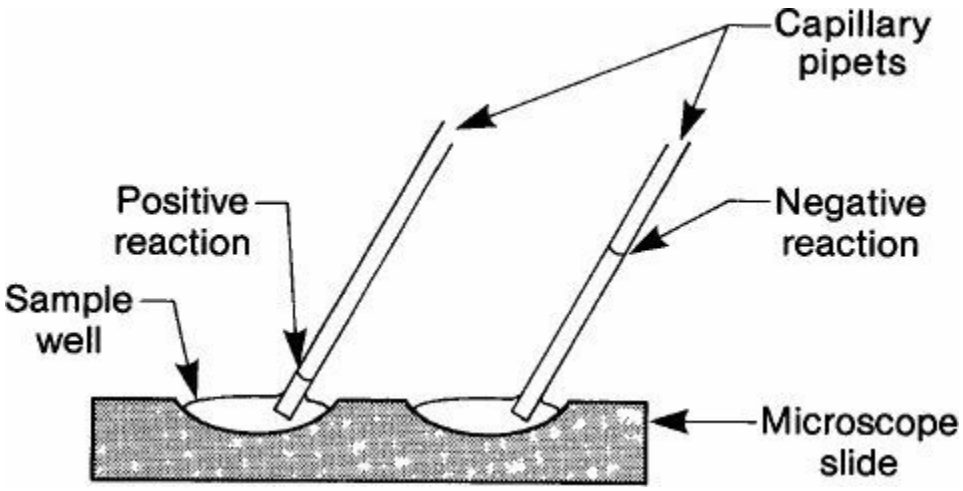

Figure 6. Examples of positive and negative LAL reactions using the improved capillary pipet procedure.*

During 1978, Harada and co-workers (26) reported that of 20 peptide-4-methyl-coumarin amides (MCA) newly synthesized for fluorogenic substrates of blood clotting factors, only Boc-Leu-Gly-Arg-MCA and Boc-Ser-Gly-Arg-MCA were found to be specific substrates for the LAL clotting enzyme. Several p-nitroanilide chromogenic substrates (PNA) having a COOH terminal Gly-Arg peptide sequence (e.g., Tos-Ile-Gly-Gly-Arg-pNA, Bz-Vol-Gly-Arg-pNA, Boc-Vol-Leu-Gly-Arg-pNA) were also hydrolyzed by horseshoe crab clotting enzyme.

The reaction mixture consists of 50 μl of substrate, 50 ml of Pregel lysate reagent, and 100 μl of sample and must be prepared in test tubes cooled in ice. Test samples are incubated at 37°C for 30 minutes and the reaction stopped by adding 12.5% acetic acid. The major disadvantage of this system is the speed of the reaction, which requires the use of ice-cooled test tubes and limits the number of sequential tests that can be done at any given time. However, endotoxin levels of from 5 pg/ml to 50 ng/ml can be accurately defined. The sensitivity of the lysate system can be increased when fluorometry is used with MCA. These systems are 10 times more sensitive than the clot end point when lysate of the same sensitivity is used to conduct parallel tests.

More recently, an LAL chromogenic substrate assay was reported by Lindsay (27) that requires mixing 0.1 ml of LAL with 0.1 ml of test sample and preincubating the contents for eight minutes. After incubation, the artificial substrate is added (0.5 ml) and the solution is incubated for three minutes. The reaction is then stopped by adding 0.1 ml of acetic acid and the optical density is read at 405 nm, using a spectrophotometer. When the preincubation time is increased, the sensitivity of the test system can also be increased significantly as can be seen in Figures 7 and 8. Although the chromogenic substrate method is extremely accurate over a wide range of endotoxin concentrations, the speed of the reaction prohibits conducting large numbers of LAL tests without introducing

significant variation between samples prepared early in testing and those completed later in the test sequence. The nature of this test may allow it to be automated and provide a hard copy readout.

7. *Microdilution Assay*

Prior and Spagna (28) developed a LAL microtiter assay which requires only 50 μl of LAL, one-half the conventional amount used in most LAL assays. Conventional microdilution U-bottom plates and 50-μl microdiluters are used. The latter are rendered pyrogen-free by flaming the tips in a Fisher burner. Microtiter plates are covered with plastic lids, test samples mixed, and then incubated at 37°C for one hour. End points are determined by adding 50 μl of 0.005% aqueous crystal violet to each well. In samples negative for endotoxin, the stain permeated throughout the reaction mixture, but in those samples forming a solid clot, the stain remained on the top of the clot, thus permitting a simple end point determination to be made. No significant difference in sensitivity was found between parallel clot end point and microdilution end point assays.

Although the authors claim the method is economical, they did not present cost comparison data. They also state that 25 μl of lysate could be used to further reduce the cost of performing the assay, but data were not included to substantiate the suggestion.

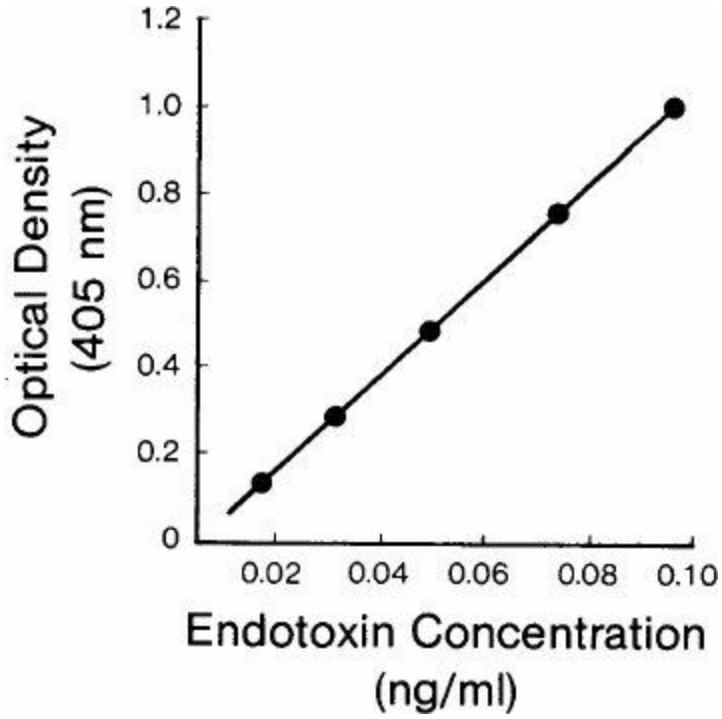

Figure 7. Chromogenic assay standard curve using an 8-minute preincubation and a 3-minute substrate incubation.

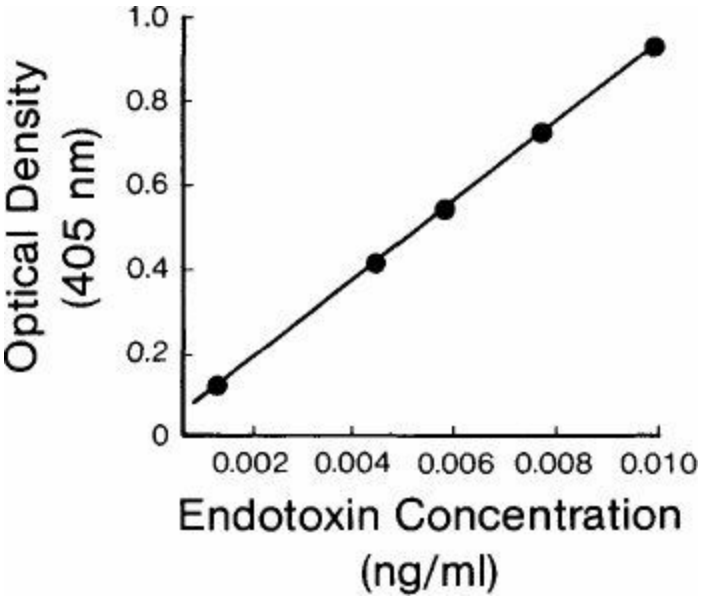

Figure 8. Chromogenic assay standard curve using a 20-minute preincubation and a 3-minute substrate incubation.

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DISCUSSION SESSION II

Q. by C.W. Bruch – USA

What percentage of sterilized supplies in Swedish hospitals are resterilized? Do the Swedish authorities encourage the reuse of sterlized disposable supplies?

A. by B. Nyström – Sweden

I did not talk about resterilization of disposable supplies. I talked about resterilization of nondisposable supplies like surgical instruments and nondisposable conventional operating room textiles. We do not approve of resterilizing things that have been marked to be disposable.

Q. by A. Skopek – Australia

Have you generated any data on the efficacy of cold sterilization of nondisposable surgical instruments using, for instance, activated gluteraldehyde solution?

A. by B. Nyström – Sweden

There is work in progress on evaluating a low temperature steam and formaldehyde process. There is no work in progress in my country, as far as I know, as to evaluation of any gluteraldehyde process.

Q. from the floor:

If you had to work in an ETO contaminated environment, what ETO level, in air, would you accept for your own safety? (8 hours per day, for 5 days a week, for 25 years).

A. by J.E. Willson – USA

This question has not really been solved yet. My own opinion would be that I would certainly want to be below 10 parts per million on **a** time weighted average.

Q. by R. Garrison – USA

The data on very long real expiration lives in commerce are heartening. Would you comment on the possibility that novel packaging development would be stymied by lack of similar long term data, an unwillingness to depart from the known?

A. by G.B. Phillips – USA

No, I do not think so. I think that none of us feel that we have perfect packaging. I think that we all realize the hazards that occur in breathable packages and I think we understand the liabilities that packages can be punctured inadvertently creating contamination. We have all heard the stories of goods being left out on the loading dock and having gotten wet and later dried. Moisture penetrating the package may carry organisms into it also. So, I would hope that our observations on shelf life or the maintenance of sterility being an event-related rather than a time-related thing would not thwart the packaging people in doing a better job and continue to give us better packages. Incidentally, just from the FDA's point of view, if you look at recalls over the years, what has been recalled for sterility purposes or for nonsterility, I believe you will find that approximately 85% of the recalls have been, not because of proven nonsterility, but because of open packages.

Q. by W. Bradbury – USA

In the three year blister package stress test, what was the microbial challenge? Was this monitored and/or controlled in the other studies mentioned?

A. by G.B. Phillips – USA

I am repeating some data that was given to me. As far as I am aware, there was no microbial challenge other than just stressing the package under the physical conditions of the temperature and humidity that I mentioned. There was no microbial challenge. These were just safety testing of the product.

Q. by G. Whittaker – USA

How do you insulate the high voltage CRT from the flammable gas system? What guidelines of GMP are available for validating microprocessor programs for sterilizers?

A. by A.N. Parisi – USA

We do use pure ethylene oxide. All the interactions with the vessels are automatic by the air pressure lines. There is actually no electrical interfacing with the vessel. As for thermocouples, they go through an entrance safety barrier. It is all approved by our insurance carriers. There is a validation procedure. There is a formal procedure for validating the computer program for the operation of the various cycles.

Q. from the floor:

Would you not agree that thermal sterilization kinetics is only a first order process only when a single species of bacterium is involved? That it is not a straight line semilog death curve for mixed microbes?

A. by F. Halleck – USA

I agree.

Q. by J.L. Whitby – USA

Will the new model Cobalt 60 irradiator deliver a wide range of doses or only say 0.5 Mrad increments? This has implications in the need for accurate dose determination.

A. by J. Masefield – USA

The master timer, the timer that sets the dwell time of the carrier in each position, can be set so as to deliver any increment of dose depending on the mechanical speed limitations of the irradiator. Starting at increments of 0.2 Mrad, you can set the timer for whatever increment you require. But from there on, each carrier must receive a multiple of that increment until you reset the timer setting. Thus, I would envisage a rounding up of doses. With the dose setting strategy presented, you might arrive at a dose of 2.37 Mrad. We would not choose that as a minimum dose. We would increase it (say, to 2.5 Mrad) so that the total dose becomes a multiple of some convenient increment, taking into account the dose requirements of the total range of products being processed in the irradiator at the time.

Q. by P.M. Schneider – USA

What are the consequences and what action should be taken if a positive sterility test is observed on a sample obtained during a distribution center audit? Should one relate the positive to shipping conditions, storage, or a false positive; ignore or recall?

A. by G.B. Phillips – USA

Having not been part of the company that did, I can only conjecture that what should be done would perhaps relate to the age of the product. If it was a Johnson & Johnson gauze package that was perhaps 45 years old, that would be one thing. But if there were various products that perhaps were still in the field, then there would be an obligation to look into it. Obviously, in most of our laboratories, the first question we would probably ask ourselves: "Is that a false positive … is this within the realm of laboratory accident? Perhaps the speciation of the organisms would give you some information on that.

Q. by P.E. Vidal – France

What is the knowledge about ethylene oxide sterilization and resterilization by gamma rays of plastics containing chlorine?

A. by J.E. Willson – USA

I am not sure of the exact date, but this whole question was originally raised by a letter, I think that appeared in a British journal [Cunliffe, A.C. and Wesley, F. Hazards from plastics sterilized by ethylene oxide. British Medical Journal **2**: 575-567, 1967] many years ago, that apparently gamma irradiated plastics that had been subsequently sterilized with ethylene oxide were purported to contain more ethylene chlorohydrin residue. Some work was done under the aegis of the old Sterile Disposable Device Committee of the Health Industry Association. I believe there was a round robin study done to corroborate the British observation and they were unable to show that this was the case. Subsequently, Dr. O'Leary did some work, I believe, at Ethicon in which he confirmed that they were unable to show significantly increased levels of ethylene chlorohydrin.

Finally, there was a publication that came out of Mount Sinai by Dr. R.B. Roberts, entitled "γ – Rays + PVC + EO = OK" (Respiratory Care **21**: 223-224, 1976). That was his equation.

Q. by C.W. Bruch – USA

What percentage of the sterile disposable device manufacturers place a sterilization date on their products? What reason does the industry have for not sterilization dating their products?

A. by G.B. Phillips – USA

My impression is that those who put expiration dates on their packages merely to indicate the expiration of sterility are very few. Obviously there are many other products, and I mentioned some of them, for which, for other reasons, they must give an expiry date. For example, if there is a battery that has a specific life, or if there is a radioisotope that has a specific shelf life. These are the other reasons.

Comment by C.W. Bruch – USA

That does not answer the question. The question relates to sterilization dating, not expiration dating.

The industry is not doing a service to the hospitals who are trying to maintain inventory control. The hospitals should know that the product that they have purchased has not been sitting in a warehouse for a couple of years before it was finally shipped to them. I am rather sensitive to this issue that the industry will not sterilization date their sterile disposables.

Comment by F.E. Halleck – USA

Many of the manufacturers, instead of sterilization dating, use a manufacturing code. If you contact some of them, I am sure they will reveal their coding to determine the manufacturing date. You would get the same information from that batch code as you would with a sterilization date. The manufacturing or batch code gives the user the date at which all the quality control tests were completed to allow the release of the product from quarantined storage. A sterilization date would give the date of operation of only one quality control parameter.

Comment by G.B. Phillips – USA

One of the problems that has occurred is just a mechanical problem. We have always recommended a first in, first out kind of a system. But, the way that products are distributed in this country, the customer may receive a product with a certain date and his next shipment may be from a date of a week earlier. That is very hard to explain. I think it is better to have the hospitals and the users practice as we do in our component inventory—a first in, first out system.

Q. from the floor:

Could I get a clarification on this radiation—ETO story? I think the question was asked about radiation followed by ETO sterilization. I think the data that was offered to say it was OK was for ETO sterilization followed by radiation. The problem, if there is a problem, seems to me would occur if you irradiate first, then ETO sterilize afterwards. When you irradiate PVC, you are going to create a lot of active sites and I question whether the question that was asked was really the question that was answered.

A. by J.E. Willson – USA

You are correct. The answer to the question related to ethylene oxide sterilization *after* irradiation. The original question was ETO sterilization *followed* by irradiation. The question was assumed to be misstated since there is almost no chance of the latter occurring, because apparently there is only one hospital in this hemisphere that has a cobalt unit.

Q. by Z.R. Glaser – USA

On your last slide, you indicated a future US ETO occupational exposure standard of 10 parts per million. Is it not possible that the agency responsible for setting such a standard might come to a different value in the light of new toxicity results? Is the future standard already established? I am not so aware. Also, I am sure that you are aware that the STEL of 75 PPM was only a recommendation of NIOSH? I do not believe that OSHA has yet acted on that recommendation. Am I in error?

A. by J.E. Willson – USA

The figures that I projected in the last slide were taken from the handbook of threshold limit values of the American Conference of Governmental Industrial Hygienists. These, in general, have been adoted by OSHA. The ten part per million future figure is already listed in that booklet as an intended change; generally, intended changes are carried by the Association for two years and then become standard. So these are already pegged into the 10 part per million. Now, certainly, any government regulatory agency has the authority to peg it lower, if they wish.

Comment by Chairman R.W. Campbell – Canada

Mr. Cobis has a very important observation to make on the reuse of disposables and I invite him to present it at this time.

Comment by Mr. Cobis – USA

In 1978, the Veterans Administration issued a strong directive to its 176 hospitals, that no presterilized, disposable products would be reprocessed without written reprocessing procedures given by the device manufacturers. Because of the severe economic impact on our medical care budget, coupled with documented proof by several health care facilities that they had indeed been successfully reprocessing some disposables without any associated patient adverse reactions, that strong policy was soon modified. Our relaxation was restricted to renal dialysis and inhalation therapy items for use on the same patient and required conformance with FDA recommendations of January 27, 1977. As time progresses, we are becoming more convinced that the associated risks are considerably less than originally estimated and that savings or cost benefits are susbtantial. Our experience thus far has been tremendously successful. While we are still cautious in expanding the policy, we are convinced that reprocessing can be successfully accomplished, at considerable cost savings, with limited risk. The advantage of utilizing the costs savings to expand patient care programs far outweigh the associated risks.

Comment by J.L. Whitby – Canada

I think quite a lot of resterilization, of what might be considered disposable, goes on in hospitals. What do we use our gas sterilizer for? We use them for sterilizing heat labile articles, most of which, as far as the manufacturers are concerned, are disposables. We first get them in that way. Tubing is a good example, for instance, endotracheal tubes and tubing for connecting people to respirators, or anaesthetic apparatus, etc. We resterilize quite of number of things and some articles we pasteurize and thus do not even sterilize. I think this is a trend which is going to continue. However, nothing should be reprocessed unless the reprocessing is evaluated. If the evaluation shows that risks of reprocessing are acceptable, I think it would be wrong for somebody in authority to regulate reprocessing out of patient care.

Comment by Mr. Cobis – USA

I might add one more point. At times it would appear that some health care industry manufacturers may have jumped on the band wagon of popular opinion. We know, in fact, that we have had many devices for which we had been given reprocessing instructions by manufacturers in the past. The product has not changed; however, they have been reclassified as disposables and the manufacturers will not give us reprocessing instructions. But the fact is we are still getting the identical product that we had previously been told we could resterilize. Could this attitude be associated with increased profits.

Comment by G.B. Phillips – USA

I think not. One thing that would be foremost in the mind of a manufacturing concern offering you reprocessing instructions, is the liability incurred in so doing.

Q. by C.W. Bruch – USA

I want to commend Mr. Cobis. I read your article and I respect you for taking a public stance on this issue. I heard recently that with cardiac catheters people were marking the catheters and resterilizing them up to four times without any problems. From your first remarks, you indicated to your users, hospitals, that if they come up with some data, you are willing to let cardiac catheters go in this direction. Is that correct?

A. by J. Cobis – USA

We have had some requests from health care facilities, with documented evidence of successful reprocessing of heart catheters, for permission to reprocess and resterilize. These requests have been refused. Our medical staff feels that, although we may reach that point in the distant future, heart catheters are among the more sensitive items of patient care that should be excluded at this time. As our experience grows in reprocessing the less sensitive items, we may approach the more sensitive ones. At the present time, however, we have no intention of allowing heart catheters to be reprocessed. We will entertain any request to reprocess other disposables that are accompanied with evidence of proof of successful reprocessing.

Comment by C.W. Bruch – USA

We are moving into what I call a double standard which industry has brought about. Reprocessing is occurring in industry (during initial production), but they are not telling anybody. Yet, industry will turn around and tell hospitals that disposable supplies cannot be reprocessed. We all have fear of liability. I am not belittling that fear. Industry, in my mind, cannot play it both ways, reprocessing themselves and then telling hospital people that they cannot reprocess.

Comment by F.E. Halleck – USA

Many of you probably do not know that when hospitals talk about reprocessing, they are not talking about medical products that have been used and are bloodied, full of mucus and dirt. They want to reprocess those items that have been opened and never used which had no contact with the patient.

Comment by D. McLeod – USA

I can only say that the Army has taken the position that we will not resterilize single-use disposable items. However, we also have been looking at, because of our mobilization posture, the possible reuse of our single-use items. I have written to industry and I have gotten comments back from different industrial concerns telling me the items that could, if needed, be reprocessed. So I feel that in that respect, we are getting cooperation from industry in the event that we would have to reprocess some of the items.

Comment by C.C. Mascoli – USA

To expand a little bit on Dr. Halleck's point, there was a relatively recent article published on the reuse of cardiac catheters. I think it was in the Massachusetts General Hospital. The problem is not merely that which has been mentioned. In this case, they were not being washed properly and there was accumulation of endotoxin and there was endotoxin shock and fever and reaction experienced by the patients. There is more than meets the eye to the question of reprocessing.

Comment from the floor:

I do recall a case in Canada that pointed up the danger of resterilization. Apparently, the resterilization process the hospital was using had caused dislocation of the tubing to the degree where the patient was wrongly connected and it was a fatal occurrence. These are the kind of things, I think, the unforeseen that will occur when any kind of resterilization process is applied. We are concerned about it in Canada, but we have not really resolved the question one way or another as yet.

Comment by F.E. Halleck – USA

I would like to add another point to that. There is inconsistency in the federal agencies. The Center for Disease Control has stated that scopes can be disinfected. The first patient of the day gets a sterile scope. After that, disinfection is utilized for all subsequent patients. How can you compromise such a procedure endorsed by one federal agency with the statement that you cannot reuse and reprocess disposables?

Comment by J.L. Whitby – Canada

This is exactly the problem we face. The pasteurization, for instance, of cystoscopes is not a sterilization process. But in terms of MSI, it probably delivers a safer product than the use of gluteraldehyde or chlorhexidine or any other chemical that might be chosen. I think some of the differences arise because of differences in clinical practice. In the United States, the patient receives a bill for a surgical operative procedure and everything that can be identified as used in the operation is itemized and put on his bill. This practice favors single use items. In Canada, the hospital receives a per diem for the management of patients based on average costs so that the hospital looks at its budget in a different way than when costs are recovered by billing a patient, and reprocessing of items is more likely to be considered. Nonetheless, whatever the budgetary practice, the evaluation of any reuse process must consider whether the product is still properly usable after it has been through the resterilization process.

Kilmer-Award Presentation

Robert A. Fuller

Johnson & Johnson
New Brunswick, N.J.

COMMENTARY

Dr. Jocelyn C. Kelsey

The Second Kilmer Award was presented to Dr. Jocelyn C. Kelsey for his many contributions to sterilization philosophy and technology and to environmental microbiology.

Dr. Kelsey received his education in England in St. Lawrence College, Ramsgate; Clare College, Cambridge University; the London Hospital Medical College and the London School of Hygiene and Tropical Medicine. He held numerous medical appointments before joining the Central Public Health Laboratory in 1960. For the ten years preceding his retirement in 1977, he served as Deputy Director of the Public Health Laboratory service of Great Britain.

Dr. Kelsey has been active in committees and working groups of the Medical Research Council, the Department of Health, the Royal College of Pathologists and the Council of Europe; delegate to the International Atomic Energy Agency and consultant to the World Health Organization. During the Second World War Dr. Kelsey served as a Major in the Royal Artillery.

Dr. Kelsey is the author of some 50 publications. He is perhaps best known for his paper entitled "The Myth of Surgical Sterility." This paper is reproduced, by the courtesy of *The Lancet* and follows the remarks of Dr. Kelsey.

In 1976 I retired after working for nearly twenty years in the rather sordid field of sterilisation and disinfection, and if my name should ever be remembered it will be in that context. From time to time I have been asked how I came to that sort of work. Was it, I was asked, part of a planned career? Was it a long-felt want and ambition from the time when at my mother's knee, to make my name known in the field of germs and how to kill them? Today I can reveal all; the sad fact is that it was quite by chance that I got involved in the whole business and despite all my efforts once hooked I could not get away.

Somewhere in the United Kingdom, in the fifties, I was working in a hospital as a medical microbiologist. From time to time it was my custom to help with an autopsy, and one day I was asked to help with an autopsy on a young woman who had just died, as another hospital, of tetanus. She had been pregnant and a member of the medical staff had inserted a hollow needle into her chest to draw off some fluid, which he though he had found there. He got no fluid, but she got tetanus and died of it within a few days. I was able to grow tetanus from her chest, from the needle the doctor had used; when we examined the steam steriliser it was only too clear what had gone wrong. The drain was blocked, no air had escaped by gravity, and there was a telltale line of rust a third of the way up the

chamber. This lower third of the chamber had only contained air, not hot enough to kill the spores of tetanus, and unhappily the needle had been in that part of the chamber. It was a classic case of a faulty steriliser, and I sat throughout the inquest in great distress lest I be called, and be forced to tell the truth. To my surprise not only was I not called, but at the end of the inquest, when a verdict of death from misadventure had been arrived at, the lawyer representing the deceased rose and made a little speech thanking all at the hospital for their care for the late patient.

I was very distressed and I broke all the rules I had been taught as I had been trained as a doctor; I allowed myself to become emotionally involved and I decided that I would do all in my power to stop anyone else from dying from tetanus, or indeed anything else that could be prevented. I started a personal program of fussing and bothering all those people who knew about sterilising and cared that others should know. At this time quite a lot of work had been done, but it had been published in obscure journals as an obscure subject. I fussed to my old chief, Graham Wilson and to his successor, James Howie, to Robert Knox, to Michael Darmady, a pathologist at Portsmouth, to James Bowie at Edinburgh. Together we all fussed to our Medical Research Council, who were already concerned; finally the M.R.C. called a Conference, which set up a Working Party. I got put on it, and became its Secretary. Thus was started a way of life which lasted from 1957 to 1964, and produced three Reports. We met, we talked, we planned, we did practical work, we dreamed dreams and we saw visions. We made many acquaintances, some friends, and a few enemies; this was not only at home in the United Kingdom, but in Europe and the United States of America. As a result of my going to conferences all over the world I have been invited to be here today. When I retired I was the Director of a special laboratory set up at the Central Public Health Laboratory near London, and I was the Chairman of the British Central Sterilising Club.

Sterilisation, in the sense of freeing things from all living creatures, is a rather strange topic. Basically it is extremely simple; if you get anything hot enough for long enough it becomes sterile. In practice it can be extremely complicated; so much depends on what apparatus is used and how it is operated. When the Medical Research Council first looked into the matter it was handled by very low-grade, and very poorly paid, people. Those worked in the bowels of the hospital with virtually no skilled supervision. The older of you will remember side-rooms attached to wards where instruments were boiled, for uncertain times. Even some operating rooms used to boil their instruments. Our Working Party had to take off its jackets and show how unreliable this boiling was, and how most spores can survive exposure to boiling water. I was once very nearly killed when I was inside a huge steriliser cleaning the drain; the door was slammed shut and only by banging extremely hard with my massive spanner could I stop the steam being turned on and my cooked body being revealed when next they loaded the machine.

The last twenty-five years have seen changes in the practice of sterilisation within hospitals that seems with hindsight to be small miracles. From a set of procedures with very little hard scientific basis and even less skilled supervision, we now have Central Sterilising Departments set up and highly trained operatives within them. Scientific expertise is indeed not only important but essential, and we are very right to stress it, and check it; however, in my own experience, it was a simple failture to understand simple things that counted. We all know the Bowie-Dick test for the removal of air from high-vacuum sterilisers; as late as five years ago I found operators who thought it was a test for how hot for how long. In one of our best centres for neurosurgery the continual sepsis of wounds with gram-negative bacteria was only solved by a thorough microbiologist spending the night in the

room where patients were prepared. He found that in the middle of the night a retired and elderly porter used to shave the heads of the patients. Unhappily he did this with a shaving brush which he kept in a jar of a well-known liquid disinfectant, well-known for its ability to select out and grow gram-negative organisms. When the surgeon cut the skin some hours later he did it through a low-grade infection of that skin. This practice was stopped, and so did the infection.

Dag Hammarskjöld, the former Secretary-General of the United Nations, once made a telling remark in his collected thoughts, published in English as "Markings". He said this: "Time passes, reputation increases, ability declines. "It is less than five years since I retired; yet I am already out of date with a great many things. I was once a bit of an expert on steam sterilisers, hot air, ethylene oxide, irradation, chemical disinfectants and many other things. On these matters I hope I have the sense to keep my mouth shut and my ears open.

May I end by repeating the list of the steps that seems to be involved in giving advice about sterilization and disinfection, or indeed about anything:

1. Check that it is in fact your problem. If not, to whom do you refer it and do you need to see that future enquiries go to the right place?
2. Check that it is a real problem. It may need any reassurance, a reminder of well-known facts, or an exhortation to carry out good advice already available.
3. Define the problem. Get the problem accurately and completely stated in as much detail as possible.
4. Check if an answer exists. This may be either unofficial instructions, in the scientific literature, in unpublished work, or from the experience of others. If such an answer exists, give it and ask for feed-back so that the validity of the answer may be checked.
5. If there is no known answer, ask whether one can be found in a reasonable time, and at a reasonable cost in relation to any benefit that may be expected. If work has to be done, can the questioner wait or is an interim reply, however tentative, needed?
6. If experimental work is required, what will need to be done, who will do it, and who will pay for it?
7. When an answer is available, who else needs to know about this and how do you tell him?
8. Does this problem suggest another related problem which you, or someone else should be working on?

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THE MYTH OF SURGICAL STERILITY

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TRAGIC events caused by contaminated intravenous fluids have focused attention on sterile products used by physicians and surgeons. There has been a flurry of activity throughout the health service; official reports have been published, departmental circulars issued, government inspectors have gone into orbit, and sterilising arrangements in hospitals and pharmaceutical houses have been subjected to a closer scrutiny than they have borne for many years. Users of such products have rightly demanded that they should be unequivocally safe. Perhaps this is the moment to consider what is meant by the concept of surgical sterility, whether it can in fact be assured, and if not what alternative exists. Sterility is defined by the Shorter Oxford Dictionary as "freedom from microorganisms" and this is clearly an absolute concept. Either they are there or they are not. Any attempt to ensure that products labelled sterile are in fact sterile must take one of two forms. Attention may be focused on the product or on the process.

PRODUCT CONTROL

At first sight product control, which in fact means sterility testing, is most attractive. It is easy to understand and if there is doubt about the sterility of an object the reasonable thing seems to be to test it and see. Unfortunately, this is not easy to do in practice. The idea of sterility testing arose from checking whether the microbes in an individual vaccine bottle had in fact been killed; and there it was valid. But it is not suitable for general application. There are many difficulties; cultural, technical, and statistical. The cultural difficulties spring from the fact that a valid test must ensure that a single cell of any organism will yield detectable growth within the time of observation in any special circumstances that may be present; it is thus necessary in defining a sterility test to define the media to be used, the temperature of incubation, and the time for which it is to be maintained. At present, there is no single medium which will allow the growth of every microorganism and, although an attempt may be made to surmount this difficulty by using several different culture-media, a large number would be required to ensure with reasonable certainty that any bacterial contaminant will grow. Were viruses to be sought this would involve complex systems involving tissue-culture, eggs, or animals. Furthermore, a multiplicity of culture-media introduces other difficulties of a technical and statistical nature into the test. The choice of medium is essentially a compromise to ensure that reasonable numbers of the organisms in which we are interested are likely to be detected. Similarly, there is no one temperature at which all organisms will grow and, unless the test is to be yet further complicated by incubating several tubes of each medium at various temperatures, a compromise must be accepted here also. The time of incubation is yet another compromise. Some organisms, such as the tubercle bacillus, may require 6 weeks for growth and there are published reports of spores yielding growth only after incubation for 3 years. There is also evidence that organisms damaged by sterilisation processes may have special requirements for their recovery; unfortunately these requirements are not the same for all organisms. For example, heated spores may be more easily recovered in medium containing starch, organisms that have been irradiated may do better in the presence of antibiotics or low temperatures, and small variations in the culture-medium may produce quite different effects on

different organisms. If the product to be tested contains antimicrobial agents used as preservatives, or if there are antimicrobial substances in the rubber or metal of closures, these too will interfere with the growth of organisms. Thus any realistic tests, such as those of the *British Pharmaceutical Codex* (*B.P.C.*), are based on a reasonable compromise in which a "good broth" is incubated at 30–32°C for 7 days. This will ensure that most common contaminants are detected but cannot ensure that there were no viable organisms present.

The technical difficulties in sterility testing depend on the fact that it is not always easy, or indeed possible, to transfer a sample to a culture tube without accidentally contaminating it on the way, no matter how careful the technique. The basic (or technical) contamination-rate is thus related to the complexity of the operation and is estimated by the use of deliberately "over-sterilised" objects being subjected to the standard sterility tests. For example, with ampoules, which are relatively easily sampled, a technical-contamination rate of about 0·1 % may be found, whereas with more difficult items such as dressings the rate may be as high as 5%. Therefore in interpreting the test one must be careful to distinguish testing the product from testing the tester. The *British Pharmacopoeia* (*B.P.*) allows for this by permitting two retests; failure is indicated if the same organism is isolated on more than one occasion. The statistical difficulties depend on the problems of sampling. Using simple statistical methods it is easy to demonstrate that the sensitivity of the test rises with sample size but that less assurance of sterility is given than is sometimes supposed. For example, in 20 random samples taken from a batch which is 10% contaminated, the chances are 1 in 8 of the samples being sterile and the batch accepted. For a 5% contaminated batch the chances are 1 in 3 of acceptance. To ensure a 95% assurance of detecting a batch which is 5% contaminated, 80 samples would be needed. With retesting the chances are very much worse. As previously discussed, if a reasonable assurance is required that all sorts of microorganisms will be detected, many culture tubes incubated at many temperatures would be required. This obviously increases the technical-contamination rate and statisticians have demonstrated that if the basic-contamination rate is 1 % and four tubes of culture-media are used, it is probable that every batch will be rejected and none left available for use even though they were all in fact sterile. Furthermore, sterility testing is essentially destructive in that the samples are not subsequently available for use. It is never possible to test the actual product used; any assurance given is essentially by analogy to what it is hoped is an identical product. Sterility testing thus involves a compromise choice of conditions for a limited objective. It detects significant number of important pathogens or common contaminants. It is a very crude technique indeed for assuring the sterility of medical products.

PROCESS CONTROL

The alternative to product control is to control the process. The problem is to decide what sterilisation process can be relied upon to produce sterility in all circumstances. Traditionally this is done by examining the resistance to, say, heat of some challenge organism which is known to be difficult to kill; here again decisions have to be taken about what organisms are to be used, how many of them are to be included in the challenge, in what circumstances the challenge is to be made, and how the organisms are subsequently to be recovered. The recommended times and temperatures for steam sterilisation commonly used in the U.K. are those prescribed by a working-party of the Medical Research Council. These were based on data obtained from published reports and from specially conducted experiments. If a graph is plotted of the logarithm of the time required to kill a reasonable

challenge dose of resistant organisms (the thermal-death time) against the temperature used, the result is a series of straight lines (fig. 1). Recommended times and temperatures may then be deduced from the graph so that no microbes are likely to survive if these procedures are used. Although challenge doses of gas gangrene, of tetanus, and of mesophilic test organisms will be killed by the recommended procedures with a reasonable safety margin, thermophils (such as are found in soil and as are dealt with by food manufacturers) will not necessarily be killed by the lower temperature recommended, even at the prescribed time, and organisms found in soil are most unlikely to be killed. The recommendations of the M.R.C. and of the *B.P.* are certainly safe but they certainly cannot be regarded as ensuring a sterile product within the strict definition of the term. The recommendations merely represent what informed opinion judges as a safe process and they have indeed borne the test of time. Commercially available spore-strips, incorporating spores of *Bacillus stearothermophilus*, a commonly used thermophilic test organism, have survived well-controlled exposure to 115°C for 30 minutes, which is a *B.P.* recommendation. Reference to fig. 1 shows that there is nothing surprising in this observation.

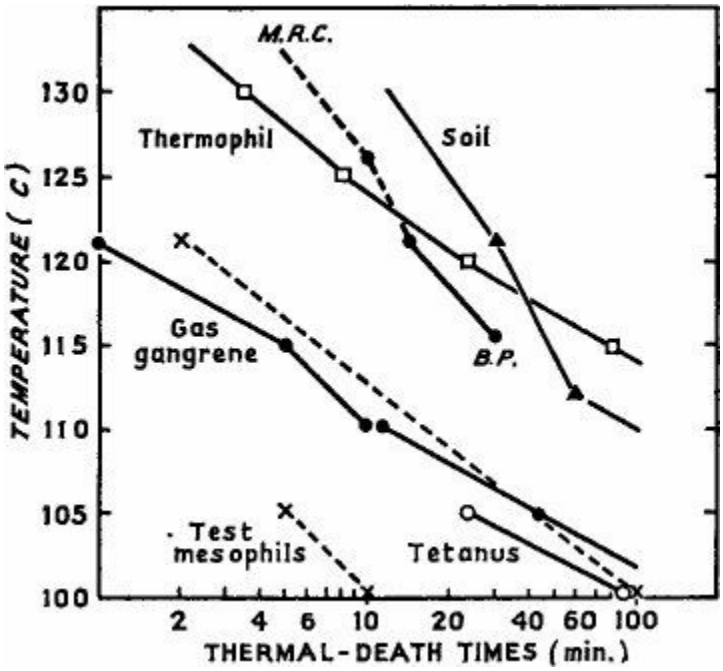

Fig. 1—Thermal-death times plotted against temperature for various groups of microbial spores.

The times and temperatures recommended by the M.R.C. working-party and the *B.P.* for steam sterilisation are shown.

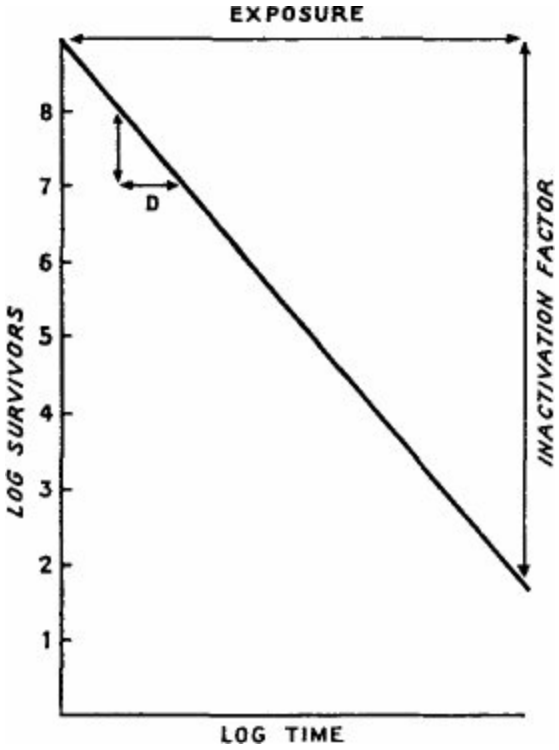

Fig. 2—Thermal-death curve obtained by plotting log survivors against exposure time, so showing derivation of decimal reduction time (D) and also inactivation factor for a given exposure.

A more elegant approach is to take a culture of some selected challenge organism and to plot a killing-curve in which the number of survivors is noted at various times of exposure. If the kill is truly exponential a straight line results as shown in fig. 2. From such a curve can be deduced the decimal reduction time—i.e., the time required at some particular temperature to ensure a tenfold drop in count; for a given exposure the inactivation factor may also be deduced—that is, the proportion of organisms killed by some particular process. If the total contaminating load on the items before sterilisation is known, then a probability can be given of a single contaminated item surviving the process. Here too it is necessary to define the type of organism to be used and how it is to be presented. For example, spores dried from saline which are trapped in crystals of salt are almost impossible to kill by ethylene oxide, and organisms may be protected against radiation if they are enclosed in proteinaceous material. It is also by no means certain that the killing-curve is always or even usually a straight line. “Shoulders” and “tails” representing deviations from such a line are usually found in practice and lend a degree of uncertainty to the interpretation of such experimental work. Current negotiations for international standards for the radiation sterilisation of medical devices are largely about the difficulty in securing agreement on these matters.

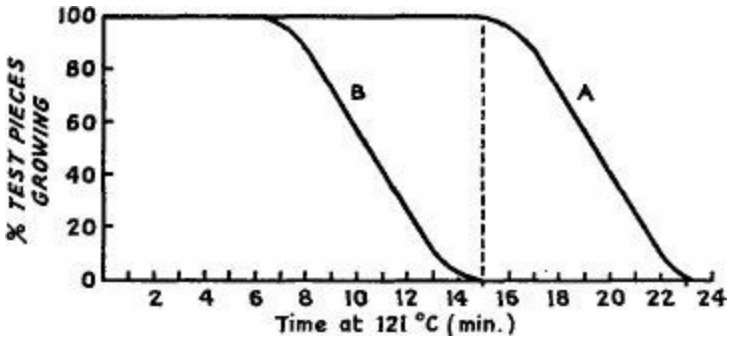

Fig. 3—Thermal-death curves for B. stearothermophilus test papers, giving percentage of papers growing after various exposure times at 121°C.

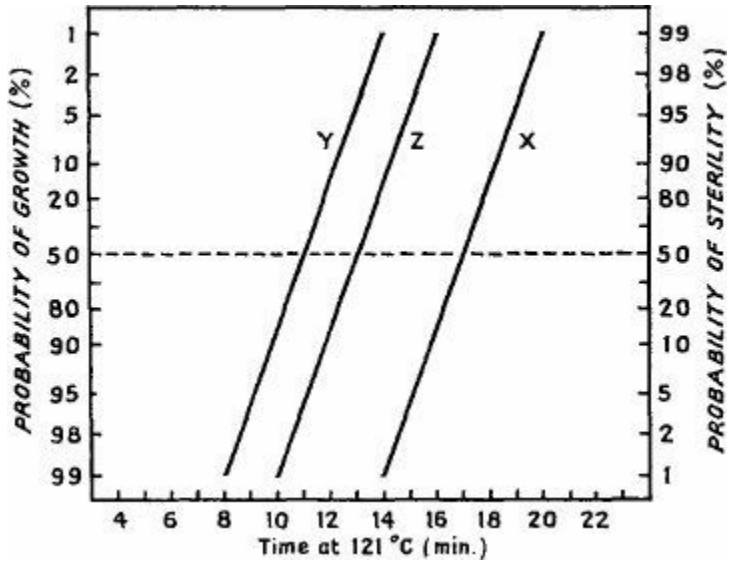

Fig. 4—Probit plot for B. stearothermophilus test papers showing percentage probability of growth (or sterility) after various exposure times at 121°C.

Sometimes, bacteriological test-pieces are used in an attempt to appraise a sterilising process by those who have to operate it. The simplest example is that of the commercially available spore-strips containing *B. stearothermophilus* which are exposed to a steam sterilisation process and subsequently cultured to see if any of them contain viable organisms. Apart from the difficulties of deciding what culture-media, time, and temperature to use, such test-pieces must be carefully calibrated to allow exposure to be correlated with "kill". In addition, the use of simple "go-no-go" test-pieces is difficult to interpret. Resistance will vary with the type of spore, the method of preparation, the number on each test-piece, and the cultural conditions provided for recovery. If batches of test-pieces are heated for various times at a fixed temperature, the result is a typical sigmoid biological dose-response curve (fig. 3). A range of conditions will produce a family of curves of different resistances. We therefore face a dilemma. If the test-piece is tough enough (e.g., curve A) to ensure that there is no significant killing until the desired exposure has been given, there will be false positives wrongly indicating failure unless an excessive exposure is given, which may damage the product. A less stringent curve (e.g., curve B) which avoids false positives may allow underexposures to go undetected. This approach may be made more precise by using dose-response

data to produce a family of probit plots (fig. 4). Here the probability of growth or of sterility is given for various exposure times at a standard temperature. For plot X, if only 14 minutes' exposure has been received, there is a 99% probability of growth and thus a good assurance that under-processing would be detected. However, after 20 minutes 1% will still grow and after 18 minutes 10% will grow, so that there will be false positives. If the likelihood of false positives is reduced by using plot Y, then exposures of only 8 minutes will be accepted. A possible compromise, which gives a reasonable assurance of an adequate exposure but which permits positive cultures to be meaningfully interpreted, is to choose a plot with an $L.D._{50}$ near to the desired standard and to use groups of test-pieces with a proportion of positive cultures being allowed. Thus using plot Z and accepting growth in, say, 1 of 5 test-pieces, a reasonable assurance is given that at least 14 minutes' exposure at 121°C has been achieved. Thus, "go-no-go" test-pieces should be calibrated by plotting a dose-response curve using fixed cultural conditions standardised in terms of an $L.D._{50}$ and used in batches rather than singly.

CONCLUSION

Although sterility is in theory an absolute term, in practice it may only be regarded as at best relative and at worst misleading. It is a philosophical concept that can never be unequivocally demonstrated in a real world. Experience has shown that it is virtually impossible, even if it is honest, to change the definition of a term that has been in use for many years *;* we may need a new term to indicate "the state of having been sufficiently freed from microorganisms to be deemed safe for some special purpose by some competent body". The abandonment of the term "sterility" and the acceptance of some other term would remove confusion and enable the important matter of providing microbiologically safe medical products to be more rationally and realistically considered.

THIRD SESSION

Regulatory Affairs
Chairman
Alan Tallentire

University of Manchester
Manchester, England

Observations on Regulatory and Industry Affairs in Western Europe relative to Sterile Single-Use Devices

Patrick T. Doolan

Becton Dickinson, Europe
Meylan, France

My observations relate to markets in Western Europe and to what are sometimes referred to as "European regulatory problems". I propose to identify some of the complexities of these problems.

Firstly, there is no "European market". There are many markets each corresponding roughly to a country; each with its own language, laws, culture and philosophy of regulation. Each market can be, and generally is, as different from the others as it is different from the United States and Canada.

Secondly, these differences are primarily of interest to companies doing business in more than one country. I wish to draw attention to the fact that there are, within industry, a number of subpopulations of interest to us. One of these subpopulations is made up of companies doing business in more than one country and another important one is the national companies which trade only in their own country. This distinction is significant as we will see later.

Thirdly, there are the problems of gathering information and understanding requirements in the countries into which a product is to be introduced or in which it is being marketed at present. The variety and complexity of regulations and of other requirements frequently make understanding them and complying with them a lengthy process. For the newcomer to a market in Europe, wishing to be compliant with the requirements, this can pose a problem. The variety of forms of requirements which may be found include, for example, government orders, specifications, monographs, decrees, arretes, guidelines, practices, "voluntary" controls, etc. These, in turn, are applied or enforced in various ways. Products may be subject to a notification or registration process. Product samples may be required for testing initially. Imported products may have to be tested for sterility and pyrogenicity, lot by lot, before release to the market. Manufacturing facilities may be subject to inspection and/or approval, or to registration.

Figure 1. Kinds of Regulatory Instruments Affecting Sterile Single-Use Devices.

	France	Italy	Spain	Belgium	Nether-lands	U.K.	Germany	Sweden	Denmark	European
Population x 10^6	52	54	34	10	13	56	62	8	5	
Laws	+	+	+	+	+	+	+	+	+	–
Regulations, Orders, Decrees, Etc.	+	+	+ 2104-1976	+	Draft	–	–	–	Draft	–
Pharmacopeia Mongraphs	+	+	Last (9th) Edition 1954	–	–	–	–	–	–	+
Specifications, Guidelines	–	–	–	–	Draft	+	–	+	–	
Registration	–	+	+	+	–	–	–		–	
Other: Approval									+	
Notification	–	–	–	–	–	–	–	+	–	
Voluntary						–			MEDU	

In Figure 1, some of the regulatory instruments are indicated which affect sterile single use devices across nine of the countries of Europe. Some of the differences between the countries have been highlighted. In general, as indicated in the top row, there is enabling legislation, or an "umbrella" law, in all of the countries. There are many different modes of application existing under the enabling legislation. Regulations are issued in certain countries; orders or decrees in others; and, as may be seen, the first four countries, France, Italy, Spain and Belgium regulate through orders, decrees, etc., as well as to some extent through pharmacopoeia monographs, as their methods of controlling single use sterile products. The Netherlands has draft regulations and Denmark is also preparing a draft at present. It is of interest that the countries on the left of Figure 1 (France, Italy, Spain), sometimes described as the Latin countries, have certain common features in their legal codes and in the status of the profession of pharmacist under the law.

Each country's requirements are published in their own language or languages. This means that in general (apart from the United Kingdom) documents are not available in the English language until a translation has been prepared.

Specifications for Sterile, Single-Use Products

In general there are no statutory product specifications; however, purchasing specifications are used widely in a number of countries. In some countries the use of product standards published by the national standards writing organization is mandatory for purchases whose funding is wholly or partially provided by the government, e.g., in France.

Guidelines

Guidelines have been issued in various countries, for example; for sterilization in Sweden; for GMP (Good Manufacturing Practice) in the United Kingdom. Draft documents are being reviewed, for example; in the United Kingdom, for a manufacturer approval/registration scheme; in the Netherlands, for sterile devices.

Other methods of control which are applied include registration, approval, notification or voluntary systems. Some registration requirements are in force, for example, in Italy, Spain and Belgium. Approval by a nongovernmental body in Denmark, the MEDU, is virtually essential. In the United Kingdom, of course, there is a voluntary system; but it is backed up by purchasing requirements and lists of approved suppliers. So, although there are no statutory requirements, control can be exercised.

Figure 2, gives an indication of some of the types of requirements for sterile single use medical devices. It is not intended to be comprehensive because, in fact, it is already rather over-crowded. However, there are a few things worth noting. It appears, first of all, that GMP requirements in fact only exist in final published form as a guideline in the United Kingdom. Sweden has a draft document under review.

Figure 2. Requirements for Sterile Single-Use Medical Devices.

	France	Italy	Spain	Belgium	Nether-lands	U.K.	Germany	Sweden	Denmark
GMP	–	–	–	–	Draft	+	–	Draft	–
Routine release:									
—Biological indicators									
ETO*	+	+	+	+	+	+	–	+	+
Irradiation	+	–	–	–	–		–	–	–
—Sterility test									
ETO	+	+	+	+	+	+	–	+	+
Irradiation	+	+	+	+	–	–	–	–	–
—Bioburden limits									
ETO	–	–	–	–	–	–	–	–	–
Irradiation	–	–	–	–	–	–	–	+	+
—Dosimetry release	0**	0	–	0	–	+	–	+	+
ETO residue limits	+	+	–	–	–	+	–	–	+
Expiration dating	+	+	+	–	–	–	–	–	–
Language of country	+	+	–	–	+	–	–	+	–
Other labeling	+	+	+	+	Draft	+	–	+	–

*Ethylene oxide.
**Dosimetric release not accepted; product and/or biological indicator tests required.

The next section in Figure 2, shows the routine release requirements for sterile products. For ethylene oxide sterilized products virtually all countries have requirements for the use of biological indicators. However, only France has requirements for biological indicators to be used routinely in radiation sterilization.

The product sterility test is still required widely for ethylene oxide sterilized products, but dosimetric release is accepted in the United Kingdom, Sweden and Denmark for products sterilized

by irradiation. In relation to bioburden limits, the only attempt to set numbers has been made in Sweden and Denmark. The zeroes on the bottom line indicate a decision not to accept dosimetry release but to require product and/or biological indicator tests for sterility.

ETO residue limits have been specified in four countries for certain products. Expiration dating of some or all sterile single use products is required in France, Italy and Spain. The language of the country is required on the labeling in France and Italy. In the Netherlands draft, a language requirement is included and in Sweden it is required for the directions for use for complex procedures. Countries having other labeling requirements, which are spelled out as detailed requirements, are identified on the bottom line.

Any attempt to relate effectively to the regulators, in the writer's view, requires an appreciation of where they are coming from. Their requirements in every case appeared good and necessary to them at the time of promulgation and constituted a solution to a real problem or a response to an identified need. At this time the difficulties facing the regulators are considerable. Sterile single use products are only one group of the many new product groups appearing on the health care scene. The complexities of the new (or old) technologies cannot be understood and controlled by a wish and they may require special training and/or additional staff. New products demand different processes and process control measures. The existing regulatory structure and budget limitations may not lend itself to introducing suitable flexible controls. I think this is, perhaps, most noticeable in countries where they are attempting to use the pharmacopoeia as the legal instrument for regulating devices.

Professional groups, because of their history of statutory responsibility and important roles in health care in a country, may see sterile single-use devices as an area in which they should be involved. This has complicated the problem of finding satisfactory or reasonably flexible methods for regulating devices.

In relation to requirements for sterilization processing, these pressures may contribute to the predilection of the regulators for the overkill approach to achieving a satisfactory Sterility Assurance Level. Regulators differ on this question even within an individual country. The required sterilization treatment may vary depending on the class of product and the ability of the agency to assure satisfactory control. For instance, even though it is not special to the United Kingdom, a situation exists there, in that the Supplies Division, Scientific and Technical Branch of the Department of Health and Social Security has a requirement for single-use plastic products that "the radiation dose shall be a minimum of 2.5 Mrad" (Mar. 1972). The Medicines Division, on the other hand, which is part of the same Department, states in the December 1978 Control of Intra-Uterine Contraceptive Devices (guidelines), issued to licence applicants, that "where sterilization is carried out by gaseous methods or gamma irradiation at doses of less than 2.5 Mrad, data will be required to validate the procedure proposed…".

Now let us go back to the industry's problems for a moment and look at some of the difficulties facing the industry wishing to interface with the regulators. One is struck every time by the extent and complexity of the problems. They tend to be treated inadequately largely because they have to be considered on a country by country basis. The resources required, therefore, are substantial if one is to do a good job, even in the larger markets.

It seems that some of the things which are most difficult for the managements of companies outside of Europe to understand (this comment is addressed particularly to American companies) is that the regulatory processes are so different from those in the USA. They are not always so overt; that they

may lack a formal or informal consultation stage. The process may consult with certain professions only or may consult with parts of industry only. This varies from country to country, of course.

Reaction to published regulations tend to have little effect on their impact. Reaction at this pont is generally too late to gain any relief. It is necessary to try to develop a proactive option.

Trade Associations

Can they help provide solutions? Trade associations at the national level are often numerous and uncoordinated. Just to give an example, a list of 37 trade associations in the United Kingdom dealing with devices and diagnostics contains about sixteen which deal with sterile single-use medical devices.

Our interface with the regulators, if it is to be managed through trade associations, suffers for a lack of resources. It depends, in most cases, on voluntary donation of time by companies' executives, scientists or technical staff. Generally, because the associations are small in membership and budget, they are unable to see their way to having any paid staff. As a result, there is, at best, an inefficient process for developing industry positions on key issues.

The three essential sequential steps in dealing with a regulatory problem, of

1. Gathering the relevant information,
2. Developing an industry position and
3. Taking action

are rarely followed in sequence. In addition, the staff resources appropriate to the task of dealing with or resolving these difficulties are rarely assigned to them by national or transnational companies. Many companies, operating in several European markets have neither dedicated resources nor coordination or counseling support for their part-time regulatory affairs people.

What has been done in Western Europe to deal with this situation? Meetings have been held which have demonstrated an interest in doing something. What has resulted? A number of attempts have been made to organize confederations in the belief that in this way it is likely that a more unified voice may be developed.

These have resulted in the formation of organizations such as the European Diagnostic Manufacturers Association (EDMA), and the European Confederation of Medical Suppliers (ECOMED)*. Each of these is struggling with complex and extensive tasks which face them. They lack the power in most of the countries at this time to interface effectively with the governments in those countries. At a national level some trade associations have developed slightly better links with the regulators. Here the dichotomy of national and transnational company's interests may inhibit progress. National companies, doing no exporting, may not wish to help transnational competitors gain easier access to the national market through the intervention of the national trade association.

In sum, the problems in Western Europe are complex, but they can only be helped by developing effective communications on technical/regulatory matters with the regulators.

“In appreciation of the international significance of the Kilmer Conference the following summary was submitted by Dr. Sataro Jitsukawa to Dr. R.W. Campbell, General Chairman of the Conference.”

Viewpoint of the Regulated on Sterilization in Japan

Sataro Jitsukawa

Department of Operation Center, Osaka University Hospital Osaka, Japan

I received a letter from Dr. Campbell requesting me to submit a paper on the sterilization field in Japan from the regulated viewpoint.

In 1968, I became a director of the Medical Instrument Society of Japan (MISJ), and took the initiative in forming a Research Committee on Sterilization (RCS) as one of the most important subcommittees of MISJ, aiming at making a contribution to the community. As the chairman, I am responsible for encouraging R & D activities on sterilization technology in Japan.

MISJ corresponds to the Association for the Advancement of Medical Instrumentation in the United States, and is a group which consists of representatives of both the regulated and regulator sides: University hospitals, hospital operating room nurses, medical industries and governmental officials, and functions to promote development of medical standards in Japan.

I would like to summarize the subject focused on my experiences as the chairman of the Research Committee on Sterilization. Because I am a member of the MISJ, it is very difficult to simply define that I am entirely on the same boat as the regulated. However, I shall be happy if this report could be somewhat informative to you in understanding the status of the development of sterilization technology in Japan.

We, the Japanese, are in a different situation from the other countries. We lag behind most advanced countries in the world in the area of sterilization. The most serious problem is that, until very recently, we could not appreciate enough the merit of sterilized, disposable surgical products. The reasons are:

1. Japan has been backward in studies on chemical sterilization techniques such as utilization of nuclear energy and ethylene oxide until long after World War II.
2. Secondly, the medical treatments in Japan are supported by the National Health Insurance scheme. However, the funds of the National Health Insurance have been in tight financial condition because the coverage of reimbursement for expensive drugs, such as antibiotics, is extraordinarily high. Due to the tight condition of the National Health Insurance funds, application of the National Health Insurance scheme to medical devices, especially medical disposable products, has still been limited.
3. Thirdly, use of antibiotics largely contributed to the therapies of infectious diseases. But, the massive use of antibiotics has invited a serious phase by its abuse. Consequently, the necessity of infection control was disregarded, which has resulted in the induction of numerous resistant microorganisms.

Presently, Japan is in the phase of reconsideration with regard to the use of antibiotics and the people concerned have been making continuous efforts to restore the delay in research and development on aseptic techniques. As a result, we recognized that heating, which has been exclusively used in the past, is not reliable enough to sterilize medical devices and that it is necessary to apply either gamma radiation or ethylene oxide as a sterilizing agent.

Research on sterilization and thereby control of medical devices has been greatly increased during the past twelve years. The transition of this tendency is presented in Figures 1 and 2.

As seen in Figure 1, the breakdown of the published papers by sterilization methods over the twelve years shows the rapid increase in number with every passing year. It indicates notably that studies on sterilization methods have been conducted covering a wide area.

Figure 2 illustrates the institutions which have carried out these studies. It is distinctive that the number of studies performed at universities and hospitals accounts for a large share. This means that the awareness of the necessity for sterilization of surgical devices has been, in a large scale, increasing every year in the institutes where sterilized, disposable products are used in actual practice for surgical treatments.

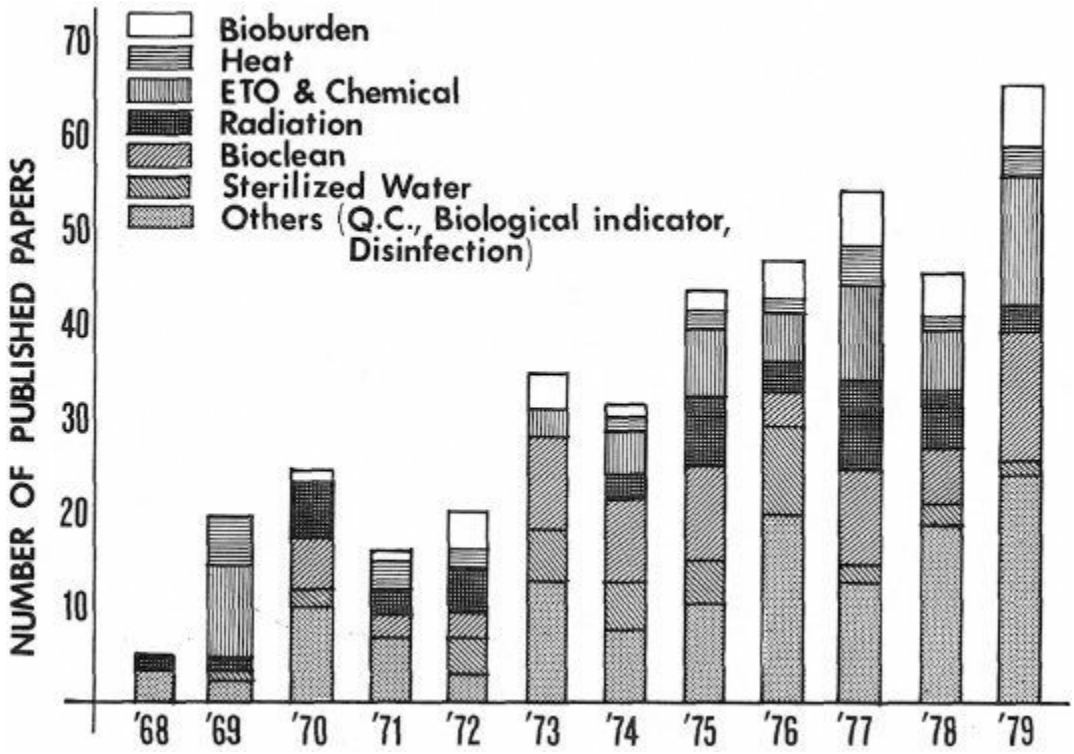

Figure 1. Published papers by sterilization method.

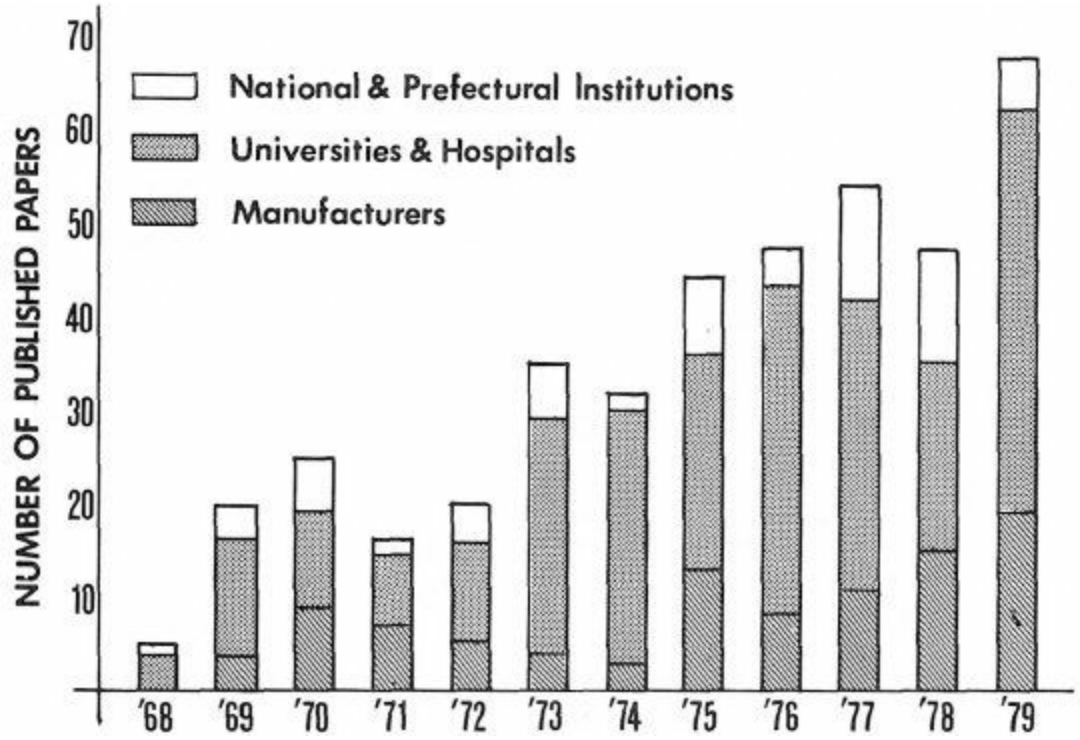

Figure 2. Published papers by institution.

Both the regulated side and the regulators have been striving to catch up with the other advanced countries in the utilization and development of disposable surgical products, where Japan has stood far behind, and thus to establish standardization in the quality of sterilized products.

Next, I would like to touch upon the present sterilization status in Japanese hospitals. Most Japanese hospitals are faced with two big problems.

Expenses for medical devices spent in hospitals are limited due to the budgetary restriction, in relation to the National Health Insurance System mentioned before. So, hospitals have been forced to, in the first place, manage their financial budgets with regard to sterilization related projects. In case of sterilization by heating, hospitals have narrowly escaped the financial problem. However, implementation of sterilization by ethylene oxide, especially the standardization of residual ethylene oxide content, has put hospitals into a very difficult situation.

The other problem is that governmental guidelines for in-house sterilization by using various agents have not yet issued in Japan and that technical standards of hospitals to assess the safety of sterilized disposables have not been established. As to products which have been sterilized either in hospitals or at manufacturers, hospitals have no way to check the safeness, whether they have properly been sterilized or there is no need to worry about their residual toxicity. A very few, special hospitals have technicians to quantitatively analyze the residual ethylene oxide content and related by-products. Therefore, hospitals in general, either refer to experiences and published papers of authoritative hospitals or technical information provided by manufacturers. Although the latter case is still limited, it is gradually increasing.

I, through my paper, would like to express sincere thanks to the overseas experts who have rendered their assistance and knowledge to us in order to improve the sterilization situation in Japan, participating in our Research Committee on Sterilization. The names of the people who have contributed by giving us lectures in our committees are listed in Table I.

Table I.–

The 33rd Conference: May 25, 1976, Osaka

Prevention of Infection in the Operating Room

Dr. Harold Laufman, Montefiore Hospital, USA.

The 44th Conference: March 31, 1979, Tokyo

Quality Control in Sterilization Procedure along with Device GMP Concept—Part I

— Sterilization and State of Control

Dr. Charles P. Truby, Becton, Dickinson Co., USA.

— A Practical Approach to Validating Sterilization Processes

Dr. Anthony Parisi, Pharmaseal Div., American Hospital Supply Co., USA.

— Parametric Considerations in Sterilization using Gaseous Ethylene Oxide

Mr. Robert R. Ernst, Deseret Co., USA.

The 45th Conference: April 14, 1979, Osaka

Quality Control in Sterilization Procedure along with Device GMP Concept—Part II

— The Contribution of GMP's to Sterility Assurance

Mr. Christopher G. Grenshow, Portex Co., UK.

— 1) Determination of the Term of Sterility Validity

2) Toxicity Tests after Sterilization

Dr. Charles Artandi, Ethicon Inc., USA.

— Governmental Policy on Medical Treatment in Japan

Mr. Hiroyuki Yanai, Ministry of Health and Welfare

Viewpoint of the Regulated on Sterilization - United States

George Heinze

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In commenting on the regulatory scene in the United States, I would like to address myself to the regulation of the sterilization process rather than the regulation of sterility. I believe most people active in the field now accept the scientific validity of the concept of sterility being a probabilistic function, albeit that the legal liability for each item labeled as "sterile" is an absolute one. Nonetheless, the industry is faced with the practical problem of operating processes which are designed to render medical products sterile and then designing some program to produce a judgement as to the suitability of releasing the entire lot of product, which has passed through this process, to the market place with an assurance that the label claim of "sterile" is valid; this, short of destructively testing the entire lot, might be viewed by some (such as company treasurers) as being counter-productive.

Indeed, the general thrust of Food and Drug Administration (FDA) and, therefore, regulatory programs in the United States over the last 15 years has been generally twofold, that being: (1) the ongoing program of testing finished product samples acquired either as the result of complaints or because of surveillance programs for sterility or (2) the program of Good Manufacturing Practice (GMP) implementation which stresses control of the sterilization process and the associated quality control programs.

With respect to the development of the GMP portion of the regulatory programs and the associated concern for control of the sterilization process, I believe we in the industry who have been concerned with sterilization have been involved with the other aspects of GMP regulation. I say this because it is my thesis that, in general, the genesis of sterilization regulation in the United States has been more soundly based on scientific and technological discussions and rationality than those regulatory thrusts in almost all other areas of GMP, which have sprung more from either quasi-legal or procedural initiatives. The personnel involved in the evolution of these regulations on both the side of the regulator and the side of the regulated have derived more from the scientific community rather than the legal, which is undoubtedly a saving grace.

In order to support this hypothesis, I would like to review some aspects of this evolutionary process. The sterilization procedure that is probably most responsible for our present approach to the entire function is the one involving ionizing radiation. Meetings as early as 1966 were specifically directed at the radiosterilization of medical products, with a symposium on the subject held in Budapest in 1967. Because this process readily lends itself to the determination of the controlling parameters and the subsequent development of meaningful specifications for process control, it became the bellweather for all other sterilization procedures. The concepts of quantitative bioburden and correlated resistance arose as working requirements during the development of radiation sterilization because of our ability to directly correlate these parameters to the dose of radiation necessary to produce a given level of sterility assurance. Given quantitative estimates of product contamination (i.e., bioburden), we can develop calibrated biological indicators which, when staged through the sterilization process, can provide excellent simulation of the fate of the natural bioburden.

Fortunately, the above approach and parallel efforts in other sterilization processes such as steam and ethylene oxide were the subject of a number of meetings and forums whose participants were scientists drawn from both industry and the regulatory agencies. As a result of the exchange of viewpoints between scientific personnel at these meetings, guidelines and regulations have evolved which have been primarily based on either scientific rationale or empathic compromise. While at times a spirit of friendly rivalry was certainly in evidence, there was, and continues to be, a notable lack of the usual adversarial divisiveness that so typically tends to characterize industry/regulatory interfaces.

Because experimental results and data have been so openly shared, we have seen a steady progression in how the sterilization process should be viewed. Again, a recitation of chronology might be in order. The use and characterization of biological indicators was one of the issues which eventually evolved into the elucidation of the bioburden approach, which in turn has lead to the concept of process release for sterilized product, rather than finished product testing and release. In turn, the concept of process release has led to the development of the qualification, validation and verification approach for the establishment of process reliability, particularly as it applies to radiation and steam sterilization.

I believe a specific illustration of this development will be useful as an insight as to how the process works. In mid-1979, officials within the Bureau of Medical Devices of the FDA expressed some concern that certain aspects of the sterilization process were being subjected to varying interpretations by people in both industry and the FDA and they asked the device GMP advisory committee to look into the situation and make recommendations. As a result of its deliberations, a committee of scientists from all sectors, working under the auspices of a professional association, the Association for the Advancement of Medical Instrumentation (AAMI), and in conjunction with FDA, have produced a draft document setting forth proposed guidelines for the qualification, validation and verification of ethylene oxide sterilization. This proposed guideline will be the basis for a conference to be held in Washington on December 2 and 3, 1980, jointly sponsored by AAMI and the FDA, whose prime purpose will be to provide a forum for all interested parties to discuss and comment on these guidelines. A final version of this document should then evolve from this input. It is this line of approach, which leads to a much more rational basis for action on the part of the regulatory sector.

Another aspect of this same approach is the input provided by the predecessor conferences to this one. I refer to the two conferences on sterilization by ionizing radiation held in Vienna in 1974 and 1977 and the First Kilmer Conference held in 1976. All of these enjoyed very broad support and the input of people representing a number of different constituencies. From conferences such as these has distilled the essence of mutual understanding and consensus which has formed the scientific foundation for progress in sterilization processing. In addition, the mutual respect engendered during this time has been critical in developing the present regulatory atmosphere, which, as noted earlier, I believe to be rather unique in the entire regulatory spectrum.

In summation, I find the regulatory climate in the United States insofar as sterilization is concerned, to be fairly balanced; that while, like most aspects of the human condition, it can be improved, pragmatically it must be viewed as a positive model for the development of future regulation and an area where the participants, whether their derivation is industrial, academic or governmental, can take pride in their achievements.

Canadian Regulatory Viewpoint — Sterility

Michael T. Cooper

Bureau of Medical Devices
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The Microbiological Survival Index (MSI), its development and the evolution of the concept to its present stage has been one of the major concerns of Canaduan regulators in the Bureau of Medical Devices over the past two years. Many other projects have, of course, been pursued besides the routine work of problem reports and their follow up. Various standards are being developed, a clinical trials register is now in operation and work has been done on biocompatibility assessment.

Sterility is a requisite quality for many medical devices and when this whole area was reviewed after the formation of the Bureau of Medical Devices it became apparent that the sterility testing being used and the evidence supporting claims of sterility were in many cases quite inadequate for the device use proposed.

The usual test of sterility had been the procedures set down in USP XIX. This had the advantage of being standardized and it ws quite adequate for many products for which it was intended. But now some method of giving an assurance of sterility much better than that possible with USP XIX, or other end product testing methods, had to be found.

Especially with devices made only in small quantities, the answer had to be in validation and control of the whole manufacturing/germ excluding/germ killing process. This rather clumsy phrase is necessary because although most processes now include a germ killing step, it may be unnecessary with certain types of process and we must allow for this. The idea of developing a clinical hazard index for each device use was looked at. It was decided that this could well be worthwhile, but it was far too large a project to tackle as a whole. The development of one part of it: MSI, the microbiological survival index, would be much more practical and useful as it could be developed in a shorter time and it would be one factor for use in the clinical hazard index, when this becomes a possibility.

The Bureau has in fact been using the basic concept of MSI for some time in its review of premarket submissions (Part V) and other selected device submissions where the microbiological state of the product is critical. Devices differ and processes differ, often very considerably, and yet comparisons must be made. Differently manufactured and processed devices may be destined for the same device use and may be intended to produce the same result. These different devices must be evaluated in such a way as to make comparisons and decisions about their suitability for any particular device use. The only way to do this efficiently is to reduce the information to a degree or level of "sterility" and this is in effect what has been done in the Bureau.

It is much simpler for everyone concerned to have a method which gives a yes or no regarding sterility, but it is not reasonable nor is it safe because it gives an often false sense of security and it also inhibits progress in developing better systems.

There are two basic questions which must be answered in the evaluation of this aspect of a device submission.

1. Is the MSI appropriate to the end use intended?

This question must ultimately be answered by the user of the device and this is where

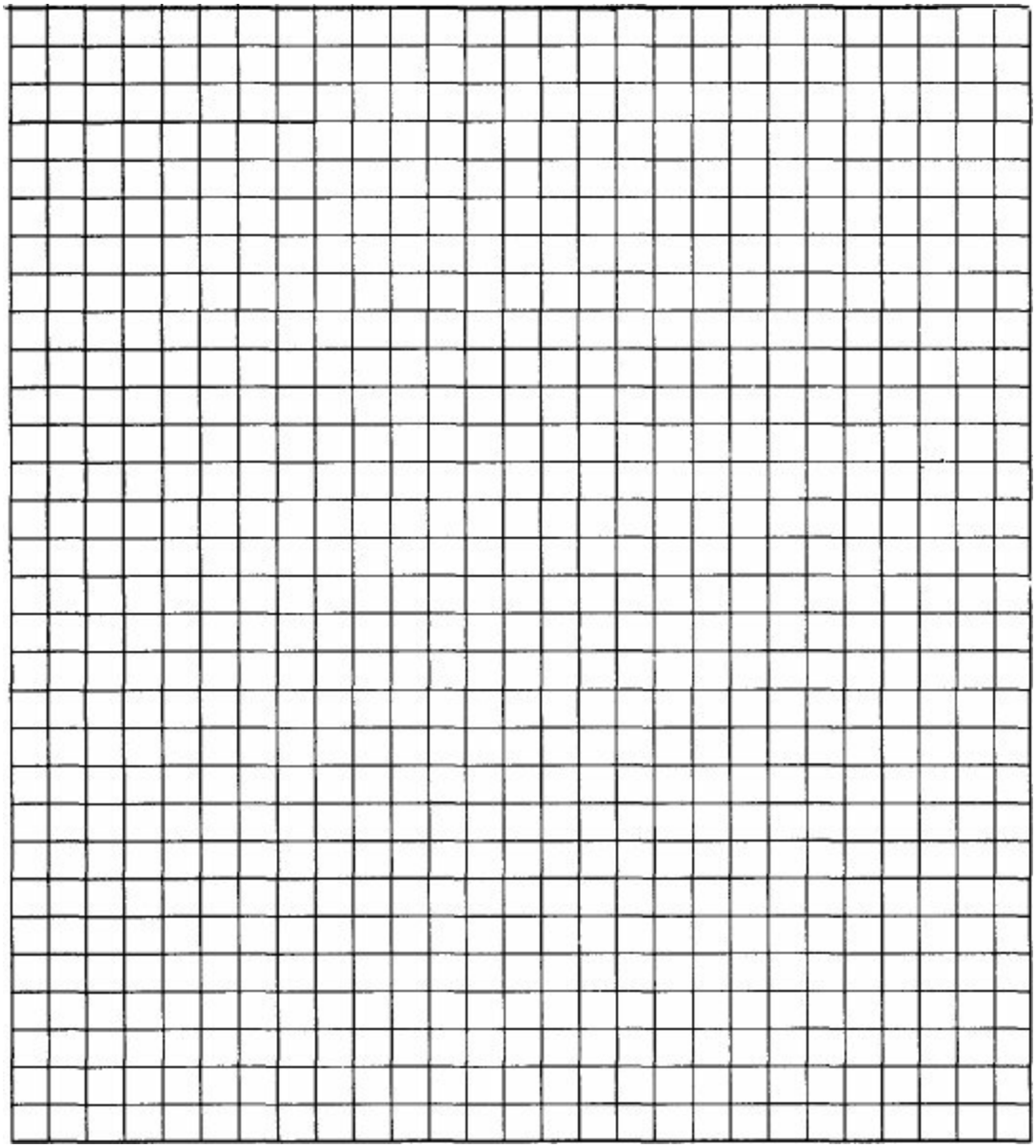
discussion between user, manufacturer and regulator can be useful and result in a level of assurance acceptable to all.

2. Will the process proposed achieve the MSI claimed?
   The problem of course is to decide how one does assess different approaches, different methods of sterilization and process control, to arrive at a number, or level of assurance, or an MSI which can be used to compare different and differently processed products.

A great deal of research, thought and work has been devoted to this problem by the Bureau— mainly by Mrs. Judith Dowler. The result has been consistent with our approach to regulation in general— a draft guideline setting down the sort of information that we feel is necessary to evaluate the manufacturing/germ excluding/germ killing process and to decide what level of assurance of microbiological safety can be justifiably claimed.

The intent of the guideline is to provide a framework for the evaluation which the Bureau must make and also to aid manufacturers in producing a submission which will be acceptable to the Bureau for this evaluation.

The Medical Devices Regulations are designed to protect the public— to ensure safe and effective medical devices. We consider that this aim requires a strict and rigorous approach to microbiological safety assurance— but a strictness in evaluating the evidence submitted to support claims for an acceptable product, not a dictatorial strictness in laying down rigid methods and processes.



UK Regulatory Viewpoint - Sterile Medical Products

Marilyn Duncan

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At the first Kilmer Conference in 1976 Alex Bishop gave a paper on the control of sterile products in the UK and on rereading it my first reaction was that on the face of it things have not changed. In fact there was little change for 20 years or so. The same statutory control of medicinal products continues and has not be extended to include further products in the devices area. The same "voluntary" controls exist for the majority of sterile dvices with the only notable happening in this area being the publication in 1979 of the Guide to Good Manufacturing Practice for Sterile Single Use Medical Devices—a joint industry/government exercise.

What then has been happening for the past four and a half years? Despite the appearance of outward calm we have been struggling with some of the most scientifically challenging and bureaucratically difficult problems in the field of sterilization and sterile products which I personally have experienced. These have been partly a spin-off from the increasing regulatory activities of other countries—notable in North America—which has promoted the new growth industry of microbiological investigation, and partly our own scientific curiosity and pursuit of greater and greater accuracy in the test methods upon which we base our requirements. I'll touch briefly on some of the major areas of our concern.

In the steam sterilization processes the problem areas we have received have included the integrity of containers and closures for sterile fluids; chemical indicators; microbiological test pieces; steam quality; accurate placement of temperature probes, and last but not least, the use of the F_0 concept in sterilizing fluids. In the area of irradiation we have the perennial problem of minimum dose; the recurring problem of the deleterious effect of irradiation on some materials; and, after being alerted by an occurence of defective batches, a prolonged look at dosimeters and their quality control. A new minor problem is the introduction of different coloured irradiation markers on imported products—with potential for user confusion.

In the complex area of gas sterilization we continue now with some success to seek for reproducible standard microbiological test pieces whilst "looking over our shoulders" constantly at the looming shadow of carcinogencity/mutagenicity questions for both ethylene oxide and formaldehyde.

We have now got so far advanced with our work on validating low-temperature steam and formaldehyde as a sterilizing method as to find the prolonged toxicological debate as irritating as the gas itself. I hope we shall see some common sense entering that area—we could not after all apply *any* method of sterilization if the medium used were not toxic to biological systems.

In the area of good manufacturing practices we have achieved much but are already deeply involved in reviewing our guide so that its practical implementation delayed due to lack of resources, can have more universal application when used as the basis of any approval or registration scheme. In the (for us) most immediate area of physical quality control of sterile devices, we have given much time to the safety and interchangeability of luer type connectors and, in a related exercise, to the design of noninterchangeable administration systems for intravenous, irrigation and enteral fluids.

Out of such a wide field of activity I cannot in the time available discuss all or indeed any of these topics in detail but I would like to focus on the two major challenges. The first has been the attack on our definition of sterility and the second is our perception of the shaky ground on which some of our sterilization requirements are built because of a national and international failure to thoroughly research, standardise and control the biological and, to an extent the physical/chemical monitors, by which we initially measure the achievement of satisfactory sterilization processing.

First, the challenge to our definition of sterility has come not only from North American industry who, apparently mainly for commercial reasons, want to change the ground rules, but also from their Governments organisations. In this country (the US) the acceptance of sterilization cycles based on "bioburden" studies and in Canada the legal acceptance of different levels of probability of sterility, has caused us much heart searching. The commercial challenge has come from the introduction of the F_0 concept of sterilization for large volume fluids, and in the field of irradiation from the strong pressure to reduce the minimum dose of irradiation. I have struggled through the mathematical maze of supportive data for both proposals and I find in both, areas of uncertainty and questionable basic foundations. The most important areas of doubt in my mind are first the use of a one in 10^6 probability of sterility based on bioburden studies when the original use of these figures related to the inactivation of highly resistant test organisms. Second, the basing of a much lower safety margin on pre-sterilization microbiological counts whose statistical relevance must be open to question and third, and perhaps most important in my mind, is that these concepts are aimed at achieving a minimum safety factor rather than at striving to improve the safety levels. It has always been our aim to encourage improved manufacturing conditions and reduction in sources of contamination caused by materials and people because a positive aim of that kind creates an awareness of the importance of sterility in the end product. It is expensive to change sterilization processes once determined and if the bioburden is a key figure in the calculations then it seems to me that effort will be to retain the *status quo* rather than improve on it.

Some time ago I saw a report which quoted the Bureau of Medical Devices as stating that so-called "overkill" sterilization can cause manufacturers laxity on presterilization practices. My field experience is exactly the opposite—it appears that the microbiologists whose life is given over to sampling, counting numbers and working out mathematical formulae get hypnotised by their laboratory results and fail to see what is happening around them. I have been told quite seriously that results show no evidence of increased bioburden from cobwebs on the ceiling or operators hair dangling over the product. This apparently made these bad manufacturing practices acceptable. They will never do so in my book, and they serve to confirm my fears of the statistical relevance of the sampling work done.

I'm afraid I believe that a concept so highly dependant on microbiological sampling and testing is in biblical terms a "house built on sand". While the traditional methods of sterilization used in the UK may be called into question as producing unnecessary "overkill"—and that can be challenged scientifically—the fact is that in relation to current proposals they are simpler to operate, simpler to police and equable for all manufacturers.

Turning to the Canadian system of qualifying the term "sterile" with a figure relating to the probability of sterility, I understand that the reasoning behind such a requirement is that to claim sterility is scientifically invalid and gives rise to a false sense of security in users. No one who is in any way professionally involved in sterilization is in any doubt that the term "sterile" used in the

medical products field is not an absolute term and knowing the limitations, would, I suggest, question the validity of the numerical system proposed.

As someone who had devoted some twelve years to the control of sterilization procedures and products I would also question whether the "sense of security" felt by users is indeed false or whether this new proposal would make it any less so. I *am* certain that it will lead to confusion and uncertainty amongst users as to which sterility assurance level should be used in different situations and how they are to equate the levels to the products they have themselves processed by for example glutaraldehyde or other "sterilizing" solutions.

Which is worse I wonder, a false sense of security, or a true sense of insecurity? I should feel remarkable insecure if I was offered a product with an MSI based on a sterilization procedure developed from bioburden data. Unless, of course, that data was generated from statistically significant sampling of all the production lines at different periods of the day, week, and season and of any other variable that could contribute to the microbiological contamination level—and then I doubt if I could the afford the product. I certainly couldn't afford the time to check the data and that as a regulator would have to be my task. At a time of scarce resources we cannot neglect to consider the cost effectiveness of any changes in our control systems. I have defended our standard sterilization methods—steam sterilization at 121°C for 15 minutes or at 134°C for 3 minutes—irradiation at a minimum dose of 2.5 Mrads; and ethylene oxide based on killing a minimum number of specified spore preparations, but the second major challenge which has concerned us has been the control of the control systems.

The *B. subtilis* test pieces used for routine control of ethylene oxide and the *B. stearothermophilus* test pieces designed to validate low temperature steam and formaldehyde cycles have in practical experimentation demonstrated both the need for and the difficulty in ensuring the reproducibility of biological monitors. Many papers have been written about the variations in resistance of spore preparations depending on their growth media and environment, the methods of preparation and exposure and the final test procedure. The lack of reproducibility of results between laboratories has confirmed our belief that a central reference laboratory at which manufacturers and users can have their test pieces standardised may be the only way in which we can be confident in continuing to use biological methods. However all is not well in the area of physical/chemical monitoring either. Despite or perhaps because of our use of a central laboratory for cross checking irradiation dosimeters we have been alerted to both the need for straight-forward quality control procedures to be applied more carefully, and to the fact that we needed to know more about the effects of environmental conditions—temperature, humidity, etc. on dosimeter responses. These are areas now well in hand and the variations have not warranted major changes but they served initially to raise the question in our minds of the validity of some of the original work on which our processes are based.

Having one's faith shaken is no bad thing if you get the right answers in the end. National irradiation dosimetry systems are being standardised internationally. I'd like to propose that eventually when national standards for biological monitors are finalised, we try to arrange for international cross standardisation so that *however* we describe our sterile products there is some international correlation between the sterility assurance levels achieved.

Scandinavian Regulatory Viewpoint —Sterile Medical Products

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In Scandinavia the quality of drugs is regulated by the European and Nordic Pharmacopoeias, regulations and recommendations issued by authorities of Health and Welfare. In Denmark, Norway and Sweden there are different approaches to the regulatory aspects of industrially sterilized medical devices.

In Denmark, a Drug Decree is in force since 1976. A paragraph on medical products is included in the Decree, which gives the right to issue statutory provisions that the law, or part of it, shall apply to products intended for use in the prevention, diagnosis or treatment of disease. It is reasonable to believe that the present voluntary registration of approval will be succeeded by a compulsory registration.

In Norway approval and control of special medical devices have been required, i.e. blood donor and infusion sets. Provisions governing the control of other medical products are in preparation.

Swedish Regulations

In the Swedish code of statutes is an act governing the control of industrially sterilized single-use medical devices. This law came into force on 1st January, 1976. This statute shall apply to the manufacture of products which are intended for single-use and to be sterile when used in medical care and which are sterlized in conjunction with manufacture. The statute does not apply to products which are to be regarded as drugs under the Pharmaceutical Products Decree, i.e. single dose syringes used as containers for drugs, infusion sets, eye drop applicators. Persons manufacturing industrially sterilized single-use medical devices shall take those precautionary and other measures which are necessary in order that the products shall be sterile when used and which can be considered reasonably called for in order to prevent or counteract that they cause injury when used. These paragraphs shall also apply as appropriate to persons who import and deal in these products.

Notification

Persons manufacturing or importing industrially sterilized single-use medical devices shall notify the National Board of Health and Welfare of this fact.

The points of the notification besides notifier/manufacturer are;

—product, which includes appearance and function, short description of the manufacturing process

—particulars of material

—packaging

—sterilization

—information.

The notification of sterilization includes facts about;

—bioburden including frequency of sampling

—name of sterlization plant

—method of sterilization

—method of controlling the process

—test for sterility including frequency of sampling

—name of control laboratory.

Labeling

In the law there is also a paragraph about labeling: Industrially sterilized single-use medical devices shall be suitably labeled with particulars which are of importance for their use, from the point of view of medical care or shall be accompanied by such particulars when supplied to consumers.

The National Board of Health and Welfare has given directions for labeling of industrially sterilized single-use medical devices (labeling of individual ward and transport packs).

The individual product pack shall be labeled with the following;

—name according to accepted Swedish usage, details about type, model, size, number of items (where appropriate)
—applications and directions for use in Swedish (where necessary)
—name of manufacturer
—sterile
—a statement that the product is intended for single use
—batch identification
—expiring date (if less than 5 years)
—special conditions of storage.

The wardpack shall also be labeled with address of manufacturer/importer and method of sterilization. The transport pack for wardpacks with the same contents shall be labeled as wardpack except method of sterilization and statement of single use.

Guidelines—Sterility

The National Board of Health and Welfare may issue directions, e.g. "Guidelines for control of the sterility of industrially sterilized single-use medical devices" with the following points;

—sterility
—autoclaving
—dry heat sterilization
—gas sterilization
—ionizing radiation
—documentation of the sterilization procedures
—test for sterility.

The *definition of sterility* is given in these guidelines as;

—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.

This is the only regulatory definition of "sterility" in the Scandinavian countries. From the medical view-point this low probability of contamination may not be necessary for all groups of products traditionally required to be sterile, for example not more than one living microorganism per thousand units may be acceptable. For some articles now offered as sterile on the market one may even question whether a microbiological quality of single microorganisms per unit would not be acceptable. It may be difficult to find suitable names for these clean products.

In the guidelines are also stated that the pack shall be so designed that the microbiological quality of the products is maintained. The devices shall be sterilized in the sealed product pack.

The *reference of conditions* of various sterilization methods are:

For *saturated steam* it is 120°C for 20 minutes. The physical parameters of the autoclaving process are to be automatically registered for each batch sterilized and regular controls with biological indicators (*B. stearothermophilus*) should be done.

Dry heat sterilization is 160°C for 2 hours. The physical parameters are to be recorded for each sterilization batch and regular controls with biological indicators (*B. subtilis*) should be done.

Ethylene oxide sterilization consists of sterilization at suitable gas concentration, temperature and time and under conditions ensuring a homogeneous gas mixture and suitable humidity in the products sterilized. Regular controls of bioburden are important. The physical parameters are to be controlled and recorded for each batch. The reliability of the sterilization procedure is to be controlled using biological indicators detecting insufficient humidity and gas concentration in each batch (*B. subtilis*).

Formaldehyde. Ethylene oxide sterilization is very seldom used in Scandinavian hospitals. To prevent occupational hazards there is an official limit in Sweden of less than 10 ppm in the working area. Instead, we use formaldehyde in steam at subatmospheric pressure (78-80°C). The process should be controlled by biological indicators detecting insufficient humidity and gas concentration (*B. subtilis*—humidity) (*B. stearothermophilus*— formaldehyde concentration).

Ionizing radiation consists of irradiation in accordance with the recommendations issued by the International Atomic Energy Agency, 1967, in Code of Practice for Radiation Sterilization of Medical Products. The microbiological efficiency of the apparatus shall be established before it is put into operation and after any essential alterations to the equipment. Regular determination of bioburden and the radiation resistance should be done. The dose shall be chosen to attain a probability of not more

than one living microorganism per million units with regard to the bioburden and radiation resistance documented. In practice commonly used doses are 2.5 and 3.2 Mrad for products with bioburden of less than one or less than 50 microorganisms per unit, respectively.

Sterility testing of the final products is not generally required for every batch. For products sterilized by ethylene oxide, however, each batch ought to be submitted to test for sterility according to the European Pharmacopoeia. Process control may be more useful, that is, determinations of bioburden, resistance, sterilizing parameters by physical, chemical and biological indicators.

Guidelines—GMP

We are now working with Good Manufacturing Practice. The GMP contains points about personnel, buildings, equipment, documentation, raw materials and package materials, manufacturing, reprocessing of materials, components and products, quality control, handling in trading and distribution, complaints and recalls.

Guidelines—Syringes

The directions and general advices for industrially sterilized single-use syringes are expected to be confirmed in a year and contain directions concerning materials used, labeling, graduated scale (capacity and scale intervals), tolerance limits on any graduated capacity, leakage, plunger resistance, particles, transparency, harmful contents, sterility, pyrogens, abnormal toxicity, and general advices concerning unit container, graduated scale (dimensions), lubrication, dead space, appearance and dimensions of the syringe and its components.

Guidelines—Product Deficiencies

The National Board of Health and Welfare has given directions to report deficiencies in products which could lead to serious medical consequences and to report products suspected to have inflicted damage to treated patients.

Common Quality Requirements

Although the official regulations are different, the quality levels applied by the authorities are very similar in the three countries. The standards for production hygiene and control, sterilization procedure and control are identical or almost identical. Sterile medical devices are defined in the same terms. The official biological indicators are the same preparations and distributed by the National Medical Institutes.

“In appreciation of the international significance of the Kilmer Conference the following summary was submitted by Dr. Sataro Jitsukawa to Dr. R.W. Campbell, General Chairman of the Conference.”

Japanese Regulatory Viewpoint - Sterile Medical Devices

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In our country, all medical devices which are to be manufactured and/or imported are subject to obtaining approvals for manufacture and/or importation from the Ministry of Health and Welfare. Approvals are granted after the investigation by several committees, based on the Pharmaceutical Affairs Law.

The organizational structure of the Ministry of Health and Welfare is shown in Figure 1.

Application for approval is submitted to the Bureau of Pharmaceutical Affairs in the Ministry of Health and Welfare, who is responsible for checking as to whether or not the submitted application form is completed as requested. And then, the Central Pharmaceutical Affairs Council examines the details of submitted application data. For further professional inspection, there is the Special Committee of Medical Devices in the Central Pharmaceutical Affairs Council.

Under the Special Committee of Medical Devices, there exist seven investigation subcommittees including the Sterilization Investigation Subcommittee, as shown in Figure 2.

Each subcommittee consists of technical experts of between seven and twelve persons. The main roles of each subcommittee are, to evaluate medical devices with originality, out of all medical devices which have been applied for licenses by applicants, and to lay down necessary national standards in order to control safety of medical devices in use. National standards which have been established since 1965 are shown in Table I. These regulate the use of medical devices which are placed in direct contact with human blood and/or human tissue, and disposable devices.

Figure 1. Organization of the Ministry of Health and Welfare

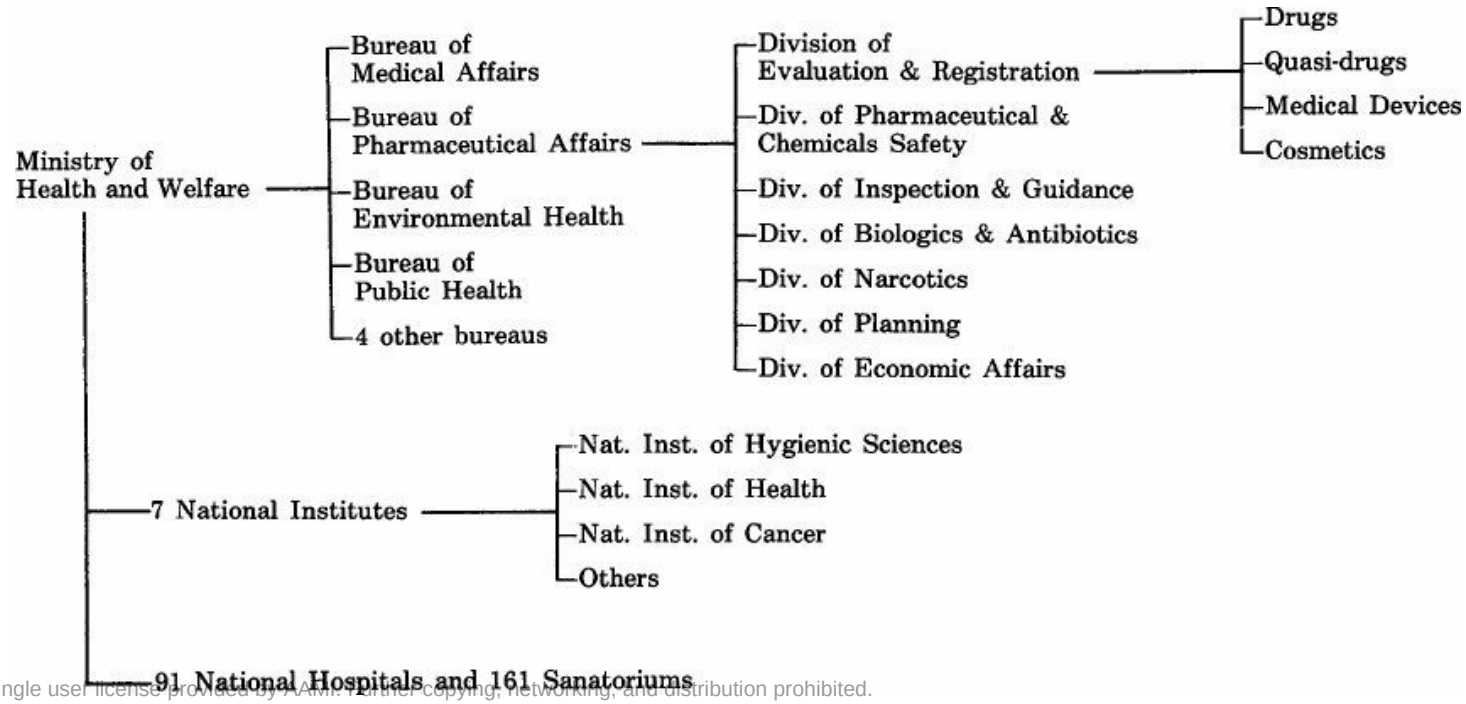

Figure 2. Structure of Various Committees relating to Licensing Approvals

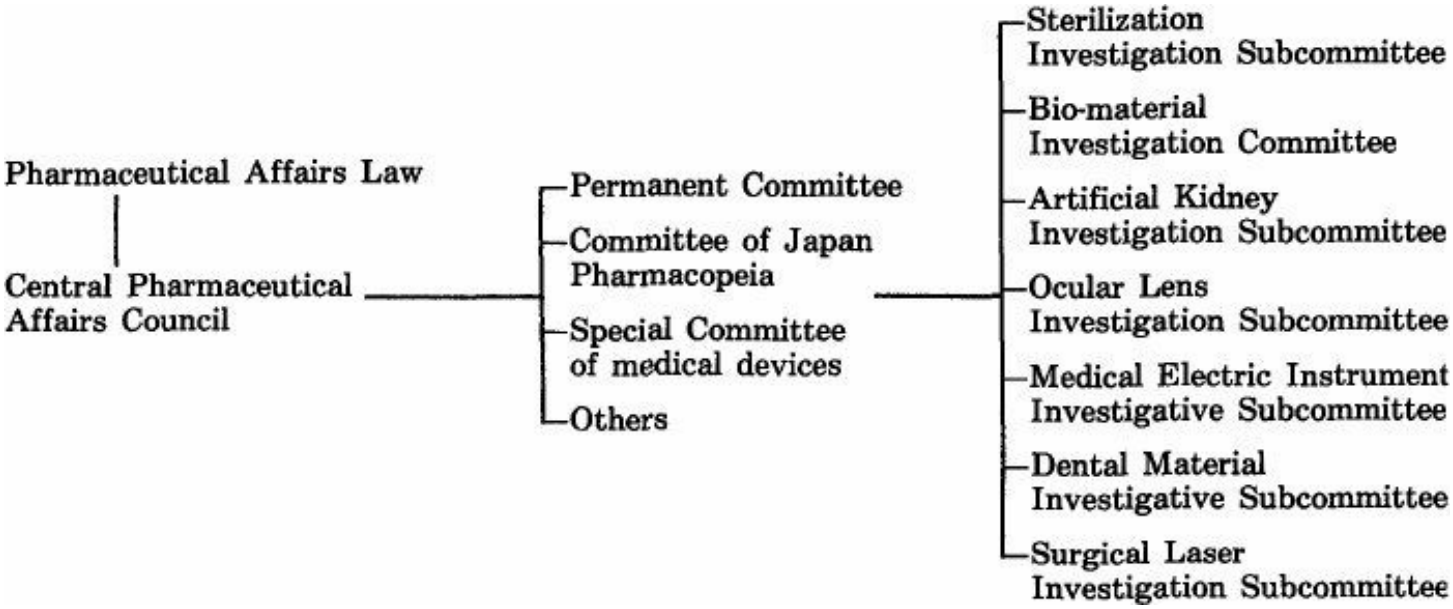

Table I.—List of Medical Devices controlled by Respective National Standards

Medical devices	Since
* Blood bag made by PVC	1965
* Disposable needle	1970
* Disposable syringe	1970
* Disposable transfusion & infusion assembly	1970
* Artificial blood vessel	1970
* Contact lens	1970
* Plastic surgical suture	1970
* Disposable artificial heart & lung assembly	1971
* Artificial heart valve	1972
* Cardiac pace maker	1976
* Silk surgical suture	1965
* X-ray equipment for medical use	1976
* Artificial kidney for hemodialysis (dialyzer, blood tubing, blood access fistula needle)	1980 (projected)

The Sterilization Investigation Subcommittee examines submitted data of products. Items to be inspected by the Subcommittee are as follows:

1. Number of microorganism at the presterilization site.
2. Method and condition of sterilization.
3. Determination of D-value.
4. Number of air born microorganisms in the working environment.
5. Comparison of physical properties of the product before sterilization with those after sterilization.

6. Sterility control during the manufacturing process.
7. Sterility test results on final products.
8. Package materials.

As to application for approval of products sterilized by ethylene oxide, the manufacturers are additionally requested to submit data on product aeration and residual ethylene oxide content.

Even after a product has been approved, a follow-up inspection is made. Products are picked up at random, and checked by our Institute to see whether or not these products are produced or handled in accordance with their application data. Unless a product will pass the inspection, its manufacturer is charged with penalties according to degree of contravention of its data.

When all submitted data on product have passed through the investigation of the Sterilization Investigation Subcommittee, the Ministry of Health and Welfare shall grant an applicant a license for manufacturing or importing the medical devices.

A person who sells medical devices which have not been approved by the Ministry of Health and Welfare, or who advertises exaggerated statements exceeding the limit of the approved descriptions concerning efficacy of medical devices, shall be liable to penalties.

In Japan, the Good Manufacturing Practice (GMP) for drugs has been legislatively in effect since 1976. However, that for medical devices has not yet been established, and is, presently, under preparation. Therefore, the Ministry of Health and Welfare gives instruction to manufacturers of medical devices corresponding to GMP for drugs.

Within one year, the Ministry of Health and Welfare will announce the guidelines on "Residual ethylene oxide content and toxicity of ethylene oxide". The following are the results of studies which our Institute has so far conducted for this purpose:

Our Experimental Results

1. Hemolysis *in vitro*

Ethylene Oxide (ETO) aqueous solution	40 μg/ml, negative 80 μg/ml, positive
Ethylene Chlorohydrin (ETCH) aqueous solution	2 %, negative
Ethylene Glycol (ETG)	20 %, negative

2. Mutagenicity in *E. coli*

ETO aqueous solution	30 μg/ml, negative 50 μg/ml, positive
ETCH aqueous solution	2000 ppm, negative 4000 ppm, positive
ETG aqueous solution	22 %, negative

3. When 500 ml of saline solution was circulated for 5 hrs. in PVC blood tubing (200 g) adsorbing ETO, concentration of ETO, ETCH and ETG in the solution were as follows:

Residue ETO in tube	ETO in saline	ETCH	ETG
1100 μg/g	120 μg/ml (32%)	8(1/15)	3
580	60 (27%)	6(1/10)	1
40	2 (13%)	1(1/2)	0

the temperature of between 50 and 60°C for twenty-four hours. Therefore, I would like to stress the importance of the temperature rather than ventilation.

5. Head space method of gas chromatography is recommended to quantitatively analyze ethylene oxide.

I have made a brief description of the current licensing status on medical devices, especially relating to sterilized products, as well as future projects by the Ministry of Health and Welfare in Japan.

Australian Regulatory Viewpoint—Sterility Testing

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Introduction

The National Biological Standards Laboratory (NBSL) in collaboration with other Sections of Department of Health is responsible for controlling therapeutic goods in Australia. Therapeutic goods include sterile pharmaceutical preparations and sterile devices. With regard to sterility the laboratory approaches control in two ways which are interrelated.

The first and probably most effective form of control is through the development of Codes of Good Manufacturing Practice and inspection of manufacturers for compliance with these Codes. However, this approach applies only to local manufacturers. The second approach is through the examination of protocols for sterile manufacture and sterility testing which companies are required to submit in order to obtain approval for general marketing or for pharmaceutical benefit listing. Protocol examination is applied to both local manufacturers and to goods imported from overseas. The National Biological Standards Laboratory also carries out sterility testing on samples of sterile goods.

In the area of imported goods we find that we are not in full agreement with many overseas companies and governments on requirements for the sterility test although we feel that our experience justifies our stand.

It is not possible in a short paper to cover the general field of regulation and control of sterile goods in Australia so I would like instead to speak briefly about two key aspects of the sterility test where our requirements differ from those applied in other countries.

Let me say before discussing the sterility test that we believe the primary reliance for sterility must be placed on the control of the sterilization process including monitoring of the presterilization microbial load, packing, validation and monitoring of the sterilization process, proper record keeping, storage and segregation of stock.

However, even with the best controls there will sooner or later be problems which arise from technical malfunction, human error, or mix-ups between sterilized and unsterilized goods. The possibilities are almost limitless. The sterility test should be seen as having an important function at this level—to detect the occasional batch of product which is grossly contaminated.

Although limited in its ability to detect low levels of contamination, the test, if carried out and interpreted correctly, is also useful as a tool in detection and correction of intermittent low grade problems.

The sterility test therefore remains a valuable part of sterility assurance.

It appears that there is general agreement on the media and principles of the test methods although there are some variations in sampling schedules. However the incubation period and the interpretation recommended in Australia is at variance with many others.

Table I gives the incubation periods recommended by various authorities. It can be seen that 14 days is being increasingly accepted as the incubation period required for direct inoculation, but most compendia still suggest that a minimum of 7 days is adequate for membrane filtration.

Table I.—Sterility test incubation periods (days recommended by different authorities).

	Membrane Filtration	Direct Inoculation
British Pharmacopoeia 1973	7	7
1980	7	14

United States Pharmacopoeia XVIII	7 or 14	14
XIX	7	14
XX	7	14
National Formulary XIV	7	10 or 14
XV	7	14
Code of Federal Regulations 1979	7	7
European Pharmacopoeia 1971	7	7
1978	7	14
Japanese Pharmacopoeia		7 or 10
Australian Standards	14	14

It appeared to us that there was insufficient evidence to establish that these periods were adequate. Indeed there is a small body of published literature which shows that treatment of organisms with sublethal doses of radiation, heat or chemicals will result in increased lag periods and delayed growth (1,2,3,4). This is precisely what we would expect of an organism which has survived exposure to a bactericide or to a sublethal sterilization process. Extended rather than shortened periods of incubation would therefore appear to be indicated.

To determine whether extended periods of incubation have any useful effect we have incubated many sterility tests for extended periods. In the past five years we have detected 38 positives in tests using membrane filtration and 133 positives in tests by direct inoculation. The results give in Figure 1. show the cumulative percentage of positive tests against the time at which growth becomes visible.

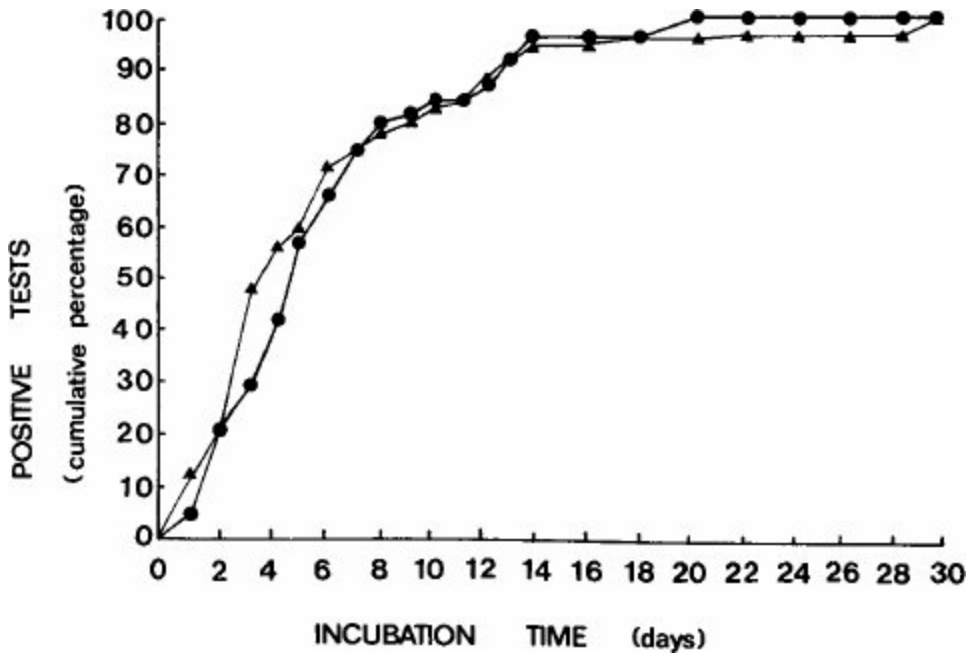

Figure 1. The cumulative percentage of positive sterility test results against the time at which growth becomes visible.

• – • Direct Inoculation
Δ — Δ Membrane Filtration.

Two interesting conclusions emerge. First there is no difference between membrane filtration and

direct inoculation in the time of appearance of growth. There appears to be no justification for regarding 14 days as essential for one method but accepting 7 days for the other. Secondly, it can be seen that if incubation is stopped at 7 days a substantial proportion of tests which would eventually become positive will be missed. Stopping the test at 7 days in fact leads to missing 25% of contaminants.

These results cannot be explained away as due to media of poor quality or as being due to subsequent contamination. All media were as described in the USP and all batches were subject to fertility tests. All NBSL media must show obvious growth of small numbers of added organisms within 48 hours and the tests are carried out both before and after addition of the product. Contamination subsequent to the test is also unlikely as all media are in screw capped bottles and we have extensive data to show that these do not cause problems.

Sterility testing is expensive and time-consuming. A substantial increase in the number of contaminants detected, by simply incubating cultures for an additional week, would seem to be sound practice. However, many manufacturers argue very strongly for a 7 day test period. In the absence of hard data to prove that 7 days is sufficient we can only conclude that the argument is an economic one. Many who support the 7 day incubation period have never incubated test for 14 days.

I would now like to briefly discuss the interpretation of the test.

Both the US Pharmacopeia XX and the European Pharmacopoeia permit a retest if a positive is detected in the first test. The retest is carried out with the same number of test containers and, if contamination is detected in the second test, the organisms are compared. If the organisms appear to be similar then the batch is rejected. If a different oganism is detected then the assumption is made that the growth was due to an adventitious contaminant introduced during sterility testing and a third test is carried out with double the sample size.

The corollary of this assumption is that products which are truly contaminated will only be contaminated with a single type of organism.

Microbiological monitoring of filling areas or of goods prior to sterilization would demonstrate the occurence of a variety of organisms. Our own observation of unambiguous multiple contaminants in “sterile” goods reflects this occurrence.

Where a positive result is obtained in a sterility test it is not uncommon to find two or three different organisms. In one extreme case we found over 20 different organisms associated with a single batch. Finding a different organism in a second test is no proof that the batch was contaminated during the test.

We recommended that if contamination is detected in a test, a retest should be carried out with double the number of test units. If contamination occurs in the second test, the batch should be rejected unless there is evidence in control tests that the test was invalid. This interpretation reduces the probability that a contaminated batch will be accepted as being sterile.

Sterility testing at its best has only a low probability of detecting contamination when the contamination rate is low. Rather than conveniently rejecting contaminants as artefacts, introduced during testing, the efforts should be directed towards improving the quality of sterility testing to reduce the chance of adventitious contamination. With trained staff and proper attention to detail it is possible to achieve rates of adventitious contamination which make it most unlikely that two consecutive tests would give rise to false positives. For example in our own test program we find a background contamination rate of 1 in 3000 transfers. Rather than excusing poor technique the

emphasis should be placed on improving the quality of the sterility test.

We believe that sterility testing, despite its limitations, has an important role. We also believe that the test can be strengthened by increasing the incubation period to a minimum of 14 days and by applying an intepretation which limits the probability that a contaminated lot will pass the test.

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Overview of Control Activities for Sterilization of Medical Devices in the United States

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The interrelationship of various activities concerning the control of medical device sterilization in the United States was reviewed. The role of the U.S. Pharmacopoeia (USP) in defining legal and non-legal information on sterilization was discussed, and the acceptability of alternate procedures utilizing biological indicators or process control release in lieu of USP finished product sterility test was high-lighted. Voluntary-standard setting organizations and the Food and Drug Administration (FDA) inspection programs have contributed to upgrading industrial sterilization practices. The Association for the Advancement of Medical Instrumentation (AAMI) has recently published guidelines on ethylene oxide and ionizing radiation sterilization practices. Other activities include the role of good manufacturing procedures (GMP) previously promulgated by FDA on sterility assurance and validation, the submission of sterilization procedures and sterility assurance methods in premarket notifications (510k), investigational device exemption applications and the use of different levels of probability of survivors.

DISCUSSION SESSION III

Q. by M. Coleman – USA

Considering that the United States sterility test can only provide assurance to $10^{-1.3}$, how can you justify the statement that developing a cycle based on the probability of 10^{-3} or 10^{-6} gives a false sense of security?

A. by M. Duncan – England

I am not sure I understand the question, but I will try to base my answer on what we do in the U.K. Since we do not rely on sterility testing, but on sterilization overkill, we do not feel we have a full sense of security. We feel we have a reasonable sense of security. I can see problems in countries where they do depend on the sterility test. I would certainly understand the reasoning behind what has gone into finding alternate methods.

Comment by M. Coleman – USA

I got the impression that you felt the sterility test was a very important factor in release of a sterile product. You mentioned a situation where you went in and you inspected a plant where they said they were doing bioburden testing, but the plant still looked sloppy and they were using that as an excuse. That to me is an obvious excuse and one that should not be accepted.

Comment by M. Duncan – England

I am sorry if I gave the impression that we relied, in any way, on sterility testing, because in fact, we do not, except for ethylene oxide where in common with most people, we still retain sterility testing in batch release. I think perhaps I should try and draw the line between what worries me about bioburden figures and what we expect in terms of control of presterilization microbial count levels. We have never set a figure, formally anyway, on what we were looking for in terms of background count before sterilization. But we have always expected that the companies did in fact know the kind of levels that they were getting on their product and that they were keeping them as low as they possibly could and that they were identifying any high levels and doing something about it.

But that said, we also expect, as part of our requirements, that there shall be a microbiologist in the plant and we expect that microbiologist to go around with his eyes open and spot things which could cause high levels of contamination and put them right. You do not need to test to see that something is wrong, you can see it and you can do something about it.

Q. by G.E. Heinze – USA

As a follow up to that, you mentioned that you were using, and you felt comfortable with, overkill processes. What is the level of sterility assurance on your overkill processes?

A. by M. Duncan – England

The overkill processes were worked out using the most resistant organisms that people could come up with at the time when we were all challenging or trying to work out what were acceptable doses. Now bearing in mind that irradiation came to the U.K. in 1960, and we have been using the same procedure ever since, the dose of 2.5 Mrad was then accepted, knowing the kind of kill that that could produce in resistant organisms. We then set, or tried to set, lower and lower bioburden levels from that, giving us certainly an order of 10^{-6}, but probably a good deal better than that, because in saying 10^{-6} we were assuming that the bioburden organisms were all as resistant as those on the test pieces. We were talking about figures around 10^{2} per item and inactivation factors, as we used to call them, of about 10^{8} or 10^{9}.

Q. by J.E.W. Nygard – USA

Since Sweden requires suppliers of medical devices to prove a 10^{-6} margin of biological safety for products sterilized by both ethylene oxide and ionizing radiation, what is the reason for continuing a requirement for sterility testing each lot of ethylene oxide sterilized products?

A. by L. Sjöberg – Sweden

I should say it is not quite a requirement. It is open for discussion. The guidelines now are in the revising stages and we are still waiting to discuss their effect in the Common Market. They can really develop their own requirements and therefore we have to move slowly. I think that you will find soon that there is no sterility testing for ethyene oxide either.

Q. by P. Schneider – USA

Do you have any concerns that adherence to fixed radiation doses may in effect lead to a suppression of sterilization technology development in your country?

A. by M. Duncan – England

No, and I cannot say that I regard varying the dose up and down as falling into that category. Certainly, we have looked at alternative methods of sterilization. We are looking at low temperature steam and formaldehyde in the traditional way by using resistant spores and making sure the cycle will kill them. We have been giving it a very close scrutiny before we are prepared to recommend it to our hospital service.

Comment by P.T. Doolan – France

I think that perhaps one of the frustrations which industry, or parts of the industry, finds in dealing with the Scientific and Technical Branch of Department of Health, is that they sit on the fence in relation to the definition of sterility, and the widely accepted 10^{-6} Minimum Sterility Assurance Level is not espoused by them. This, I think, frustrates, to some extent, the design of projects to investigate improved methods of sterilization, or dose setting or cycle reduction, because the end point remains vague and wooly. I would appeal to them to come off the fence.

Q. from the floor:

Please give the British definition of "sterile."

A. by M. Duncan – England

I can answer both questions at once. I think if we have to put figures on it, then it is reasonable to say that we regard 10^{-6} as the minimum that we would be prepared to accept. But we know we can get better. I would rather have better. We still do not know enough about microbiological monitors, nor about the resistance of the bioburden that we are trying to deal with, and there are a lot of other factors. Our definition of sterility may sound unscientific, but we accept a firm's claim that their product is sterile and they can label it sterile, if they have subjected it to one of the processes which we have laid down, which includes for radiation a minimum dose of 2.5 megarads, combined with the correct manufacturing conditions and a demonstration that they are keeping their bioburdens low. For ethylene oxide, we tie it down to our biological indicators and for steam sterilization, we have a temperature cycle laid down. People who have processed their product by one of these methods and claim their product is sterile, we would say that it is. If they have not processed it that way, we are free to query whether they, in fact, are producing a sterile product.

Comment by Chairman A. Tallentire – England

I shall depart from the impartiality expected of the Chair to say that I feel there is a slight inconsistency here in that there are cycles which are less than compendial cycles accepted within the Department of Health and I wonder whether or not there ought to be in fact an analogous acceptance of less than a rigid stipulated radiation sterilization dose.

Comment by M. Duncan – England

I think it is true, as Mr. Doolan pointed out, that under legislation for medicinal products, there is an allowance that a lower than compendial dose or lower than the traditional method could be accepted, provided that data is supplied. I suppose the fact is that, if somebody ever did produce data, and we had the time to look at it, we might indeed accept a lower dose. But then cost-effectiveness comes into play. We are not in a legislative position. We are in a supply department which is concerned with purchasing and the customer can buy what he wants.

Q. by K.H. Morganstern – USA

Based on your curves, why not a 21 day incubation period to pick up all positives?

A. by D. McKay – Australia

A 21 day incubation period would not pick up all positives. Purely by accident we left some cultures in the incubator and forgot them and we had positives at up to 42 days. But if we suggested to industry that we should be going to 28 or to 42 days, they would have a fit. So we will go a little way and say 14 days for a start. When we get that accepted, perhaps we can look at it again.

Q. by C.C. Mascoli – USA

What, if any assurance, do you have from your experimentation that the media were adequately sterilized? Also what assurance do you have that the closures and the ambient relationships were such that there was not an air current contamination of the medium while the test was being incubated?

A. by D. McKay – Australia

All batches of media, have the usual quality control on their sterilization processes. All bottles of media are incubated for a minimum of 14 days before use, after which they are held at room temperature, which probably varies between 15° and 20°C. That is a minimum of a fortnight before use, and an average of a month holding time. In the last five years, we have had perhaps several hundred thousand bottles of media and we have not had a single incident of pre-use contamination. This, I think, gives us a fair assurance that the media had been sterilized and that the closures are adequate to maintain sterility.

Comment by C.C. Mascoli – USA

You are dealing with low levels of contamination and you cannot say that the medium in which the positive occurred did not have a survivor, except on a statistical basis. You said you have had hundreds of thousands of bottles of media, representing many many different batches produced at many different times. In my experience, I know that dry powdered media can contain up to 10^{12} spores, many of them very resistant. You have a much greater difficulty in sterilizing media than you do regular products.

Comment by D. McKay – Australia

We have additional information that these positives are not the result of media contaminants. For a start, there are negative control inoculations which we find useful. Our control incoluations show a much lower frequency of contaminants and these are incubated for the full period and undergo the full test procedure which includes opening and closing bottles, movement in and out of the sterility test suite, handling during observation and so on.

Comment by C.C. Mascoli – USA

We have considerable experience with different kinds of closures of media tubes and find that, in fact, over a time, it is quite possible for wind currents to carry spores and organisms into even fairly recessed closure areas.

Comment by D. McKay – Australia

We certainly would not permit any cotton wool plug closures. We use screw cap closures in which the seal is an integral part of the molded plastic. We believe that these caps are not subject to contamination problems for the reasons indicated.

Comment by C.T. Hudson – USA

I would like to address the point Dr. Mascoli was making. We did a study about two years ago with some product containers that had to be tested by direct inoculution. We were using a container that is similar to something you would see in a grocery store. It was an olive jar with a screw cap top. We seemed to have a higher incident of positives with this particular type of closure. So what we did was to conduct some experiments on this particular closure and the same closure on which we put aluminum foil over the cap and held it in place with a rubber band. What was happening was that there was some turbulence during incubation, especially in large walk-in type incubators. We saw a big difference in the two types of closures, which leads me to conclude that contaminants can be introduced in a sterility test during incubation.

Comment by A.S. Outschoorn – USA

Just a point about the incubation of media. The period of seven days after membrane filtration in the USP sterility test was based on the results of a collaborate study organized many years ago by Frances Bowman of the FDA who was also at that time a member of the USP panel on microbiology. The results of this combined FDA/USP collaborative study, where a number of different kinds of microorganisms were inoculated into the media, were quite clear in that nothing that appeared up to ten days in direct inoculation failed to appear on the membranes in seven days. Secondly, the membranes disclosed a few fastidious types of microorganisms which did not appear at all on direct inoculation. The second point here is that, in the past, 14 days may have been required after membrane filtration. This was with Sabouraud Medium and not the Soy Bean Casein Digest Medium, which is now the official medium described in the USP. I would like to stress the point made by Dr. Mascoli, that before drawing conclusions that one requires as much as 14 days after membrane filtration, one must remember that this is just not a case of direct inoculation. One must make some assessment of the growth sustaining properties of the various media. The seven day period is based on Soy Bean Casein Digest Medium of the quality and properties which are generally commercially available at this time from various manufacturers in the United States. I do not know what sort of results would appear if media with different properties or similar media were used.

Comment by D. McKay – Australia

I would like to answer that question. The Bowman paper which is referred to has some deficiencies. I think the only way one can measure the time required for growth of organisms damaged by a sterilization process is to use organisms which are damaged by a sterilization process. The paper you referred to used laboratory cultures of organisms. These were fresh, healthy, thriving organisms which were adapted to laboratory media. Even under these conditions, it took from a few days in some cases up to ten days for the growth to occur. Damaged organisms might require longer. As I pointed out in my paper, we incoluate all our media with approximately ten microorganisms. We aim for ten; we do not accept any more than 50 microorganisms. If we do not get obvious growth in 48 hours we consider that there is something wrong with that batch of medium. I believe that the fertility of the media we are using is at least as good and probably better than that which was used in the FDA/USP study. We are using Soy Bean Medium, not Sabouraud Medium.

Comment by G.E. Heinze – USA

I think perhaps we are putting an emphasis where it does not belong. We are spending a lot of time talking about incubation time. I remember discussions of this sort on this subject 10 years ago — even 15 and 20 years ago. I think the whole point that Dr. McKay made was that they were using essentially finished product testing in his laboratory, which we are talking about here, with a prolonged incubation period, because he felt it was useful in catching that odd contaminated load. I do not think he is disputing the data that has been presented over the years, that regardless of whether you take 10 samples or 20 samples or 40 samples or whatever from a sterilizer load and finish product test them with an incubation period of 10 or 14 or 21 or 52 days, that you are not going to have any real information about low levels of contamination. The only thing it is going to yield is information on grossly contaminated loads — something in the order of 15%. What a number of us are suggesting is that the whole ritual of finished product testing, regardless of whether it be done along the lines Dr. McKay suggested or the USP line is really counter-productive, or nonproductive, if the sterilization process is carried out in the manner that has been suggested by a number of speakers at this meeting.

Comment by D. McKay – Australia

I would like to emphasize that a finished product sterility test definitely has its limitations and it is only capable of detecting gross contamination with reliability. However, I would like to take issue with one small point — that it is of no value when looking at products where there may be only low grade problems.

It has been our experience that the sterility test has been able to detect low grade problems and has led to a solution of these problems. I can give you an example. It is not an example of a sterile device, but I do not see that it should made a great deal of difference. We had a product which periodically turned up a positive result — maybe one in every three or four batches. We would never get a positive on a repeat test. If there was anything there, it was obviously at a low level, and yet, when we compared the results with the contamination rate in our control tubes, it was roughly double the control rate. For an individual batch it was never possible to establish that that batch was not sterile. Yet over a period of time, a statistically valid history did indicate that this product from this manufacturer did have a problem.

By going to the manufacturer and investigating the methods of manufacture, it was possible to put our finger on what we thought was the likely source of the problem and rectify it, whereupon the low level of positives vanished. Sterility testing, if it is carried out properly and interpreted properly, does have value in tracking down some of those lesser problems.

Q. by H. Markinson – USA

Please comment on the Bureau's position on voluntary standards?

A. by C.W. Bruch – USA

I think the industry here in the United States is aware that we support voluntary standards as a general principle, but the Bureau of Medical Devices will not publically endorse a voluntary standard.

Comment by R.W. Campbell – Canada

We looked at this question very closely when we first started setting up regulations and consulted our legal people in Canada and the answer they gave me will apply in the States as well, because both of us are basically common law countries. The principle which they quoted to me is the principle of "Delegatus non potest delegare," that is, once power is delegated by the Government to a specific body, that body does not have the power to delegate that power on to somebody else. If we were to endorse the standards of an outside body, we are in fact delegating the standard writing power to that body. It was on this principle they told us that we cannot simply endorse a standard or reference a standard in our regulations. There is nothing whatsoever to prevent us stealing the whole thing, word for word, and writing it into our regulation, but it then becomes a regulation under the Food and Drug Act. It is not constitutionally possible for us to endorse a standard written by an outside body because we are passing on power that was given to us.

Comment by M. Duncan – England

I would like to say that this is the situation in the United Kingdom as well. We cannot simply endorse voluntary standards from other bodies.

Q. by R.W. Campbell – Canada

Miss Duncan has suggested that sterilization might best be controlled by the techniques of a pharmacist of the 1897 vintage. Has she heard anything in these two days that would modify this view?

A. by M. Duncan – England

No. I am not really sure that I have. If you look at that paper of Kilmer, I am not at all sure that he was not turning out dressings with a higher probability of sterility than we are getting at the moment.

Q. by C.C. Mascoli – USA

Are you aware of the several publications and presentations in the U.K. and worldwide which address the concerns that you express: bioburden, sterility assurance for parenterals of 10^{-6}, F_0 and its correlation to lethality, both biological indicators and bioburden? Many of these studies were carried out on products in vessels by subprocess dosing (fractionals). Also many studies encompass a total quality assurance approach to product sterility and would indicate that state of the art should now give less concern for sterility assurance for steam sterilized products. Much the same kind of studies have been carried out for ethylene oxide and gamma radiation. Also are you aware of the relationship between say an F_0 of 8 and the equivalent kill offered by such a cycle in terms of commonly encountered bioburden prior to sterilization (numbers and resistance)? Commonly this is greater than or equal to 10^{25}.

A. by Mr. Duncan – England

I have read many of the papers that have been referred to. I think we still come back to the problem that concerns me most — nearly all the papers use sample bioburdens in the equations and bioburden is not necessarily amenable to statistical sampling. We know that the bioburden can vary at different points in a production line; at different times of the day— shift changes, etc.; at different times of the year— changes in temperature, humidity, wind direction, etc. Unless such variations have been thoroughly researched and are understood, the application of statistical sampling cannot be accurate. I have said already that 10^{-6} is our minimum and if we can get a better sterilization probability, then we are extremely happy to do so. We know no good reasons for reducing a probability that we have been achieving for a very long time.

Comment by C.C. Mascoli – USA

I was referring to, and I thought you were referring to, overkill cycles which do not, in my mind, require statistical sampling for bioburden. In fact, the studies I referred to do carry out bioburden analysis, a multiple total quality assurance approach to analysis. But I was referring to an overkill cycle, and therefore, I do not believe bioburden plays as much of a role or whether a statistical sample plays as much of a role. More important is the relationship between commonly encountered bioburden prior to sterilization. Admittedly it is a sampling. But across time it is a good representation of the total process and if, with filtration, which is commonly used, that bioburden is either zero or of one order of magnitude and rarely, if ever, contain spores, then indeed a sterilization cycle of a F_0 of 8 would deliver an order of magnitude 25 or greater in terms of that bioburden.

Comment by M. Duncan – England

Yes, I accept that. I think that is a reasonable statement. Indeed it has long been a surprise to me how very far we have gone along the lines of reducing the bioburden in the world of pharmaceuticals. Very many of the solutions, which are presented for total sterilization, are indeed sterile before they go near the cycle.

Q. by C.W. Bruch – USA

How did you arrive at the F_0 of 8? Was that directed to you or did you derive that on your own?

A. by C.C. Mascoli – USA

I just used that as an example. But, in fact, I believe in the U.K. some of our registered products have a requirement of an F_0 of 8.

Q. by C.W. Bruch – USA

And the product still survived the processing? Is it still usable?

A. by C.C. Mascoli – USA

Those in fact which were submitted and required to go through F_0 of 8 do. But I doubt if some of the products which are considered heat sensitive, would stand up too well under such processing.

Comment by J.O. Dawson – Scotland

The U.K. Gamma Panel would like me to express its views. I think the U.K. understands quite clearly what is meant when we use the term "sterile." Providing good manufacturing practice has been applied, there has been parametric dosimetry and the dose of 25 kilograys has been administered, the product may be called sterile without question. No sterility test is required. Now, when it comes to a sterility assurance of 10^{-3} as against 10^{-6}, I am reminded of a little piece of paper I saw many years ago on a certain piece of furniture in an American hotel, which said "Sanitized for your convenience," not sterilized, but sanitized. I think if you say something is sterile, 10^{-3} or 10^{-6}, you are confusing the medical profession. If you say one is sanitized and the other is sterilized, then the surgeon or the doctor can select the degree of safety he needs. We do not feel in the U.K. that we are stultifying development in the science of radiation.

As Miss Duncan mentioned, gamma radiation has been used commercially in our country for 20 years. It has been used in the form of electron beam in your own country for 24 years commercially. And over the last 20 years, there have been meetings of an international nature, in which the latest American thinking has been presented. The result is that in France, if you are going to radiation sterilize, you must determine the bioburden, you must put in dosimeters, you must have biological indicators, you do a sterility test and finally apply an expiry date. This has been picked up from various meetings over the years as American thought has changed.

Q. by R.W. Campbell – Canada

Does the Gamma Panel have a definition for surgically clean and sanitized?

A. by J.O. Dawson – Scotland

No. The Gamma Panel does not. I can tell you its thinking, however. It would never say 10^{-3} is sterile, as the word is used in England and Scotland.

Comment by C.C. Mascoli – USA

There are products which are heat labile, which are aseptically filled and have an assurance of 10^{-3} or slightly better. These products are being used everyday and used parenterally. I would hate to see them labelled sanitized.

Comment by R.W. Campbell – Canada

When we first put out our proposal about MSI, one of the things we mentioned in the information letter was the possibility of putting numbers on labels. This got everybody hopping mad since then the discussion has centered almost entirely on the cost of putting numbers on the labels. That is a total irrelevance. What we were offering with the MSI concept was a new language and it did not intend at that time to be more than a new language. This business of various levels of sterile should not arise. If our concept were accepted, as originally offered, then we would talk about all the things that are now called surgically clean or sanitized. It would simply be said to have an MSI of 1 or an MSI of 2 or an MSI or 4, or whatever it happens to be. We would not use the words sterile or sanitized or clean or any of those words. It would make it possible to determine on a reasonably scientific basis, even if our statistical sampling was not to Miss Duncan's satisfaction, what degree of microbiological safety was required for that product in the light of its intended use and then set an MSI level on that basis and process it to a specific level, without saying "I think that is clean enough," which is not what we are saying just now.

Comment by M. Duncan – England

Could I just say that, obviously, we are very concerned about the work that is being done in the USA and are waiting for more information to appear. We may be wrong, but we believe that the upsurge in work on irradiation was prompted by the scare about ethylene oxide. We had just got over the ethylene oxide business and we were congratulating ourselves that we have moved into low temperature steam and formaldehyde as an alternative, when the paperwork started to appear querying that compound as well. Our own medical people tend to be less concerned, at least at this stage. We have been using formaldehyde for so very long without any really clear indication of any problem arising. It has been around for very much longer than ethylene oxide has. That may be a totally unreasonable feeling of ease about it. We are very anxious to see the information which is coming out of the test work which is being done.

Q. by S. Marcus – USA

Why does no one refer to the US Army experience with radiation sterilization of food which has been given up because of the appearance of carcinogens, if I recall correctly?

A. by G.E. Heinze – USA

As a matter of fact, most of the early work done in this country on radiation sterilization was done on foodstuffs. I am aware of papers on this in the middle or the late 1950's. As to the specific point of carcinogens, I was aware that there was a concern that with the radiation levels being used, there was indeed some proof of molecular rearrangements and some splitting of proteinaceous materials and the concern that some of the materials being produced would have mutagenic and/or carcinogenic effects. However, I am unaware of any definitive work that was ever done to demonstrate that indeed this occurred. I think that what happened at the time was that there was a fear that they might be dangerous and, as a result of all the testing and work that would have to be done to remove that fear, a decision was made not to pursue the process basically because of the economics that would be involved, in order to prove it essentially negative.

Comment by P.T. Doolan – France

I think it might be of interest to the attendees if I were to take a little further the view of what is happening in Europe and what industry responses have emerged. I mentioned several recently formed associations or confederations, and I outlined the concern felt and the steps being taken by industry, in general, to organize itself, to deal with their lack of understanding, in some cases, and in other cases deal with the lack of having a satisfactory interface with the regulatory agencies. This led to a number of concerned companies getting together, embarking on a study and on the preparation of a white paper. This should summarize, on a country by country basis, on the one hand the status of regulations and the current trends in the activities of agencies such as the Department of Health, pharmacopeia, standards bodies, and on the other hand what is going on in the way of an industry response. We should be able to see if, in fact, the nucleus for a better performing interface can be identified. The work started at the beginning of 1980 and it is hoped to conclude it about the end of the first quarter of 1981, with a report. Apart from that particular exercise there is the activity of formation of ECOMED (European Confederation of Medical Suppliers) which has, to a large extent, been focused on sterile devices. They have working groups studying the regulations relative to GMP, to product labelling and to sterilization in the countries of Western Europe. These are, perhaps, the most significant activities at the present time.

Q. by Chairman

Can you tell us about the availability of that report?

A. by P.T. Doolan – France

The circulation in the first instance will be for the participants. No decision has been taken about publication and obviously, the level of interest in having it made available would influence a decision on that.

Comment by M. Duncan – England

I think I have been under reasonably strong attack here for the U.K. situation, but can I just point out to everybody that we have here on the panel, a representative from Scandinavia which has much more stringent overkill requirements. Across the rest of Europe we have far far more stringent requirements, in fact in terms of overkill, than there are in the U.K. We are somewhere down the middle of the line. Believe it or not.

EULOGIUM

The following was delivered by Mr. Masefield in accepting on behalf of Charles Artandi the Pioneer Award on the occasion of the Second Gamma Processing Seminar of the Atomic Energy of Canada Ltd., held two and one-half months after the death of Dr. Artandi.

Coinciding with a Revolution

John Masefield

Isomedic, Inc.
Whippany, New Jersey, USA

Born in Hungary where he achieved his PhD in the Royal Hungarian University of Budapest, Charles Artandi was a gentleman, a gentle man, and a man with a vision for Irradiation Sterilization.

It is rumored that irradiation sterilization first came to use in Australia, where it was used by a carpet company to irradiate goat hair used in the manufacture of carpets. Charles Artandi too first came to Johnson & Johnson in Australia, prior to his transfer to its Ethicon subsidiary in 1953. Ethicon, serious about the acquisition of new sterilization technology, assigned Charles the task of developing reliable sterilization techniques.

At that time, he had a choice of two sources for irradiation sterilization: (1) the high-energy electrons produced by various types of accelerators, and (2) radioisotopes like cobalt-60. Since accelerators were more available and less costly than cobalt-60, Charles led a team which produced major studies on the effects of radiation on, (a) one hundred different types of microorganisms, and (b) suture materials and a great variety of packaging materials.

He also completed vital biological safety tests, clinical studies, and NDA filings on irradiated sutures.

Still he was seeking a more reliable sterilization method, with greater safety than that afforded by linear accelerators. In 1960 when the price of cobalt came down significantly and the technology for its use became available, he recommended a move away from the complex electronic equipment required for other sterilization methods to a simpler, more easily controllable process. He began to switch to cobalt.

His extensive studies showed that:

1. Gamma radiation significantly improved the sterility assurance and quality of vital hospital products.
2. It provided the opportunity for the complete redesign of suture packages, and improved access to the sutures in the operating room.
3. It offered an opportunity to eliminate the use of glass tubes in the sterilization process and replaced them with more convenient and less expensive packaging materials like aluminum foil and various laminates.
4. It eliminated high-temperature heat sterilization and the associated aseptic fill-and-seal operation, simplifying Ethicon's own manufacturing operation from batch-orientation to a continuous process. Today, since irradiation sterilization is the only truly continuous sterilization process, it fits well into the flow of many manufacturing operations.
5. It was the most easily controlled process too, a factor which is recognized by the FDA's acceptance of the concept of "Dosimetry Release."
6. In addition, irradiation sterilization presented no potentially toxic residues—and created added space and inventory savings.
7. Finally, such improved quality and increased convenience helped to establish Ethicon's

leadership role in the suture industry.

Charles Artandi was awarded the Johnson Medal for Research and Development in 1978—the highest honor the company offers—for his leadership in the development of irradiation technology. In the same year he received the American Nuclear Society's Radiation Industry Award for his pioneering achievements.

And that's still not the end of his story. In the two years since those awards, he led the fight to win other major battles as well.

He helped support the movement towards the world-wide acceptance of irradiation sterilization and of flexible sterilizing dose, established by responsible manufacturers or custom sterilizers on the basis of initial contamination, end use, and the infection causing potential of the system. He also envisioned a national network of sterilization facilities which, he believed, would save companies large and small the cost of capital and the cost of maintaining technical staff and laboratories in a variety of disciplines, and could provide the opportunity for sharing the cost advantages of much larger and more flexible sterilization plants. This network now also exists.

These advantages are being achieved today through the work of Charles Artandi.

The Somerville radiation plant which he established became the Mecca for people from all over the world with an interest in sterilization. The successes he achieved there stimulated many to try this exciting technology in their own countries.

Yet, of all this he said, "The timing of my sterilization studies (merely) *coincided with a revolution* in the healthcare industry which lifted the heavy burden of cleaning, maintenance, and sterilization off the backs of hospital personnel."

What lies in the future? I studied his recent speeches and writings to see what he might have said to us all. Here is his partial answer.

"The moral of my story is," he wrote in June of 1978:

1. "Take risks, because all benefits of a new technology cannot be predicted by early cost analyses."
2. "New technology often reaps unexpected benefits. It may provide an opportunity for new product designs, for the use of less expensive materials, and so on. Remember, therefore, that new technology 'per se' does not have to be cost effective."

In 1978 Charles Artandi was named the "Father of Irradiation Sterilization." And we are his sons and daughters.

To those who are at this table, nay, to all of us, Charles would say he was fortunate to work in this endeavor. "Whatever success I had," he insisted, "was largely due to *others* who did fundamental work here and abroad… to companies with vision and universities." For us, he had one wish, as expressed when accepting the Johnson Medal: "It is with deep gratitude, I wish you all continued success, for the real reward of innovation is to see the contributions of our work develop over a period of time."

Today, Charles receives posthumously the Atomic Energy of Canada Pioneer Award for his achievements in the field of dosimetry, radiation sterilization, standards and methods.

During his lifetime this movement advanced from a carpet factory in Australia to become the finest sterilization procedure ever known. I accept this award on behalf of Charles Artandi, and for his wife, Susan, who like him, is a gentle woman, delicate, and compassionate. May our endeavors also

“coincide with a revolution.”

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Note: Numbers in italics represent figures, those in bold face type represent tables.

*R.J.L. Bondara, *et al*. Properties of *Limulus* amebocyte lysate and the turbidimetric assay for the quantitative determination of gram negative bacterial endotoxin. Worthington Biochemical Corp. 1978.

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*Editor's note: name changed to EUCOMED in May 1981.