

# STERILIZATION OF MEDICAL PRODUCTS

# STERILIZATION OF MEDICAL PRODUCTS

## Volume IV

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# Johnson & Johnson

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Medical Products Disinfection and Preservation**

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# Preface

This volume represents the proceedings of an international conference on the subjects of sterilization, disinfection, and preservation. The conference was a gathering of technical professionals in the sterilization field representing 18 countries around the world and totaling approximately 300. The number of participants far exceeded attendees at the previous five Johnson & Johnson sponsored international conferences on this subject.

During the meeting it was appropriate to recognize, by a special award, two world leaders in the science of environmental microbiology and sterilization. The award was established ten years ago to honor the memory of Dr. Fred B. Kilmer, Johnson & Johnson's first Technical Director and an early pioneer in the fields of sterilization and environmental microbiology. Previous awards have been made to Dr. Charles R. Phillips and Dr. Saul Kaye who laid the foundation for the use of ethylene oxide as a sterilizing agent and to Dr. Jocelyn Kelsey, Deputy Director emeritus of the Public Health Laboratory Service of Great Britain for his many contributions to sterilization and the control of microorganisms in the environment. At the conference banquet, Kilmer Awards were made to the following scientists:

Professor Liu Yu-jing of the Institute of Microbiology and Epidemiology of the Academy of Military Medical Sciences for his numerous contributions to the control of organisms in the environment.

Professor Irving J. Pflug, Professor of Microbiology and Food Technology in the University of Minnesota, for his work in the area of moist heat (steam) sterilization.

The non-Chinese participants would like to express their appreciation for the courtesy extended by the Chinese sponsoring organizations and the warm reception by the Chinese participants.

E. R. L. Gaughran  
R. F. Morrissey  
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# Banquet Address

**Deputy  
Chairman:**

**Mr. Yang Jing-ren**

*Chinese People's Political Consultative  
Conference  
Beijing, China*

# **Address at the Banquet sponsored by the Chinese People's Political Consultative Conference and the Ministry of Public Health of the People's Republic of China**

**Mr. Yang Jing-ren**

*Deputy Chairman*

*Chinese People's Political Consultative Conference*

*Beijing, China*

Honorable guests and dear friends:

On the occasion of the banquet which is given just before the opening of the Beijing International Scientific Conference on Sterilization of Medical Products, Disinfection and Preservation, please allow me, on behalf of the National Committee of the Chinese People's Political Consultative Conference and the Ministry of Public Health of the People's Republic of China, to extend my warm welcome to all the participants from different countries in the world and to wish a complete success of the conference.

The Beijing International Scientific Conference on Sterilization of Medical Products, Disinfection and Preservation, which is going to start from tomorrow, is initiated and supported by the Medicine and Health Subcommittee by the Chinese People's Political Consultative Conference National Committee and the Ministry of Public Health of the People's Republic of China. It is organized and sponsored by the China National Center for Preventive Medicine, Johnson & Johnson Company of the United States and the China Medical and Health Corporation. The Johnson & Johnson Company provided the fund for this conference from the Johnson Foundation. I would like to express my gratitude to Johnson & Johnson Company for their kindness and I would also express my warm welcome to the Johnson & Johnson delegation headed by Mr. Clare, President of Johnson & Johnson, who is here specially for attending the opening session on tomorrow.

China is a developing socialist country. Before liberation, the mass of Chinese people suffered seriously from poverty, infectious diseases were highly prevalent and there were very limited health facilities. As a result, the health of Chinese people was in a very poor situation. After liberation, the Chinese government has paid great attention to the development of medicine and public health undertakings. Not only a series of policies fit to the situation in China were promulgated, but also basic construction on medicine and public health have vigorously come out. As a result of the efforts in the last 30 years, the health

situation of Chinese people has been greatly improved. The average life-span of Chinese people has been increased from 35 years old before liberation to 68 years old at present. However, in the field of research on medicine and public health, including the sterilizing technology, our works were started rather late. So, as compared with the advanced countries, we still have a long way to go. With the development of the economy and science and technology, our works in this field will also be improved. We hope we will get help from the experts in sterilizing technology from various countries. We believe that this International Conference on Sterilization will greatly speed up development of the science and technology of sterilization in China.

The open door policy is now a fundamental national policy of the Chinese government. Based on the principles of maintaining dependence and equality and mutual benefit, we will continue to strengthen our trade contacts and technical exchange with other countries. In this respect, medicine and public health is one of the important fields.

Although our Conference is of short duration, our friendship will be long lasting. For sure, this Conference will enhance our mutual understanding and friendship.

In the National Committee of the China People's Political Consultative Conference, there are many top Chinese scientists of medicine and public health. We hope, during this Conference, we will expand our contacts with friends in the field of medicine and public health from other countries and also wish to facilitate the development of medicine and public health undertakings in the whole world through our joint efforts.

Finally, I would propose a toast for the development and continuance of the friendship between peoples of China and other countries in the world, for the complete success of this Conference, and for the health of all our guests, friends and colleagues.

# Opening Ceremony

**General  
Chairman:**

**Prof. Chen Chun-ming, M.D.**

*Director: Chinese Academy of Preventive  
Medicine  
Beijing, People's Republic of China*

# Opening Remarks by Dr. Chen Chun-ming

## General Chairman

Vice Minister, Dr. Chen Min-zhang, Dr. Shen Qi-zhen, Mr. David Clare, Ladies and Gentlemen, Colleagues:

The 1985 Beijing International Conference on Sterilization of Medical Products, Disinfection and Preservation is opening. On behalf of the Organizing Committee, I warmly welcome all the participants from 20 countries and my Chinese colleagues from different parts of China. On behalf of the China National Center for Preventive Medicine, I thank all of the foreign scientists for coming and making friends and sharing your knowledge and experiences with my Chinese colleagues.

Sterilization, disinfection and preservation, as those of the most important measures for disease prevention, has been well-developed, and is proved to play a more important role in improving people's health. Twenty-seven distinguished scientists have been invited, among whom many are internationally known, as speakers at the Conference. Their presentation, as well as the discussion, will certainly be of great help in exchanging experiences and future development of medical sciences. So, the success of this Conference is predictable.

What I would mention at this Conference is that we have 170 Chinese scientists from the hospitals, health and epidemic prevention centers at universities and research institutes of all the provinces, autonomous regions and municipalities.

The Conference gives the opportunities for the Chinese scientists to communicate directly with the scientists from 20 countries. Even though the sterilization work in China has had good progress after the founding of new China, especially during the past five years, there is still a big gap to meet the needs of public health practice. Since a further collaboration project will soon be organized, I would say this Conference is just in the nick of time, so we hope to express our eager desire to open up the way of international support with collaboration in this respect.

At this ceremony, we are honored having the Vice Minister of the Ministry of Public Health, Dr. Chen Min-zhang, the Chairman of the Medical and Health Subcommittee of CPPC, Dr. Shen Qi-zhen, and the President of Johnson & Johnson, Mr. David Clare, to whom we should express our gratefulness for their enthusiasm in supporting the Conference.

Finally, besides the success of the meeting, I wish all of you good health and to have a pleasant stay in China.

Thank you.

# Address by Vice Minister Chen Min-zhang

*Ministry of Public Health*

Respected Chairman, Professor Chen Chun-ming, Respected Mr. Shen Qizhen, Head of the Medical and Health Subcommittee of the Chinese People's Political Consultative Conference National Committee, Distinguished Mr. Clare, President of Johnson and Johnson, Honorable Scientific Consuler of American Embassy to China, Ladies and Gentlemen:

The 1985 Beijing International Conference on the Sterilization of Medical Products, Disinfection and Preservation brings together the international experts and professionals on this field.

Please allow me, on behalf of the Ministry of Public Health, to extend my warm welcome to the participants, ladies and gentlemen.

The purpose of this conference is to strengthen the information exchanges on sterilization of medical products, environmental disinfection and food preservation, to promote the development and research on this field of various countries, hence the sterilization work can play a more important role and make greater contribution to the prevention and treatment of diseases, to the health and guarantime and upgrading the people's health standard.

It is well known that sterilization involves many disciplines and plays an important role in prevention and treatment of diseases. After the establishment of new China, much has been achieved in preventive sterilization monitoring, sterilization in hospitals and hepatitis B, testing sterilization effectiveness, sterilization agents, and sterilization by physical means, etc.

Comparing with the advanced countries, there are certain distance and problems in research and adoption of new methods and technologies although we have made some progress in recent years. For improving our work in this field, we'll learn the merits consciously from foreign countries, strengthen the scientific research, investigation and management, and adopt new methods and technologies actively. We are planning to work out a practical and realistic regulation on sterilization that will meet China's conditions and needs.

We hope to take the opportunity of this international conference on the sterilization of medical products, disinfection and preservation to learn from each other through experience exchanges, so that we can develop and bring forth new ideas on sterilization work and make greater contribution to treating diseases and epidemic prevention.

**I wish the conference full success.**

I wish you ladies and gentlemen good health.  
Thank you.

# Address by Mr. Shen Qi-zhen

*Director, Medical and Health Subcommittee of the Chinese People's Political Consultative Conference National Committee*

Madame Chairman, Ladies and Gentlemen:

The International Scientific Conference on the Sterilization of Medical Products, Disinfection and Preservation is started today. The conference is sponsored and organized by the China National Center for Preventive Medicine, Johnson and Johnson Company of the United States and the China Medical and Health Corporation. And it is also initiated and supported by the Medicine and Health Subcommittee of the Chinese People's Political Consultative Conference National Committee. Please allow me on behalf of the Medicine and Health Subcommittee of the Chinese People's Political Consultative Conference National Committee to warmly welcome all the specialists and scientists in the field of sterilization.

Last evening, at the banquet given by the Chinese People's Political Consultative Conference and the Ministry of Public Health of the People's Republic of China, Deputy Chairman, Mr. Yang Jingren mentioned the progress made in the field of medicine and public health in China as well as that in preventive medicine, including the advances in the science of sterilization. Now I would like to add a few words. With the development of our national economy, the improvement of the medical and health conditions and the popularizing of sports, China is no longer the so-called "Sick of the Far East". The Chinese people begin to march gallantly towards the goal of four modernizations; i.e., the modernization of industry, agriculture, science and technology and national defense in the 21st century.

Deputy Chairman Yang Jingren also dwelt on the open-door policy of China and expressed the wish to promote friendship between the Chinese People's Political Consultative Conference and peoples of all nations in the world. As a permanent part of the National Committee of the Chinese People's Political Consultative Conference, the Medicine and Health Subcommittee will also do its best to make a useful contribution in this connection.

In our Medicine and Health Subcommittee, there are many well-known specialists and scientists. We often organize field visits and discussion meetings on some important aspects of medicine and health. We made comments and suggestions to be passed on to various government departments for their reference in making policies and programs. We put special emphasis on preventive medicine and have made investigations on certain diseases and made some relevant suggestions and proposals which were much

appreciated by the leaders of our party and government.

We hope to further mutual visit, exchange information and promote mutual understanding with friends of all nations, in order to contribute to the peace, progress and prosperity of mankind.

Madame Chairman, Ladies and Gentleman: I would like to take this opportunity to express our warm welcome to Mr. Clare, the President of Johnson and Johnson, and his associates who have accepted the invitation from the Medicine and Health Subcommittee and are now attending this opening session. I would also like to express our appreciation and gratitude to Johnson and Johnson for sponsoring this conference.

I believe this International Scientific Conference on Sterilization will speed up the development of the science and technology of sterilization in China.

I wish a complete success of this conference and all our friends the best of health.

Thank you.



But my involvement pales next to that of our company as a whole. Since its founding almost 100 years ago, Johnson & Johnson has been a pioneer in the sterilization field. A great deal of the credit for this goes to the company's first scientific director, Fred B. Kilmer, who served the company for 45 years. Part of his work was conducted in the nineteenth century, including his classic article "Modern Surgical Dressings," published in the American Journal of Pharmacy in 1897. Much of it still seems current, especially regarding microbiological control of the environment and confirmation of the effectiveness of sterilization processes.

The basic premise of Dr. Kilmer was that antiseptic dressings represented a monumental improvement in surgical practice. He published his findings in "Modern Surgical Methods of Antiseptic Wound Treatment" in 1888. This book went through five editions by 1893. More than one and one-half million copies were ultimately distributed.

Kilmer's work was intended to disseminate the findings of Sir Joseph Lister and was a compilation of reports by eminent surgeons of the time. It was the only clear and accessible explanation of the new surgical concept of asepsis.

At the time Kilmer's book was published, Johnson & Johnson was applying the concept to its products by manufacturing moist Lister-type dressings. Along with Kilmer's publication, that marked the real beginning of antiseptic surgery in the U.S.

Kilmer also observed, however, as did Lister, that surgeons were contaminating their own patients by operating ungloved, in blood-spattered clothing, and with nonsterile instruments. There was a need for more than just antisepsis.

In 1897, Johnson & Johnson published "Asepsis Secundum Artem," on the practical application of asepsis to the preparation of surgical dressings. It included instructions on how to keep rooms aseptic, and how to train employees in asepsis and methods of hermetic sealing.

This illustrates another way in which the company has helped to bridge the gap from antisepsis to sterility. Aside from its products, Johnson & Johnson has been responsible for numerous educational publications and conferences on the subject virtually since its inception.

Also in 1897, the company developed another major contribution to surgery with an improved sterilizing technique, using dry heat, for catgut sutures.

Surgical dressings needed to be completely free of microorganisms. Kilmer believed that dressings should be as ready for surgery as the surgeon himself—and, as a result, he conceived the idea of sterile patient-ready dressings. In applying these principles, Johnson & Johnson began treating Lister-type cotton and gauze dressings with moist heat to yield not only an antiseptic product but also a *sterile* one. The process was carried out in America's first two-door industrial steam autoclave. It was the start of the transition from antiseptic to aseptic surgery.

At about the same time, aseptic rooms were built and bacteriological tests applied to all raw materials. The company in 1892 successfully met the requirements for a sterile product through a process of keeping dressings continuously under aseptic conditions during manufacturing and then subjecting them to repeated sterilization.

The company also was one of the early industrial users, and played a role in the

development of chemical sterilization, including ethylene oxide, which now is the most widely used sterilization method for surgical products. The ethylene oxide technique initially was used in the early 1940s in industry—the success there led to acceptance in hospitals.

A Johnson & Johnson Division, Ethicon, started research on radiation sterilization in the late 1940s. By 1956, it had developed a radiation-based process employing a Van De Graaff generator to sterilize sutures. In fact, the first product to be successfully sterilized by radiation on an industrial basis was the Ethicon surgical suture.

In 1961, Johnson & Johnson commissioned, in England, its first cobalt-60 sterilization facility. The company became the first successful large-scale user of this technique. Johnson & Johnson now operates 13 cobalt-60 irradiators worldwide—at locations in North and South America, Australia and Europe. We are one of the world's largest users of atomic energy for peaceful purposes.

As was the case with ethylene oxide, radiation sterilization began in industry and was especially useful because of the growth of plastics, which are unable to withstand the high temperatures needed for heat sterilization. Radiation was also desirable because of its proven ability to kill microorganisms, and the reliability and reproducibility of process conditions.

Johnson & Johnson still is searching for more effective and practical methods of sterilization, and for better sterile products for postoperative care as well as for surgery. That search began a long time ago and, among other things, led the company to pioneer the use of sterile patient-ready dressings in hospitals. The search must be tireless if people are to have the best products and medical care possible. We are dedicated to continue this effort.

Nor is it enough for products to be sterile—the conditions in which the products are used must be free of infectious agents too. That includes operating theaters and other parts of hospitals. And by applying asepsis to postoperative care, we can help patients recover faster. That not only improves survival rates but also achieves needed savings for health care systems. I note with pleasure that “Disinfection and Preservation” is included in the title of this conference. That shows you realize the value of an entire program directed at preventing infection.

It is satisfying to me that Johnson & Johnson is involved with this outstanding conference, and I wish all of you well with it. I'm sure it will be successful with such knowledgeable people as participants. I can't think of any topic that has more direct effect on the health and well-being of people around the globe. It should be a source of great satisfaction to each of you to be part of such an effort. I thank you very much for giving me the opportunity to be here and speak with you today.

# Address by Dr. Jack L. Gosnell

*Scientific Attache of the U.S.A. Embassy in Beijing*

Madame Chairman, Dr. Chen, Dr. Shen, Ladies and Gentlemen. I'm very honored to be here today to represent the United States Embassy and to represent our Ambassador who originally planned to be here today but unfortunately could not make it.

I can assure you of the support of the Embassy and the U.S. Government in this wonderful conference. This conference is, I believe, an outstanding example of the way in which relations between China and the other nations have been developing over the last ten or fifteen years.

Let me explain the reasons for that connection. The first reason is to take it from a historical viewpoint. Dr. Chen is from the Beijing Union University. There is also the Beijing Union Hospital. That hospital was formed many years ago from the work of concerned foreigners, concerned Chinese to improve healthcare in China—foreigners and Chinese working together to improve the healthcare.

The second way in which this represents China's recent development is that this conference represents China's efforts at national modernization and the open-door policy.

This kind of science and technology conference and other conferences that are going on now and will go on between now and the end of the year have increased markedly in the last several years and have now grown to such an extent that I know of no Embassy in Beijing which is capable of following all of the conferences and all of its national participants who are here in these conferences. You have become too big for us.

This conference also provides the heavy participation and support of the American private sector. As U.S. Vice-president Bush said here less than two weeks ago, the United States, both in the private sector and in the public sector, is eager and pleased and willing to work with China toward the national modernization.

In this regard, the conference is supported by Johnson & Johnson. This is an American firm which has brought its outstanding capabilities, its interest in top-quality research and its immaculate, wonderful reputation for good business here to this conference. We in the American Embassy are very proud that Johnson & Johnson is making such a contribution to international medicine and to the bilateral relationship.

Thus, in view of the history, in view of international science and technology, and in view of international trade, I believe I can represent the American Embassy—I hope I can represent all Embassies here in Beijing, to congratulate the hosts, the sponsors and all the participants of this fine international conference. Thank you.

# Session I

**Chairman: Prof. Liu Han-ming**

*Director, Institute of Epidemiology and Microbiology  
China Academy of Preventive Medicine  
Beijing, People's Republic of China*

# Sterilization Concepts and Methods of Sterilization Employed by the Hospital and Industry

Richard J. DeRisio

*Johnson & Johnson Sterilization Sciences Group  
U.S.A.*

I am very pleased and deeply honored to participate in this international scientific conference. The science of sterilization offers challenges and opportunities to individuals in a variety of professional disciplines. This symposium will afford an opportunity to share our knowledge and experience, and to increase our awareness of the various methods used worldwide to assure medical product safety and sterility.

## Methods of Sterilization

In reviewing sterilization practices in hospitals and industry, we find the principal methods used are those shown in Table I. Steam and gaseous chemicals such as ethylene oxide and formaldehyde are widely used in both hospitals and industry. Certain processes unique to industry, such as radiation sterilization and sterile filtration with aseptic fill, require large capital expenditures for facilities and equipment.

**Table 1. Principal Sterilization Methods**

---

<u>Hospital</u>	<u>Industry</u>
Steam	Steam
Gaseous Chemicals	Gaseous Chemicals
Liquid Chemicals	
	Radiation
	Dry Heat
	Filtration

---

nature of the item being sterilized. Figure 1 lists several methods of sterilization and for each an indication of the process parameters requiring control.

Time must be controlled for all sterilization methods. The chemical methods have several other variables that affect the chemical reaction rate and the permeability of the sterilant. Thermal methods are somewhat less complicated and require control of relatively few factors in order to assure reproducible heat transfer to the product. Radiation techniques are potentially the least complex of all to control—often simply time and bulk density affect the process. Filtration may be more complicated than indicated because of the need for aseptic conditions.

CONTROLS REQUIRED	STERILIZATION METHOD					
	GASEOUS CHEMICAL	LIQUID CHEMICAL	STEAM	DRY HEAT	FILTRATION	RADIATION
TIME	■	■	■	■	■	■
TEMPERATURE	■	■	■	■	□	□
PACKAGING	■	■	■	■	□	□
BULK DENSITY	■	□	■	■	□	■
PRESSURE/VACUUM	■	□	■	□	■	□
HUMIDITY	■	□	■	□	□	□
CONCENTRATION	■	■	□	□	□	□
pH	□	■	□	□	□	□

Figure 1. Sterilization process variables.

## Steam Sterilization

Of the principal methods used today in hospitals and industry, steam sterilization is by far the oldest and has been the most extensively studied. During the mid-nineteenth century, tremendous progress was made in the study of microorganisms. Louis Pasteur developed bacteriological methods that led to his studying the destruction of microorganisms by heat. Robert Koch proved that disease was caused by microbial species, and his development of techniques for antiseptics and sterilization had a profound effect on infection control in

hospitals.

Steam sterilization is often the method of choice in industry and the hospital because this technique has several advantages. Steam penetrates and heats porous materials rapidly, and gives up its heat of vaporization readily upon contact with solid surfaces. Resistant bacterial spores are destroyed in brief exposure periods. There are no toxic residues remaining on materials after the sterilization process, and product quality and process lethality can be controlled easily and reliably. Also, steam is an economical sterilizing agent.

There are, however, some disadvantages to steam sterilization. Steam cannot be used for products damaged by high temperature and moisture levels. Failure to completely eliminate air from the sterilizer could render the process ineffective, as does the presence of superheated steam. Also, steam sterilization is unsuitable for products such as anhydrous oils, greases or powders where complete contact with moist heat cannot be assured.

Steam processes have several applications in the health care industry including sterilization of parenteral solutions, gauze pads, adhesive bandages, metal surgical instruments, culture media, and equipment for aseptic filling and sterility testing.

Perhaps the most important aspect in assuring a safe, effective cycle is the removal of all air from the sterilizer and, for porous materials, from the product load. Air heats slowly, has a very low heat content compared to saturated steam, and can act as an insulator around the product.

An air-displacement cycle, such as that used for vials and ampoules, is shown in Figure 2. During the come-up time before the exposure period, air is forced out of the sterilizer from the end opposite the point at which steam is entering to bring the load up to the sterilizing conditions of temperature and pressure.

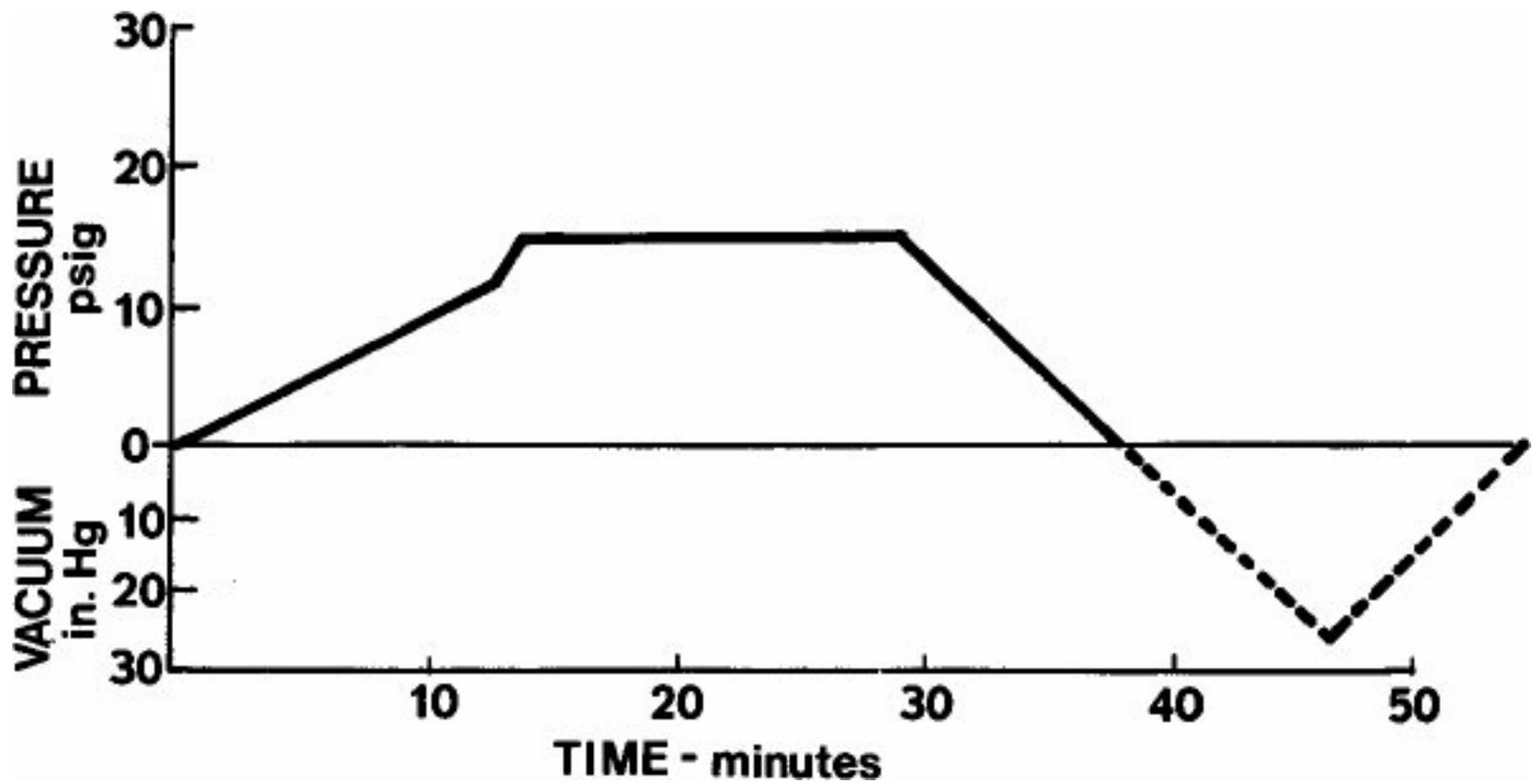


Figure 2. Pressure/vacuum pulsing cycle.

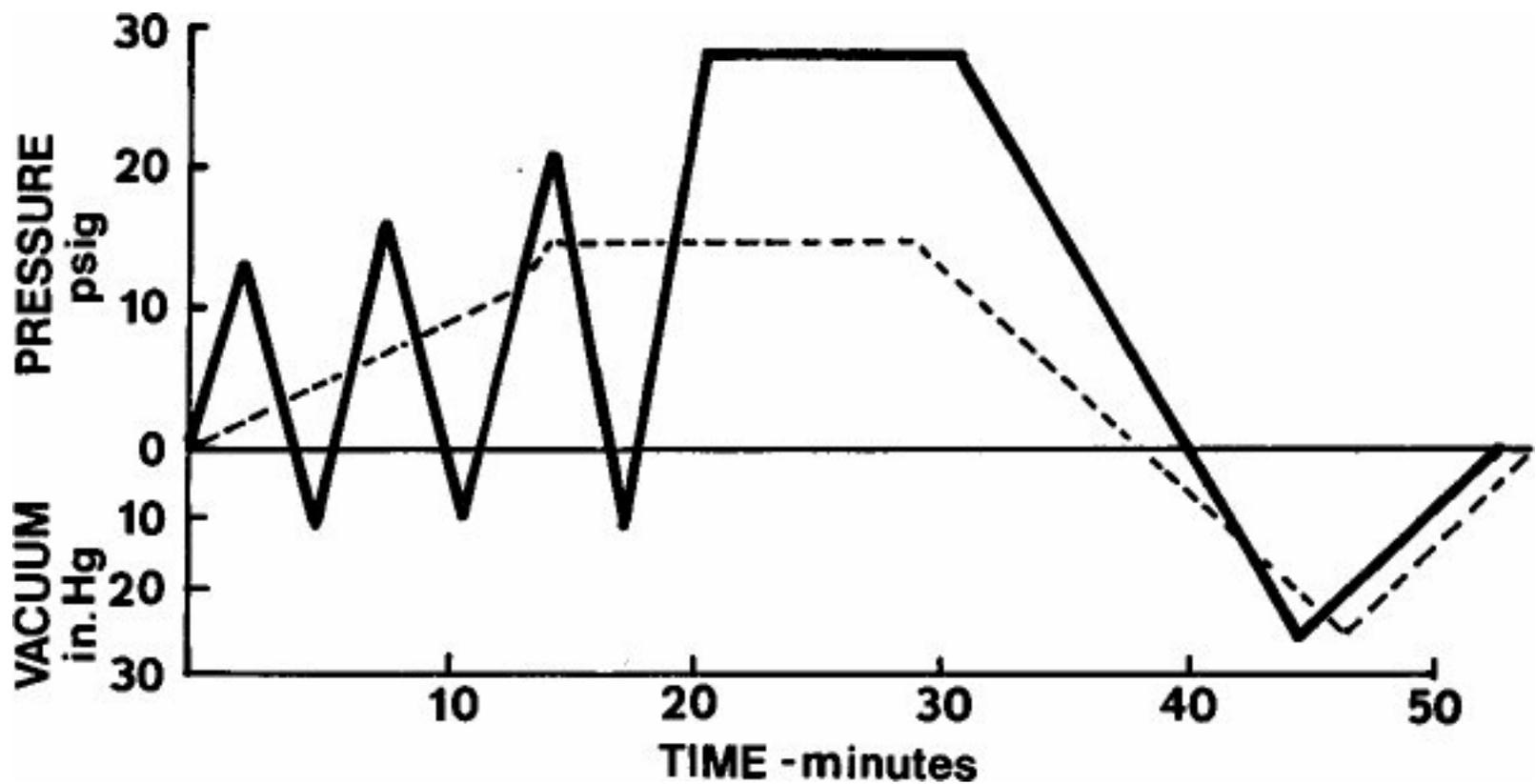


Figure 3. Air displacement cycle.

Shown in Figure 3 is a pressure/vacuum pulsing cycle. Multiple steam pulses with intermittent evacuations ensure complete air removal from porous loads while bringing the products up to the specified temperature. The use of steam pulses between evacuations

reduces the drying and cooling effects that the vacuum cycles might have on the load. A final vacuum phase facilitates drying.

Steam sterilization processes are developed using biological indicators and physical parameter measurements. The rate of biological inactivation is compared to the physical estimate of process lethality which is integrated from actual product temperature data. This is expressed as the equivalent number of minutes exposure at 121.1°C. Routine process control must assure that heat transfer to the product is the same as that observed during cycle development studies.

Investigations into the mechanism of thermal death indicate that microbial destruction results from protein denaturation. Studies of the energy requirements for breaking chemical bonds have demonstrated that the energy levels for both inactivation of spores and denaturation of proteins are similar<sup>1</sup>.

Microbial death generally follows the first-order kinetics of a monomolecular reaction, therefore the death rate can be represented by a straight line when the logarithm of the number of survivors is expressed as a function of exposure time. Perhaps it would be worthwhile to discuss, in general, this relationship between microbial inactivation and the sterilizing exposure time or dose, as shown in Figure 4.

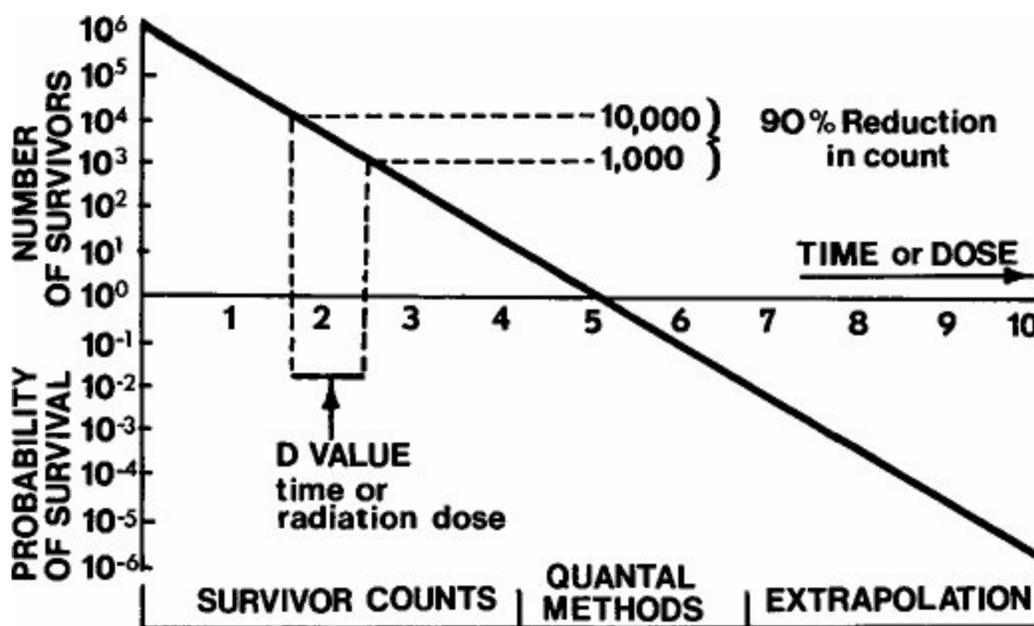


Figure 4. Microbial survival vs. process dose.

This example could apply to thermal, radiation or gaseous methods. The initial count at zero exposure time is one million spores of a homogeneous microbial challenge such as a spore strip, inoculated product, or other biological indicator. Also, one can evaluate the destruction of the presterilization bioburden which is defined as the number of microorganisms on those surfaces of the product intended to be sterile. Of course, the bioburden can include several different species each with a different resistance to sterilization.

The decimal reduction value or D value is the time required under defined exposure conditions to reduce the population by 90% or one logarithm cycle. Using a calibrated preparation of spores, one can use the D value to compare different sets of process

conditions. Alternatively, the resistances of several different species can be compared using defined exposure conditions.

At low process doses, the number of survivors can be counted to establish points on the survivor curve. At somewhat larger doses, one would expect a fraction of the samples to contain surviving organisms. This area is called the quantal zone. In this region, one uses mathematical approaches such as the most probable number (MPN) method<sup>2</sup> or the Spearman-Kärber method<sup>3</sup> for calculating D values based on the fraction of replicate units that test sterile among samples exposed to several time increments within this region. Dr. Irving Pflug has published practical methods for gathering and interpreting data in this region<sup>4</sup>.

At higher doses beyond the quantal region, recovery of survivors exceeds the sensitivity and practicality of a sterility test. One must extrapolate to the probability of an organism surviving. The probability of survival that corresponds to the process exposure time or radiation dose is referred to as the sterility assurance level (SAL). In practice, the SAL for most terminally sterilized products is  $10^{-6}$  or one in one million. This estimate of the probability of a survivor is conservative inasmuch as the process is often based upon microbial challenges more difficult to kill than any pathogenic species that might be present in the bioburden. Moreover, the sterility assurance level is conservatively estimated at the lowest lethality location in the load with process parameters at or below the minimum specified settings.

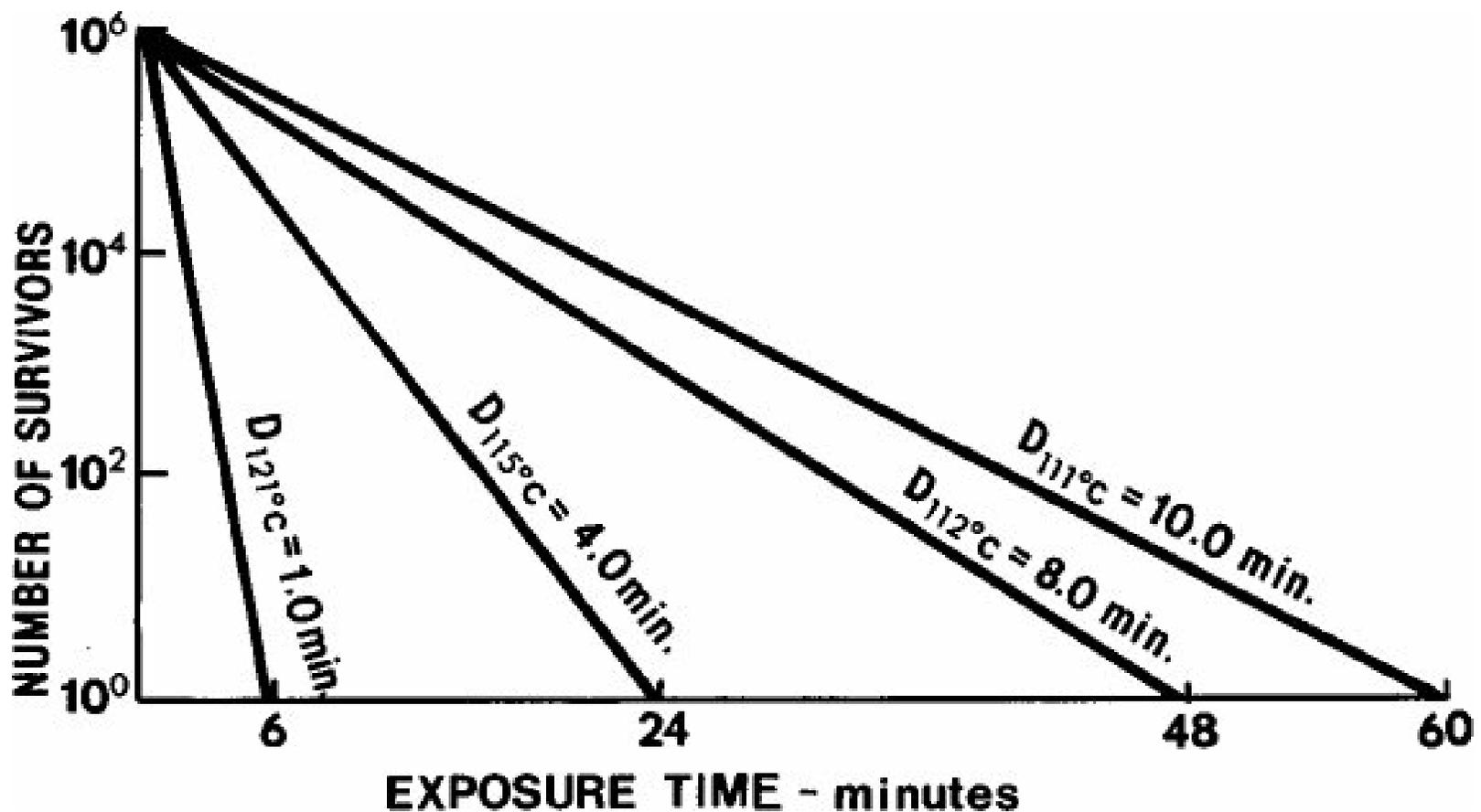


Figure 5. Times required to achieve a six log count reduction at various exposure temperatures.

An actual example of survivor curves for a heat resistant microorganism is shown in Figure 5 reflecting the temperature dependence of the decimal reduction time. Between the temperatures of 111° and 121°C, the D value (or time to achieve a 90% reduction in count) decreases by a factor of 10 thus shortening from 60 to 6 minutes the time for a six-log reduction in count. Obviously, the process must be based on the slowest heating product in the load, otherwise the process time could be greatly underestimated.

## Surviving Fraction

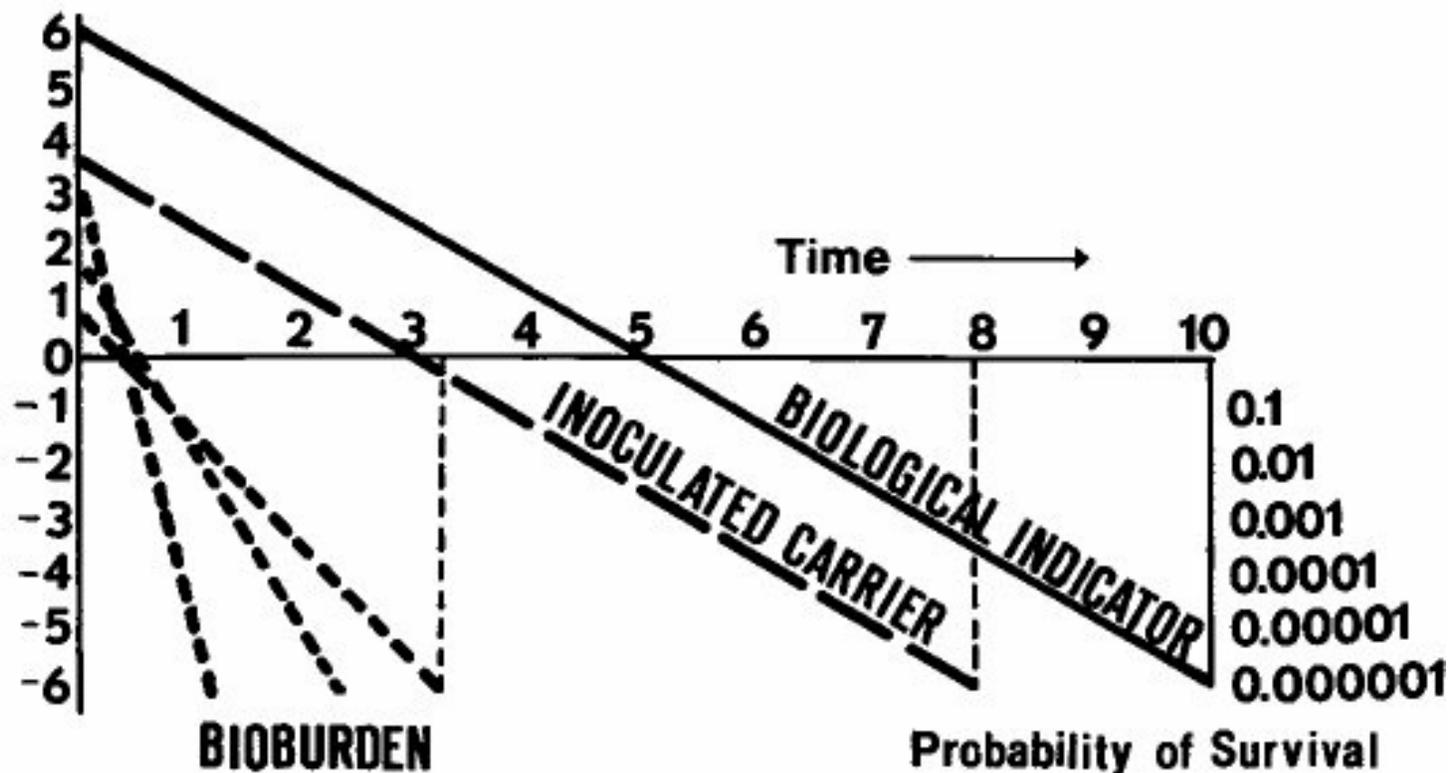


Figure 6. Microbial inactivation curves.

The microbial inactivation curves shown in Figure 6 can be used to compare three methods of process establishment, overkill, bioburden-based, and the combination biological indicator-bioburden method. For the overkill approach, the microbial challenge contains a large number of microorganisms known to be much more resistant to the mode of sterilization than the naturally occurring bioburden, represented here as three species with different resistances.

Demonstration of overkill involves reduction of the challenge to 10<sup>0</sup> or one organism, and then adding an appropriate safety factor to achieve the desired level of sterility assurance. In this example, the indicated cycle time is 10 hours. In practice, many users look for total destruction of the spore challenge in a half cycle which could lead to needlessly long cycle times for products with low bioburden. Typically, overkill cycles are used where there are likely to be substantial and sometimes unpredictable variations in load configuration, product bioburden, or equipment performance.

The bioburden-based cycle requires a thorough knowledge of bioburden with strict routine control. Usually there is a step such as filtration that almost entirely removes presterilization bioburden. Cycle development work involves either exposing the product to

short subprocess cycles and counting survivors, or the determination of the D values of resistant bioburden isolates.

For the combination biological indicator-bioburden approach, the product or carrier, such as a paper strip, is inoculated with a resistant bacterial species at the known product bioburden level as shown by the inoculated carrier line that indicates an 8-hour cycle. The biological indicator used for routine monitoring will be consistent with the challenge used during cycle development studies.

## Ethylene Oxide Sterilization

The most common chemical used for sterilization is ethylene oxide (EtO), a colorless gas with a density greater than air and very high diffusivity. Because it is flammable and highly explosive in its pure form, EtO is usually mixed with an inert gas such as Freon-12 or carbon dioxide when used for sterilization.

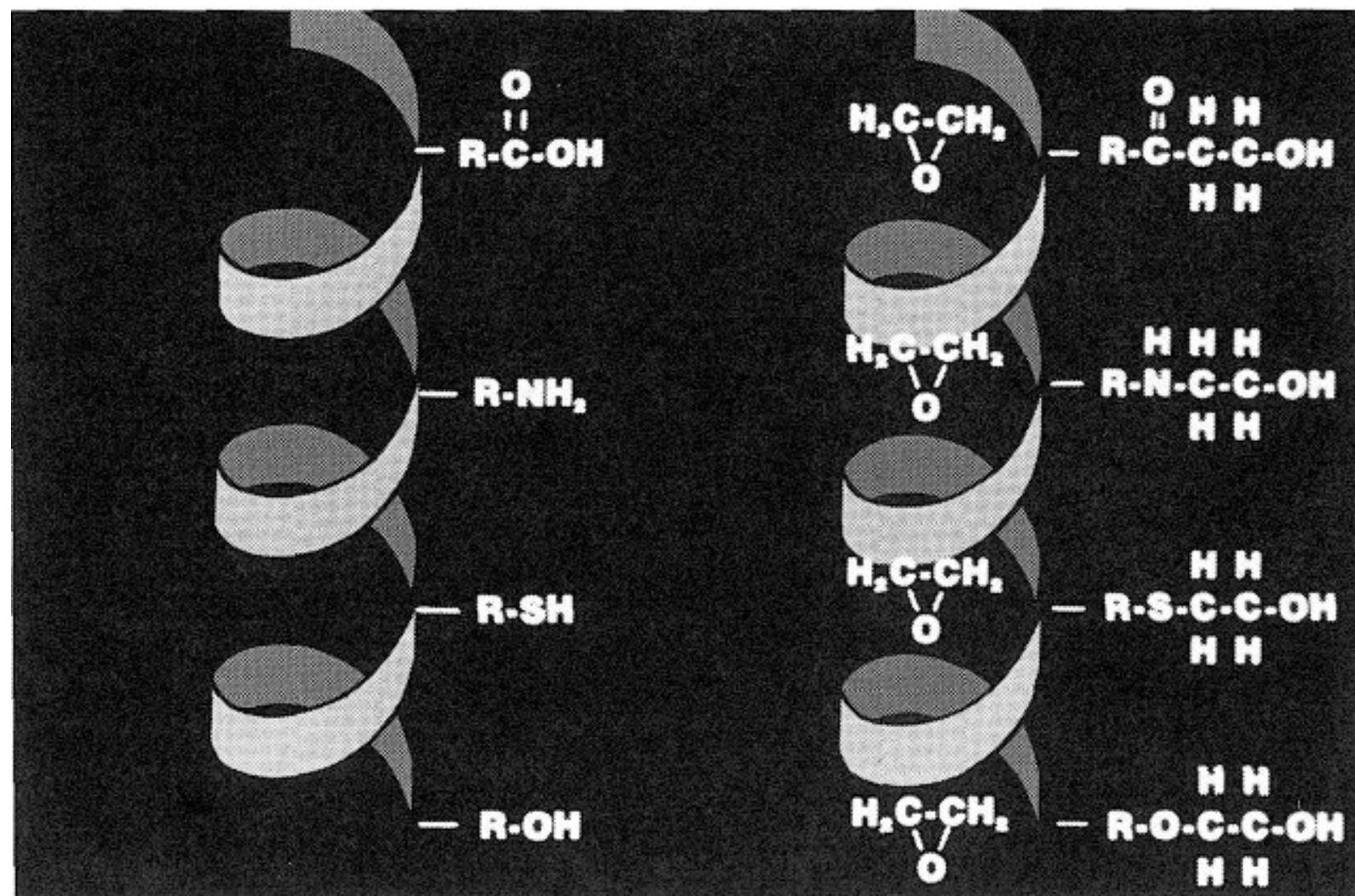


Figure 7. Interaction of ethylene oxide with organic radicals.

Figure 7 depicts the attack of EtO on DNA. The simplest epoxy compound ( $\text{CH}_2\text{CH}_2\text{O}$ ), EtO is extremely reactive. In the cells of microorganisms, EtO can react with the carboxyl, amino, sulfhydryl, hydroxyl, or phenolic radicals in all proteins and amino acids. Because the

microorganism cannot use these newly-formed moieties in metabolism or reproduction, the cell dies.

There are several advantages to using EtO sterilization. The moderate levels of temperature and relative humidity permit sterilization of a wide range of component materials without physical damage. Because of its high diffusivity, ethylene oxide penetrates readily through shipping cartons and product packaging. With careful selection of equipment and controls, the process can be operated reliably, and nonexplosive mixtures of EtO are readily available worldwide.

There are, however, some disadvantages to EtO sterilization. Unfortunately, some of the attributes that make EtO an effective sterilant—particularly its reactivity and diffusivity—render EtO hazardous to humans. As a result industry, hospitals, and regulatory agencies have taken steps to limit worker exposure and control environmental emissions.

The EtO sterilization process is complex. Proper design, effective control, and validation are needed to obtain the assurance that all products in the load are adequately sterilized. Compared to steam processes, the total cycle time is considerably longer. In addition, controls are required to minimize EtO reaction by-products. Most countries have maximum limits for EtO residue levels on medical devices based upon the product's intended use.

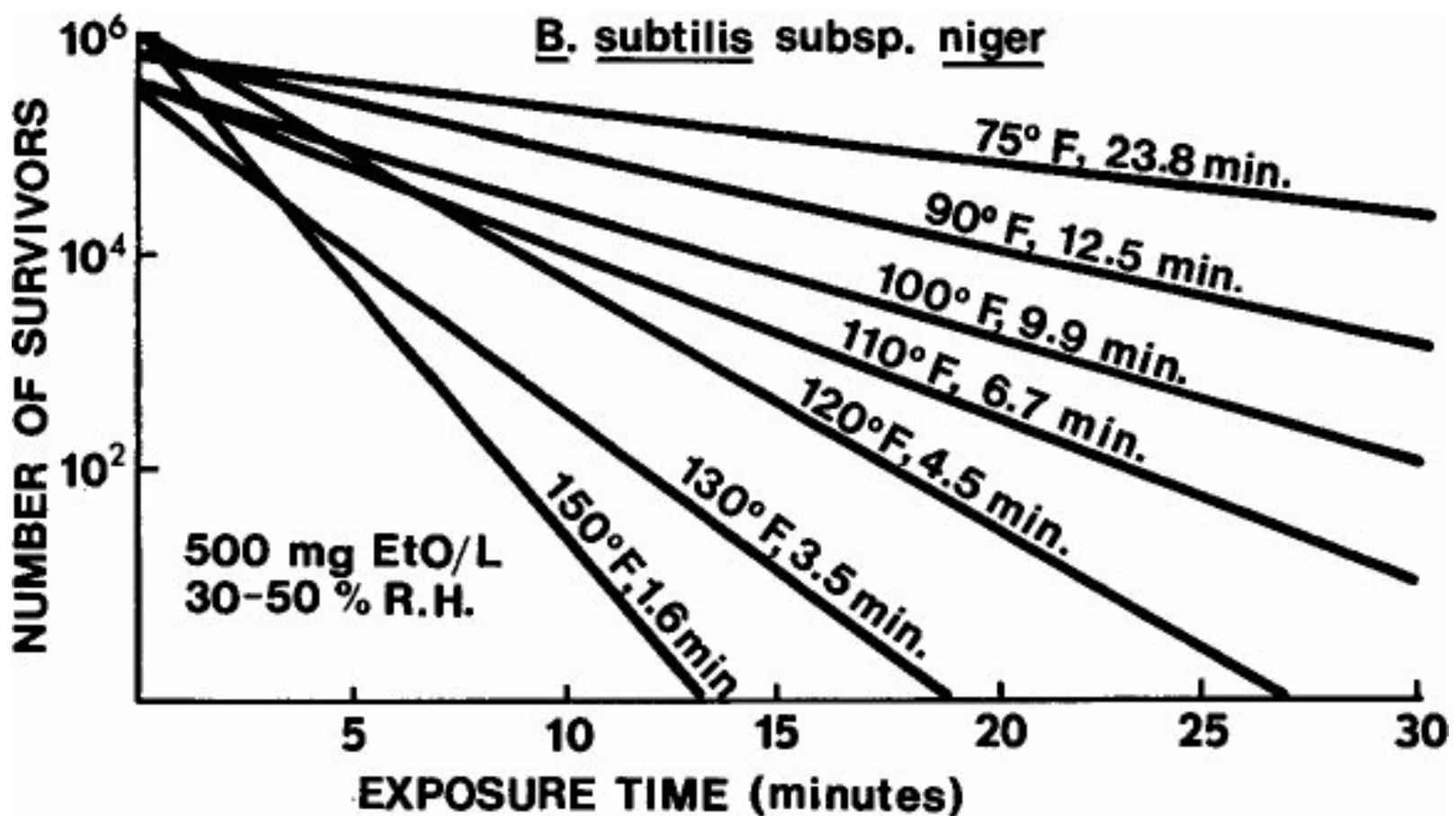


Figure 8. Effect of temperature on the D value of *Bacillus subtilis* subsp. *niger*.

The lethal alkylation process is a temperature-dependent chemical reaction. The survivor curves shown in Figure 8 demonstrate the effect of temperature on the D value of *Bacillus subtilis* subsp. *niger* inoculated onto a paper carrier<sup>5</sup>. For each 20°F increase in temperature, the rate of biological indicator kill approximately doubled. For example,

increasing the temperature from 90° to 110°F reduced the time to obtain a one log reduction in count (the D value) from 12.5 to 6.7 minutes. The implications are clear: the ability to run cycles at higher temperatures can significantly reduce exposure time.

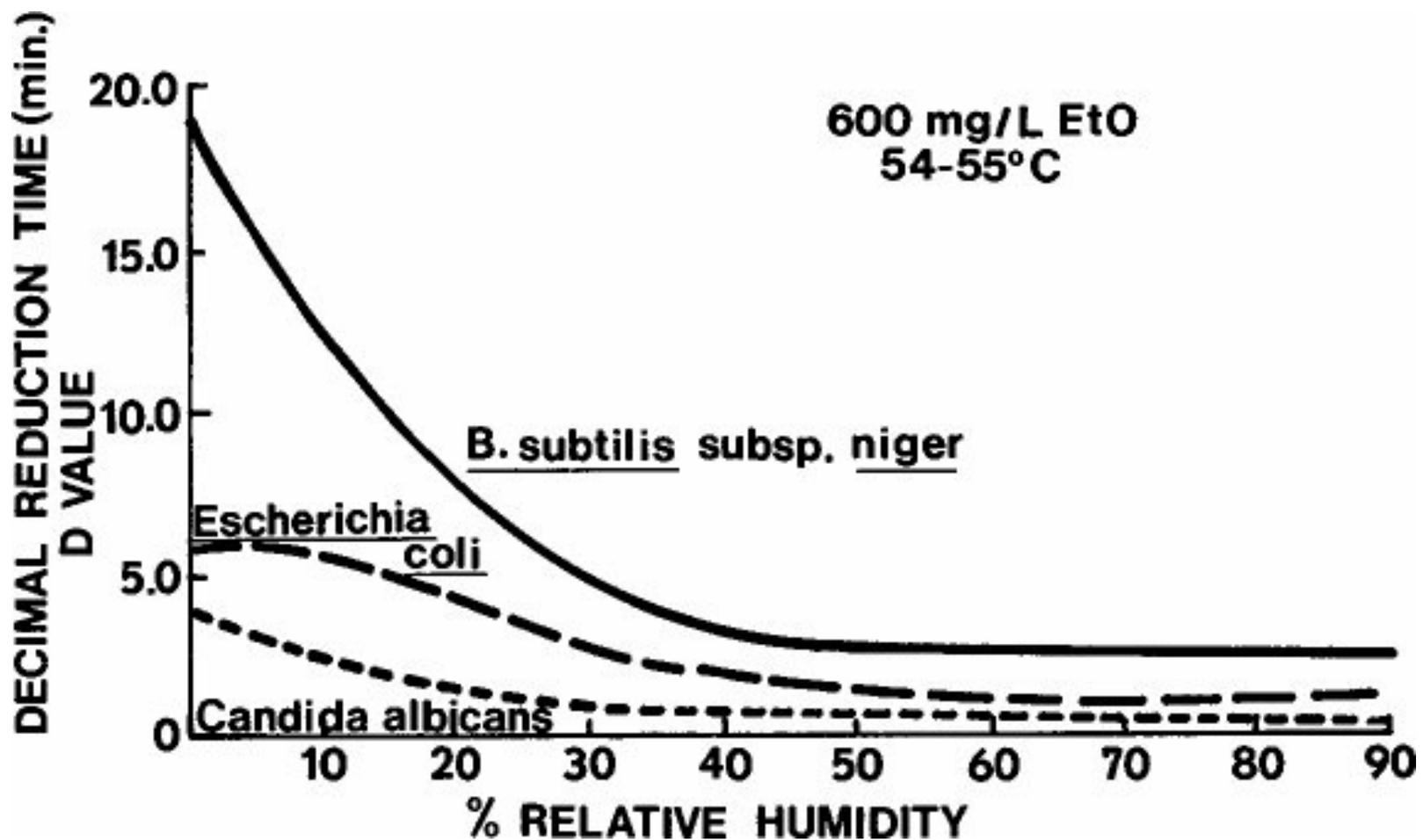


Figure 9. Effect of relative humidity on microbial D value.

Another EtO process variable whose control is essential to assuring product sterility is relative humidity. Although laboratory experiments have demonstrated that 33% relative humidity is optimal, a higher level (40 to 50%) is ordinarily used to establish a driving force between the sterilizer environment and the product inside its packaging. In Figure 9, the striking effect of the relative humidity level during exposure to ethylene oxide on the D value of three species of microorganisms is shown<sup>6</sup>. It is apparent that at relative humidity levels below 30%, the D values increase to an extent that a significantly longer exposure time would be needed in order to achieve the desired microbial reduction and sterility assurance level.

Materials must be humidified before exposure to EtO and are usually held for several hours at elevated temperature and relative humidity. In addition, steam is added to the sterilizer at the beginning of the cycle prior to gas charging. In-chamber humidification is most effective when done under vacuum so that there is relatively little air to act as a barrier to moisture transfer.

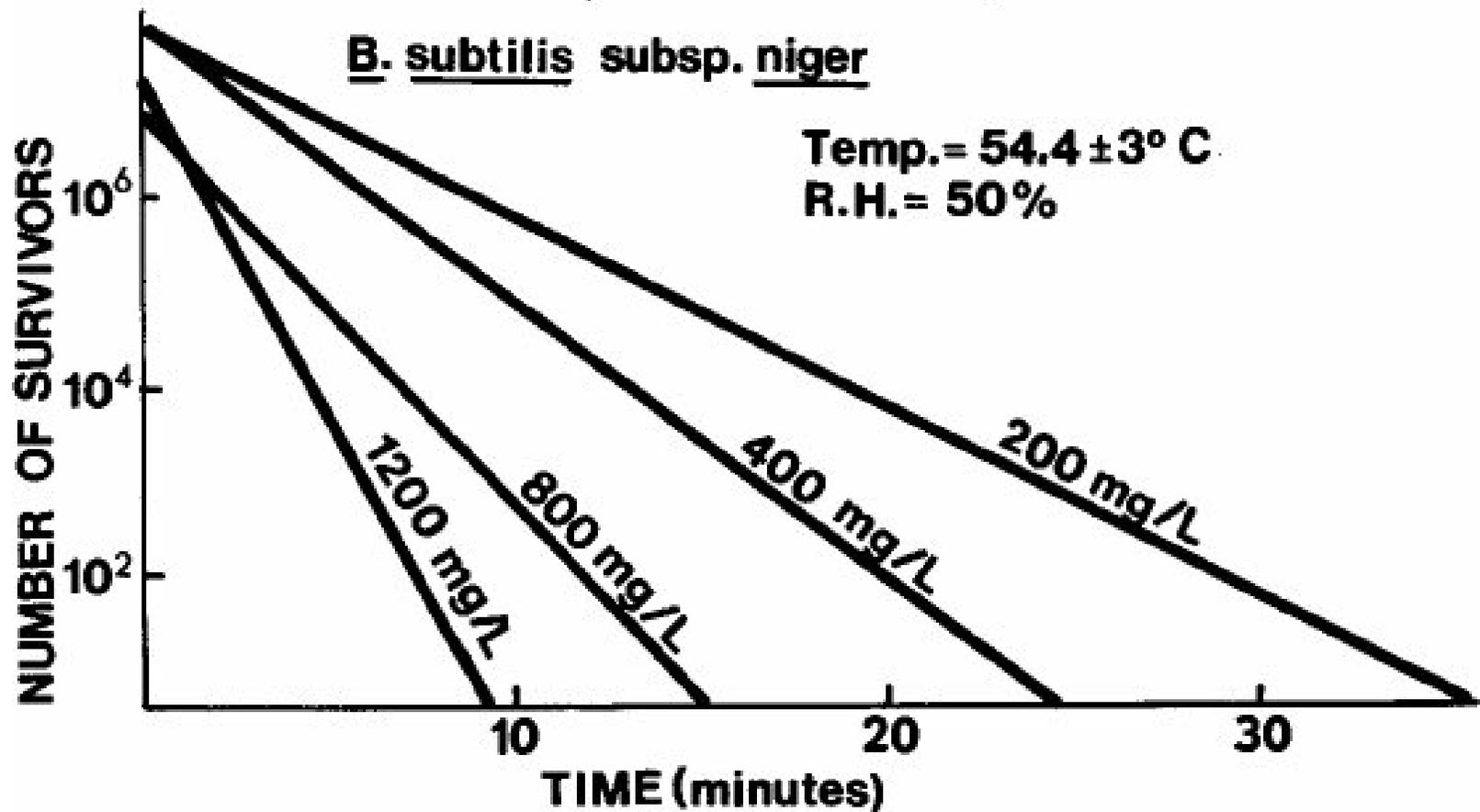


Figure 10. Effect of ethylene oxide gas concentration on resistance of *Bacillus subtilis* subsp. *niger*.

The rate of sterilization also depends upon the concentration of ethylene oxide gas present during the exposure phase. It is common practice to sterilize using EtO concentrations at or above 400 mg/L. Studies have shown that a significant increase in lethality occurs between 200 and 400 mg/L<sup>7</sup>. Supporting data for spore strips exposed directly to the sterilizing environment are shown in Figure 10. In actual practice, where EtO penetration through the package is a rate-limiting factor, increases in concentration to very high levels might not be necessary. Of particular importance to hospitals was the observation that the resistance of spores dried in physiological saline increased, thus requiring the use of higher gas concentrations (800 mg/L) to achieve sterilization. This occlusion simulated inadequate precleaning of resterilized items.

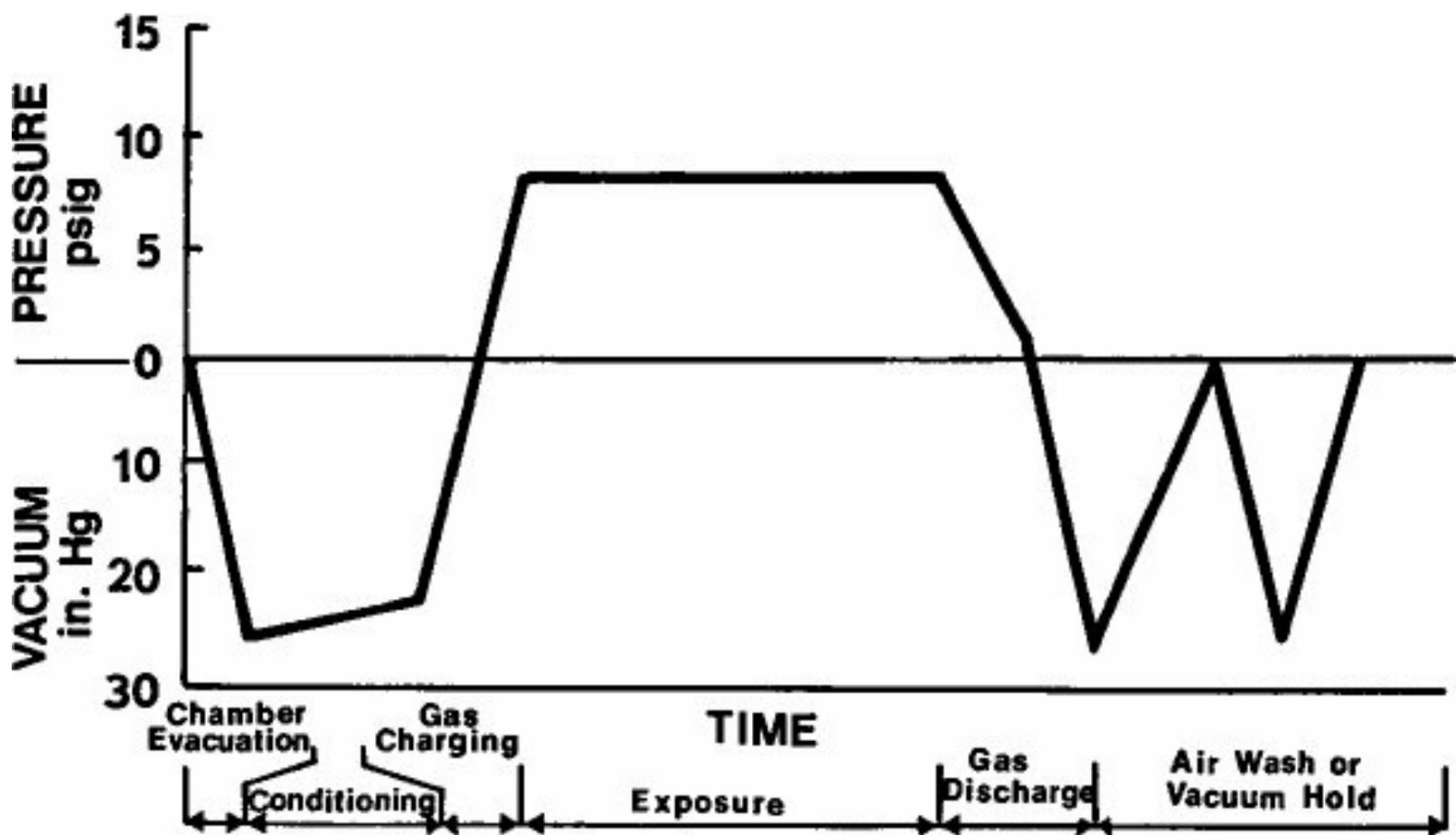


Figure 11. McDonald process.

A widely used EtO sterilization cycle is the McDonald Process<sup>8</sup> shown in Figure 11. Following an initial chamber evacuation, steam is added to increase the temperature and relative humidity in the load during the preconditioning phase. The indicated pressure rise can be monitored to ensure process control during this step. Preheated gas is then charged into the chamber and exposure commences when the specified chamber pressure is reached. At the end of exposure, a vacuum is drawn in the chamber to accelerate removal of residual gas from the load, and filtered air is used to return the sterilizer to ambient pressure.

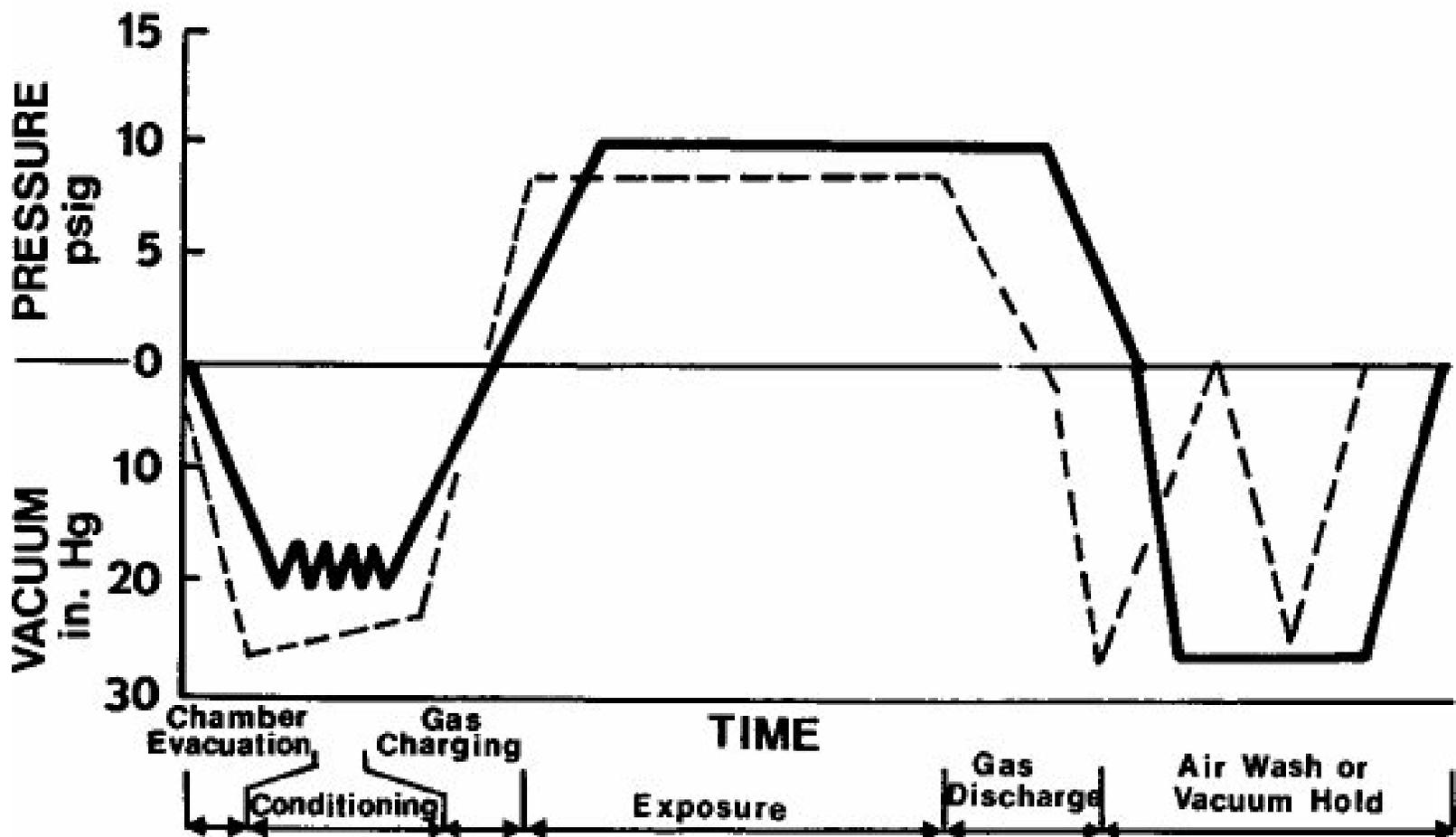


Figure 12. Dynamic environmental conditioning.

Shown in Figure 12 is a Dynamic Environmental Conditioning or D.E.C. cycle that incorporates steam pulsing with vacuum to improve the effectiveness of load conditioning. In practice, the vacuum pump is operated either continuously or intermittently, and steam is pulsed in at controlled intervals. Other aspects of the process are similar to that described above.

## Radiation Sterilization

Recent increases in the use of radiation sterilization have been stimulated by interest on the part of both users and manufacturers of medical products who recognize that radiation-sterilized articles retain a high level of product and package integrity, and are free from sterilant residues.

The radiation received by the article being sterilized can be in the form of gamma rays, x-rays, or accelerated electrons. The lethal effects of these forms of ionizing radiation on microorganisms have been attributed to two distinct types of reactions. In one mechanism, radiation impinges directly on a target molecule within the cell. It is likely that the primary cause of microbial inactivation in this mode is the disruption of molecular bonds within DNA. A second mechanism involves cell destruction by free radicals and other radiation-produced compounds such as peroxides. These lethal chemical compounds are formed both within

and adjacent to the cell.

Cobalt-60, the most extensively used radionuclide source, is supplied in stainless steel encapsulations or "pencils" that are held in a source rack. In most commercial-scale systems, product cartons are loaded into large tote bins and moved by conveyor into the irradiator, around the source in several passes, and then out to a holding area.

There appears to be renewed interest in the use of accelerated electrons for sterilization of medical products. In the past, the primary disadvantage of electron-beam sterilization was the comparatively shallow penetration of electrons into the product cartons. This has been offset, in part, by the development of commercial accelerators capable of operating at higher energies. Also, methods for irradiating both sides of a carton using novel conveyor systems have increased the capability of the accelerator to process larger shipping containers. In contrast to gamma sterilization, dose rates are much higher with accelerated electron beam units, thus reducing the time to deliver the sterilizing dose.

There are several advantages of radiation sterilization that have made it attractive for medical product sterilization. The penetrating ability of radiation permits product sterilization in sealed packages and shipping cartons. Because radiation sterilization has fewer variables, reliable process control can be readily achieved. For the materials commonly irradiated, there are no hazardous residual compounds generated. The temperature levels during exposure are moderate, thus providing for safe irradiation of many common component materials such as thermoplastics.

There are some disadvantages of radiation sterilization. The physical and chemical changes caused by ionizing radiation have been studied extensively and include effects such as discoloration and embrittlement. A considerable amount of research has been directed toward identifying and developing radiation-stable polymers for use in product and packaging materials. Another disadvantage is the high startup cost for the facility, and equipment, and for gamma sterilization, the radionuclide source. The need for shielding to prevent a biological hazard increases the cost and complexity of the unit.

<i>log (SAL)</i>	<i>Average Bioburden Entire Device</i>												
	2	5	10	<b>50</b>	100	500	1000	5000	10 <sup>4</sup>	5 × 10 <sup>4</sup>	10 <sup>5</sup>	5 × 10 <sup>5</sup>	10 <sup>6</sup>
-3	0.60	0.71	0.80	1.00	1.10	1.32	1.42	1.66	1.76	2.01	2.12	2.37	2.49
-4	0.88	1.00	1.10	1.32	1.42	1.66	1.76	2.01	2.12	2.37	2.49	**	**
-5	1.19	1.32	1.42	1.66	1.76	2.01	2.12	2.37	2.49	**	**	**	**
<b>-6</b>	1.52	1.66	1.76	<b>2.01</b>	2.12	2.37	2.49	**	**	**	**	**	**

Figure 13. Gamma sterilization dose setting table. Association for the Advancement of Medical Instrumentation, method B1.

Radiation sterilization is widely used to sterilize disposable medical products such as syringes, catheters, sutures, blood filters, and laboratory supplies. The primary consideration regarding the use of radiation for sterilization, assuming an in-house or

contract facility is available, is product stability. The application of radiation sterilization to a wide range of medical products has been facilitated by the recent development of very sophisticated yet easy-to-use techniques for establishing the sterilizing dose<sup>9</sup>. In one method, the bioburden of ten medical devices from three independent lots is measured. The overall average (or in some cases the highest individual lot average) and the desired sterility assurance level are used to select the process dose from the table shown in Figure 13.

For example, the table indicates that a dose of 2.01 Mrad (20.1 kGy) is required to obtain an SAL of  $10^{-6}$  for devices having a bioburden averaging 50 colony-forming units (CFUs). There is a provision for verifying that the actual bioburden fits the radiation resistance distribution upon which the tabular data are based.

In a second method, the bioburden resistance is evaluated directly by exposing devices to incremental doses from 0.2 to 1.8 Mrad and using the proportions of nonsterile units to mathematically set a process dose. A unique provision of these methods is an audit program in which devices are given a subprocess dose and then sterility tested. Increases in the number of positive units compared to the original dose-setting experiment could indicate changes in bioburden counts or resistance which might require an increase in process dose.

Radiation sterilization has fewer variables than any of the other predominant methods. Exposure time is based on the particular requirement for absorbed dose and must be periodically adjusted to account for radionuclide source decay. Exposure time for machine-generated radiation sources is controlled by regulating conveyor speed. One must have confidence in the location of the low dose point (with regard to sterility assurance) and the high dose point (with regard to material integrity) in order for dosimetry measurements to be meaningful. Maintaining a constant dose distribution requires control of bulk product density and load positioning with respect to the source. Biological indicators are only rarely used in radiation sterilization for commissioning, cycle development, or routine monitoring reflecting the reliability of dose measurement systems.

Once cycle development or dose-setting for the selected process has been completed, evidence is needed that the sterilization equipment can successfully deliver the process in the hospital or manufacturing facility. This important quality assurance activity is process validation.

## Process Validation

Validation demonstrates that the process does what it is supposed to do and has been formally defined as follows<sup>9</sup>:

“Process validation is establishing documented evidence which provides a high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality attributes.”

There are several terms associated with validation and routine process control. *Validation* comprises the *qualification* of each process in the total system. The subsequent

*certification* is an administrative approval process affirming that the qualification steps were completed successfully. *Requalification* is the periodic repetition of selected aspects of the original validation program to ensure that the process has remained in a state of control. *Verification* is an ongoing activity whereby a company reviews production run data using a method such as trend analysis to establish that the process is not drifting toward the specified control limits.

There are three elements in a sterilization validation program:

*Installation qualification* establishes that the equipment as installed meets its design specifications. The equipment is visually inspected; instruments are calibrated; and all systems are evaluated to ensure that the sterilizer can be operated properly.

*Performance qualification* incorporates multiple runs (usually a minimum of three) to demonstrate that the process can operate successfully to completion without deviating from the pre-established control limits. The items processed in these runs are evaluated to assure that they meet product release requirements.

Physical parameters and microbial challenge data are obtained from more locations in the load than will be monitored routinely. The resulting data gathered during these studies will be used to justify the specific sites at which parameters are monitored routinely.

The last element of the validation program, *certification*, constitutes the formal review and approval of all process data and test results.

For radiation processes, the term *commissioning* ordinarily refers to all of the above validation activities, when carried out for the first time in a new installation.

Companies and health-care providers do not validate their processes simply to meet the requirements of regulatory or accreditation agencies. Rather, it is recognized that validation has several important benefits. Among these are the increased assurance of product safety and functionality. Because it challenges the process across the full range of specified operating limits, a well-designed validation program will give management proof that the process will consistently manufacture a product that is safe and effective. The rigorous investigation of process capability during qualification may permit deletion of those finished product tests that are far less sensitive to malfunctions than the instrumentation used to control and monitor the process itself. A well-validated process can result in reduced expenditures for testing and quarantine, as well as elimination of the cost of rejects and rework.

Perhaps the most important benefit of validation is the proof to a company or hospital that its products are safe and fit for use. If there is an allegation that a product has not performed properly, then the validation data along with routine production control information will provide the foundation for the defense of the product's quality.

## Where Do We Go From Here?

There are many opportunities in the sterilization sciences to advance the state of the art and the level of technology, and improvements can benefit diverse groups. The investigation and development of new technologies is vital if we are to ensure that there are methods available to safely and effectively sterilize newly-emerging products while preserving their

functional properties. Sterilization process optimization not only lowers the cost of health care products, but also improves overall product quality.

Process validation can help prevent cycle deviations and reduce the resulting costs of product rework and rejection. Attention to process reliability through design, equipment qualification, and control provides the patient with the assurance that each sterilized item is free from microbial contaminants.

These opportunities will continue to provide a worthy challenge to all of us associated with the science of sterilization.

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# The Current Status and Prospect of Research Work of Disinfection and Sterilization in China

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## Introduction

Under the leadership of the China Ministry of Public Health, the nationwide work of disinfection and sterilization has been carried out by the China Academy of Preventive Medicine and the Hygiene and Epidemic Prevention Stations at different levels including provinces, cities, as well as counties. Similar disinfection systems have been founded in divisions of the army and railway systems respectively.

The disinfection of food is undertaken by Departments of Food Hygiene and Epidemic Prevention Stations at different levels, while the management of drinking and polluted water is carried out by the Environmental Health Services. It is conducted and supervised by Departments of Disinfection of the Hygiene and Epidemic Prevention Stations at different levels. The administrative measures of disinfection were taken by every province or city and regulation for preventive disinfection and nosocomial disinfection (including household) were worked out.

## Surveillance of Preventive Disinfection and Sterilization

This aspect includes the community production and living, for example, the disinfection and sterilization of food. The Regulation for Food Hygiene was issued by the Chinese government. The food industry and diet-drinking services have performed according to the regulation under the conduction and supervision of the Hygiene and Epidemic Prevention Stations at different levels. The restaurants (including canteens, dining rooms) generally have used the boiling methods to disinfect tableware. Others have utilized sodium dichloroisocyanurate, sodium chlorophosphate, etc. The commercial detergents have been used for disinfection of tableware and fruit. Infrared-disinfection is being developed.

**Drinking water:** The waterworks in all of the middle or large cities have been equipped with facilities for filtration, sedimentation and chlorination. Besides, sodium hypochlorite is generated by electrolysis. This method is suitable for use in middle and small waterworks. At present, ozone is used to disinfect drinking water in some cities. Recently, a better waterworks for drinking and living was built up in Beijing and Shanghai. Not only is it equipped with facilities of conventional treatment of water from deeper layers, but also a device to generate ozone when it reacts with water. Some plants use high-pressure ultraviolet lamps, in which the anode generates hot mercury gas. The output is 500 W. Two tubes could be used jointly, for disinfecting water. The chlorine products such as sterilants containing sodium dichloroisocyanurate (SDIC), are used to purify and disinfect the open well water in rural area. The quality of the water is up to the standard.

**Disinfection of contaminated water:** Disinfection of polluted water is usually achieved by chlorination according to GBJ 48-83 national standard. Some hospitals have utilized electrolysis of sodium chloride to form sodium hypochlorite which has a powerful germicidal effect in contaminated water. The advantage of this method is that sodium hypochlorite ( $\text{NaClO}_2$ ) is easily obtained by electrolysis, the procedure of operation is simple and its effect is stable.

**Disinfection of air:** The method of fumigation with formaldehyde and peracetic acid is one of the effective measures used in the ward and maternity rooms (lying-in-ward), but all of the patients and staffs have to leave temporarily. On the other hand, the air of the wards could be sterilized by indirect irradiation with ultraviolet light, and at this time, the patients and the staff could still stay within their wards. The number of bacteria in the air of the wards would be decreased by burning several "healthy" incense sticks which are made of some flavor drug and wood dust. Studies of using different ways to collect samples of air and the methodology have been carried out. Up to now, several types of sampling machines are available for detecting bacteria and viruses in the air. The procedure of using them is simple and the results obtained are reliable. Therefore, they contribute to the study of bacterial aerosol in the air.

**Disinfection of fur and wool:** Since the 1970's, in order to disinfect the possible presence of anthrax spore in fur for exportation, the wrapped furs are placed into small or medium plastic tents which were sealed and disinfected with ethylene oxide (ETO) or its gas mixture using  $0.4 \text{ kg/M}^3$  for 24 hr at  $20\text{-}30^\circ\text{C}$ . After such treatment, the requirements for perfect disinfection are reached.

The experience showed that the effect of this method is satisfactory. Recently, the use of sterilization by ionizing irradiation for wrapped wool and fur is performed for the size of  $100 \times 80 \times 50 \text{ cm}$  of wool, a dose of 110 thousand Roentgen units through bilateral irradiation could kill *Brucella*.

**Sterilization of drugs:** Some pharmaceutical factories have applied a test of LAL (*Limulus amoebocyte lysate*) to detect whether or not pyrogens (bacterial endotoxin) are present in drugs. Pyrogens can be produced by the presence of bacteria. In order to prevent contamination, in particular the fungi, a lot of studies and practices have been carried out. The results showed that application of ETO or by  $\text{CO}^{60}$  irradiation was quite effective. After irradiation, the nature and effect of traditional Chinese medicinal herbs remained essentially

the same as before. However, the effect of the liquid form of some medicine might be unstable after irradiation.

## Nosocomial Disinfection and Sterilization

The routine work of isolation and disinfection, required in a nosocomial regulation is formulated by the authorities of provinces and cities. The requirements and measures for laboratories of hepatitis, enteric infections, respiratory infectious diseases as well as the central division of supply are formulated in detail, respectively. It is very important to prevent nosocomial infection and cross infection. The department of disinfection and sterilization of Hygiene and Epidemic Prevention Stations is responsible for conducting and supervising nosocomial disinfection and in inspecting and checking the effectiveness of disinfection. Under the leadership of the director of the hospital, a group has been set up, which is composed of the representatives from the health care department, nursing department and wards, medical affairs, laboratories and logistics department. The group is in charge of formulating, inspecting, and implementation of the regulations for disinfection and isolation, providing new methods, formulation of the regulations for isolation and disinfection, surveillance of environmental contamination, effectiveness of disinfection, training of personnel, exchange of information, etc. At present, the implementation of the regulations of disinfection work is being pushed forward through appraisal of comments. However, some stations are short of enough personnel and equipment. The surveillance and inspection of contamination there is being carried out actively. It is rather unfortunate that there is as yet no nationwide regulations for disinfection issued by the Chinese government. So the responsibility of nosocomial infection as a result of rather serious environmental pollution cannot be revealed studied on the basis of legal requirements.

Nosocomial and environmental infectious cases: Due to insufficiency of supervision and disinfection, all kinds of enteric infections and intoxications occurred in a number of hospitals. For example, the situation of hepatitis virus B (HBV) contamination has been a serious problem, because of the incubation period of such patients might be so long that the source of infection is difficult to determine. The result of investigation showed that the contamination rate of HBV is 5-10% in some restaurants, canteens, hospitals where the hygienic conditions were bad. The drainage systems (sewage disposal) of a few hospitals did not meet the standard of hygiene, meanwhile, *M. tuberculosis* and *S. dysenteriae* were detected in a few hospitals.

The reference parameter of bacterial count in hospital has been worked out in certain regions. As follows:

< 1500  
germs/M<sup>3</sup>

< 16 *Streptococcus* groups A and B/M<sup>3</sup> in fresh and clean air in summer

> 2500  
germs/M<sup>3</sup>

> 36 *Streptococcus* groups A and B/M<sup>3</sup> in polluted air in summer.

< 4500

germs/M<sup>3</sup> < 24 *Streptococcus* groups A and B/M<sup>3</sup> in fresh and clean air in winter.

> 7000

germs/M<sup>3</sup> > 36 *Streptococcus* groups A and B/M<sup>3</sup> in polluted air in winter.

The results of actual detection showed that the air was polluted in many hospitals.

It is important to develop a simple and effective method of disinfection. The regulation for Food-Hygiene has to be thoroughly implemented and the nationwide regulations for disinfection and sterilization remain to be formulated.

The methodology of disinfection in hospital: The conventional apparatus and dressings are mainly sterilized by autoclave. The heat labile substances should be disinfected with ethylene oxide or formaldehyde vapor. The surfaces of the body should be sprayed or swabbed with peracetic acid. Maternity wards and operating rooms are disinfected with ultraviolet rays. All syringes and apparatus for dentists should be sterile before use each time. For other equipment such as endoscopes, respirator machines, anaesthesia apparatus, special regulation should be established. A number of studies on disinfection of hepatitis B virus have been carried out. As to the disinfection of the household of patients where infectious diseases occurred, final disinfection and isolation should be required.

## Disinfection of Hepatitis Virus B

This subject is one of the most active research works on disinfection in China. That is because: (1) The percentage of HB<sub>s</sub>AG carriers is as high as 8% (1979-1980). The incidence of the active HBV patients is rather high. Moreover, it is known that hepatitis B is often associated with hepatic cancer. The contamination of the environment with hepatitis B virus is a very serious problem. (2) Eliminating HBV from the environment is difficult. (3) The cultivation of the HBV virus remains difficult. Due to the lack of appropriate susceptible animals at present in China, loss of HB<sub>s</sub>AG antigenicity is being used as a parameter of detecting the effectiveness of its disinfection. Many experiments have shown that the elimination of HB<sub>s</sub>AG is even more difficult than that of the HBV infectivity.

Recently, it is evident that the antigenicity of HBV is destroyed under the action of 0.2-0.5% peracetic acid for 10 min. Active chlorine, 500-1000 ppm, is capable of destroying its antigenicity rapidly. Use of an evaporating method (3g/M<sup>3</sup> of the mixture of formaldehyde-chlorine or 2% glutaraldehyde) and the microwave with an output 500 W, 3 min, may result in elimination of its antigenicity. Many reports revealed that the effect of routine disinfectants on HBV is not good. The experiments of the virucidal effect have been carried out with DNA polymerase as a parameter of the effectiveness of disinfection. The results suggested that those disinfectants in routine use were effective. A number of disinfectants are said to be without effect, this might be in part due to the use of destroying antigenicity as a parameter of successful disinfection.

# The Biological Indicator Microorganisms and the Monitor in Evaluating Efficacy of Disinfection and Sterilization

Studies on this aspect have been carried out actively. Thus the disinfection of type B hepatitis virus with a product containing chlorine 500 ppm for 120 min has been tested. The effectiveness of inactivation of HB<sub>s</sub>AG is similar to that of coli-phage f<sub>2</sub>. It is recommended that the inactivation of coli-phage f<sub>2</sub> as an indirect index of detecting hepatitis B virus and enteric viruses in polluted water of hospitals or the spore of *Bacillus anthracoides* be used as the biological indicator microorganisms.

Examination of the effect of autoclaving is usually made by the use of the melting method with sulphur (melting point 114-116°C) or benzoic acid (melting point 121-123°C), but these methods cannot be used to express the duration of disinfecting effect. The auto-recording method by warm thermocouple and a monitor (changeable color of the card) which has been widely used in other countries are ideal methods, but this technique has not been widely used in China. At present, the biological means of determination, i.e., the use of *C. stearothermophilus* spores as the indicator microorganism, is available.

Disinfection of spores of *Bacillus anthrax* on fur with ethylene-oxide: The indicator microorganism used is *Bacillus subtilis* subsp. *niger*.

An attempt to study with the aim of obtaining a chemical indicator has been made and elementary success is being obtained.

The comparative experiments regarding the effectiveness of disinfection with CO<sup>60</sup>gamma irradiation have been carried out. The result suggest that the resistance of *Bacillus cereus* spores to the irradiation is very great. Generally, 500 thousand Roentgen units result in death of over 99.9% of the spores.

With ultraviolet and disinfectants, if applied to the target bacteria, *E.coli* and *Streptococcus* are selected as the indicator microorganisms for determining the disinfecting efficacy. Besides, there are two methods, i.e., an ultraviolet intensity meter and chemical indicator with a disinfective dose. Both could be used to detect the effectiveness of disinfection.

## Disinfectants

There are many disinfectants and detergents such as: ethylene oxide, chloro-products; chlorine dioxide, sodium dichloroisocyanurate, chloro-trisodium-phosphate as well as sodium hypochlorite, glutaraldehyde, iodophor, etc. have been studied in China nowadays.

Sodium dichloroisocyanurate (SDIC) is one of the chloro-disinfectants. Its trade name in China is Yuloujing and its active chlorine liberated is about 62-64%. The experiments showed SDIC made in China is a very effective disinfectant. Its toxicity is rather low and it has no accumulative effect and also lack of inducement. *E. coli* and *B. cereus* spores could be killed at 100-250 ppm and 350-600 ppm in 20 min respectively, and for hepatitis B virus at 500 ppm in 5 min. Acidochlorofumigate as a new type of disinfectants is a mixture of SDIC, potassium permanganate and an acidic-synergistic which can be put into practical

use.

Chlorobromisocyanurate is a chloro-product with 65% bromine-chlorine which could kill HBV at 400 ppm. Chlorinated trisodium phosphate ( $\text{Na}_3\text{PO}_4\text{NaOCl}\cdot 11-12 \text{ H}_2\text{O}$ ) is a compound resulting from the interaction of sodium phosphate and the water solution of sodium hypochlorite. *E. coli* and *B. cereus* spores can be killed at 40 ppm and 1500 ppm for 10 min, respectively.

In recent years, most of the detergents which have been produced and in practice are used in China are chloro-products as mentioned above. A generator for sodium hypochlorite has been designed and is being produced in Shanghai and Guangzhou. Through electrolysis of sodium-chloride, a great deal of sodium hypochlorite could be produced. The apparatus could be used in medium or small waterworks. The experiments showed that for the generator, model SX-1 made in Shanghai, ionizability is low in the acidic condition. A large amount of HClO was produced, and bactericidal effect is strong. The output of chlorine was 120 g/h-200 g/h, respectively, by means of the generator made in Guangzhou. When it is put into the pool for 1-2 h after sedimentation, the treated water can be drained and the bactericidal rate would reach to 99.8%. The number of *E. coli* is less than 500 germs/1. Since 1981, there have been more than 20 hospitals using the generator to disinfect the polluted water. According to the report given by Guanzhou Institute of chromogenic metals, the generator with titanium as positive electrode could produce sodium hypochlorite. Meanwhile, the contents of some trace elements such as Lead (Pb), Chromium (Cr), Arsenic (As), Mercury (Hg), etc. were below the limits provided by national standards for drinking water and its duration of the life span for effective use was 12,860 h. It was found that 1000 mg/1 HClO could kill spores of *B. cereus*.

At present, peracetic acid has been widely used in China. A concentration of 0.2-0.5% of the disinfectant could kill *M. tuberculosis*, bacterial spores and could inactivate HB<sub>s</sub>Ag in 10-30 min. Peracetic acid is a broad-spectrum disinfectant with many advantages. For instance, its bactericidal effect is rapid and high, even at low temperatures it remains highly effective. The use of this disinfectant would not result in community effects with a pollution problem. However, its disadvantages were shown to be: unstable, with irritant smell which might render it unsuitable for further use. In order to overcome the above shortcomings, acetic acid, peroxide of hydrogen and sulfuric acid should be put into the containers separately to be mixed before use. Because peracetic acid has a high disinfective effect on HB<sub>s</sub>Ag, it is recommended for use in disinfection in hospital and household.

A study of the mechanism of bactericidal action of peracetic acid was carried out and followed by analysis of amino acids, RNA and DNA after leakage from the spores of *Bacillus subtilis* subsp. *niger* by means of incorporation of tritium ( $\text{H}^3$ ) into its spores. The experiment showed that the drug could destroy the permeability of the spore resulting in breakdown and dissolution of the core, and finally cause the death of spore due to the leakage of substances with large molecules, such as DNA, RNA and protein from the interior. The destruction on the spores was a result of the action of peracetic acid itself, not by activation of bacteriolysin. It is mainly due to the double effects of acid and activated

oxygen from peracetic acid. However, it seemed to be that ethylene oxide has been used in the disinfection of exported fur by fumigation within large and medium sizes of plastic enclosures as early as 1960s.

It was evident that a defined concentration of ethylene oxide was needed to destroy the spores of *B.anthrax* at different temperatures.

A grain depot contaminated with anthrax spores had been disinfected with ETO. To determine the residual dose of ETO, a method was devised with the upper-air-gas chromatography. ETO has also been used in the disinfection of fine medical instruments, the artificial circulation machine for heart-lung operations, various specula, medical cannula and other instruments, as well as traditional Chinese herb medicine for the elimination of contamination by fungi. The residual dose is so small as compared with its great efficacy that people have paid attention to its use. Pure ETO is a combustible and explosive substance, therefore, use of a mixture of ETO and some inert gases, such as Freon (F12) etc. must be used to overcome this shortcoming. Besides, the effect of killing spores, glutaraldehyde is rapid, being ten times as that of formaldehyde. The irritation and corrosive effect of glutaraldehyde is very low, so it has been widely used for disinfection and sterilization of medical apparatus and fine instruments in other countries. The experiment showed that the bactericidal activity and destruction of HB<sub>S</sub>AG by 2% neutral glutaraldehyde made in Shanghai is similar to that of basic glutaraldehyde made abroad with a Tradename of Cidex. Moreover, its effect of killing spores is even greater than an imported fortified acidulated glutaraldehyde (Sonacide).

Iodophor is a complex compound which is composed of a surfactant as solvent and a carrier. Its solubility is 16 times greater than that of iodine and its bactericidal effect is also greater than that of iodine, but its toxicity for humans is very low. A Chinese named DARMEI detergent has been made in Shanghai.

Various types of bactericidal incense materials used for disinfection of air in closed containment are made of Chinese herb medicine. By means of fumigation over 90% of the bacteria in the air of a room could be eliminated. Ozone is very effective for disinfecting bacteria in air and water. Its side effects on humans is very low, therefore, the manufacture and utilization of the ozone ion-generators should be promoted.

## **Disinfection by Physical Means**

Nowadays, the method is most commonly used in disinfection of medical instruments in hospitals, Hygiene and Epidemic Stations and Institutes is the autoclave. Because of following reasons, such as inadequate time for exhaustion of air inside of the apparatus, inappropriate operative procedures as well as the use of containers with air-tight- aluminum boxes with medical instruments inside, the purpose of disinfection would be defeated. To solve the problem, a prevacuum equipped autoclave and the use of aluminum boxes with holes in them should be recommended.

Ultraviolet irradiation is one of the conventional methods for disinfection of laboratories and the operating room in hospitals. An apparatus for generating ultraviolet rays and a

facility to exhaust polluted air were invented to control the laboratory infection and to provide an approach of preventing bacterial aerosol infection.

Disinfection by microwave: Since 1970's microwave has been used to disinfect and treat pills of traditional Chinese herb medicine, ampoules and canned foods. In the recent years, it has been used in the disinfection of 'Renminbi' (the Chinese paper currency), the tickets for meals, canteens, case histories, experiment sheets and reports, dressing package, fur and operating instruments wrapped with moist cloth, glasses as well as enamel ware, etc. Experiments suggested that it is effective and applicable. Microwave sterilizers made in China have been provided for practical use. HB<sub>S</sub>AG could be destroyed at 75°C for 3 min.

## **The Future Prospect of Disinfection and Sterilization Work in China**

The disinfection work has played a very important role in preventing infectious diseases in China and further efforts should be undertaken to fortify the strength of the leading and technical aspects. We are preparing a report to the Chinese Ministry of Public Health, suggesting that the Chinese Ministry of Public Health should establish a special committee for disinfection work in China and to formulate regulations for disinfection work as well as to fortify scientific research works in this field. Based on universal standards in determining the effectiveness of disinfection and in detecting for contamination, overall control on disinfection should be carried out.

Along with the rapid development of the national economy and elevation of peoples' living standards, requirements of various packages should be improved. It is recommended that the packages for drug, food and utensils etc. should preferably be sterile or used once. This improvement would prevent contamination and cross-infection as a result of inadequate disinfection and sterilization. Meanwhile, the side effects caused by using disinfectants would appear much lower. X-ray-apparatus for disinfection, new disinfectants and effective simple instruments for disinfection are in urgent need in China.

We believe that along with the realization of the modernizations, under the implementation of an open-door policy to other countries and vitalization of the national economy in China, great progress in disinfection work will have to be made. During the course of the present international symposium, we are going to learn a great deal from you and as a result, we shall be able to develop and further strengthen scientific cooperation and friendship between China and all other countries in the world. Hoping that all of you will contribute greatly to our work in disinfection work.

# Wet-Heat Sterilization, Including Both the Design of the Process and Equipment Used to Sterilize Product<sup>1,2</sup>

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Wet-heat sterilization is one of the simplest and most efficient ways of producing sterile products, whether they be foods, drugs, hardware or devices. We use the term “wet heat” to indicate the presence of a saturated condition, either the presence of water (in the liquid state) or the presence of saturated steam ( $a_w = 1.00$ ).

The equipment required for wet-heat sterilization, the laboratory or industrial autoclave or retort, are relatively simple devices, easy to control, and extremely effective in carrying out the sterilization operation.

In this report, we will concentrate on the wet-heat sterilization operation. This presentation can be considered to be in two parts:

- (a) Requirements in developing an optimum sterilization process and
- (b) Equipment and procedures used in the plant in wet-heat sterilization.

Heat sterilization is not an area in which there is rapidly changing technology and a great deal of new scientific information. It is a slowly evolving area, so while there is change, the change is relatively slow. There are many excellent review articles that are a part of textbooks or reference books that are applicable to the heat sterilization area. Most of these include very complete bibliographies. Therefore, in this report I will not exhaustively review the literature and cite all the references in the area. What I will do is try to point out particularly good review articles that are widely available that do have rather complete bibliographies. In one sense, this report is an update of the report of Pflug, 1973, on the heat destruction of microorganisms.

While the wet-heat sterilization operation has changed very little in recent years, as far as the process and equipment are concerned, there are some major changes in how we view the process and our approach to explaining sterilization to the person newly entering the field. The treatment or the philosophy of the use of models in the sterilization process is

new in this report. We have used models for many years. However, we believe that our method of describing them has been improved and will give the reader a better feel for the overall use of models.

In recent years, we have been teaching a workshop on the Microbiology and Engineering of Sterilization Processes for the Parenteral Drug Association (Philadelphia, Pennsylvania). Our interaction with the students in these workshops has produced a continual change in the way we present our concepts of sterilization. Many of the ideas presented in this report have been developed in these workshop teaching programs and are taken from the textbook used in teaching this workshop (Pflug, 1982).

## Symbols and Their Definitions

$a_w$	Water activity, attribute of a microorganism that is usually assumed to be equal to the relative humidity of the atmosphere surrounding the cell.
$D_T, D_{T_{ref}}$	The cotangent of a straight-line semilogarithmic microbial survivor curve, which is the time for the population to decrease by 90 percent.
ERH	Equilibrium relative humidity.
$f_h, f_c$	Time required for the asymptote of the heating or cooling curve to cross one log cycle that is, the time required for a 90 percent change in temperature on the linear portion of the curve.
$F, F_T, F_O, F_C$	Sterilization value; $F_T$ equivalent time at a specified temperature for a specified z-value; $F_O$ , equivalent time at 121.1°C (250°F), for a z-value of 10°C (18°F); $F_C$ , equivalent time at 120°C, z-value of 10°C.
HP	Heat penetration measurement, an input in the heat sterilization process design.
$J_h, J_c$	Ball lag factor of a heating or cooling curve.
k	Reaction rate constant.
MI	Microbiological input in a sterilization process design.
$N_O, N_F$	Number of Microorganisms per Unit— $N_O$ is the initial number, $N_F$ is the number after a sterilization process (F).
NPT	Nominal-sized pipe in inches, USA.
PC	Processing conditions, inputs in a sterilization process design.
PNSU	Probability of a nonsterile unit in a lot of sterilized product.
$T, T_1, T_{ref}$	Temperature; $T_1$ , heating medium temperature (autoclave, retort temperature); $T_{ref}$ , reference temperature of z-value curves.

UHT	Ultra high temperature sterilization processes carried out in heat exchanger.
$Y_n$	Spore-Log Reduction— $Y_n = \log N_O - \log N_F$
Z	Temperature coefficient of microbial destruction, degrees of temperature (C or F) for a ten-fold change in the F- or D-value; the degrees of temperature for the thermal death time or thermal resistance curve to traverse one log cycle.

## Wet Heat vs. Dry Heat

It has long been known that microorganisms are killed quickly and at comparatively low temperatures in the steam autoclave (121.1°C for 15 or 30 minutes) compared to the dry heat oven (150-160°C for 1 to 2 hours). The underlying phenomenon has been delineated only comparatively recently. The work of Murrell and Scott (1957) showed that the microbial death rate is a function of the water activity,  $a_w$ , or equilibrium relative humidity, ERH. The results of this and other work led the way to a general awareness of those working in the sterilization area that wet heat is a single specific condition where the  $a_w$  or ERH is 1.00. Since the effect of water on the heat destruction rate of a microorganisms is covered by only two conditions, wet and dry heat, if wet heat is a single condition where  $a_w$  or ERH is 1.00, then all  $a_w$  or ERH conditions between 0 and 1.00 are dry heat conditions! Therefore, in dry heat we can have any level of  $a_w$  or ERH between 0 and 1.00. Since  $D_T$  is a function of  $a_w$  or ERH, the value of the  $a_w$  or ERH of the water in the microorganisms that are being killed is a critical parameter in the dry heat sterilization system. The general relationship of  $D(T)$  and  $a_w$  or ERH is shown in Figure 1.

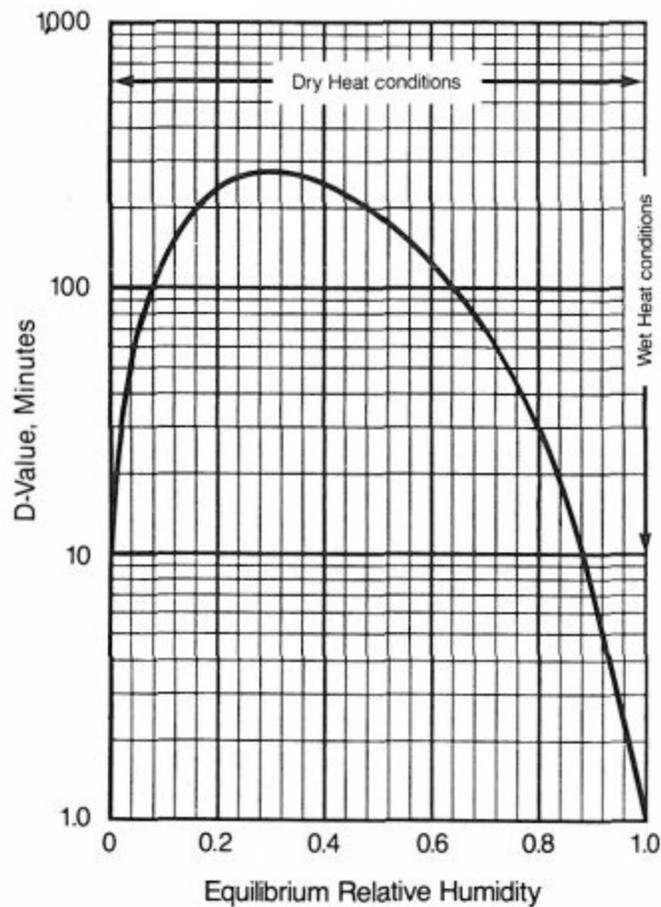


Figure 1. The Effect of the Equilibrium Relative Humidity of the Environment Surrounding the Spores on Their D-Value; the Commonly-used Terms, “wet-heat conditions” and “dry-heat conditions,” are Identified.

## Overview of the Sterilization Area

The area of sterilization is very broad in that it includes both a scientific and engineering area. In the scientific area we have both microbiology and statistics. The engineering area is primarily process engineering, with heavy emphasis on steam processes, heat transfer and instrumentation.

It is natural for us to think of sterilization as one large, broad entity. However, I believe there are certain aspects of it that are better understood if we can think about sterilization in terms of scientific endeavors and certain engineering endeavors.

The division of sterilization into scientific endeavors and engineering endeavors may seem artificial and needless. However, I believe we can better understand the sterilization area, its problems and how to solve them, if we separate the search for basic data (science) from designing and carrying out the sterilization operation in the manufacturing plant (engineering).

I will start by listing the definition (New College Ed., Am. Heritage Dictionary, 1976) of science and engineering.

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Science: The observation, identification, description, experimental investigation and theoretical explanation of natural phenomena.

The application of scientific principles to practical ends as the design,  
Engineering: construction and operation of efficient and economical structures, equipment and systems.

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**Table I. Some of the Specific Areas that are Part of Sterilization Science and those that are Part of the Sterilization Engineering Endeavor:**

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*Sterilization Science*

- (1) Carrying out Research Directed Toward Developing an Understanding of Microbial Death Kinetics
- (2) Developing Microbial Destruction Data for Specific Microorganisms in Specific Products and Sterilization Systems.
- (3) Determining the Microbial Bioburden on Products to be Sterilized.

*Sterilization Engineering*

- (1) Designing, Developing and Installing Sterilization Equipment
  - (2) Designing the Sterilization Process
  - (3) Developing and Maintaining Test Equipment, Including Standards; Calibrating Measuring Equipment in the Plant
  - (4) Establishing Equipment and Process Operating Parameters
  - (5) Validating the Equipment and the Process
- 

In Table I are listed some of the specific sterilization areas that are part of the scientific endeavor and also some of the areas that are part of the engineering endeavor.

Since we are involved in both science and engineering in the sterilization operation, we will be involved with both scientists and engineers! These two groups of individuals have radically different basic training. They are pointed in two different directions. The scientist is trained to search for new knowledge, to search for "truth." The engineer is trained to get a job done. To get a job done, we have to make accommodations, we must improvise, synthesize, make allowance for variation in known conditions, and provision for compensating for unknown conditions. The objective of the engineering working in the sterilization area is to produce sterile product. The interest of the scientist is to learn about and understand sterilization.

The sterilization project team must include both scientists and engineers who recognize their individual roles and appreciate both disciplines.

## **Models in the Sterilization Area**

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There are several different uses for models in the sterilization area as shown in Table II.

The scientist who gathers microbial destruction data uses models to reduce and correlate data. It is much more efficient to report the results of a major microbial destruction experiment in terms of one or two parameters and their statistical limits rather than reporting raw data either in tabular or graphical form. In this situation we will choose that model which best fits the data. Many different models have been used to correlate microbial destruction data.

The scientist does a second type of modeling, which is to try to determine if experimental results follow some accepted law of science. Rahn (1945) was working to develop a model that fits general scientific principles that would also fit microbial destruction data.

In this presentation we will say very little about the scientific aspects of sterilization. Our primary thrust will be in the engineering area. We must think of the engineering design sterilization model as different from the model used by scientists to correlate data. The engineering model has to meet the objectives of the engineering design, but it will be synthesized from the scientific data gathered regarding microbial destruction.

**Table II. Use of models\* in the sterilization area for both microbial destruction rate and temperature effect**

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*Sterilization Science*

- (1) Used to Correlate and Simplify Experimental Data (Data Fitting Using Simple and Higher-Order Polynomials)
- (2) Derived from Basic Scientific Principles to Explain Microbial Death Kinetics (Theoretical)

*Sterilization Engineering*

Sterilization Process Design Engineering (Simple with Great Utility)

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\* Definition of "Model": A System of Postulates, Data, and Inferences Presented as a Mathematical Description of an Entity or State of Affairs (Webster's New Collegiate Dictionary, G. & C. Merriam, Springfield, MA 1977).

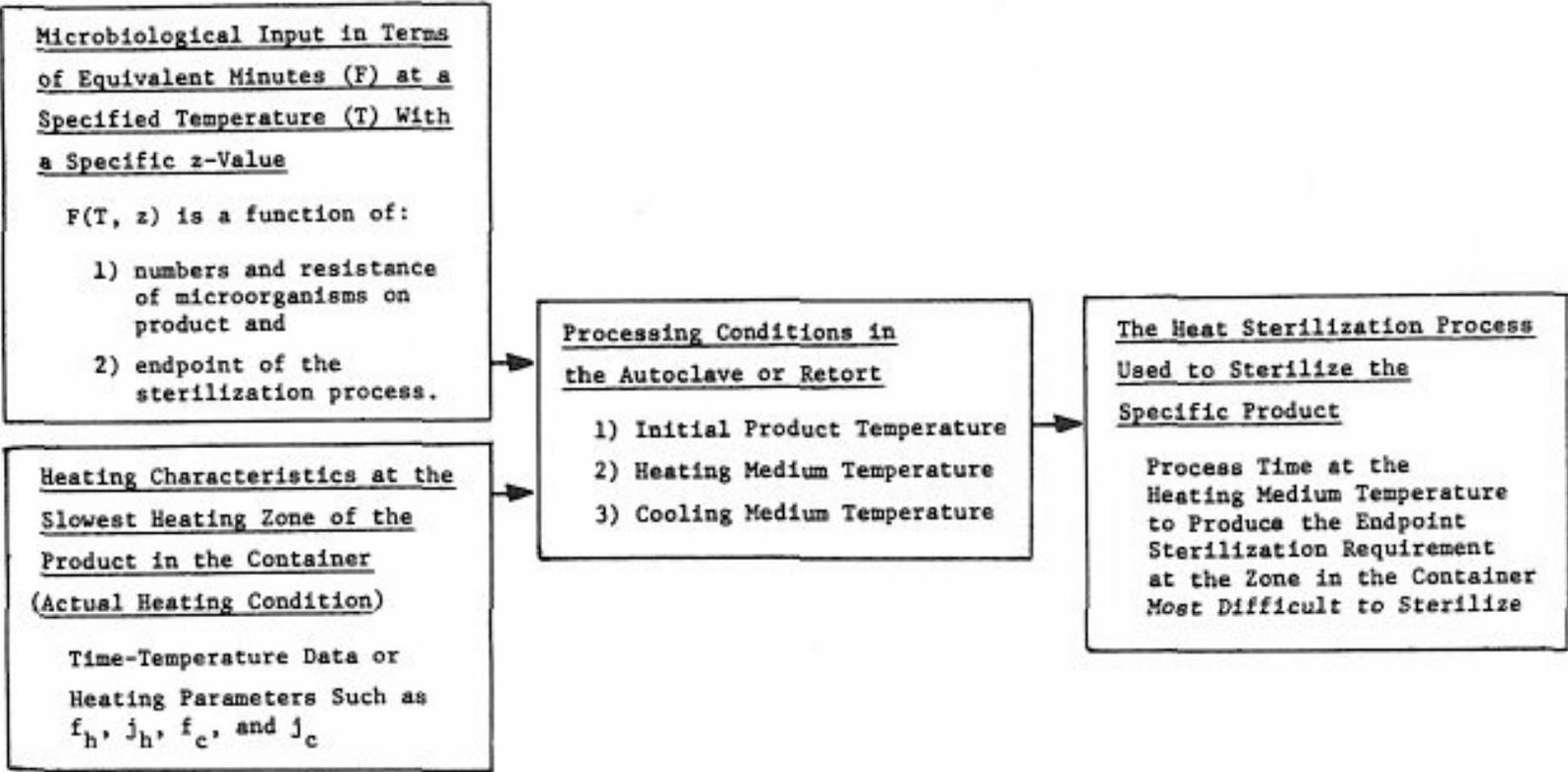


Figure 2. Data Flow in Sterilization Process Design.

# The Engineering Operation of Sterilizing Objects and Products

Sterilization is an engineering unit operation. In designing a sterilization process we make use of both engineering and microbiological data. We can think of the sterilization process design as being based on all the factors that influence the movement of heat to the critical zone in the container plus the destruction characteristics of the microorganisms located at this critical zone.

The objective of a sterilization process is to kill the resistant microorganism on the product. There are a number of steps we must take, starting with the raw data (such as the number and resistance of the microflora of the product), before we obtain the time-and-temperature condition used by the operator of the autoclave in the manufacturing plant to produce sterile product. A generalized diagram of data flow in sterilization process design is shown in Figure 2. The sterilization process engineer must take the microbiological inputs (MIs), the heat penetration data (HP), and the processing conditions (PCs) and establish the sterilization process for the product. This is the information used by the autoclave operator. Designing the sterilization process would be direct and simple if the values of MIs, HP and PCs were all known or measurable. We select or know the processing conditions; we can measure the heat penetration characteristics of the product in the specified-sized container. However, establishing the value of the microbiological input is the difficult part in the design of sterilization processes. The microbiological input is the “sterilization process equivalent time value (F)”; we must determine the F-value that will produce the desired level

of microbial destruction. One effective way of accomplishing this is to have an engineering design model for calculating the sterilization process time (F).

The requirements for an engineering sterilization process design model are quite different from those of the scientist gathering basic microbial destruction kinetic data. Some of the characteristics of an engineering design model are:

- (1) a best-fit approach in modeling the scientific data is used (accurate to 90 to 95 percent);
- (2) the design system model includes provision for error (safety factor) so results should give a safe performance, even when the fit of the model to the scientific data is poor; and
- (3) the engineering design model must be simple enough to be usable by the people in the field.

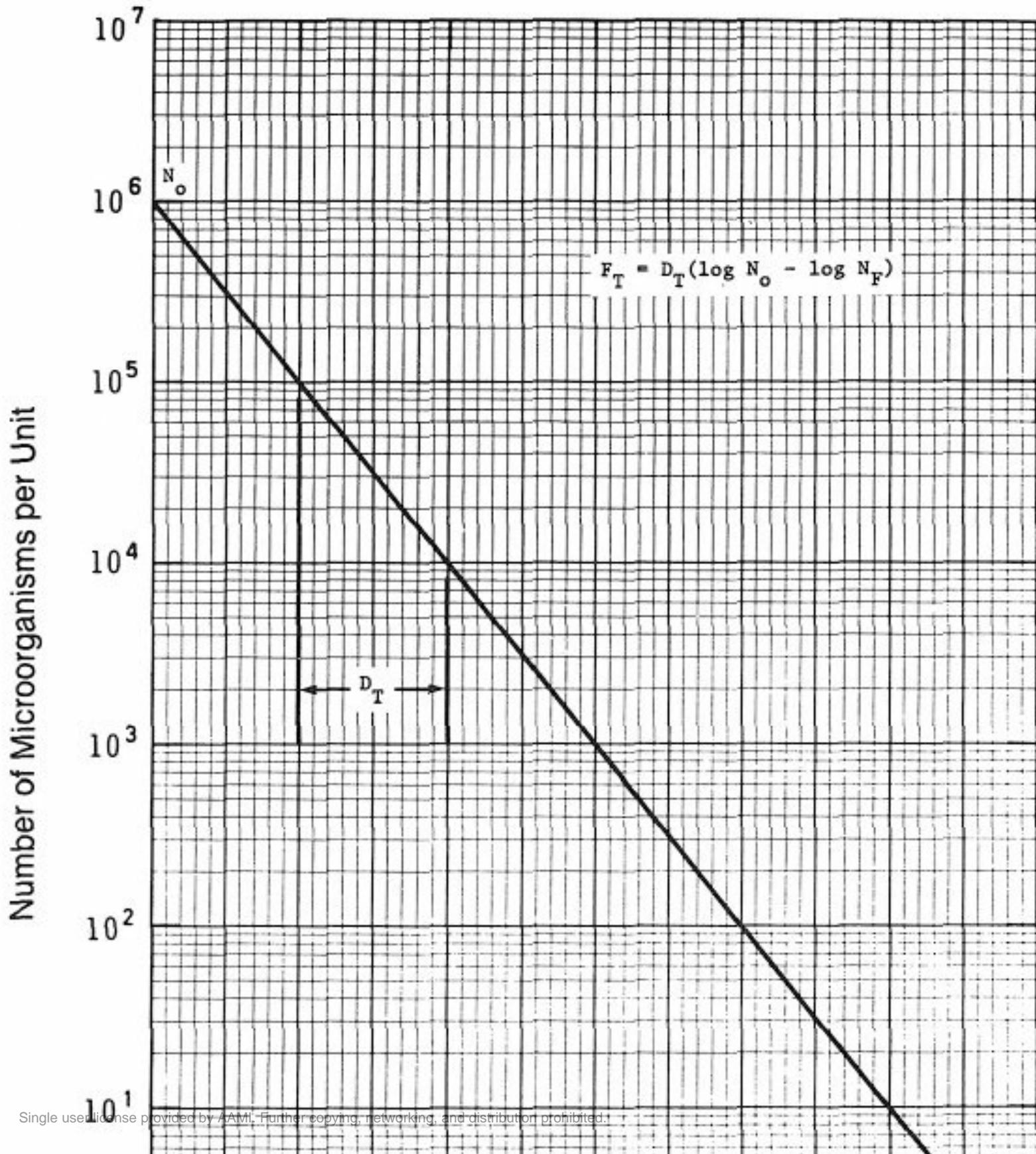
A review of the literature on wet-heat destruction of microorganisms, while showing that microbial destruction curves of almost all shapes are possible, indicates that in about 40 percent of the heat destruction tests, the data form an approximate straight-line semilogarithmic survivor curve. In another approximately 40 percent, the data form a curve where the initial portion either shows a much lower destruction rate (concave downward) or a much larger destruction rate (concave upward) followed by constant-rate straight-line destruction (Pflug and Holcomb, 1983). To meet the characteristics we describe above as desirable in an engineering design model, with emphasis on the necessity of keeping the model simple, we conclude that the simple straight-line semilogarithmic model shown in Figure 3 is the best compromise. The appropriate parameters,  $N_0$  and  $D_T$ , of the model can be adjusted so the model represents a worse case condition (the greatest degree of safety) based on actual microbial destruction data. The equation that describes the model is:

$$\log N = -F_T/D_T + \log N_0 \text{ or, rearranged in terms of } F_T,$$
$$F_T = D_T(\log N_0 - \log N_F)$$

where  $N_0$  is the initial microbial population;  $D_T$  is the microbial destruction rate, the time to reduce the population by 90 percent at Temperature T; and N is the population after F minutes of heating. The model relates the length of the heat stress period at a specified temperature with numbers of survivors.

The  $D_T$ -value is a unique term, both in concept and use, and warrants further explanation. When we have a straight line correlation on a semilogarithmic graph, it is possible to have a very meaningful measurement of the rate of change if we choose the right parameter. If, on the semilogarithmic graph, the logarithmic scale is on the y axis and the arithmetic scale is on the x axis; the change, y, for the line to cross exactly one log cycle will always be 1.0. ( $y = \log N_2 - \log N_1$ ; when  $N_2$  and  $N_1$  are one log cycle apart and the line has a negative slope, as in the case of a microbial survivor curve, the difference,  $\log N_2 - \log N_1$ , will always be -1.0.)

The slope of a straight line is Tangent  $\theta/y/x$ . A straight-line survivor curve graph of  $\log N$  vs.  $F$  will have a slope interpreted in terms of a  $y$  of one log cycle,  $1/F$ ; the units will be  $1/\text{Minutes}$ , the numerical value requires a calculation for direct interpretation. The term,  $1/\text{Slope}$ , which is  $x/y$  and is equal to the Cotangent,  $\theta$ , has units of minutes for the line to cross one log cycle, it is directly interpretable; it is the time for a 90 percent change in the number of surviving microorganisms.



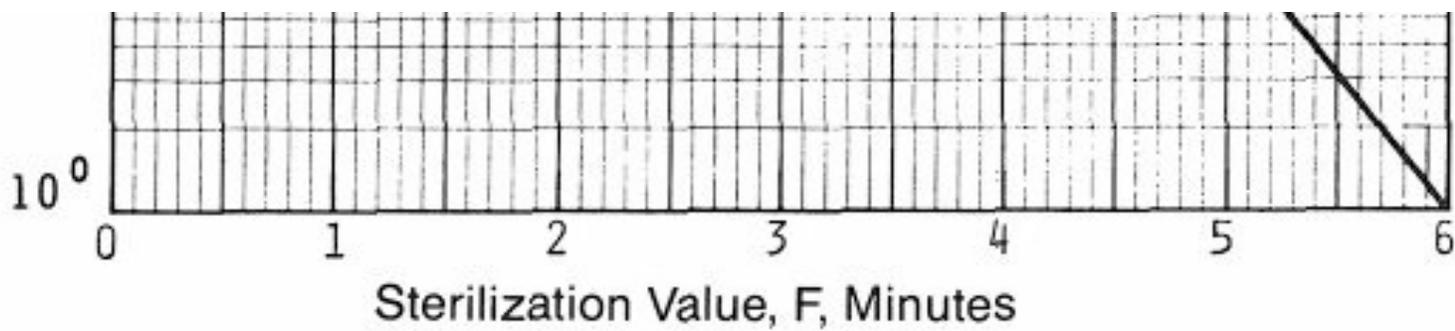


Figure 3. Simple Logarithmic Model Used as a Sterilization Engineering Design Model.

Taking advantage of these relationships, the sterilization microbiologist has named  $1/\text{Slope}$  ( $y/x$ , the cotangent of theta) as the D-value, meaning decimal reduction time since it is the time for a one-log or 90 percent reduction in the number of microorganisms.

It is necessary to have a temperature coefficient to adjust the sterilization value to different temperatures and also to integrate microbial kill over different temperatures when the sterilization takes place during changing product temperatures (heating or cooling process). We use the Bigelow (1921) model because it has the attributes to quality as an engineering design model. The equation for the thermal resistance curve (D vs. T) is:

Number of Microorganisms per Unit

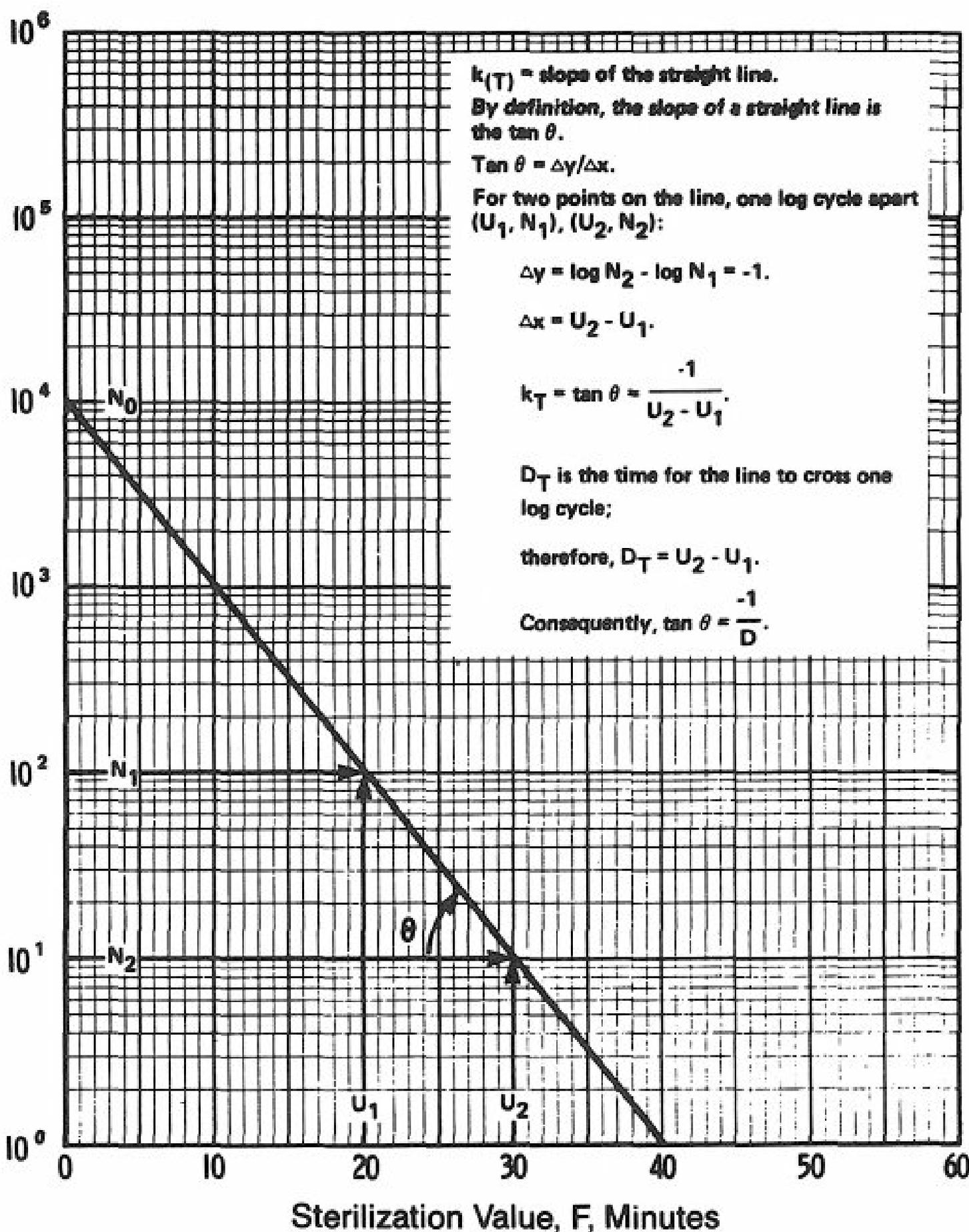


Figure 4. Semilogarithmic Survivor Curve Relationships.

$$\log D_T = \frac{-1}{Z} (T - T_{ref}) + \log D_{T_{ref}}$$

In the model the change in the microbial population, due to the sterilization process,  $F_T$ , is:

$$F_T = D_T (\log N_O - \log N_F);$$

the logarithmic change in the microbial population due to the sterilization process is called the spore-log reduction ( $Y_n$ ).

$$Y_n = \log N_O - \log N_F$$

therefore,

$$F_T = D_T Y_n.$$

## The Sterilization Process Endpoint

The endpoint of the sterilization process is a difficult problem in process design, both in philosophy and magnitude, that must be solved before we can proceed to use our model in the design of the process.

The fact that the order of death of microorganisms is logarithmic (geometric progression) is in direct conflict with the practical idea that we should produce sterile foods, drugs, and implanted medical devices. "Sterilization" is any process, physical or chemical, which will destroy all forms of life, as applied especially to microorganisms, including bacterial and mold spores, and the inactivation of viruses. The terms, "sterile," "sterilize," and "sterilization," in a bacteriological sense mean the absence or destruction of all viable microorganisms. These terms indicate an absolute, not a relative, condition. Any specific item or unit is either sterile or it is not sterile. We can go into the laboratory and determine if an item is sterile or not sterile.

The philosophical problem is, "How do we reconcile the fact that the death of the individual entities of a microbial population, subjected to heat, radiation, or chemicals, proceeds as a geometric progression (where only with an infinite treatment can we be absolutely sure that all microorganisms have been killed and therefore all units are sterile) with the practical problem that the consumer wants "sterile" items and thinks in terms of an absolute condition.

The problem of the dichotomy of having an absolute term, "sterile," and a microbial death rate that is a geometric progression has been resolved in the USA where the Food and Drug Administration (FDA) will allow the term, "sterile," to be used to describe the microbial condition of the product if there is one or fewer nonsterile units per one million ( $10^6$ ) units for products manufactured under the good manufacturing practices (GMP) regulations. We can interpret this specification or any similar specification using the straight-

line semilogarithmic model to yield a basis for the F-value design of the sterilization process. When only one unit in one million units is nonsterile, the probability is high that there is one surviving viable microorganism. (If two viable microorganisms survive in  $10^6$  units, there would be two nonsterile units.) Consequently, in an analysis based on the specification, "one nonsterile unit per lot of  $10^6$  units," we can use one microorganism as the endpoint number of microorganisms surviving in the  $10^6$  unit "lot" of product.

We will use the term, " $N_0$ " to identify the initial number of resistant microorganisms (spores) per unit of product.

We can treat these data in either of two ways; we will show that both ways yield the same result: We will use only the spore-log reduction ( $Y_n = \log N_0 - \log N_F$ ) part of the model in this analysis.

(1) We will make the analysis using the number of units cited in the specification. If the specification is one or fewer nonsterile units per  $10^6$  units, we will make the analysis on the basis of  $10^6$  units; if the specification is one or fewer nonsterile units per  $10^9$  units, we will make the analysis on the basis of  $10^9$  units, etc.

On the specification units number basis, we will use as the starting resistant microbial population, the initial number per unit ( $N_0$ ) times the number of units in the specification.

The number of resistant organisms surviving the sterilization process will be one as discussed above. When the specification is one nonsterile unit per  $10^6$  units, the spore-log reduction will be:

$$Y_n = \log (N_0 \times 10^6) - \log 1.$$

When  $N_0$  is 100, the spore-log reduction,  $Y_n$ , will be 8.

(2) We will make the analysis on the basis of one unit. The initial number of critical microorganisms per unit is  $N_0$ . After the sterilization process, there will be one microorganism surviving in  $10^6$  units. The spore-log reduction will be:

$$Y_n = \log N_0 - \log (1/10^6).$$

When  $N_0$  is 100, the spore-log reduction will be 8.

Once we understand the principle behind the analysis, we believe it is better to talk about the sterilization process in terms of the individual unit. The initial microbial load is  $N_0$  and after sterilization, the result is the probability of a nonsterile unit (PNSU). We will discuss the unit concept further using the graphs in Figure 5.

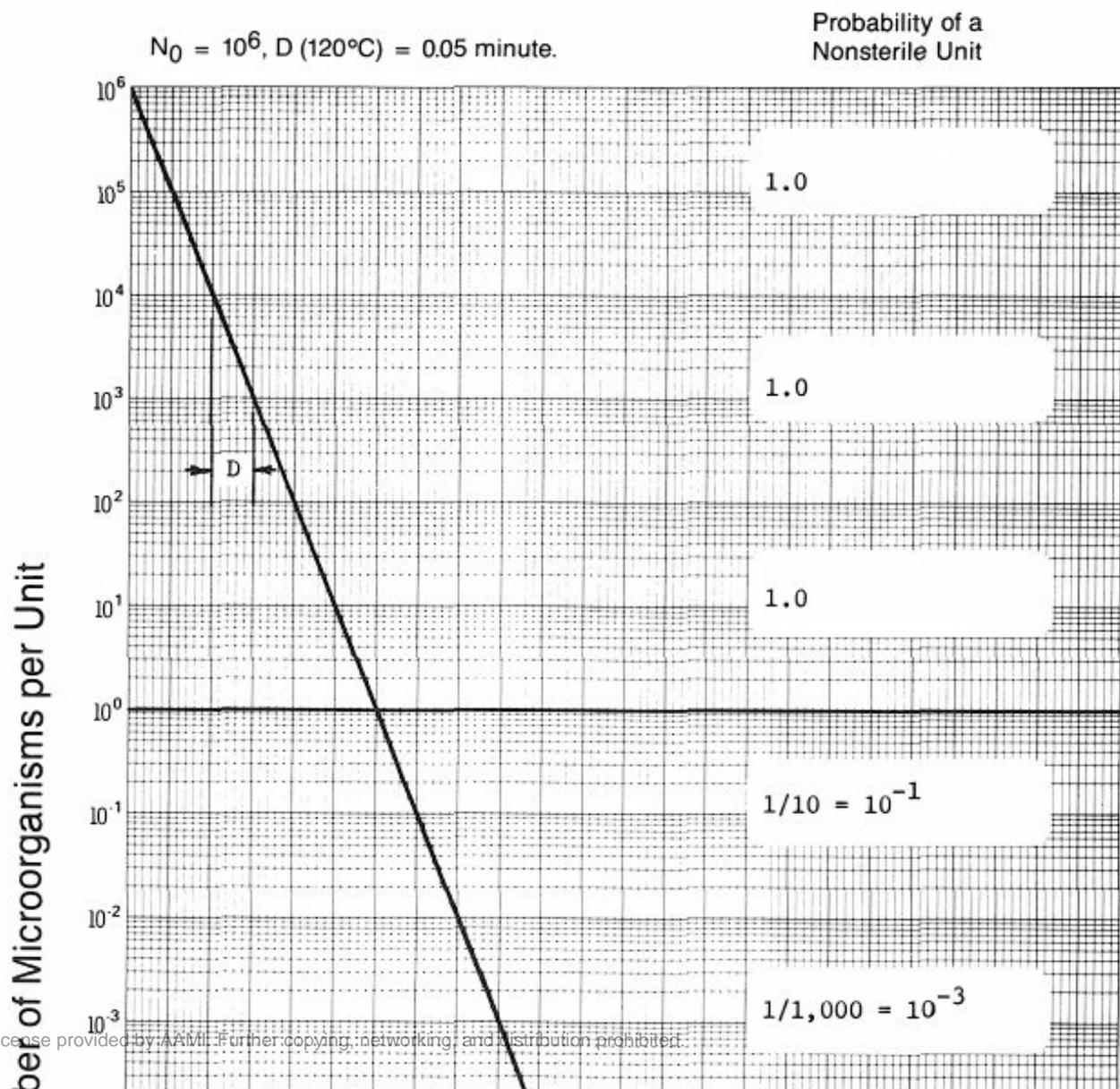
In the graph in Figure 5, we have extended our y axis scale to a survival level of microorganisms per unit of  $10^{-9}$ . Obviously, we cannot have survival of either 0.1 or 0.01 microorganism, even if our model indicates this survival level. (We cannot have a fraction of an organism!) We interpret a calculated survivor level of 0.1 in terms of the survival of whole microorganisms, the survival of one whole microorganism in ten units. A survival level of 0.01 is a survival level of one organism in 100 units; a survival level of 0.001 is the survival

of one organism in 1,000 units. This progression can be extended as shown in Figure 5.

We have reconciled the two divergent conditions by saying that we will accept a product for commercial use and call it "sterile" if the probability of the unit being nonsterile is very low. It is generally agreed in the USA that for pharmaceutical products terminally sterilized as part of the manufacturing process there should be fewer than one nonsterile unit in one million units.

In view of the absolute nature of the definition of "sterile," there is a need for a definitive term to identify the microbial status of a pharmaceutical, drug or food product that has been subjected to a process designed to reduce to a low level the microbial contamination of the product to a low level. The need is for a term that will indicate the probability level of a viable microorganism remaining in a unit of the product, thereby being in agreement with our present thinking regarding microbial destruction. Therefore, we will avoid the conflict of trying to use the absolute term, "sterile," to describe the actual status of viable microorganisms in the product.

A direct approach toward the development of a definitive term is to call it what it is, the "probability of a nonsterile unit (PNSU)." If this terminology is adopted, then "PNSU" could be used to indicate, in abbreviated form, this definition.



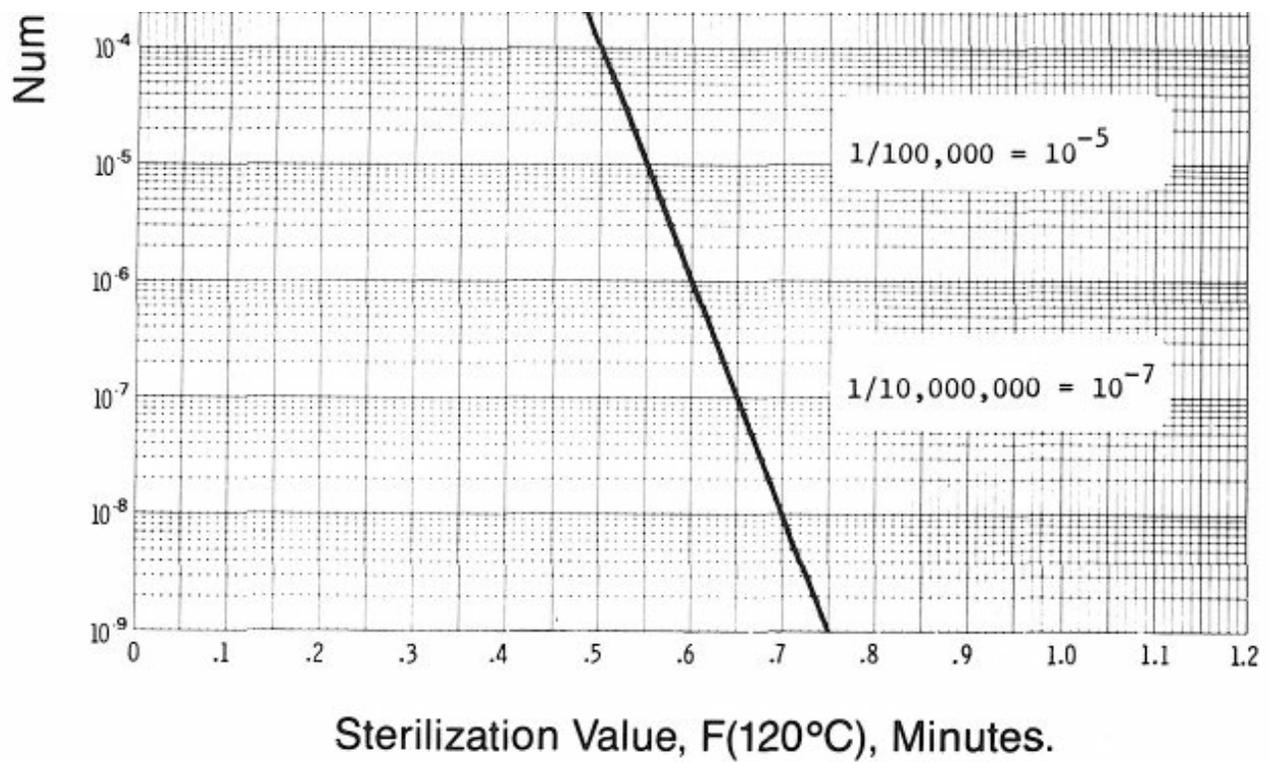
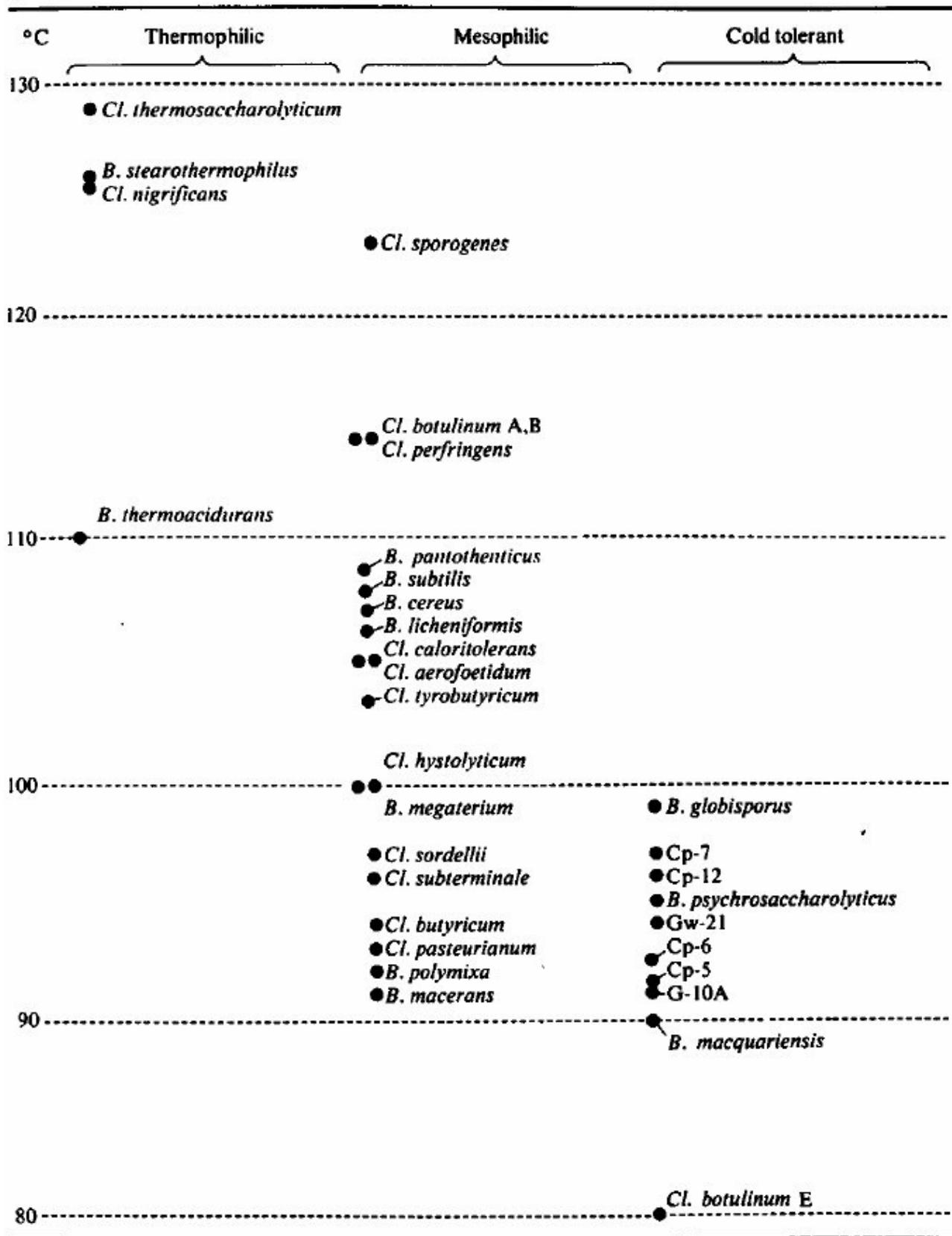


Figure 5. Graph of Sterilization Engineering Design Model Based on a Unit Where  $N_0$  is  $10^6$ ,  $D(120^\circ\text{C})$  is 0.05 Min., and Extended to a Probability of Survival of  $10^{-9}$ .

In our present thinking, most products that incur nonpathogenic contamination should receive a treatment that will reduce the “probability of a nonsterile unit (PNSU)” to less than one in one million, PNSU  $10^{-6}$ . In the food industry, for the pathogen, *Clostridium botulinum*, there should be fewer than one unit containing a *Clostridium botulinum* spore per  $10^9$  units (PNSU  $10^{-9}$ ), and for other mesophilic spore-forming bacteria in sterilized food, a PNSU of  $10^{-6}$ . The use of PNSU is a good approach since it has direct meaning, is easy to read, and is meaningful.

**Table III.**

Approximate heat resistance\* of spores of thermophilic, mesophilic and cold tolerant sporeformers, with D-values expressed as the temperature (in °C) at which D = 1 min



\* The calculated  $D$ -values are based on data of Ingram (1969), Russell (1971) and our own data. When no  $z$ -values were available, we have calculated  $D$ -values with assumed values of  $z = 10^\circ\text{C}$ . For *Cl. thermo-saccharolyticum* we assumed  $z = 15^\circ\text{C}$  and for *B. macquariensis*  $z = 8^\circ\text{C}$ .

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## $D_T$ Value to be Used in Sterilization Process

In the engineering design model, the sterilization value,  $F_T$ , equals  $D_T$  times  $Y_n$ . If we assume that for pharmaceutical products manufactured under good manufacturing practices, the  $Y_n$  will be of the order of 8 (log  $N_0$  will be approximately  $10^2$  and the final sterilization endpoint will be a probability of  $10^{-6}$ ), the  $F_T$ -value will be of the order to  $8D_T$ . If we think about the species or organisms that are going to be a problem in our pharmaceutical products and the care under which the products are manufactured, on a practical basis it is probable that the  $D_{121}^{\circ}\text{C}$ -value will be somewhere between 0.5 and 1.0 minute.

The bacterial spore is the most resistant microorganism that must be killed. In wet-heat sterilization processes, we are normally concerned with the destruction of the mesophilic spore-forming organisms, those organisms that can grow at ambient temperatures. Emphasis is usually placed on the very resistant spore-forming species, but only a few species have  $D_{121.1}^{\circ}\text{C}$ -values of one minutes. Table III taken from the report of Michels and Visser (1976), visually gives an overview of bacterial spore resistance by species. Only one mesophilic species is shown to have a  $D_{120}^{\circ}\text{C}$  of more than one minute.

A  $D_{121.1}^{\circ}\text{C}$ -value of 1.0 minute with a spore log reduction of 8 would mean that our sterilization process at  $121.1^{\circ}\text{C}$  ( $F_0$ ) will be 8 minutes. This value has been recognized by the U.S. FDA in their preliminary guidelines for large-volume parenteral solutions (1976) and in USP XX (1980). This process specification,  $F_0 = 8$  minutes, is commonly referred to as an overkill process. (The  $F_0$  of 8 minutes is measured at the slowest heating point inside the product.)

It is probable that the resistant microorganisms associated with the product will more nearly have D-values of the order to 0.6 or 0.7 minutes, which indicate an overkill  $F_0$ -value of 5 or 6 minutes.

For a more accurate design of the sterilization process, the actual D-value of the critical microflora of the product or of a suitable indicator organism in the product will be used (Pflug, 1982).

## **Discussion of the Difference in Approach to Measurement and Control of Sterile Products Compared to Measurement and Control of General Contamination Problems**

This is a discussion of the differences in the approach to measurement and control of sterile products where the probability of a nonsterile unit should be of the order of  $10^{-6}$  compared with measurement and control of general contamination problems where there will be more than one contaminating entity per unit in the final product. The points in the discussion will be made using the data in Table IV. We will first consider the situation where there is more than one contaminating entity per unit and then discuss the situation where there is fewer than 0.1 contaminating entity per unit.

If the average contamination level of a product is 100 entities per unit (line 1 of Table IV), for practical purposes, every sample selected will be contaminated (line 1, Column B). If we

randomly select one container of this product and assay it, we will find between 80 and 120 contaminating entities, 95 times out of 100 (line 1, Column D). The conclusion we can draw is that when we have a contamination level of 100, whether they be molecules, particles, or other entities, the results of the analysis of a single unit will give a good estimate of the contamination level of all units in a "lot" of product.

**Table IV. Expected Variation in the Amount of Contamination in Units of a Lot<sup>a</sup>.**

	<b>A</b>	<b>B</b>	<b>Probability of finding at least one contaminated unit after analyzing all of the units in a random sample. (Number of units in a sample)</b>			<b>D</b>
	<b>Concentration of contaminating entities per unit</b>	<b>Probability of any one randomly selected unit being contaminated</b>	<b>C<sub>1</sub></b> 3	<b>C<sub>2</sub></b> 20	<b>C<sub>3</sub></b> 100	<b>Interval, with probability at least .95, for the number of observed contamination entities in a unit (the probability is included in the parentheses).<sup>b</sup></b>
<b>1</b>	100	≈ 1	≈ 1	≈ 1	≈ 1	≈ 80 – 120 <sup>c</sup> (≈ .95)
<b>2</b>	1.0	.63	.95	≈ 1	≈ 1	0–3 (.981)
<b>3</b>	0.1	.095	.26	.86	≈ 1	0–1 (.995)
<b>4</b>	0.01	.0099	.030	.18	.63	0 (.990)
<b>5</b>	0.001	.00099	.0030	.020	.095	0 (.999)
<b>6</b>	0.000001(10 <sup>-6</sup> )	≈ 10 <sup>-6</sup>	.30X10 <sup>-5</sup>	.19X10 <sup>-4</sup>	10 <sup>-4</sup>	0 (.999999)

<sup>a</sup>Calculation based on Poisson Model

<sup>b</sup>This is the range in which about 95% of additional plate counts of entities in a unit should fall. The numbers in the parentheses are the actual probabilities.

<sup>c</sup>This interval is an approximation and is the mean plus and minus two standard deviations.

On Line 2 is shown information for an average level of contaminating entities of one per unit. For this condition when a single sample is evaluated, only 63 times out of 100 will contamination be found (Line 2, Column B) and the number of entities found will vary between zero and three 98 times out of 100 (Line 2, Column D). When multiple samples are tested, the probability of having one of the samples show contamination increases: if three samples are tested to 0.95 and if there are 20 or more samples, the probability approaches 1.00 (Line 1, Column C<sub>1</sub>, C<sub>2</sub>, and C<sub>3</sub>).

We will now proceed to examine a sample from a lot of sterile product (Line 6). The probability of a nonsterile unit is 10<sup>-6</sup>; therefore, the probability of any one randomly-selected unit being contaminated will be approximately 10<sup>-6</sup> (Column B). If we sample one unit, we will find zero (Column D). If we carry out a sterility-type test with 20 samples, we will find that we only have .19 × 10<sup>-4</sup> chances of finding a positive and if we use 100 samples in the sterility test, the probability of finding a positive is only one in 10,000

(Column C<sub>3</sub>).

Now let us examine, under Columns C<sub>1</sub>, C<sub>2</sub>, and C<sub>3</sub>, at what concentration of contaminating entities can we expect to usually find evidence of this contamination: If there are three units per sample (Column C<sub>1</sub>), the contaminating entity rate has to be an average of one per unit if we are going to have a positive result 95 times out of 100. If there are 20 units in the test sample, then the contaminating entity level can be 0.1 and 86 times out of 100 a unit will be positive. If the sample size is increased to 100 units, then, if the contaminating entity level is 0.01 per unit, 63 times out of 100 there will be a positive unit in the sample (Column C<sub>3</sub>).

We believe that the data in the chart lead to the following conclusions:

- (1) That using as many as 100 units per sample, we cannot sample product to verify that it is sterile.
- (2) A testing program of 3, 20, or 100 units per sample will only find gross contamination, an average contamination level of 0.01 or one contaminating entity per 100 units.

## Temperature Coefficient Model

An important relationship in the sterilization engineering area is that of the thermal death time (TDT) curve (F vs. T). As has been shown previously, the TDT value is the product of the spore log reduction ( $Y_n$ ) and the D-value ( $F_T = D_T \cdot Y_n$ ).

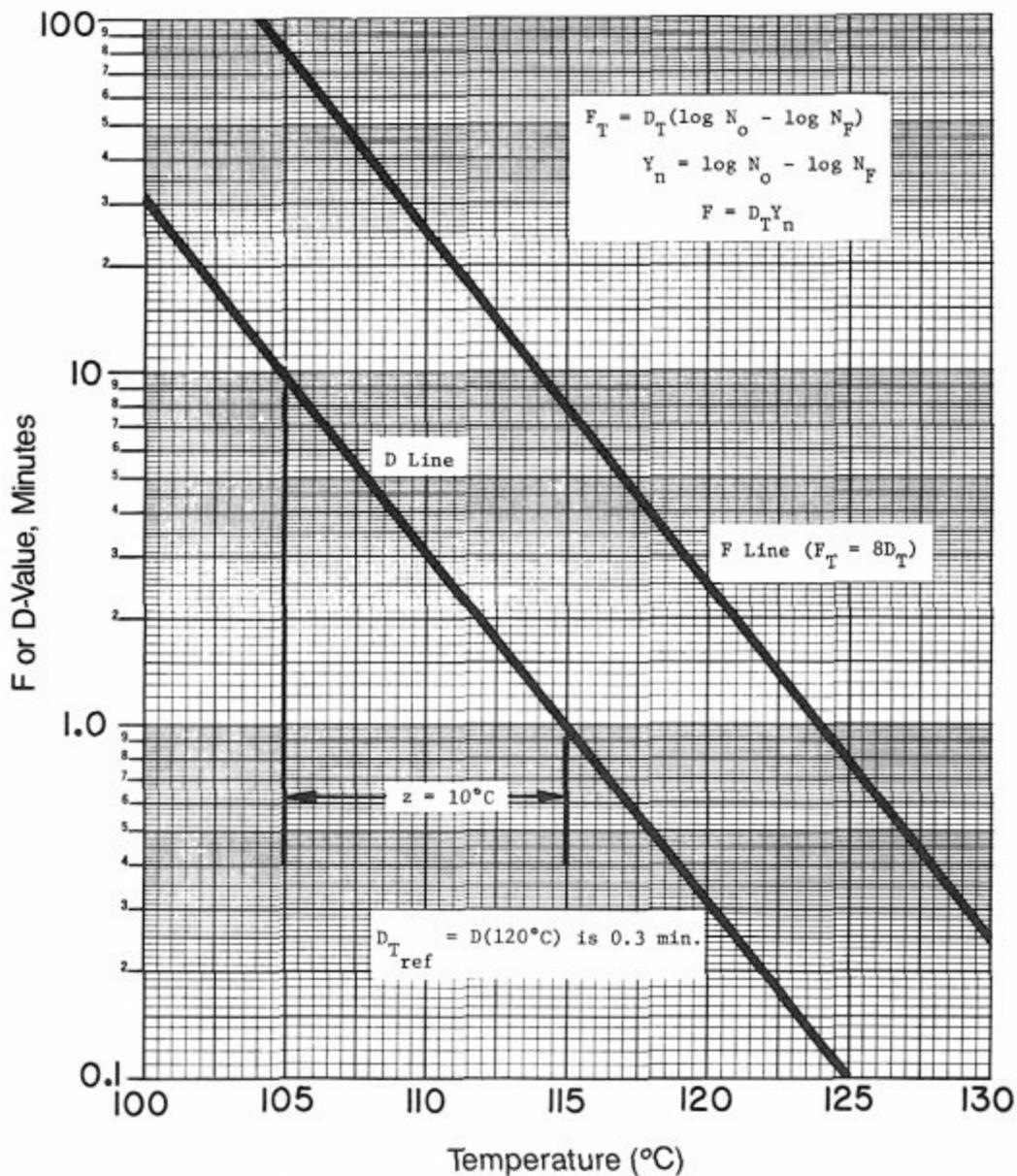


Figure 6. z-Value Graph Showing Both the Thermal Resistance Curve (log D vs. T) and the Thermal Death Time Curve (log F vs. T).

The equation for the TDT curve is:

$$\log F_T = \frac{-1}{z} (T - T_{ref}) + \log F_{T_{ref}}$$

As stated previously, z-value lines of the TDT and thermal resistance (TR) graphs for the same microorganism and substrate are parallel. The  $D(T, z)$  and  $F(T, z)$  are related in the semilogarithmic microbial destruction model. Both a TR curve and a TDT curve are shown in Figure 6.

There are two specific problems in the sterilization process area that are solved using the temperature coefficient model: (1) converting D-values and F-values at one temperature to D- and F-values at another temperature and (2) determining the equivalent sterilization value at the selected reference temperature of the sterilization process. For a temperature coefficient model to be useful, it should aid in solving both of these problems.

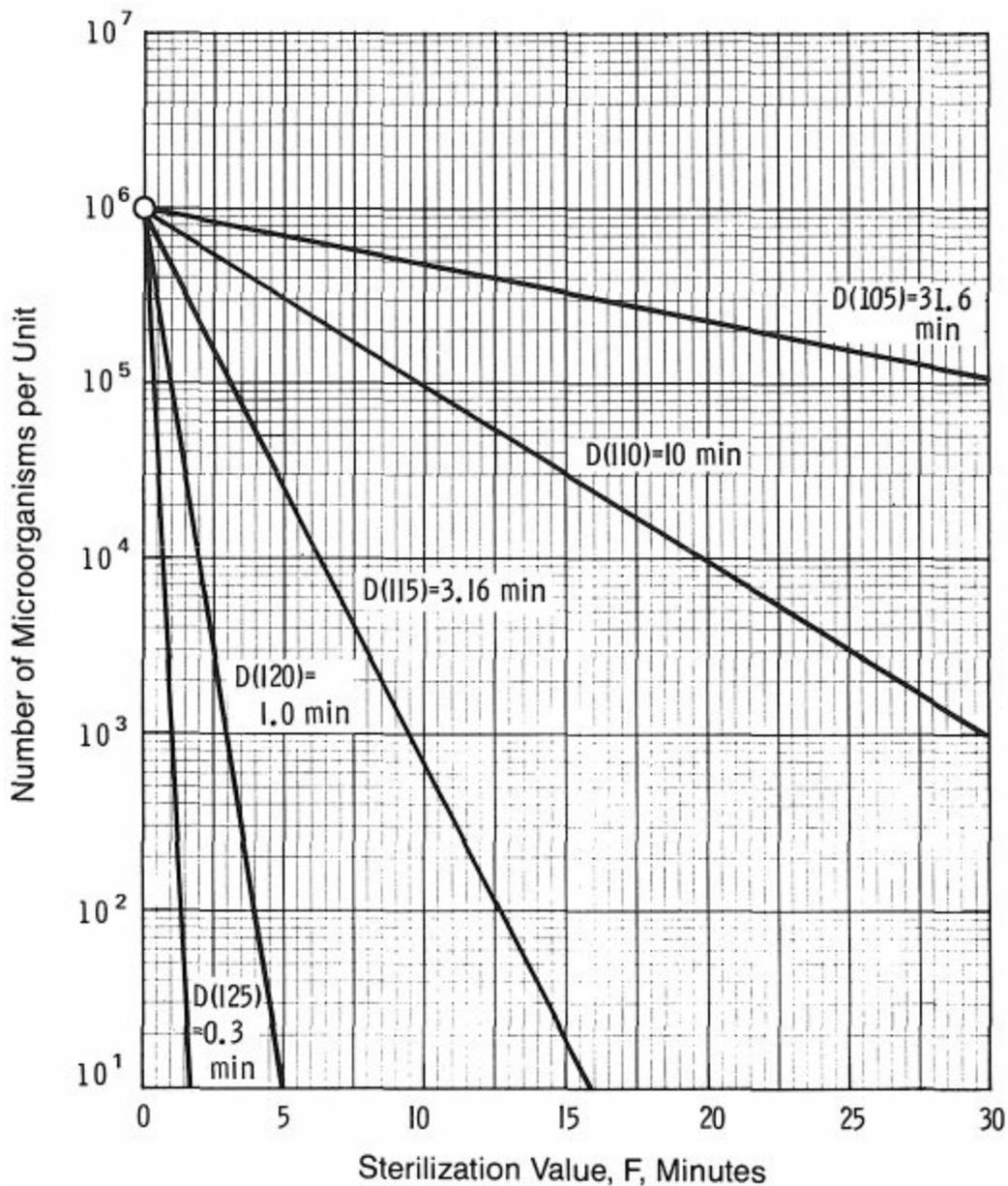


Figure 7. Results (D-Values) of Thermal Destruction Tests of a Single Spore Inoculum at Five Temperatures.

### Z-Value Graph from D-Values

The general acceptance of the D-value as a measure of the microbial destruction rate in the 1950's led to the method in use today for preparing the z-value graph; in this method the logarithm of the D-value is plotted vs. temperature. The graph of log D vs. temperature is called a thermal resistance (TR) curve.

In Figure 7 are shown survivor curves at several temperatures; the D-values are indicated on the graph. To prepare the z-value graph in Figure 8, the D-values were plotted on semilogarithmic paper vs. temperature. Conventionally, a straight line is drawn through the data points and the z-value is the degrees of temperature for the D-value to change by a factor of ten. A more accurate estimate of the z-value is obtained using a simple, least squares regression line fit to the data, log D vs. temperature. The z-value estimate is the

negative reciprocal of the slope of the fitted line. The units of the z-value are degrees of temperature; it is important to always include the temperature scale identification as °C or °F when using or reporting a z-value.

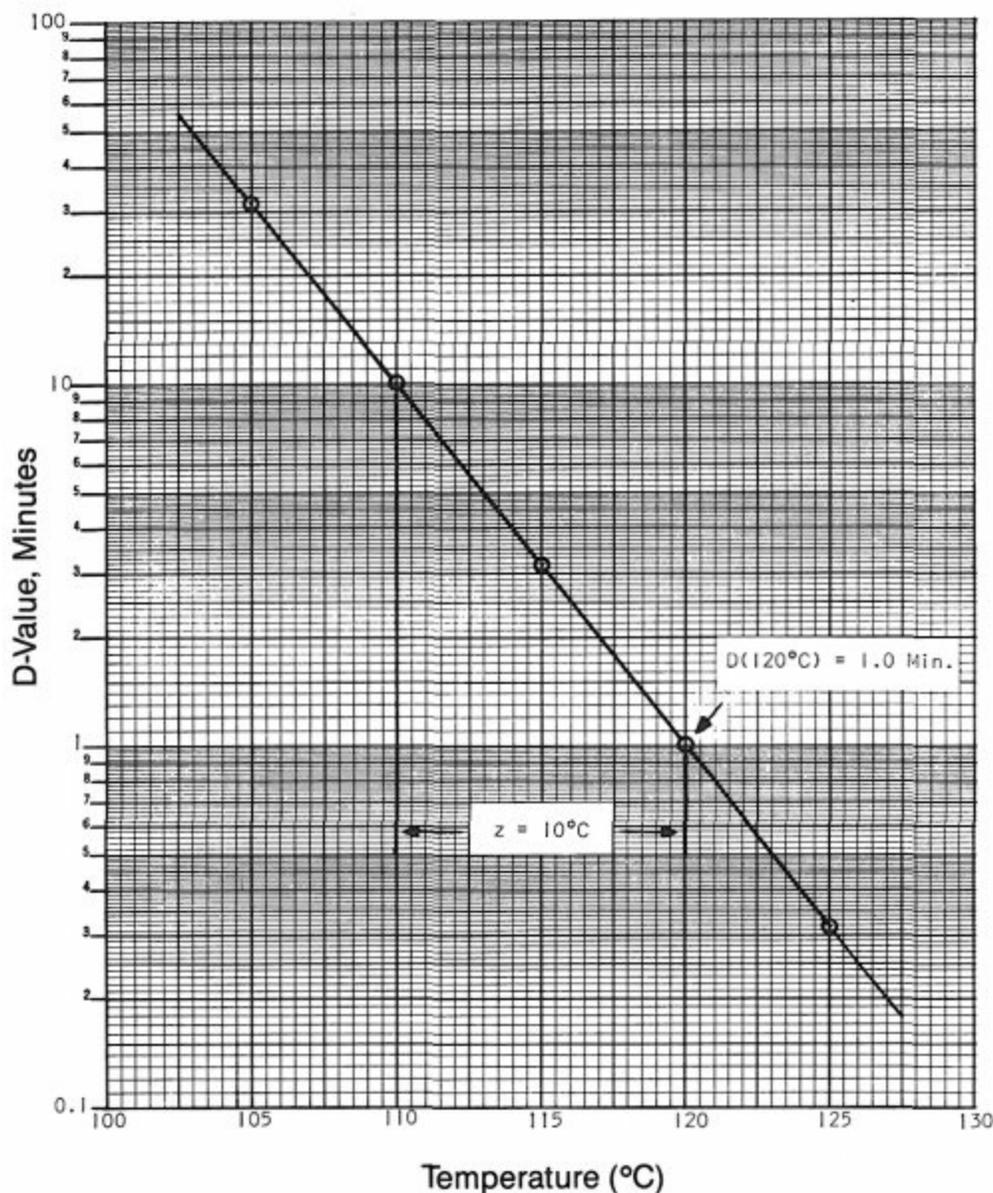


Figure 8. D(T)-Values of the Five Survivor Curves in Figure 7 Plotted to form a Thermal Resistance Curve.

Since the z-value is always the result of a series of experiments at a minimum of two temperatures and more desirably three or four temperatures, the experimenter must validate the test system to ensure that the D-value data generated in the experiments at the several temperatures can be combined. If the data at the several temperatures are for different test conditions, they cannot be combined to yield a meaningful z-value.

The Bigelow or z-value model is the temperature coefficient model that is used worldwide in designing and monitoring food and pharmaceutical sterilization processes. The method is simple and straightforward. It is accurate as any other method available today, even though it is empirical in nature.

Bigelow (1921) reported that the method is based on the fact that if the logarithms of the destruction times (F- or D-value) are plotted vs. temperature on an arithmetic scale, the

result over the usual range of temperatures of interest can be represented by a straight line. The lack of a theory as to why thermal destruction data should form straight lines when plotted in this manner has caused many researchers to search for a theory that could be used to reinforce this method of analysis or to locate or develop another method of analysis that was based on a temperature coefficient theory.

Originally, the numbers in the Bigelow model were established by endpoint methods applied to replicate units of product. Today, D-values at several temperatures are the inputs.

## **Conclusions and Recommendations Regarding a Temperature Coefficient Model**

In an attempt to reach a decision regarding which temperature coefficient model should be used in the sterilization area we should, perhaps, ponder for a moment the end results of our deliberations. Our objective is an analytical system that can be used in the laboratory and in the manufacturing plant for the design, validation, and monitoring of sterilization processes. Its major use will be as a tool in the field to help us have better sterilization processes so we have improved products. Accuracy of the method is of first importance, however, the ability of the user to understand and use the method is of equal importance. If the users have a feel for the method, if they understand how the method works, they are more likely to accept the method and then I believe it will be used more accurately and more efficiently!

On the basis of accuracy, I believe that the Bigelow model and Arrhenius model used in the chemical kinetics area are essentially equal. I believe the ultimate consideration is for a model to sufficiently describe the effect of temperature on D (or k). Regarding Bigelow vs. Arrhenius (which some also consider empirical), for a temperature range of 30°C there is negligible difference between the two models, especially when one considers the variability of D (or k). I cannot think of an applied sterilization situation where accuracy of a model over more than 30°C is necessary. The question can be raised regarding extrapolation from low to ultra high temperature (UHT). Large differences may result in extrapolation from low lethal temperatures to high temperatures as for UHT processes, depending on the model used but also on the z-value used. Regardless of the model, extrapolation should be avoided if possible, and where necessary, safety factors added to insure a safe product.

In the use of the Bigelow model as a sterilization engineering model a z-value of 10°C or 18°F is used. We can say that the sterilization engineering z-value is 10°C (18°F) and the TR curve is taken to be a straight line. Experimental TR curves for several species of bacilli have been found to be curves, the z-value decreasing the increasing temperature.

## **The “General Method” of Calculating the Sterilization Value (F) of a Heat Sterilization Process**

The sterilization value (F) of a heat process is, by custom, the equivalent time at a base or reference temperature. In the USA, it is usually equivalent minutes at 250°F. If a z-value of 18°F is used, the sterilization value at 250°F is called the F-value. The subscript (0) indicates that the temperature is 250°F and the z-value is 18°F. Since the scientific community throughout the world is committed to the metric system, the development and some of the examples in this section are carried out using all metric units. In parts of this section, we are using as our reference base the temperature of 120°C and identifying the sterilization value for this reference base as  $F_c$ . (When unspecified,  $z = 10^\circ\text{C}$ .)

During the last fifty years, many scientists have worked to develop improved methods of calculating the lethality of heat processes. Methods developed include: The “General Method” graphically (Bigelow et al., 1920) and numerically (Patashnik, 1953); “Formula Methods” (Ball, 1923 and 1928, and Ball Olson, 1957; “Nomogram Method” (Olson and Stevens, 1939); and “Computer Method” (Sasseen, 1969). The “General Method,” in its original graphical-use form, was laborious; hence, the development of other methods. In these developments, accuracy of the method and ease and efficiency in application have been the objectives and it was usually not possible to satisfy both requirements. The advent of the digital computer makes possible wide use of the “General Method.”

## Development of the Lethal Rate (Lethal Ratio) Concept

Determining the sterilization value of a heat process in terms of the equivalent time at a reference temperature, for example, 120°C, means adding up the sterilization value at each temperature. The use of the lethal rate (or lethal ratio) concept makes it possible to do this in a direct way.

The summing up of the lethal effects at different temperatures requires a temperature coefficient model. We will use the model of Bigelow et al. (1920). It is first shown in general equation form,

$$\log F(T) = - 1/z (T - T_{\text{ref}}) + \log F(T_{\text{ref}}),$$

and then in a more useful arrangement,

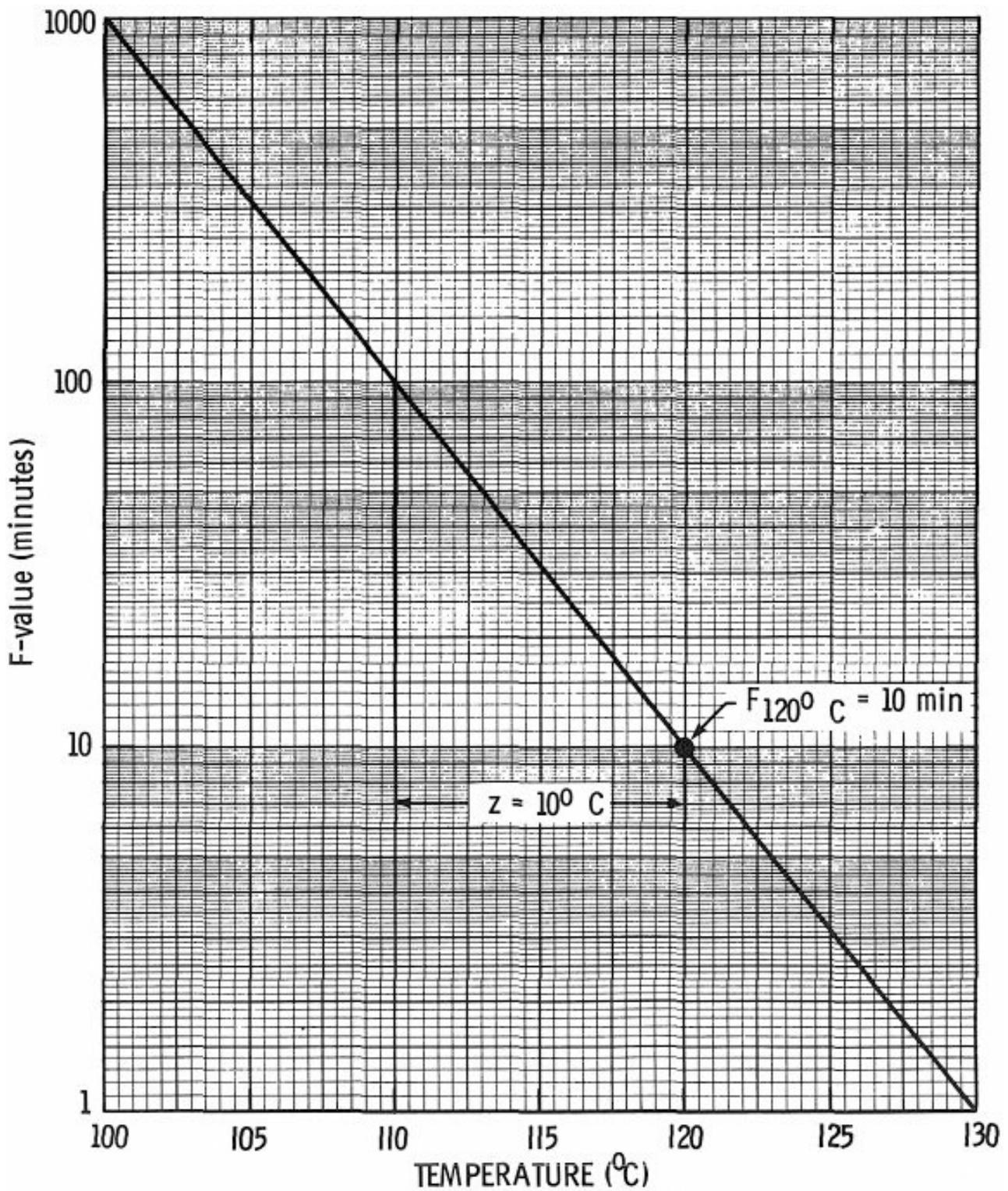


Figure 9. Thermal Death Time Curve with an  $F_{120^{\circ}\text{C}}$  of ten Minutes and a z-Value of  $10^{\circ}\text{C}$ .

$$F(T)/F(T_{\text{ref}}) = 10^{(T_{\text{ref}} - T)/z}$$

The Bigelow model is shown in graphical form for an  $F(120^{\circ}\text{C})$  reference value of ten minutes and a z-value of  $10^{\circ}\text{C}$  in Figure 9. For practical purposes, we assume that the TDT curve is a straight line when  $\log F$  is plotted vs. temperature. The line is established by specifying the slope and a point on the line. The z-value is used as the slope function,  $z = -1/\text{slope}$ , and an F-value at a reference temperature,  $F(T_{\text{ref}})$ , as the point on the line.

Sterilization processes are based on the “kill time” at a specified reference temperature ( $T_{ref}$ ). The “kill time” at the reference temperature is  $F(T_{ref})$ .

**Table V. Kill Times Relative to 120°C, and Lethal Rates Relative to 120°C for the z-Value Curve Data in Figure 9,  $F(120^\circ\text{C})$  of Ten Minutes and a z-Value of 10°C.**

(1) $T, ^\circ\text{C}$	(2) (Kill Time) $F_T$ , Min.	(3) (Relative Kill Time) $F_T/F_{120}$	(4) (Lethal Rate) $F_{120}/F_T$
100	1,000	100	0.01
110	100	10	0.1
116	25.1	2.51	0.398
120	10	1.0	1.0
130	1.0	0.1	10.0

The graph in Figure 9 relates the sterilizing value,  $F$ , with temperature. By definition, the time-temperature conditions of all points on the line produce the same microbial kill, although the  $F$ -value will vary widely with temperature as shown in Column 1 of Table V. All points on the line are equivalent to an  $F_c$ -value ( $T = 120^\circ\text{C}$ ,  $z = 10^\circ\text{C}$ ) of ten minutes. All points on the line are therefore  $F$  minutes at  $T^\circ\text{C}$ , equivalent to an  $F$ -value of ten minutes at  $120^\circ\text{C}$ .

The next part of this development will be carried out on a general basis with symbols rather than with actual temperature conditions.

The z-value graph relates time and temperature for an equal microbial kill.

The “kill time” (Column 2, Table V) is  $F(T)$ ; units are “minutes at  $T$ .”

In Column 3 of Table V are listed the relative kill times corresponding to the temperatures in Column 1. These are determined by dividing the kill times at test temperature,  $F(T)$  (in Column 2), by the kill time at the reference temperature,  $F(T_{ref})$ .

The kill time,  $F(T)$ , at temperature  $T$  is determined from the z-value graph. Next, we determine the relative kill time:

$$\frac{F(T)}{F(T_{ref})}, \text{ stated in units, } \frac{\text{minutes at } T}{\text{minutes at } T_{ref}}.$$

The values of relative kill time are a function only of the z-value of the TDT ( $F$ -value). Curves that have the same z-value will have the same relative kill time values.

The procedure of going from “kill time” to “relative kill time” has the effect of shifting the z-value curve to where it passes through the point,  $F(T_{ref}) = 1.0$ .

The units of relative kill time are (minutes at T)/(minutes at  $F_{ref}$ ). We can observe at this point that the reciprocal of the relative kill time will have units of (minutes at  $T_{ref}$ )/(minutes at T); this is a rate expressed in terms of the chosen reference temperature.

$$\text{Relative kill time is } \frac{F(T)}{F(T_{ref})}$$

The reciprocal of the relative kill time is the rate of microbial kill at any temperature (T) expressed in terms of  $T_{ref}$ .

$$\frac{F(T_{ref}), \text{ min at } T_{ref}}{F(T), \text{ min at } T}$$

The reciprocal of the relative kill time, which is the rate that is used in all sterilization process calculations, is called the lethal rate and is identified by the symbol, "L."

$$\text{The rate of microbial kill is } \frac{F(T_{ref})}{F(T)}$$

and is called the lethal rate (L).

The z-value equation is:

$$\frac{F(T_{ref})}{F(T)} = 10^{(T - T_{ref})/Z}$$

Hence, the lethal rate, L, is  $10^{(T - T_{ref})/Z}$

The lethal rate is unique in that although developed using F-values, the lethal rate is a function only of (1) the difference between the product temperature (T) and the reference temperature ( $T_{ref}$ ) and (2) the z-value. The product of the lethal rate and the time at temperature T is the kill time (equivalent minutes) at the reference temperature.

The kill time at the reference temperature is obtained by multiplying the reference temperature-based rate by the effective time.

$$\frac{F(T_{ref})}{F(T)} \times \Delta t = F(T_{ref})$$

$$\frac{\text{min at } T_{ref}}{\text{min at } T} \times \text{min at } T = \text{min at } T_{ref}$$

We have now shown in a general way how we can calculate the kill time at the reference temperature that is equivalent to process times at other temperatures. We will now go from the general solution to a solution using specific reference temperatures and z-values. The generally agreed-on reference temperature for the English system of units is 250°F,  $z = 18^\circ\text{F}$  and for the metric system of units, 120°C and 10°C.

We can now insert reference temperatures and z-values into our general equations.

*Metric System of Units*

$$T_{ref} = 120^\circ\text{C}, z = 10^\circ\text{C}$$

$$\text{Therefore, } F(T_{ref}) = F_c$$

$$\frac{F(120^{\circ}\text{C})}{F(T)} = \frac{F_c}{F(T)} = 10^{(T - 120^{\circ}\text{C})/10^{\circ}\text{C}}$$

Lethal rate,  $L$ , =  $10^{(T - 120^{\circ}\text{C})/10^{\circ}\text{C}}$ .

*English System of Units*

$T_{\text{ref}} = 250^{\circ}\text{F}$ ,  $z = 18^{\circ}\text{F}$ .

Therefore,  $F(T_{\text{ref}}) = F_c$ .

$$\frac{F(250^{\circ}\text{F})}{F(T)} = \frac{F_o}{F(T)} = 10^{(T - 250^{\circ}\text{F})/18^{\circ}\text{F}}$$

Lethal rate,  $L$ , =  $10^{(T - 250^{\circ}\text{F})/18^{\circ}\text{F}}$ .

Since lethal rates for any specific reference temperature ( $T_{\text{ref}}$ ) are a function only of the z-value and the product temperature, tables of lethal rates for a specific z-value for a range of product temperatures can be prepared. Three lethal rate tables are included: For the English system, a lethal rate table with a reference temperature of  $250^{\circ}\text{F}$  and a z-value of  $18^{\circ}\text{F}$  is shown in Table VI; for metric system use, a table of lethal rates with a reference temperature of  $121.11^{\circ}\text{C}$  with a z-value of  $10^{\circ}\text{C}$  is shown in Table VII; and for a reference temperature of  $120^{\circ}\text{C}$ , a table of lethal rates with a z-value of  $10^{\circ}\text{C}$  is shown in Table VIII.

In using the General method, we are interested in adding the overall effect where the product is at more than one temperature during the heat process. The sterilization effect at several temperatures can be summed up through appropriate procedures.

To calculate the  $F_o$ -value that results when a product is at  $T_1^{\circ}\text{C}$  for  $t_1$  min +  $T_2^{\circ}\text{C}$  for  $t_2$  min and  $T_3^{\circ}\text{C}$  for  $t_3$  min:

$$[L(T_1) \times t_1] + [L(T_2) \times t_2] + [L(T_3) \times t_3] = F_o$$

Ball and Olson (1957), on pp. 184-189, verified analytically the additive properties of partial sterility conditions at different temperatures. The attendees are directed to this reference for a more complete treatment of this subject.

## Determining the F-Value of a Sterilization Process

Time-temperature data similar to that shown in Column 1 and 2 of Table IX must be available as a prerequisite to the analytical evaluation of a heat sterilization process. Once these data are available, the F-value of the sterilization process can be determined regardless of the heating pattern of the container.

Conventionally, we are interested in the sterilization value at the slowest heating zone of the container, i.e., that zone in the container that receives the smallest F-value. (The slowest heating zone must be located before the time-temperature data gathering process can begin.)

Temperatures must be measured at small time intervals so that a clear picture of the heating and cooling pattern is available. The frequency at which temperature measurements are made during a test to gather time-temperature data depends on the rate of the heating

of the product in the container, which, in turn, is a function of container size and product physical properties, most importantly the viscosity of the product in the container. The optimum temperature measurement interval will vary with the heating rate and should be selected so to describe the heating pattern. Probably 20 data points are the minimum necessary to adequately describe the heating and cooling pattern and to calculate the F-value of the sterilization process.

**Table VI. A Table of Lethal Rates (L) for a Reference Temperature of 250°F and a z-Value of 18°F. L=Minutes at 250°F per Minute at 7°F.  $L=10^{(T - 121.11)/10}$  (Temperature T is in °F).**

TEMP. DEG F	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
200	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
201	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
202	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
203	0.002	0.002	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
204	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
205	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.004	0.004
206	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
207	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.005	0.005
208	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
209	0.005	0.005	0.005	0.005	0.006	0.006	0.006	0.006	0.006	0.006
210	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.007	0.007	0.007
211	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.008	0.008
212	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.009	0.009
213	0.009	0.009	0.009	0.009	0.009	0.009	0.010	0.010	0.010	0.010
214	0.010	0.010	0.010	0.010	0.011	0.011	0.011	0.011	0.011	0.011
215	0.011	0.012	0.012	0.012	0.012	0.012	0.012	0.012	0.013	0.013
216	0.013	0.013	0.013	0.013	0.014	0.014	0.014	0.014	0.014	0.014
217	0.015	0.015	0.015	0.015	0.015	0.016	0.016	0.016	0.016	0.016
218	0.017	0.017	0.017	0.017	0.018	0.018	0.018	0.018	0.018	0.019
219	0.019	0.019	0.019	0.020	0.020	0.020	0.020	0.021	0.021	0.021
220	0.022	0.022	0.022	0.022	0.023	0.023	0.023	0.024	0.024	0.024
221	0.024	0.025	0.025	0.025	0.026	0.026	0.026	0.027	0.027	0.027
222	0.028	0.028	0.029	0.029	0.029	0.030	0.030	0.030	0.031	0.031
223	0.032	0.032	0.032	0.033	0.033	0.034	0.034	0.035	0.035	0.035
224	0.036	0.036	0.037	0.037	0.038	0.038	0.039	0.039	0.040	0.040
225	0.041	0.041	0.042	0.042	0.043	0.044	0.044	0.045	0.045	0.046
226	0.046	0.047	0.048	0.048	0.049	0.049	0.050	0.051	0.051	0.052
227	0.053	0.053	0.054	0.055	0.056	0.056	0.057	0.058	0.058	0.059
228	0.060	0.061	0.062	0.062	0.063	0.064	0.065	0.066	0.066	0.067
229	0.068	0.069	0.070	0.071	0.072	0.073	0.074	0.075	0.075	0.076
230	0.077	0.078	0.079	0.080	0.081	0.083	0.084	0.085	0.086	0.087
231	0.088	0.089	0.090	0.091	0.093	0.094	0.095	0.096	0.097	0.099
232	0.100	0.101	0.103	0.104	0.105	0.107	0.108	0.109	0.111	0.112
233	0.114	0.115	0.117	0.118	0.120	0.121	0.123	0.124	0.126	0.128
234	0.129	0.131	0.133	0.134	0.136	0.138	0.139	0.141	0.143	0.145
235	0.147	0.149	0.151	0.153	0.154	0.156	0.158	0.161	0.163	0.165
236	0.167	0.169	0.171	0.173	0.176	0.178	0.180	0.182	0.185	0.187
237	0.190	0.192	0.194	0.197	0.200	0.202	0.205	0.207	0.210	0.213
238	0.215	0.218	0.221	0.224	0.227	0.230	0.233	0.236	0.239	0.242
239	0.245	0.248	0.251	0.254	0.258	0.261	0.264	0.268	0.271	0.275
240	0.278	0.282	0.285	0.289	0.293	0.297	0.300	0.304	0.308	0.312
241	0.316	0.320	0.324	0.329	0.333	0.337	0.341	0.346	0.350	0.355
242	0.359	0.364	0.369	0.373	0.378	0.383	0.388	0.393	0.398	0.403
243	0.408	0.414	0.419	0.424	0.430	0.435	0.441	0.447	0.452	0.458
244	0.464	0.470	0.476	0.482	0.489	0.495	0.501	0.508	0.514	0.521

245	0.527	0.534	0.541	0.548	0.555	0.562	0.570	0.577	0.584	0.592
246	0.599	0.607	0.615	0.623	0.631	0.639	0.647	0.656	0.664	0.673
247	0.681	0.690	0.699	0.708	0.717	0.726	0.736	0.745	0.755	0.764
248	0.774	0.784	0.794	0.805	0.815	0.825	0.836	0.847	0.858	0.869
249	0.880	0.891	0.903	0.914	0.926	0.938	0.950	0.962	0.975	0.987
250	1.000	1.013	1.026	1.039	1.053	1.066	1.080	1.094	1.108	1.122
251	1.136	1.151	1.166	1.181	1.196	1.212	1.227	1.243	1.259	1.275
252	1.292	1.308	1.325	1.342	1.359	1.377	1.395	1.413	1.431	1.449
253	1.468	1.487	1.506	1.525	1.545	1.565	1.585	1.605	1.626	1.647
254	1.668	1.690	1.711	1.733	1.756	1.778	1.801	1.824	1.848	1.872
255	1.896	1.920	1.945	1.970	1.995	2.021	2.047	2.073	2.100	2.127
256	2.154	2.182	2.210	2.239	2.268	2.297	2.326	2.356	2.387	2.417
257	2.448	2.480	2.512	2.544	2.577	2.610	2.644	2.678	2.712	2.747
258	2.783	2.818	2.855	2.891	2.929	2.966	3.005	3.043	3.082	3.122
259	3.162	3.203	3.244	3.286	3.328	3.371	3.415	3.459	3.503	3.548
260	3.594	3.640	3.687	3.734	3.782	3.831	3.881	3.930	3.981	4.032
261	4.084	4.137	4.190	4.244	4.299	4.354	4.410	4.467	4.524	4.583
262	4.642	4.701	4.762	4.823	4.885	4.948	5.012	5.076	5.142	5.208
263	5.275	5.343	5.412	5.481	5.552	5.623	5.696	5.769	5.843	5.919
264	5.995	6.072	6.150	6.229	6.310	6.391	6.473	6.556	6.641	6.726
265	6.813	6.901	6.989	7.079	7.171	7.263	7.356	7.451	7.547	7.644
266	7.743	7.842	7.943	8.046	8.149	8.254	8.360	8.468	8.577	8.687
267	8.799	8.913	9.027	9.143	9.261	9.380	9.501	9.624	9.747	9.873
268	10.000	10.129	10.259	10.391	10.525	10.661	10.798	10.937	11.078	11.220
269	11.365	11.511	11.659	11.809	11.961	12.115	12.271	12.429	12.589	12.751
270	12.915	13.082	13.250	13.421	13.594	13.769	13.946	14.125	14.307	14.491

**Table VII. A Table of Lethal Rates (L) for a Reference Temperature of 121.11 °C and a z-Value of 10°C. L=Minutes at 121.11°C per Minute at T°C.  $L = 10^{(T - 121.11)/10}$  (Temperature T is in °C).**

TEMP. DEG C	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
90	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
91	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
92	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002
93	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
94	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
95	0.002	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
96	0.003	0.003	0.003	0.003	0.003	0.003	0.004	0.004	0.004	0.004
97	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.005	0.005	0.005
98	0.005	0.005	0.005	0.005	0.005	0.005	0.006	0.006	0.006	0.006
99	0.006	0.006	0.006	0.007	0.007	0.007	0.007	0.007	0.007	0.008
100	0.008	0.008	0.008	0.008	0.008	0.009	0.009	0.009	0.009	0.010
101	0.010	0.010	0.010	0.010	0.011	0.011	0.011	0.011	0.012	0.012
102	0.012	0.013	0.013	0.013	0.013	0.014	0.014	0.014	0.015	0.015
103	0.015	0.016	0.016	0.017	0.017	0.017	0.018	0.018	0.019	0.019
104	0.019	0.020	0.020	0.021	0.021	0.022	0.022	0.023	0.023	0.024
105	0.024	0.025	0.026	0.026	0.027	0.027	0.028	0.029	0.029	0.030
106	0.031	0.032	0.032	0.033	0.034	0.035	0.035	0.036	0.037	0.038
107	0.039	0.040	0.041	0.042	0.043	0.044	0.045	0.046	0.047	0.048
108	0.049	0.050	0.051	0.052	0.054	0.055	0.056	0.057	0.059	0.060
109	0.062	0.063	0.064	0.066	0.067	0.069	0.071	0.072	0.074	0.076
110	0.077	0.079	0.081	0.083	0.085	0.087	0.089	0.091	0.093	0.095
111	0.097	0.100	0.102	0.104	0.107	0.109	0.112	0.115	0.117	0.120
112	0.123	0.126	0.129	0.132	0.135	0.138	0.141	0.144	0.148	0.151
113	0.155	0.158	0.162	0.166	0.169	0.173	0.177	0.182	0.186	0.190
114	0.195	0.199	0.204	0.208	0.213	0.218	0.223	0.229	0.234	0.239
115	0.245	0.251	0.256	0.262	0.269	0.275	0.281	0.288	0.294	0.301
116	0.308	0.316	0.323	0.330	0.338	0.346	0.354	0.362	0.371	0.379
117	0.388	0.397	0.406	0.416	0.426	0.436	0.446	0.456	0.467	0.478
118	0.489	0.500	0.512	0.524	0.536	0.548	0.561	0.574	0.587	0.601
119	0.615	0.630	0.644	0.659	0.675	0.690	0.706	0.723	0.740	0.757
120	0.774	0.793	0.811	0.830	0.849	0.869	0.889	0.910	0.931	0.953
121	0.975	0.998	1.021	1.045	1.069	1.094	1.119	1.146	1.172	1.199
122	1.227	1.256	1.285	1.315	1.346	1.377	1.409	1.442	1.476	1.510
123	1.545	1.581	1.618	1.656	1.694	1.734	1.774	1.816	1.858	1.901
124	1.945	1.991	2.037	2.084	2.133	2.183	2.234	2.286	2.339	2.393
125	2.449	2.506	2.564	2.624	2.685	2.748	2.812	2.877	2.944	3.013
126	3.083	3.155	3.228	3.304	3.381	3.459	3.540	3.622	3.707	3.793
127	3.882	3.972	4.064	4.159	4.256	4.355	4.457	4.560	4.667	4.775
128	4.887	5.000	5.117	5.236	5.358	5.483	5.610	5.741	5.875	6.012
129	6.152	6.295	6.442	6.592	6.745	6.902	7.063	7.228	7.396	7.568
130	7.745	7.925	8.110	8.299	8.492	8.690	8.892	9.099	9.311	9.528

**Table VIII. A Table of Lethal Rates (L) for a Reference Temperature of 120°C and a z-Value of 10°C. L=Minutes at 120°C per Minute at T°C.  $L=10^{(T - 120)/10}$  (Temperature is in °C)**

TEMP. DEG	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
C										
90	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
91	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.002
92	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
93	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
94	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
95	0.003	0.003	0.003	0.003	0.003	0.004	0.004	0.004	0.004	0.004
96	0.004	0.004	0.004	0.004	0.004	0.004	0.005	0.005	0.005	0.005
97	0.005	0.005	0.005	0.005	0.005	0.006	0.006	0.006	0.006	0.006
98	0.006	0.006	0.007	0.007	0.007	0.007	0.007	0.007	0.008	0.008
99	0.008	0.008	0.008	0.009	0.009	0.009	0.009	0.009	0.010	0.010
100	0.010	0.010	0.010	0.011	0.011	0.011	0.011	0.012	0.012	0.012
101	0.013	0.013	0.013	0.013	0.014	0.014	0.014	0.015	0.015	0.015
102	0.016	0.016	0.017	0.017	0.017	0.018	0.018	0.019	0.019	0.019
103	0.020	0.020	0.021	0.021	0.022	0.022	0.023	0.023	0.024	0.025
104	0.025	0.026	0.026	0.027	0.028	0.028	0.029	0.030	0.030	0.031
105	0.032	0.032	0.033	0.034	0.035	0.035	0.036	0.037	0.038	0.039
106	0.040	0.041	0.042	0.043	0.044	0.045	0.046	0.047	0.048	0.049
107	0.050	0.051	0.052	0.054	0.055	0.056	0.058	0.059	0.060	0.062
108	0.063	0.065	0.066	0.068	0.069	0.071	0.072	0.074	0.076	0.078
109	0.079	0.081	0.083	0.085	0.087	0.089	0.091	0.093	0.095	0.098
110	0.100	0.102	0.105	0.107	0.110	0.112	0.115	0.117	0.120	0.123
111	0.126	0.129	0.132	0.135	0.138	0.141	0.145	0.148	0.151	0.155
112	0.158	0.162	0.166	0.170	0.174	0.178	0.182	0.186	0.191	0.195
113	0.200	0.204	0.209	0.214	0.219	0.224	0.229	0.234	0.240	0.245
114	0.251	0.257	0.263	0.269	0.275	0.282	0.288	0.295	0.302	0.309
115	0.316	0.324	0.331	0.339	0.347	0.355	0.363	0.372	0.380	0.389
116	0.398	0.407	0.417	0.427	0.437	0.447	0.457	0.468	0.479	0.490
117	0.501	0.513	0.525	0.537	0.550	0.562	0.575	0.589	0.603	0.617
118	0.631	0.646	0.661	0.676	0.692	0.708	0.724	0.741	0.759	0.776
119	0.794	0.813	0.832	0.851	0.871	0.891	0.912	0.933	0.955	0.977
120	1.000	1.023	1.047	1.072	1.096	1.122	1.148	1.175	1.202	1.230
121	1.259	1.288	1.318	1.349	1.380	1.413	1.445	1.479	1.514	1.549
122	1.585	1.622	1.660	1.698	1.738	1.778	1.820	1.862	1.905	1.950
123	1.995	2.042	2.089	2.138	2.188	2.239	2.291	2.344	2.399	2.455
124	2.512	2.570	2.630	2.692	2.754	2.818	2.884	2.951	3.020	3.090
125	3.162	3.236	3.311	3.388	3.467	3.548	3.631	3.715	3.802	3.890
126	3.981	4.074	4.169	4.266	4.365	4.467	4.571	4.677	4.786	4.898
127	5.012	5.129	5.248	5.370	5.495	5.623	5.754	5.888	6.026	6.166
128	6.310	6.457	6.607	6.761	6.918	7.079	7.244	7.413	7.586	7.762
129	7.943	8.128	8.318	8.511	8.710	8.913	9.120	9.333	9.550	9.772
130	10.000	10.233	10.471	10.715	10.965	11.220	11.482	11.749	12.023	12.303

**Table IX. Time-Temperature Data for the Slowest Heating Zone of a Container of Product Measured During a Sterilization Process (Columns 1 and 2) and the Corresponding Lethal Rate (Column 3).**

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(1) Time (Min.)      (2) Temperature (°C)      (3) Lethal Rate (Min. at 120/Min. at T)

0	48.9	-
1	49.4	-
2	56.1	-
3	69.4	-
4	83.3	-
5	93.3	0.002
6	101.1	0.013
7	106.7	0.047
8	110.6	0.115
9	113.3	0.214
10	115.3	0.339
11	116.9	0.490
12	118.1	0.646
13	118.9	0.776
14	119.4	0.871
15	120.0	1.000
16	120.3	1.072
17	120.6	1.148
18	120.7	1.175
19	120.8	1.202
20 Steam Off-Cool	120.9	1.230
21	118.3	0.676
22	96.7	0.005
23	76.7	-
24	62.2	-
25	51.1	-
26	43.3	-
27	37.2	-
28	32.8	-

---

Sum of lethal rates = 11.021,  $F(120^{\circ}\text{C}, 10^{\circ}\text{C}) = d \times (\Sigma \text{ of lethal rates}) = 1 \times 11.021, = 11.0 \text{ Min.}$

# The General Method Graphically

In this procedure, the sterilization value ( $F$ ) of the heat process is obtained using a graphical integration technique to solve the general equation,

$$F(T_{\text{ref}}, z) = L \int_0^t dt.$$

In our example problem we will use  $120^\circ\text{C}$  as the reference temperature. The procedure is as follows: lethal rates are plotted on the y axis of  $10 \times 10$  or  $20 \times 20$  lines-per-inch graph paper as a function of time in minutes on the x axis. Since the lethal rate,  $L$ , is minutes at  $120^\circ\text{C}$  per minute at  $T$ , plotted vs. time in minutes at  $T$ , the area under the curve is minutes at  $120^\circ\text{C}$ . Since the lethal rate vs. time is a curve, we cannot obtain the area under the curve directly. In this graphical procedure we must add a step, where we measure the area under the curve in area units, in our example square inches, using a planimeter, and then convert the area units to  $F$ -value units. To convert the square inch area under the curve to minutes at  $120^\circ\text{C}$  an area conversion factor is developed by determining the  $F(120^\circ\text{C})$  of an area of one square inch of the graph. The square inch adjacent to the coordinate zero is the simplest area to work with; the product of the lethal rate one inch up the y axis and time one inch to the right on the x axis is minutes at  $120^\circ\text{C}$  per square inch of graph; the product of this factor and the area under the curve in square inches is minutes at  $120^\circ\text{C}$ .

The general method lethal rate graph illustrates at a glance the relative microbial kill power of the different portions of the heat process. It dramatically points out the ineffectiveness of the first few minutes after steam-on and the importance of the last minutes before the start of cooling.

In Column 3 of Table IX are listed the lethal rates corresponding to the temperatures in Column 2. The lethal rates were obtained from Table VIII. The lethal rate data in Table II are plotted in Figure 10 to make a lethal rate graph; the area, measured using a planimeter, is 11.0 square inches. The area conversion factor of the graph is 1.0 (min at  $120^\circ\text{C}$ )/(square inch); the minutes at  $120^\circ\text{C}$  are  $11.0 \times 1.0 = 11.0$  minutes.

## The General Method Numerically (Nonmechanized)

It is possible to determine the sterilization value of a heat process using the "General Method" in a numerical mode instead of graphically. The basic principles are the same for both procedures. Consequently, the results are equally accurate. The numerical computational procedure eliminates plotting the lethal rate graph and measuring the area under the curve. The numerical methods are well-suited for use using a digital computer.

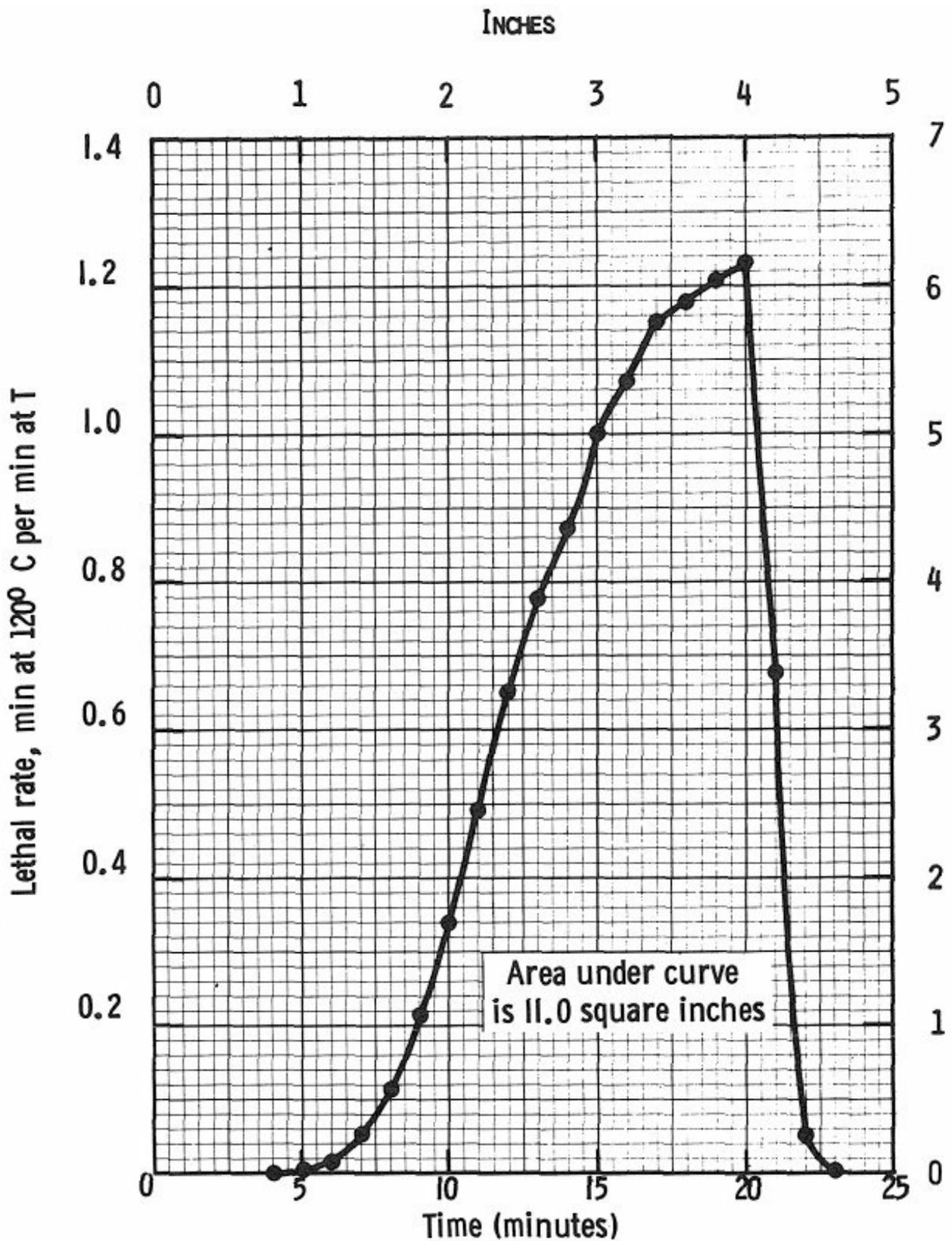


Figure 10. Lethal Rate Data from Table IX Plotted to Make a Lethal Rate Graph.

Mathematicians have developed several methods for finding the area of an irregular geometric figure; two methods; the Trapezoidal Rule and Simpson's Rule, will be described here as possible methods for evaluating the area under the lethal rate curve. In both of these procedures, it is necessary to divide the area under evaluation by equally-spaced parallel cords; the length of the cords are  $Y_0, Y_1, Y_2, \dots, Y_n$ . The constant distance between

the cords is d.

Burington (1940) states that, in general, Simpson's Rule is more accurate than the Trapezoidal Rule; however, lethal rate graphs are relatively simple geometric figures that usually can be made to start and end at zero and have a straight line base, so the difference in results using one or the other of the two methods is not great. Simpson's Rule for an odd number of Y values is written mathematically as:

$$\text{Area} = (t/3) (Y_0 + 4Y_1 + 2Y_2 + 4Y_3 + 2Y_{n-2} + 4Y_{n-1} + Y_n).$$

The Trapezoidal Rule for determining area is written mathematically as:

$$\text{Area} = t [(Y_0 + Y_n)/2 + Y_1 + Y_2 \dots + Y_{n-1}]$$

Patashnik (1953) describes the evaluation of thermal processes using the Trapezoidal Rule. In determining the F-value of a sterilization process, the length of the cord is the lethal rate (L), and the distance between cords will be the time, d, between successive temperature measurements. The area under the curve is equivalent minutes at the reference temperature used in determining the lethal rates. Patashnik points out that in evaluating a normal heat process, the data included in the analysis may be selected so the values of the first and last points,  $Y_0$  and  $Y_n$ , are zero, simplifying the Trapezoidal Rule equation to:

$$\text{Area} = d(Y_1 + Y_2 \dots + Y_{n-1}).$$

In terms of lethal rates, the Patashnik method simplifies to:

$$F(T_{\text{ref}}) = d [L(T_1) + L(T_2) + L(T_3) + \dots]$$

The results of evaluating the heating data in Table IX numerically using the Trapezoidal Rule are shown at the bottom of Table IX. In this example,  $d = 1.0$  min; therefore, the  $F(120^\circ\text{C}, 10^\circ\text{C})$ -value is one (1) times the sum of the lethal rates.

If the Patashnik method is to be used to determine the sterilization value of a portion of a sterilization process, for example, the initial 15 minutes of the process in Table IX, then we must use the basic Trapezoidal Rule equation as shown below:

$$F(T_{\text{ref}}) = d \left[ \frac{L(T_0)}{2} + L(T_1) + L(T_2) + L(T_3) \dots L(T_{n-1}) + \frac{L(T_n)}{2} \right]$$

The 15-minute temperature would be  $T_n$ ; therefore, only half of the  $L(T_n)$  value will be added. The  $F(120^\circ\text{C})$  for the first 15 minutes (Example Table 9) is calculated below:

Sum  $L(T)$  from 4 through 14 minutes = 3.513.

$L(T)$  for the 15th minute = 1.00,  $\frac{L(T)}{2} = 0.500$ .

Sum of the lethal rate = 4.0 - 13.

$F(120^\circ\text{C}, 10^\circ\text{C}) = d \times \text{of lethal rate} = 1 \times 4.013 = 4.0$  minutes

Table X.

```

PROGRAM STERILF (INPUT,OUTPUT)
C THIS FORTRAN PROGRAM APPROXIMATES THE STERILIZING VALUE F AT USER
C SPECIFIED BASE TEMPERATURE AND Z VALUE. TIME INTERVAL BETWEEN
C SUCCESSIVE TEMPERATURE MEASUREMENTS MUST BE CONSTANT THROUGHOUT
C THE DATA LIST. TIME IS IN MINUTES.
C
  DIMENSION TEMP(100),TIME(100)
C
C READ IN TEMP SCALE (IS), NO. OF TEMPS (NT), TIME INTERVAL (TI),
C FIRST TEMP, BASE TEMP (TB) AND Z-VALUE (Z).
  10 PRINT *,"ENTER C FOR CENTIGRADE, F FOR FAHRENHEIT",
    READ 20,IS
  20 FORMAT(A1)
    PRINT *,"ENTER NUMBER OF TEMPERATURES",
    READ *,NT
    PRINT*,"ENTER THE FIRST TIME AND TIME INTERVAL",
    READ*,TIME(1),TI
    DO 30 I=2,NT
      TIME(I)=TIME(I-1)+TI
  30 CONTINUE
    PRINT *,"ENTER BASE TEMPERATURE AND Z-VALUE",
    READ *,TB,Z
C
C READ IN TIME-DEPENDENT TEMPERATURE VALUES AND PLACE THEM IN
C ARRAY TEMP(I).
    PRINT *,"ENTER TEMPERATURE VALUES, ONE PER LINE"
    DO 40 I=1,NT
      READ *,TEMP(I)
  40 CONTINUE
C
C PRINT TABLE OF TIME/TEMPERATURE VALUES.
    PRINT 50,IS
  50 FORMAT(/,3X,"TIME",8X,"TEMPERATURE",/," (MINUTES)",5X,
+ " (DEGREES ",A1,")",/,1X,9("-"),5X,11("-"),/)
    DO 70 I=1,NT
      PRINT 60,TIME(I),TEMP(I)
  60 FORMAT(3X,F5.2,10X,F5.1)
  70 CONTINUE
C
C APPROXIMATE THE INTEGRAL USING THE TRAPEZOIDAL RULE.
    VALUE=0.0
    DO 80 I=1,NT-1
      XL1=10.**((TEMP(I)-TB)/Z)
      XL2=10.**((TEMP(I+1)-TB)/Z)
      VALUE=VALUE+((XL1+XL2)/2.)* (TIME(I+1)-TIME(I))
  80 CONTINUE
C
C PRINT THE RESULTS.
    PRINT 90,TB,IS,Z,VALUE
  90 FORMAT(/,3X,"F(",F5.1,A1,"Z=",F4.1,") = ",F4.1)
C
C DO AGAIN WITH DIFFERENT DATA ?
    PRINT *," "
    PRINT *,"ANOTHER PROBLEM (Y/N)",
    READ 20,IRESP
    IF (IRESP.EQ."Y") GO TO 10
END

```

ENTER C FOR CENTIGRADE, F FOR FAHRENHEIT? C  
 ENTER NUMBER OF TEMPERATURES ? 20  
 ENTER THE FIRST TIME AND TIME INTERVAL ? 4,1  
 ENTER BASE TEMPERATURE AND Z-VALUE ? 120,10  
 ENTER TEMPERATURE VALUES, ONE PER LINE

? 83.3  
 ? 93.3  
 ? 101.1  
 ? 106.7  
 ? 110.6  
 ? 113.3  
 ? 115.3  
 ? 116.9  
 ? 118.1  
 ? 118.9  
 ? 119.4  
 ? 120.0  
 ? 120.3  
 ? 120.6  
 ? 120.7  
 ? 120.8  
 ? 120.9  
 ? 118.3  
 ? 96.7  
 ? 76.7

TIME (MINUTES)	TEMPERATURE (DEGREES C)
-----	-----

4.00	83.3
5.00	93.3
6.00	101.1
7.00	106.7
8.00	110.6
9.00	113.3
10.00	115.3
11.00	116.9
12.00	118.1
13.00	118.9
14.00	119.4
15.00	120.0
16.00	120.3
17.00	120.6
18.00	120.7
19.00	120.8
20.00	120.9
21.00	118.3
22.00	96.7
23.00	76.7

F (120.0C,Z=10.0) = 11.0

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ANOTHER PROBLEM (Y/N) ? N

# The General Method by Computer or Programmable Calculator

Both the digital computer and programmable calculator are ideally suited for carrying out sterilization value calculations. Their ability to quickly calculate lethal rates and to sum the lethal rates using either the trapezoidal or Simpson's rule makes the process direct and efficient.

Available at the University of Minnesota is an interactive computer program, written in FORTRAN, that will calculate the equivalent sterilization value ( $F$  for the desired base temperature and  $z$ -value) of a heat sterilization process. Sterilization values for several  $z$ -values can be obtained at the same time. This program is listed in Table X.

We have calculated the  $F_c$ -value for the data in Table IX using the computer program. The result is an  $F_c$ -value of 11.0 minutes which is in agreement with our earlier calculated values. The interactive computer printout, including program, data input, and data output, is shown in Table X.

## Discussion of the General Method

The general method of process calculation is the most accurate method for determining the sterilization value of a heat process. In the general method, the actual time-temperature data are used and the analysis is carried out using the  $z$ -value of choice. The resulting sterilization  $F$ -value is the true value for the specific data used. The measurement method used, be it graphical or numerical, by hand or using a calculator or computer, will all yield similar results.

The general method is used as the basic method for calculating  $F$ -values to be used to compare the performance of the formula methods such as Ball (1923, 1928), Ball and Olson (1957), Hayakawa (1970), or Stumbo (1973).

## Sterilization Process Control

The design of a sterilization process must take into account where the temperature measurements are made that are used to control the autoclave temperature.

The steam sterilization process is normally a specified process time at a specified autoclave temperature. The process time is measured from the time the autoclave or retort reaches the specified operating temperature to steam-off (start of cooling). A manual or automatic timer is used to control the process time. The usual method of controlling the steam sterilization temperature is to measure and control the temperature of the steam environment in the autoclave. In laboratory and hospital autoclaves, the sensor for the temperature controller is located in the drain line of the autoclave. In the food industry, the sensor for the temperature controller is located in a well or vented pocket in the wall of the

autoclave so it is not damaged with the loading and unloading of the autoclave. We call this “environmental control,” since the temperature of the steam environment is controlled (we do not measure the temperature of the product itself).

In an environmentally-controlled sterilization process, the time-temperature conditions and the nature of the heating medium and its circulation in the autoclave are specified in the process design and are closely controlled during the sterilization operation. In the development of the sterilization process, the heating and cooling characteristics of the lowest heating zone of the containers of product are determined. These data, in addition to temperature conditions that exist at the coldest zone of the autoclave, are combined into the sterilization process design. This is a heating medium temperature-time process on the basis of (1) a specific product container size and shape, (2) product heating conditions (a function of product viscosity), (3) initial temperature condition, and (4) cooling conditions. The process design, in terms of autoclave environmental conditions, insures that all units, including those at the coldest part of the autoclave, receive the design F-value.

In the validation operation, we verify that we are able to deliver the specified process when operating the autoclave at conditions that will deliver the smallest F-value. During autoclave validation, it is necessary to (1) determine the temperature profile of the autoclave to locate the cold zone, (2) know the heating and cooling characteristics of the units being sterilized including the slowest heating zone in the container, (3) have a uniform initial product temperature or identify containers that are at the lowest temperature, and (4) have in any one load only one product and only one size of container.

To validate the autoclave system for delivery of the sterilization process to a product, three sterilization processes are carried out using the minimum operating conditions; the three consecutive processes must all yield acceptable results with small variability.

In an environmentally-controlled autoclave process, the initial temperature of the product, the heating medium temperature and its velocity or agitation rate, are critical points in delivering the designed process and, therefore, these attributes of the product and process must be monitored and controlled. When these parameters are controlled and the process adequately validated, there is confidence that the design F-value will have been delivered to the product units because the system will have been validated to do just this.

## **Removing Air from the Steam Autoclave**

The objective in operating the steam autoclave is to have 100 percent steam in the chamber. At the start of the process, the chamber will be filled 100 percent with air. Therefore, the first order of operation is to get rid of the air and replace it with steam. Probably more problems have arisen in steam autoclave operation due to failure to remove air than from any other cause. We believe the magnitude of this problem warrants a special discussion on air removal systems and their attributes.

In all autoclave systems, the problem of air has two aspects: (1) the effect of the partial pressure of the air on the autoclave automatic control system and (2) the effect of small pockets of air where nonsaturated steam conditions may exist.

We should eliminate the first problem by the design of the autoclave control system.

The temperature in the autoclave chamber should be controlled using a controller with a temperature sensor. It is correct that for saturated steam there is a specific temperature-pressure relationship. However, if air is present, the total pressure in the autoclave will be the sum of the partial pressure of the air plus the partial pressure of the steam. Consequently, if a controller with a pressure sensor is used, the pressure condition may be satisfied when there is a substantial partial pressure of air in the autoclave with accompanying lower temperature. If the steam control system is operated on the basis of a temperature sensor, then the control system will add steam until the desired temperature is reached, regardless of the presence of air. This does not mean that if there is air in the autoclave we have eliminated the air problem. What it does mean is that in the area of the temperature sensor, and usually throughout the major part of the autoclave, the temperature will be at the specified operating temperature and that further air removal will proceed from the correct operating temperature. All autoclave systems should have some type of air removal system that operates throughout the sterilization process.

I will discuss air removal, starting with the simplest and most widely-used system. I will start with a discussion of the gravity displacement of air as it is used in the laboratory, hospital and pharmaceutical plant sterilizer, then proceed to discuss the sweeping out of the air with flowing steam, as is used extensively in the large-volume parenteral industry and in the food industry, and lastly, the most positive method of air removal where the air is positively removed from the autoclave using a pump. In general, the cost of the air removal operation proceeds in the same order that I will cover them in that gravity displacement is the least expensive; sweeping air out of the autoclave with flowing steam (at least when energy is cheap); and mechanical pumping usually is considered to be the most expensive way of removing air.

## **Gravity Removal of Air from the Steam Sterilizer**

There are many factors that either accelerate or retard the removal of air from the steam sterilizer and its contents. We will first discuss these in relation to the steam sterilizer itself, and secondly, relate it to the removal of air from the sterilizer load.

### **(1) *Location of Openings for Exhausting Air***

For best results, the exhaust port should be at the bottom (lowest level) at the coldest part of the sterilization chamber. If the air exhaust port is at the top, inflowing steam will move to and flow out that port, leaving a large amount of air in the lower regions of the sterilizer.

### **(2) *Piping System for Exhausting Air***

The air exhaust piping system must be sized to take care of the required exhaust air flow rate. Not only diameter but length of pipe and restrictions to flow, such as valves and the thermostatic trap, must be considered. In most systems, once the critical flow has been reached, there will be no increase in air exhaust rate with further increases in pressure.

### **(3) *Rate of Steam Flow***

When steam enters the autoclave too slowly, it may diffuse throughout the chamber,

heating the air. When the steam enters with too much turbulence, it may entrain air. In both cases, we create a steam-air mixture, which makes air removal more difficult. For best air removal, the inflow of steam should be equalized with the outflow of the exhaust gas stream. This condition must be established by experimentation; it is often hard to achieve but will provide the best results.

#### (4) *Temperature Differences for Stratification*

Stratification of steam over air will provide the best conditions for the removal of air. Cool air is about two times as heavy as steam. However, if the air is allowed to become heated, it will decrease in density and diffuse into the steam and will be more difficult to remove.

The air in the autoclave will also acquire heat from the steam jacket; the longer air is allowed to be in contact with the hot chamber wall, the harder it will be to remove.

## **Use of Flowing Steam (Venting) to Sweep Air out of the Autoclave or Retort**

The North American canned food industry removes air from the large autoclaves or retorts by sweeping out the air with flowing steam. The operation is referred to as “venting” the autoclave or retort. Venting considerations enter into the design of the autoclave and retort piping system. The venting lines are always larger than the steam inlet to the specific unit plus there are specifications regarding the minimum back pressure permitted in the venting line during the venting operation.

The general venting procedure is to add steam at the bottom of the autoclave and exhaust or vent from the top, plus the drain line at the bottom of the unit is open. However, there is some flexibility in design so that in the bottom unloading Malo-type retort, steam enters at the top with primary venting just above the bottom door and with secondary venting through a port in the door itself.

In contrast to the gravity autoclave system discussed above where steam flow is relatively low and the mixing of steam and air is avoided, in the large food industry autoclaves, during the venting period, steam flow into the autoclave, and air plus steam exhaust is very high. The turbulence of the steam flowing through the autoclave scrubs the air from around the containers and entrains it in the stream of gas flowing out the vent.

Vertical retorts are normally 42 inches in diameter and many are 84 inches high with a total volume of 67.5 cubic feet. Typically, this type unit will have a one-inch NPT steam inlet and a 1.25-inch NPT exhaust line. The basic vent schedule is to open the steam valve wide and vent for four minutes or for whatever time is required for the mercury-in-glass thermometer to reach 105°C. The addition of divider plates or other restrictions to gas flow in the autoclave will increase venting times and may require venting with no obstructions between layers of containers.

The autoclaves and retorts in the food industry are equipped with bleeds (0.25-inch petcocks) located at the top and at the mercury-in-glass thermometer pocket on the side of the retort; these bleeds are open wide throughout the length of the sterilization process to

facilitate the removal of residual air.

## **Mechanical Air Removal**

Today, many sterilizers are equipped with mechanical vacuum pump systems designed to rapidly and efficiently remove air from the sterilizer and its load. The type of mechanical air removal system required depends upon the design and use of the autoclave system. In some cases, only a large fraction of the air in the autoclave is to be removed; consequently, the autoclave needs only to be evacuated to perhaps an absolute pressure of 200 mm Hg. In other systems, the desired endpoint is 80 mm Hg. absolute pressure. For removal of air from loads of fabric, a pump-down to less than 15 mm Hg. absolute pressure (by barometrically-compensated controls) must be met. In all cases, the desired vacuum should be reached after from four to five minutes of pumping. The rotary oil seal pump is probably the most widely-used system. In certain cases, water-sealed pumps and ejectors are used. It is important that the vacuum pumping system be protected by an efficient condenser and trap system.

## **Removing Air from Packages of Fibrous Materials**

Huge quantities of fabric-type hospital supplies are sterilized in steam autoclaves. These supplies are normally organized in packages with a suitable external cover or wrapper. It is desirable to limit the size of the largest package to  $0.3 \times 0.3 \times 0.5$  meter with a weight of 5 to 6 kg. Air removal from these bundles of fabric material, in which on a world-wide basis, cotton is undoubtedly the dominant fiber, is a major problem. There seems to be general agreement that a vacuum of 15 mm Hg or less is necessary to remove sufficient air for efficient penetration of steam to all parts of these bundles of fabric supplies.

The theory and practice in the removal of air from hospital packs has been reported by Ernst (1968). The reader is directed to this source for a more complete treatment of the subject.

The nature of the fabric material and the energy level of the molecules of air are such that there is a tendency for air in fabric materials to be driven into the material by incoming steam and to remain as a pocket in the fibrous material during the sterilization cycle. Needless to say, the existence of such an air pocket will lead to inadequate sterilization. It is only through evacuation to a level of about 15 mm Hg absolute that we will have removed sufficient air that when steam is added to the sterilizer, any remaining air molecules will mix with the steam molecules, initially forming a steam-air mixture at about the same temperature as the steam. Slowly, the remaining air will be carried out with the continuous vent or air removal system of the sterilizer. The magnitude of the air-holding effect is a function of the size of the fabric bundle and the origin of the fibers.

## **Autoclave or Retort Systems Used to Sterilize Product**

The steam sterilization system consists of a basic pressure vessel fitted with an appropriate door (two if it is a pass-through unit between a sterile and nonsterile area), piping connected to a “clean steam” supply, safety devices, and a control system. There is almost an unlimited number of ways that we can build a sterilization system. The use of the system and available funds determine the major characteristic of sophistication and size. In this report, we will discuss three general classes of steam sterilization systems. We will first cover the most basic sterilization unit, the gravity autoclave system. Secondly, we will discuss the high-vacuum autoclave system. Thirdly, we will discuss the simple nonjacketed steam autoclave or retort used for producing terminally-sterilized pharmaceutical industry preteral solutions and for sterilization of low-acid canned food.

Discussion of sterilization equipment will be limited to a general overview. For more information, the reader is specifically directed to the publications of Bock (1965), Ernst (1968), Joslyn (1983), and Perkins (1969). In the United Kingdom (UK), the Department of Health and Social Security (DHSS) has developed a great deal of data on all phases of sterilization and sterilizers. Their publication, “Health Technical Memorandum 10, Sterilizers” (DHSS, 1980), is the best steam sterilizer operating manual I have seen. A copy should be on the bookshelf of all health care organizations that operate steam sterilizers.

## **The Basic Steam Autoclave, Gravity Displacement of Air**

A single wall or jacketed pressure vessel manufactured in accordance with the pressure vessel code established by the local regulatory body is the basic part of the steam sterilization system. The pressure vessel must be equipped with some type of safety door, so the door cannot be opened while the sterilizer is under pressure. The sterilization operating system will include, in addition to the basic autoclave chamber, piping controls and auxiliary devices. The autoclave chamber must have a properly-sized overpressure safety valve. The sterilizer must be equipped with a mercury-in-glass thermometer; for gravity displacement and mechanical vacuum autoclaves, it is usually located in the drain line. There should also be an appropriate pressure gauge that shows the pressure in the autoclave chamber. The piping system must be appropriate for the use; a gravity displacement sterilizer should have appropriate traps for removing both condensate and air from the autoclave.

The sterilizer may or may not have a steam jacket; if it does not have a steam jacket, the walls of the autoclave should be well-insulated. The advantages of the steam jacket are: (a) it prevents excess wetting of the load; (b) faster operation, since more surface is in contact with steam; and (c) improved drying of materials at the end of the sterilization cycle. A disadvantage of the steam jacket system is that air in contact with the hot walls tends to become heated and mix with the steam in the autoclave, thus reducing stratification and the efficient removal of air where the gravity air removal is used. Heat added to the autoclave through the steam in the steam jacket supplies energy to the steam chamber without added moisture, resulting in drier steam.

Air is removed from the sterilization chamber by the unique flow pattern of the steam, illustrated in the diagram in Figure 11. Steam is admitted to the rear of the sterilization

chamber, usually impinging upon a baffle designed to remove as much water (condensate) as possible from the steam as it enters the autoclave. The steam will tend to move upward because it is hot and light, relative to the cooler air. As more steam is added, it continues to flow across the top of the sterilization chamber, displacing the air in that region toward the relatively cool door. With the steam stratified above the air, the increasing pressure of the steam forces the air out through the sterilization drain line and through the thermostatic steam trap. The mercury-in-glass thermometer is located in the drain line, which is the coolest part of the chamber. It indicates the temperature of the outflowing mixture of air, steam and water. Thus, when the drain-line thermometer indicates  $121^{\circ}\text{C}$ , there is assurance that the temperature in the chamber is at or above this temperature. A diagram showing temperature in a gravity air-removal autoclave is shown in Figure 12.

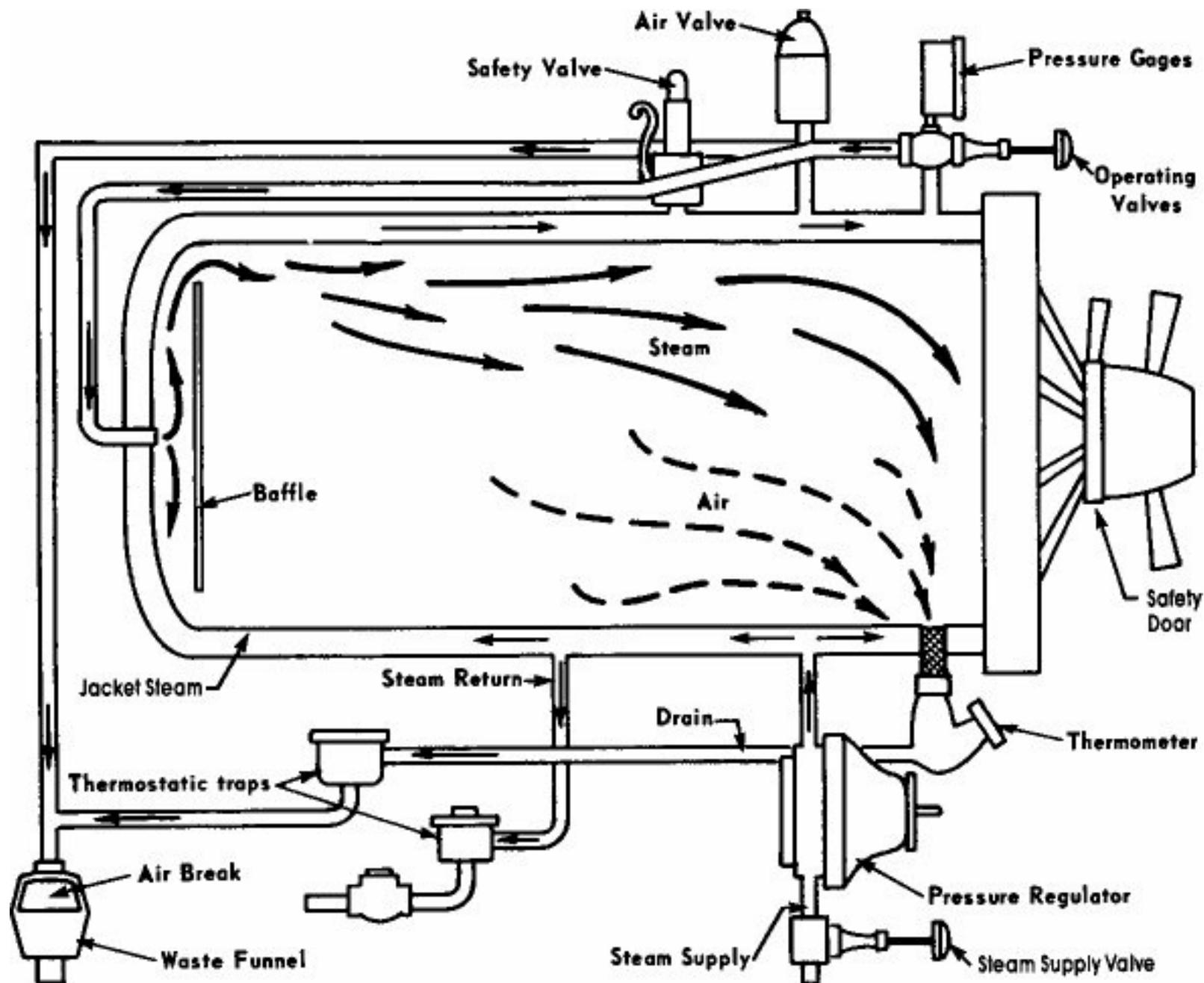


Figure 11. Diagram of a Jacketed Gravity Displacement Autoclave Showing the Essential Parts and an Idealized Flow Pattern for the Incoming Steam and the Outgoing Air.

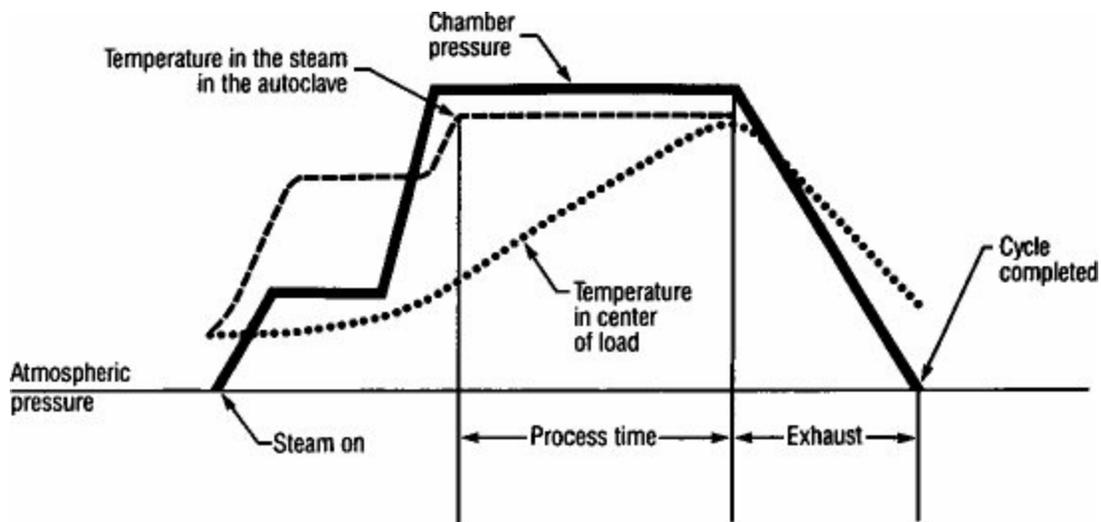


Figure 12. Pressure-Temperature Diagram for a Typical Gravity Autoclave Sterilization Process.

The principle advantages of the downward displacement sterilizer is its relatively low initial cost, relatively simple operation and low maintenance cost. Disadvantages are as follows:

- (1) For certain types of product, the time to sterilize can be long. This will depend upon the nature of the material in the load. As has been mentioned previously, packages of fabric supplies require long times for gravity air removal. In large sterilizers that are heavily loaded, there may be a lack of assurance that all parts of a load received the proper sterilization treatment.
- (2) The time necessary to dry a load of fabric supplies may be long.
- (3) Control in loading packs of fabric materials must be exercised to have reproducible sterilization.
- (4) When these sterilizers are used for hard goods or solutions, they are relatively simple and direct to operate. However, with packs of fabric materials, extra time and care must be exercised by personnel in the preparation and loading in order to have proper operation.

## Steam Autoclaves with a Mechanical (Vacuum Pump) Air Removal System

Many medium-sized and large-sized autoclaves installed in hospitals and pharmaceutical manufacturing plants today are equipped with vacuum pumps to remove air at the beginning of the sterilization cycle and to aid in drying the load at the end of the cycle. Almost all of these autoclaves are equipped with jackets so the temperature of the inside wall of the sterilizer can be controlled.

There are a great many variations in "vacuum steam autoclaves." In general, there are units that are designed to operate in the temperature range, 120 to 125°C, and those that

are designed to operate in the temperature range, 130 to 135°C. In addition to variation in the temperature range, the type of vacuum pumping system will vary with the use of the autoclave. Where hard goods are to be sterilized, a residual pressure of 80 to 200 mm Hg. absolute is acceptable, whereas when the autoclave is used with full loads of fabric supplies, the air removal system should be able to reduce the absolute pressure to less than 15 mm Hg. (measured using a barometrically-compensated instrument). In general, the pump should be sized so that the pump-down requires only four to five minutes.

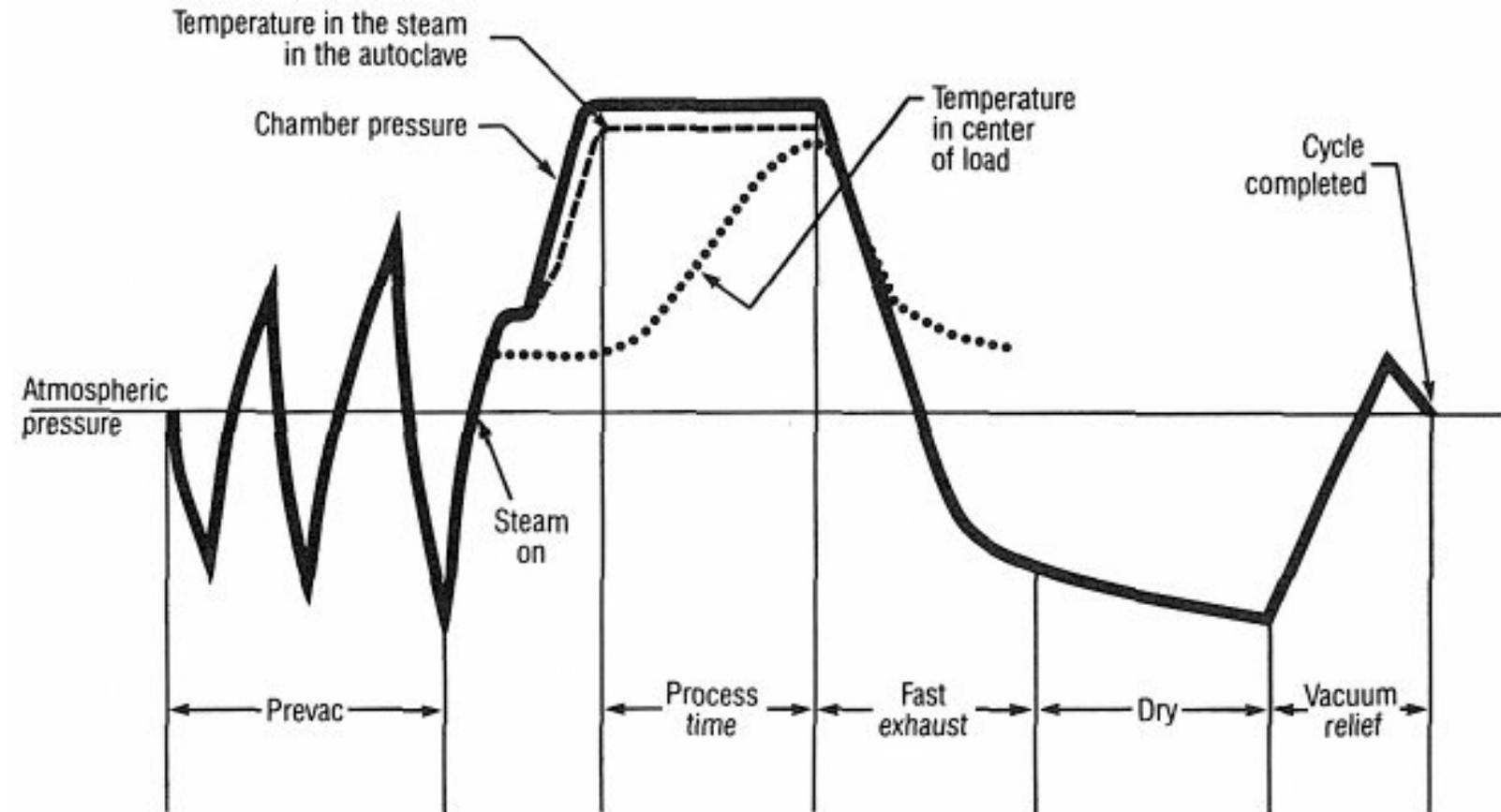


Figure 13. Pressure, Temperature Diagram for Typical Prevacuum Sterilization Process.

A diagram of pressures and temperature as a function of time for a typical prevacuum sterilization process is shown in Figure 13. A photograph showing the front and load cart of steam autoclaves in a hospital central supply is shown in Figure 14.



Figure 14.

High temperature/high vacuum sterilizers tend to be highly automated, which leads to certain disadvantages which are as follows:

- (1) Air leakage is always a problem in vacuum autoclaves. If a leak should develop in an automated system, it is often hard to locate and difficult to repair.
- (2) Automatic sterilizers tend to have high initial costs.
- (3) Highly-automated autoclaves, as with all equipment, require regular maintenance to prevent malfunctions. The level of maintenance capability of the plant in which the autoclave is located should be of the same order as the level of sophistication of the equipment.

Many vacuum steam autoclaves are equipped for pulsing. Pulsing systems are widely used to aid in the removal of air from autoclave loads of dressings sterilized in the high-vacuum sterilizer. The effect of the steam pulsing system varies, depending upon the pressure range of the pulses and the degree of vacuum attained.

## **Autoclaves and Retorts Specifically for the Sterilization of Products in Hermetic Containers**

The terminal sterilization of products in hermetic containers is one of the most important operations in the supply line to the health industry. It is used to produce both sterile drugs and nutrient products. A wide range of drug products is produced plus large-volume

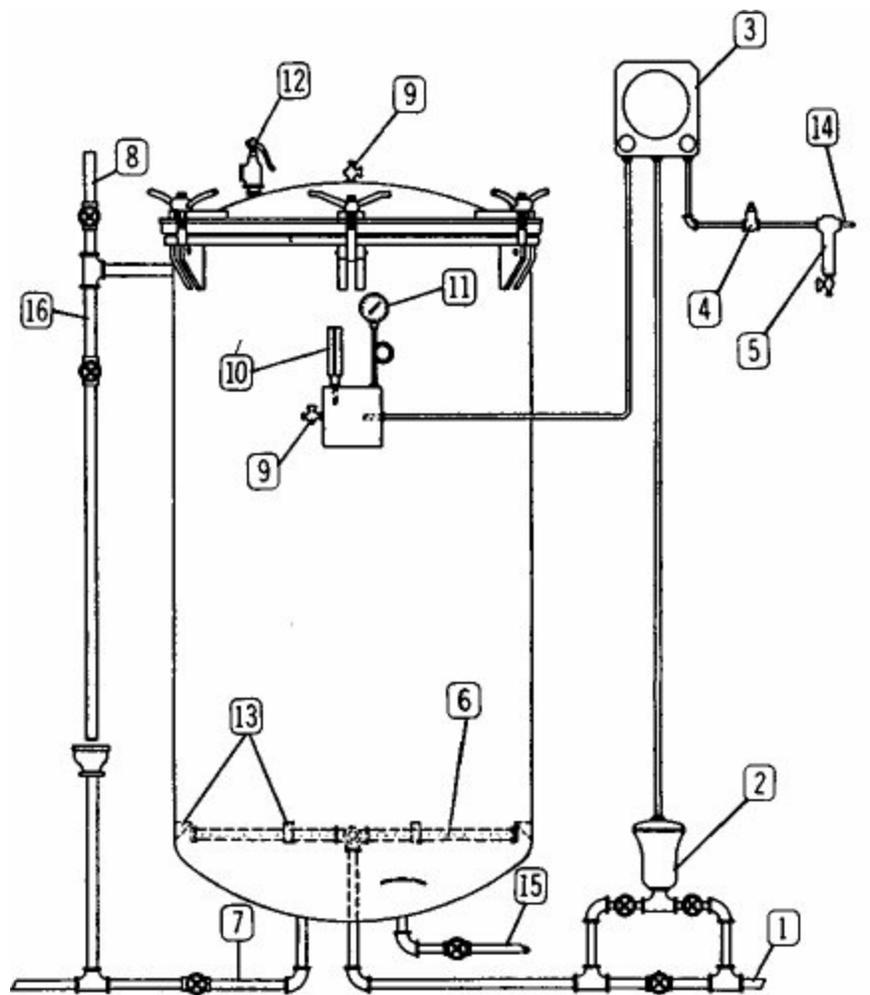
parenteral solutions (dextrose, saline, and lipids for total parenteral nutrition to mention a few), foods for tube feeding in health care facilities, as well as food for consumption by the general population.

Products may be in glass, metal, rigid plastic, or flexible, plastic containers. The nonagitating (still), noncontinuous autoclave or retort is the basic steam sterilization unit both in the pharmaceutical and food industries; it can be considered the primary piece of heat processing equipment. In some operations, batch-still processes are being replaced by continuous or continuous-agitating systems.

The still autoclave or retort may be either of a horizontal design, with the door in the end, or of a vertical design with the door at the top (Figure 15).

Horizontal retorts may be 10 to 20 ft. long, and 42 to 60 in. in diameter. Horizontal retort normally have rectangular baskets and are loaded by pushing the basket, supported by carriers with wheels, into the retort.

Standard vertical retorts are 42 in. in diameter and either 72 or 96 in. high; however, special vertical retorts 42 in. in diameter and 140 in. high are in use in some plants. Processing food in retorts is a batch operation; steel crates three feet in diameter and approximately 24 in. high with perforated or slatted sides and bottoms are used as the containers for moving the cans from the processing line into the retort and from the retort through the cooling system to the finished product line. Retort crates are usually handled in a combined system of three-wheeled dollies and an overhead monorail system. The loading and unloading of retort crates has been mechanized to the point where a crate can be filled in less than a minute with a single man directing the filling operation. One system for filling retort crates uses a hydraulic unit to elevate the removable bottom until it is level with the top, which is also level with the can line. The cans move onto the bottom steel sheet until it is filled. It is then dropped the distance of one can height plus the thickness of the divider plate and a second layer of cans is allowed to flow onto the plate. This is continued until the retort crate is filled. Normally, the crates are wheeled from the end of the filling line to the retort area. Another method of transferring filled containers to the retort crates is through the use of a magnetic lifter. In this system, the filled containers are accumulated in a circular area that has the same diameter as the retort crate. When the space has been filled, the head of the transfer machine is moved over the containers, the electrical magnet energized, and the containers moved from this accumulating space into the retort crate. A divider is placed on top of containers and the operation is repeated. The retort crates are unloaded by reversing the system.



*Courtesy of Continental Can Co.*

(1) Steam, (2) regulating valve, (3) controller, (4) reducing valve, air, (5) air filter, (6) steam distributor, (7) drain, (8) vent, (9) bleeders, (10) indicating thermometer, (11) pressure gauge, (12) pop safety valve, (13) basket supports, (14) air for controller, (15) water, (16) overflow, ⊗ manual valves.

Figure 15. Diagram of the Piping System for Sterilization of Product in Saturated Steam in a Vertical Retort. Air Removal is by Venting.

Retort crates are usually loaded into vertical retorts by overhead steam or electrical hoisting systems, although some plants use a special type of mechanical lift truck. Standard vertical retorts are designed to hold either three or four crates; however, 140 in. retorts will hold five crates.

In modern canning plants, steam produced in remote steam boilers or steam generators is the source of the heat energy required in processing operations. Steam at a line pressure of 100 to 125 psi should be available at the retorts for best results. Steam production, flow, and consumption is expressed in terms of boiler horsepower (hp); one boiler hp is equivalent to 33,479  $\beta$ tu/hr. According to Bock (1965), the peak heat demand of still batch retorts occurs during venting and for standard (42 in. x 96 in.) vertical retorts, or a horizontal unit of similar volume, will vary from 80 to 200 hp for steam inlets in the range of 1- to 2-in. pipe size. This peak demand exists for only a relatively short period during each

individual retort cycle. After the vent valve is closed and the retort reaches operating temperature, the steam consumption rate decreases rapidly to 3 to 5 hp and remains at this rate for the balance of the process. For processes up to 60 min, a total of approximately 300 lbs of steam or 8.6 boiler hp/hr is consumed with half of this amount used during venting.

The steam is fed into the retort through a system of steam spreaders or distributors designed to facilitate removal of air from the retort during venting. When steam is turned on, the void space in the retort is filled with air, which must be displaced during the venting operation. An inadequately-vented retort may contain steam-air mixtures or entrapped pockets of air at temperatures below the specified steam temperature, which result in underprocessing and spoilage. Retorts are equipped with bleeders, small 1/8- or 1/4-in. petcocks, that remain open to remove any air that may accumulate during processing.

Listed as follows are the steps recommended in NCA Bulletin 32-L (1959) for a simple steam sterilization process.

## **Preparing to Start the Sterilization Process**

- (1) Close the door or lid and check to determine if all the lugs are fastened securely.
- (2) Check the temperature recorder to insure that it is working properly — clock wound, pen inked, and chart firmly in place.
- (3) Open the vents and bleeders; close the drain and overflow (unless the overflow is used for venting).

## **Come-up to Temperature**

- (1) When the retort is ready for operation, admit steam by gradually opening both the controller and the by-pass lines.
- (2) When the correct venting temperature has been reached and the specified time has elapsed, close the vents. Never vent for less than the recommended conditions. Do not depend on agreement between mercury thermometer and pressure gauge readings as a criterion for complete air elimination, because this is not necessarily a true indication of the desired condition. If the pressure gauge is up but the temperature is low, it means there is still air in the retort and venting should be continued until agreement is reached.
- (3) Gradually close the by-pass just before the processing temperature is reached. This will prevent a sudden drop in temperature, which often occurs when the by-pass is closed too rapidly.
- (4) When the retort has reached the processing temperature desired, check the temperature indicated on the mercury and recording thermometers. It is not serious if the chart indicates a temperature slightly lower than the mercury thermometer, but it must never be higher. When the temperature is correct, start timing the process. Use an accurate clock for this purpose, not a wrist watch or the recorder chart.

- (5) At the start of the process, enter on the production record the time, the mercury thermometer reading, the pressure, and the temperature indicated by the recording thermometer.
- (6) Keep a record of the “come-up” time to make certain it has been long enough to allow sufficient venting.
- (7) With some vacuum-packed products, it may be necessary to heat the cans sufficiently to dissipate the internal vacuum before the pressure in the retort is permitted to become greater than 2 lbs., otherwise the cans may panel or even collapse.

## **Holding Period at Processing Temperature**

- (1) Maintain the retort temperature about one degree above the recommended processing temperature. This helps to compensate for unavoidable fluctuations.
- (2) As the process continues, check the temperature from time-to-time to make certain it is holding properly.
- (3) Leave all bleeders wide open during the entire process.
- (4) When the recommended time for the process has elapsed, turn off the steam and immediately start the cool.

The design of the piping and control system for retorts has been standardized and these specifications are available in the USA from instrument suppliers, container manufacturers, and the National Food Processors Association. In Figure 16, the piping and control system for a simple steam cook is illustrated.

Still retorts may be operated manually with very simple instrumentation or automatically with temperature and time cycle controls. The expenditure that can be justified for instrumentation generally must be related to the complexity of the process. Steam cooking of small-sized metal containers is a rather simple operation and warrants only one temperature controller, whereas processing glass-packed products under water with superimposed air pressure requires a pressure, temperature, and time cycle control to achieve the same efficiency.

In processing medium-sized metal cans of food in still retorts where steam is used as the heating medium and the cans are cooled with water either in the retort or in a cooling canal, it may be necessary to cool the cans under pressure so that the ends of the cans remain concave during cooling. If the retort is blown down (pressure in the retort reduced to atmospheric pressure) immediately at the end of the heating time, the internal pressure inside large-diametered cans may be sufficient to buckle the cans; therefore, it is necessary to maintain a pressure in the retort equal to pressure in the containers during the first few minutes of the cooling period. Steam may be used to maintain the pressure if the cold water is introduced into the bottom of the retort under a layer of hot water; a better solution is to use compressed air.

Products packed in glass containers must be processed under water with superimposed air pressure. The water level must be six inches above the top layer of jars during the entire

process; the retort should be equipped with some type of water level indicator or device to warn the operator if the water is below a safe level. A minimum of 4 inches for steam and air should be maintained between the water surface and the top of the retort. Both steam and air are added at the bottom of the retort and are introduced through a suitable distributing system. The superimposed air pressure should be controlled automatically so that the total pressure of 28 to 30 lbs. per sq. in. prevents venting or the loss of lids from the glass containers. This pressure is maintained throughout the process, including come-up, cook and cool. In vertical retorts, the air is added with the steam and both the steam and air are discharged by the spreader next to the wall of the retort. This procedure will cause rapid vertical movement of the water next to the wall and downward movement in the center of the retort, creating the circulation necessary for uniform temperature during the come-up and cook. Usually, a mechanical water-circulating system is used on horizontal retorts. Glass-packed products are cooled in the retort under water until the temperature of the food product has been reduced below 150°F when cans are placed in the warehouse.

## Concluding Statement

Heat is one of the most simple and most effective sterilization agents; however, even a heat sterilization operation must be done correctly. The sterilization operation is a critical point in producing sterile hospital supplies, sterile food, or sterile drugs. Failure of the sterilization process can mean an immediate public health hazard. Consequently, the sterilization scientist and engineer must approach both the design of the process and the actual sterilization operation with the critical nature of the operation in mind.

In this report, we have provided basic information on both the design of wet-heat sterilization processes as well as the operation of the autoclave or retort used to carry out the sterilization process. A properly-designed process, delivered using validated well-maintained and well-operated equipment, will result in products that will meet the design sterility requirements.

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# Gaseous Methods of Sterilization

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## Abstract:

Two toxic gases, ethylene oxide and formaldehyde, are commonly used as sterilants. Both gases are highly efficient against all microbial contaminants, when appropriate relative humidity can be achieved throughout the medical products to be sterilized.

Because of the complexity of the methods and the toxicity of the gases, ethylene oxide and formaldehyde sterilization should be used only when sterilization by moist and dry heat would damage the medical products and radiation sterilization is not applicable. Ethylene oxide sterilizers are in use for large scale procedures at hospitals. Formaldehyde sterilizers are used at hospitals. Formaldehyde is also used as fumigant for room and equipment decontamination.

Advantages and disadvantages connected to the two gaseous methods are discussed. A difference in microbicidal efficiency when standard procedures of the two methods are used for sterilization of medical devices are evaluated, and the microbial resistance spectra for the two gaseous methods are compared to the spectra for heat and ionizing radiation.

A Scandinavian model for microbiological control on gaseous sterilization is described.

## Introduction

Two toxic gases are in common use for sterilization of medical products: ethylene oxide and formaldehyde. Both gases have been used for sterilization, mainly of medical devices and equipment, for many years. Formaldehyde for about 100 years and ethylene oxide for about 50.

Both gases are toxic and can in high concentration cause acute and severe illness. Both gases are known to be mutagenic and carcinogenic and the maximal permissible concentration in the air in the working area is in several countries about 1 ppm. Ethylene oxide is in mixture with air explosive. Formaldehyde gas is neither explosive or inflammable. Formaldehyde is a potent allergen. Ethylene oxide causes no allergy problems.

Both gases are very efficient sterilants provided the relative humidity during the exposure time is high enough. All sorts of microorganisms can be inactivated by these two gases at gas concentrations, temperatures and exposure times well within the limits indicated by physical, chemical and economical parameters for sterilization of medical devices.

**Table I**

<i>Sterilization method</i>	<i>parameters to be controlled</i>
Saturated steam	temperature, time
Dry heat	temperature, time
Radiation	absorbed dose
Gas sterilization	temperature, time, gas concentration, relative humidity

Compared to sterilization procedures by means of heat or ionizing radiation a gas sterilization procedure is complex (Table I). Temperature, exposure time, gas concentration and relative humidity are important parameters.

Ethylene oxide and formaldehyde sterilization of medical devices and supplies has been used at hospitals and industry during so many years that advantages and disadvantages connected to the two methods are well known<sup>1,2</sup>. Various models of ethylene oxide sterilizers from small laboratory sterilizers to very large models for industrial sterilization of single use devices are on the market. The formaldehyde sterilizers I know about are all of comparatively modest size and for use at hospitals.

A typical gas sterilization cycle will be as follows: Conditioning of the products to be sterilized with regard to humidity, removal of air, rehumidification and adjustment of temperature at a higher than room temperature level, exposure to the toxic gas at controlled temperature, and finally removal of gas and excess water. Parts of the first and the last step in such a cycle can take place outside or inside the sterilizer. Different temperatures and different gas concentrations are in use.

It shall be mentioned that formaldehyde is used also without being confined in a sterilizer. Decontamination of biosafety cabinets, laboratory and animal rooms contaminated with dangerous pathogens is a parallel to procedures in a formaldehyde sterilizer. It is, however, difficult to control all four decisive parameters in a biosafety cabinet or a room, and the microbiological efficiency of such a decontamination is not always as good as expected. Unavoidable temperature gradients disturb the distribution of formaldehyde and water vapor.

The gas methods are flexible methods with regard to capacity of the sterilizer and influence on the products to be sterilized. Properly used the ethylene oxide—water vapor method is the most gentle sterilization available for electronic- and optical devices, and formaldehyde procedures are not much inferior to it.

# Toxicological risks

As mentioned both gases are mutagenic and carcinogenic and the permissible level in the working area has been lowered step by step in many countries and is by now about 1 ppm in some countries. The need for documented protection of personnel, patients and environment against exposure to the toxic gas add to the cost per product unit by large scale use of ethylene oxide at industry. It is however possible to keep the exposure to personnel and patients well below the current standards. At hospitals with small or medium size sterilizers a reliable control would be difficult and costly to maintain even when chemists and equipment for measurements are available for other purposes.

Neither of the two gases should be tolerated as measurable air pollution at hospitals. The toxicologic risk associated with gas sterilization give a reason to prefer formaldehyde for ethylene oxide at hospitals. The human nose can smell formaldehyde also in low concentration. The sensitivity is often at the level of 1 ppm. Defects in a formaldehyde sterilization procedure with regard to leakage during the cycle or residuals in the products can be detected by the personnel because of the unpleasant and "dangerous" smell of formaldehyde. Good chemists and sensitive chemical methods as independent control will be advisable, however continuous chemical control and alarm systems will often be too costly to be realistic advise.

Ethylene oxide has an uncharacteristic and not unpleasant smell, and most humans will not notice the smell unless the concentration is very much higher than the level assumed to be safe.

## Exposure time and temperature

The time necessary for sterilization of medical devices is about the same for the two gas methods provided the temperature is the same and optimal gas concentration and relative humidity can be achieved. Sterilization at ambient temperature is possible, however it is a time consuming procedure and the low partial pressure of saturated steam cause difficulties when complex devices are to be sterilized at ambient temperature. With an excess of humidity condensed water can trap the gas and with too low a relative humidity the procedure will be inefficient. Temperatures about 50 to 55°C are preferred for most ethylene oxide procedures. For formaldehyde procedures the preferred temperature range has been about 50 to 65°C for many years.

The time needed for a sterilization cycle at a temperature about 55°C will be from 2 to 12 hours depending on the type of product, the size of the load and the technology for exchange of air with gas and water vapor and vice versa. Differences in heat sensitivity of the medical products have therefore been of minor importance for a choice between the two gas methods. However, in recent years the experience with decontamination of medical devices by means of hot water or saturated steam in the temperature range between 80 to 95°C has had an influence on the choice of temperature for formaldehyde sterilization in the Scandinavian countries. A standard recommendation for decontamination is 80°C for 10

minutes in water or saturated steam. Only endospores of bacteria and a few viruses with high heat resistance can survive such an exposure. The equivalent time at 75°C is 30 minutes. At 75°C or higher temperatures the condensation of paraformaldehyde on products become an uncommon problem. Therefore formaldehyde procedures at 75 or 80°C and saturated steam is to be preferred of microbiological and toxicological reasons. However, this leaves us with the problem that many hospital departments are sure that 75°C for 30 minutes will cause too fast a degradation of the costly endoscopes. It is still an open question whether this suspicion is justified. None of the departments using the procedure for the various types of endoscopes have used it for a long period, and a sterilization is used only when a chemical disinfection is estimated to be insufficient. So far none of the hospitals using a 80°C formaldehyde procedure have reported about damage to the equipment.

## **Gas concentration**

In typical ethylene oxide procedures the gas concentration is between 300 and 700 mg per liter at a temperature about 55°C. In a formaldehyde procedure at 80°C the gas concentration is between 40-80 mg per liter.

Under experimental conditions an increase in gas concentration with a factor of 2 will decrease the exposure time necessary for sterilization also with a factor of two. Under the conditions prevailing in the sterilization chamber loaded with plastic devices wrapped in plastic and paper the relationship between gas concentration and exposure time is less simple. The time needed before an equilibrium of temperature, gas concentration and humidity throughout the chamber and in the load is achieved will be long compared to the experimentally achieved values for the necessary exposure time. Often an equilibrium is not achieved during a cycle. Gas and water are absorbed in the load and later on released again. Energy can be released as heat or bounded as chemical energy during the cycle and variation in pressure will give variation in temperature. Therefore the necessary exposure time for a full scale sterilization normally is several times longer than the exposure time determined experimentally, and a doubling of the gas concentration will not necessarily give an exposure time only half as long.

In procedures with high relative humidity a high gas concentration increase polymerisation of both gases. The polymerisation of ethylene oxide can give a black or dark brown pigment on metal, e.g. hypodermal needles. A small dark spot in the skin can be visible years after an injection with such a needle. Paraformaldehyde can give a grey or white layer on instruments. The release of formaldehyde from sterilized equipment is a serious problem for doctors and nurses with formaldehyde allergy.

## **Relative humidity**

The role of moisture in the inactivation of microorganisms by ethylene oxide was examined by Kaye and Phillips in 1949<sup>3</sup>. An optimum for the microbiocidal effect was demonstrated at about 30% relative humidity. The effect was greatly reduced at lower

humidity and slightly reduced at higher humidity. These data, obtained in laboratory experiments, have been the basis for some misunderstandings in the application of ethylene oxide for sterilization. It is not important that the relative humidity is close to the optimum during a sterilization cycle. If it is, it is also close to a too low relative humidity. It is, for ethylene oxide as well as for formaldehyde, preferable to have a good approximation to 100% relative humidity in the process because water may become absorbed in the load during the cycle. The microbicidal effect of both gases at a relative humidity close to 100% is excellent. Condensed water may be a problem because the toxic gases are dissolved in water and polymerisation is increased. However, reasonable heat isolation of chamber can prevent unintended temperature differences.

**Table II. Suggested parameters for gas procedures.**

	<i>ethylene oxide</i>	<i>formaldehyde gas</i>
temperature	55°C	80°C
gas concentration	450 mg/l	60 mg/l
relative humidity	80%	90%
exposure time	2 hours	30 minutes

In recent years a technology for evacuation of the air from chamber and load by repeated evacuations and fillings with saturated steam has improved the humidification. When the same technology is used for removal of the toxic gas after the exposure time a significant part of problems with remnants of gas in the sterilized products disappear (Table II).

## **Microbicidal effect**

As mentioned already ethylene oxide and formaldehyde can inactive all types of microorganisms, including bacterial spores and viruses<sup>4,5,6,7</sup>. If any exceptions of interest for the practical application of the sterilization methods exist, agents called prions are candidates<sup>8</sup>. These agents are probably a new class of infectious agents and the resistance against heat and radiation is also very high. For sterilization by ethylene oxide and formaldehyde gas genetically determined differences in resistance have so far been insignificant compared with the differences in resistance caused by dehydration and physico-chemical protection of some of the microorganisms contaminating medical products. However, with improved technology for humidification this picture may change. The gas concentration can be reduced and the relative humidity can be kept at a reliable maximum. Therefore the importance of genetically determined differences in resistance against the toxic gases is increased, and a change in test strains for biological indicators may become necessary.

Provided the toxic gas has unhindered access to the microorganisms, and the water content in the organisms is sufficient, the inactivation is a first-order reaction, the dose-response curve for a pure culture being a straight line in a semilogarithmic system (Fig. 1). Exceptions are described (e.g.<sup>6,9</sup> and may indicate that more variation in dose-response curves might be found if looked for.

Of special importance for proper use of ethylene oxide and formaldehyde gas for sterilization is the fact that dehydrated microorganisms, microorganisms covered by organic material and microorganisms included in crystals can be very difficult to inactivate by means of the toxic gases<sup>10</sup>. Such organisms with increased resistance constitute a small fraction of the microorganisms in most environments comfortable for human beings. In dust samples collected in private homes in Denmark, in laboratories and in clean areas in factories etc. they are demonstrated to be few<sup>11</sup>. Not more than 1 per about  $10^4$  or  $10^6$  of the total number of colony forming units (cfu) (Fig. 2). Under extreme conditions as in old dust from a dirty and dry store room or a factory room with high temperature and low humidity the total number of cfu per mg is low and dominated by spores. From such environments examples with 1 extremely resistant organism per. 100 are found. The extremes are of little interest for routine sterilization of medical products. The important questions are whether such dried or protected organisms occur on or in medical products prior to sterilization, and whether it can be secured that these organisms are inactivated together with the majority of contaminants, if they should occur?

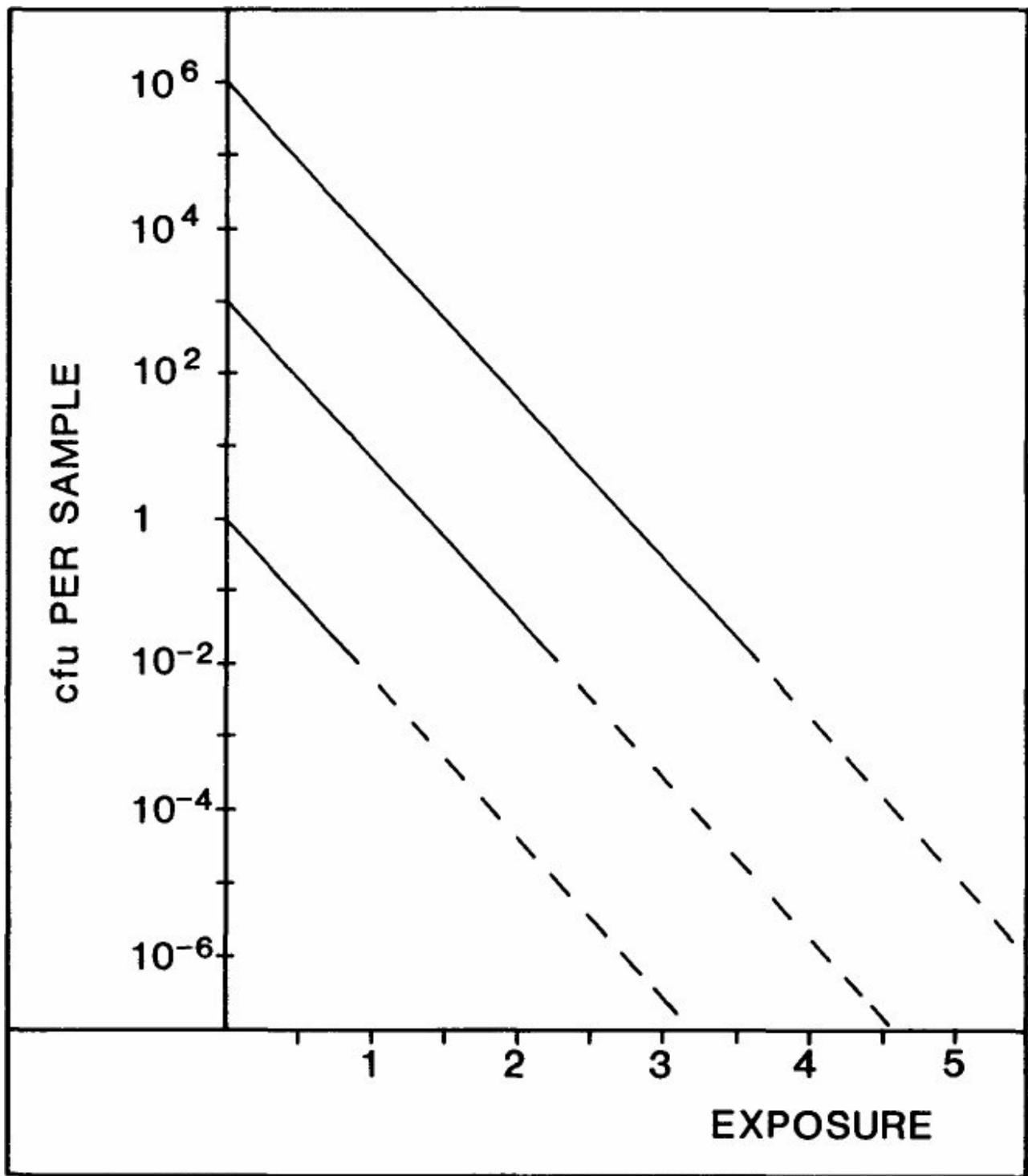


Figure 1. First order dose-response curve for inactivation of a pure culture of bacteria. The resistance can be demonstrated to be independent of concentration of cfu from about  $10^9$  cfu per sample to about 1 cfu per 100 samples ( $10^{-2}$ ). It is assumed that extrapolation is permissible.

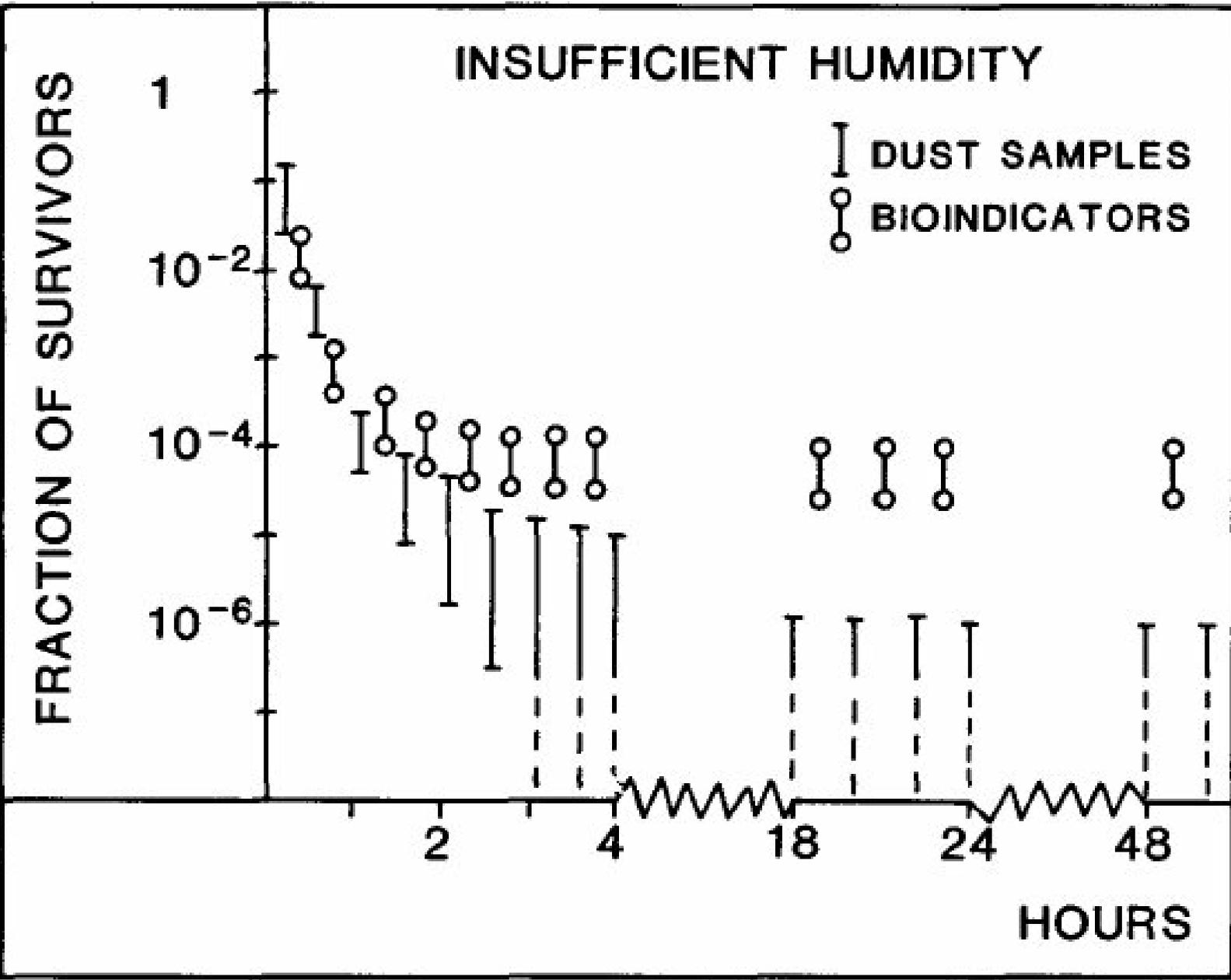


Figure 2. Gas sterilization with suboptimal humidity in the microenvironment. The dose-response curves indicated for dust samples and bioindicators respectively, are summations of about 20 years experience with dust samples from private Danish homes used as a reference from nature when bioindicators for control of gaseous sterilization are calibrated. The dust samples are not vacuum dried prior to the use as reference. The sterilization procedures include a short vacuum phase (<20 mm Hg). The relative humidity during exposure time is 50 ± 10%.

Experience with conventional sterility tests on gas sterilized products, e.g. on batches under suspicion to be unsterile, give some information about the first question. Microorganisms isolated in my laboratory in such tests on ethylene oxide exposed products are spores of bacteria or types of non-sporeforming bacteria known to survive for long periods of time in dust. When such strains are examined for resistance against the relevant gas, the resistance of the laboratory preparations are quite ordinary. It is unlikely that these organisms were on the product in the necessary very high numbers and have had ordinary

resistance prior to the exposure to the gas. It is more likely that they have been protected by dehydration, by being part of a larger particle covering the cell surface or by being placed in the product unit at a location where the toxic gas or the water vapor or both cannot come in sufficient amount during the cycle.

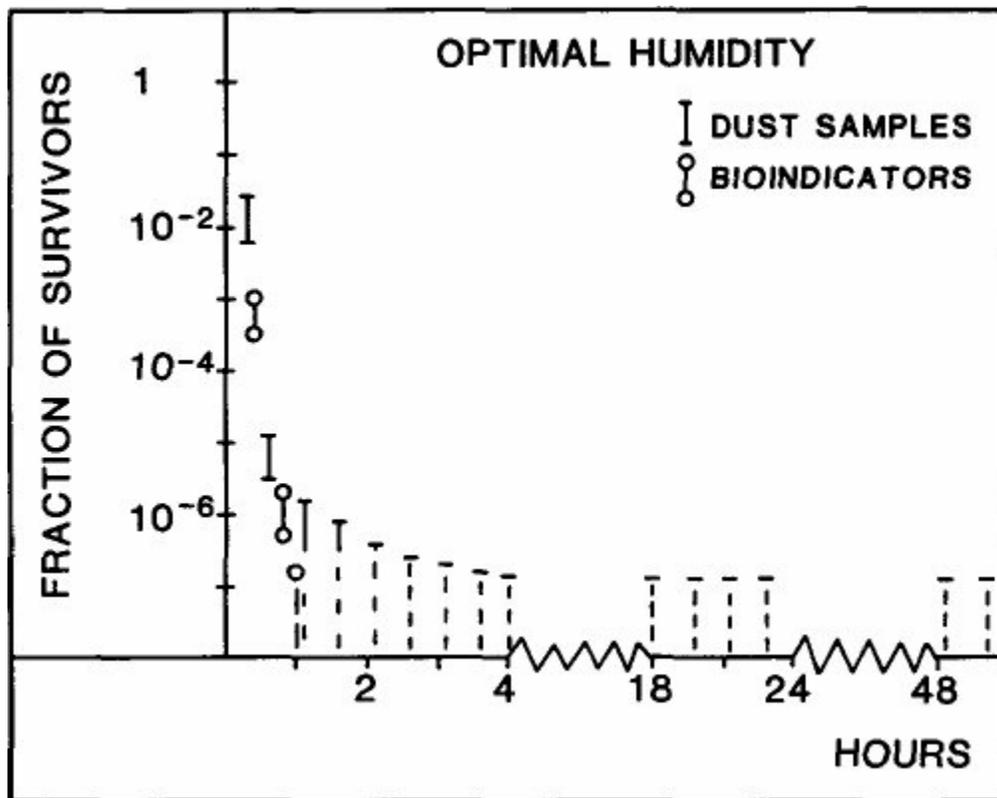


Figure 3. Gas sterilization with optimal humidity in the microenvironment.

The dose-response curves indicated are from the calibration of bioindicators for control of gaseous sterilization, and the dust samples and bioindicators are from the same batches as the samples used in the examinations demonstrated in Fig. 1. The only difference is that prior to sterilization procedure optimal humidity in the microenvironment for the microorganisms is secured by a 24 hours exposure to 100% relative humidity at a temperature slightly higher than the temperature in the sterilizer chamber during exposure time.

The last five years experience with pulsed vacuum-saturated steam, prior to exposure to the toxic gas, has demonstrated that the same influence on the microenvironment can be achieved in the sterilizer chamber within 20 minutes.

Unfortunately it is not possible to examine the resistance of the microorganisms on and in the medical devices directly unless the devices are grossly contaminated. Indirectly the resistance on medical devices can be elucidated by examination of dust samples, because at least a part of the contamination on the product units is dust particles from the air. In dust from private homes in Denmark protected organisms are numerous. In a clean area with air conditioning and very good hygiene the fraction of protected organisms probably is small. However, human clothes and skin can deliver some.

Most but not all of the protected organisms can become sensitive to ethylene oxide or formaldehyde if exposed to water or 100% relative humidity (Fig. 3). Microorganisms

confined in particles not readily soluble in water or permeable to water constitute a fraction resistant not only to gas sterilization but also to saturated steam sterilization<sup>1,10</sup>. Biological indicators can measure the microbicidal effect of a gas sterilization. They can, however, only reveal insufficient effect of the procedure on organisms protected by the microenvironment if a similar protection is built into the indicator (Fig. 2 & 3).

The importance of dehydrated or protected microorganisms can be evaluated quantitatively when the microbiological contamination on medical devices prior to sterilization is known. For products with an average of 10 cfu per product unit and about 1 protected organism per  $10^5$  organisms in the microbial contamination, surviving organisms on the sterilized product will be about 1 per  $10^4$  product unit (Fig. 4). This is well below the level of contamination detectable by sterility test on finished product units. When a sterility assurance standard of  $10^{-3}$  can be applied, the protected organisms most likely are insignificant. When the initial contamination is at a higher level, e.g.  $10^3$  per product unit, the number of organisms surviving the exposure to the gas will be at, or close to, the level where a conventional sterility test can reveal contamination. If the sterilization standard to be applied is  $10^{-6}$ , it is necessary to secure that also protected organisms are inactivated (Fig. 5). In the Scandinavian countries biological indicators with spores of *B. subtilis* have been used for control of gas sterilization procedures for many years. About 35 years ago an indicator with spores on sand, dried with some sodium chloride, was introduced as a standardized indicator for use in the control of sterilization by moist and dry heat and formaldehyde. The test strain used for control on saturated steam procedures is now a *B. stearothermophilus*, and we may change the test strain for gas sterilization too, within the next few years. However, the basic concept for preparation of indicators for gas sterilization is, and probably will be, unchanged. By a drying procedure, during the preparation of the biological indicators, a fraction of the spores are confined in sodium chloride crystals. Such an indicator is very sensitive for the amount of water available in the inactivation procedure. If the confined spores are not released from the crystals, they will not be inactivated by the gas. The inactivation curve for these biological indicators is not identical or parallel to the curve for the reference from nature, the dust samples (Fig. 2). The inactivation curve for a procedure with a humidification efficient enough to release the spores from the crystals will cross the inactivation zone for dust samples at a level of about an inactivation factor of  $10^6$  (Fig. 3). If the indicator units are inactivated it is probable that dust and contact contamination on a load of medical devices is inactivated also, provided that the bioburden is low. It is not a very accurate test system. However, it has been used with reasonable results for many years at hospitals and in microbiological laboratories and it is based upon experience with the resistance of microorganisms in reference samples from nature. In a gas sterilization procedure with relative humidity higher than 75% the indicator units behave exactly as indicator units without sodium chloride.

The resistance spectra for the two gas sterilization methods are from a quality control viewpoint influenced by two parameters in the procedure, the relative humidity and the temperature. The gas concentration and the exposure time have in most procedures, I have information about, a relative broad safety margin.

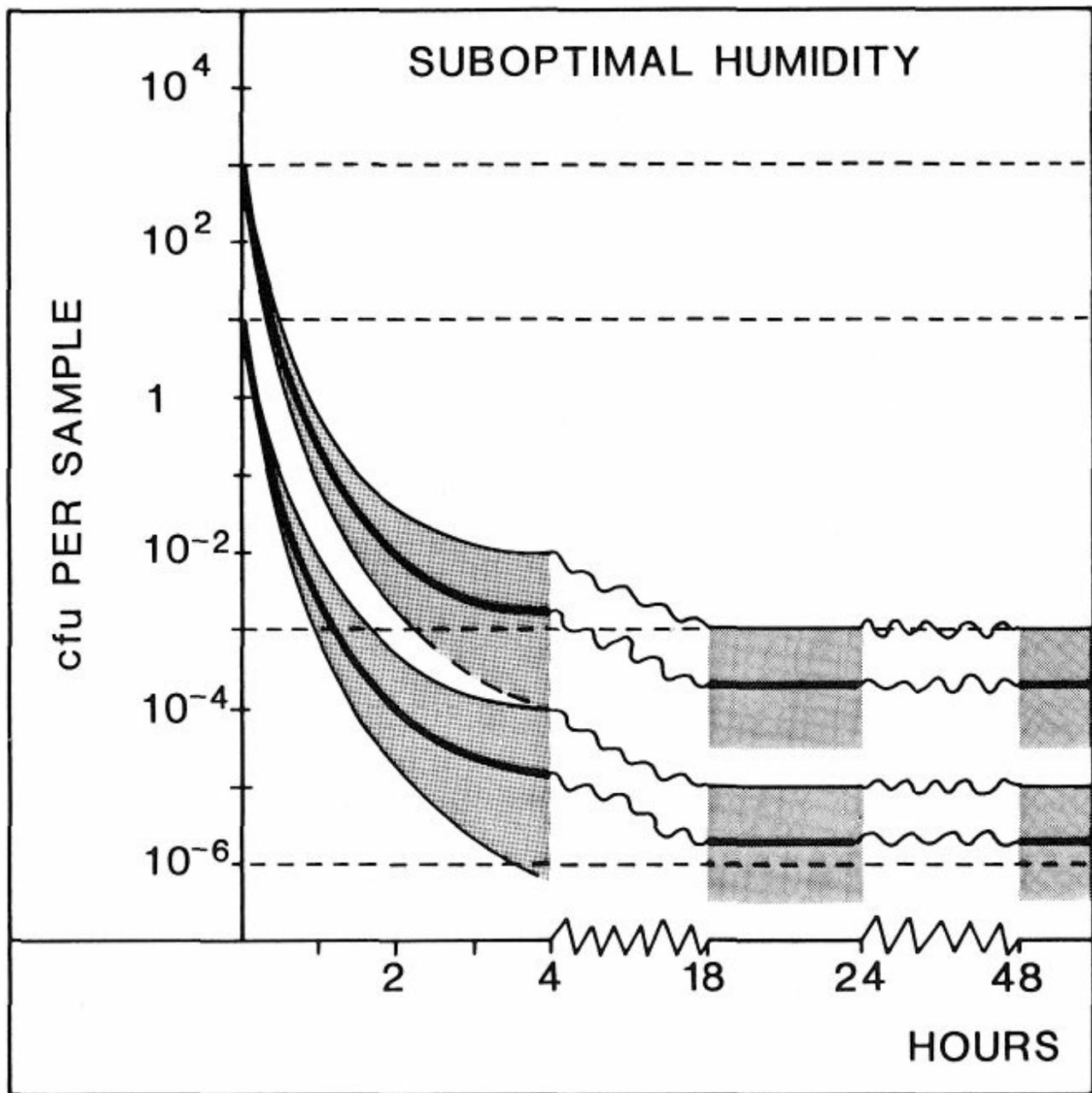


Figure 4. Influence of bioburden on sterility assurance with suboptimal humidity in the microenvironment.

The experimental data for the inactivation factor-exposure time relationship for dust samples from private homes is transferred to lower concentrations of microorganisms, 10 and 10<sup>3</sup> cfu per product unit respectively. It is assumed that the dose-response curves for various concentrations of microorganisms are parallel. The level 10<sup>-6</sup> is the basis for the sterility assurance standard used in the European pharmacopoeia. The level 10<sup>-3</sup> is about the extreme limit for disclosure of microbial contamination by means of conventional sterility testing on finished product units.

When surviving organisms after a gas procedure at 50 to 60°C are found, several types of bacteria and fungi can be represented. Sporeforming strains are most common, but also Gram-positive cocci, coryneiforme rods and certain types of Gram-negative rods can be found. This signifies that also some pathogenic microorganisms might survive if such were a significant part of the contamination prior to the gas procedure.

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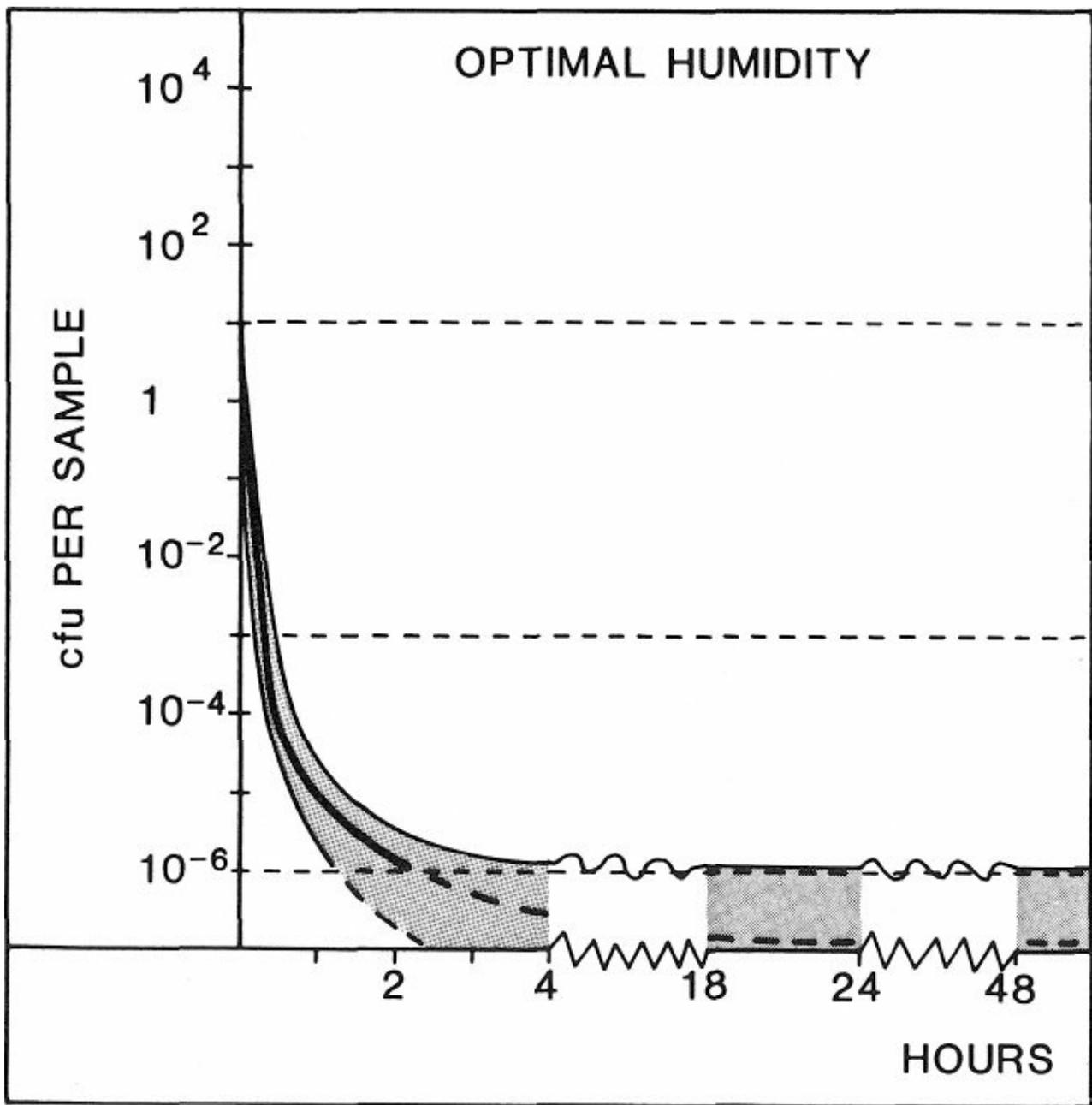


Figure 5. Influence of bioburden on sterility assurance with optimal humidity in the microenvironment.

The dose-response curve for dust samples from routine calibration of bioindicators indicate that the sterility assurance level of  $10^{-6}$  can be achieved when the microbial contamination per product unit can be kept on a low level.

At an  $80^{\circ}\text{C}$  and 100% relative humidity formaldehyde procedure we have so far only been able to pick up sporeforming bacteria. It is already mentioned that genetically determined differences between various strains of microorganisms may become more important in the not so far future. However, little is known about the variation in resistance when large mixed populations are exposed to formaldehyde gas. Compared to the resistance spectra for the sterilization methods based on moist and dry heat, the resistance spectra for ethylene oxide and formaldehyde in  $50^{\circ}$  to  $60^{\circ}\text{C}$  procedures are unfavorable for the use of the methods at hospitals. Even for heat resistant spores of bacteria the safety margin is good in the standard heat procedures at  $121^{\circ}\text{C}$  for 15 minutes and  $170^{\circ}\text{C}$  for 60

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minutes, respectively. For nonspore-forming bacteria, for all viruses and fungi the safety margin is extremely broad, and even in cases with severe defects in the procedure only spores of bacteria can survive. As the only exception of interest, I know about, prions shall be mentioned. It is recommended<sup>8</sup> that the exposure time with saturated steam at 121°C shall be 60 minutes when contamination may contain prions. Compared to the resistance spectrum for radiation a difference between the gas methods and radiation is of special importance. A gas procedure efficient against bacterial contamination will also be efficient against viruses. A radiation dose giving acceptable sterility assurance against bacterial contamination may not be efficient enough against viruses. Several viruses are reported to have D-values about 0,5 megarad, and in dried condition the D-values can be expected to be even higher. As a curiosity it can be mentioned that the D-value for the prions may be as high as 5,0 megarad (Table III).

**Table III. Microorganisms Surviving an Insufficient Sterilization**

<b>Sterilization Method</b>	<b>Microorganisms</b>
Saturated Steam	Spores of bacteria
Dry Heat	Spores of bacteria
Radiation	Gram-positive Cocci, certain strains of Gram-negative rods. Some spores of bacteria. High resistance can be demonstrated. Several viruses have high resistance to ionizing radiation.
Ethylene Oxide	All types of microorganisms may survive if failure in the humidification occur. Genetically determined high resistance cannot be demonstrated.
Formaldehyde Gas at temperatures below 70°C.	As ethylene oxide
Formaldehyde Gas at 80°C and 100% relative humidity	Spores of bacteria

I should like to comment on the phenomenon of delayed growth in sterility tests on gas sterilized products and from biological indicators exposed to ethylene oxide or formaldehyde gas. Delayed growth is defined as growth detectable only after prolonged incubation time. The length of the incubation period required or recommended therefore has an influence on the number of delayed cultures registered. However, growth after 6 or 12 weeks is obviously delayed growth, and such examples do occur in experimental work. The phenomenon is related to physico-chemical protection of microorganisms. It is not uncommon, when dust samples are examined, and occur rather frequently when samples of

soil are included in the experiments. In experiments with one or more of the decisive parameters changed to the limit for acceptable efficiency measured by means of biological indicators, delayed growth may also occur in the incubated indicator units. When a gas procedure with a narrow safety margin is found, the delayed growth in a significant number of samples can be demonstrated beyond all reasonable doubt about the reality of the phenomenon. Repair mechanisms after sublethal damage to the microorganisms, as it is known from radiation damage, might be an explanation for delayed growth in some cases. However, the correlation to the physico-chemical protection is obvious. When the gas procedure has a broad safety margin the delayed growth phenomenon is insignificant, whether the biological indicator used is designed for an incubation period of 5, 7 or 14 days. However, you may never know whether delayed growth could occur in the control of the sterilization of a certain product, unless prolonged incubation is a part of the routine validation of gas sterilization procedures.

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# Session II

**Chairman: Prof. Alfred D. Zampieri, M.D.**

*Director, Laboratory of Epidemiology and  
Biostatistics*

*Institute Superiore di Sanità*

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# Introduction by Session

Chairman, Alfred D. Zampieri, M.D.

I am extremely honored to participate in this International Scientific Conference organized by China Academy of Preventive Medicine and I wish to thank personally Madame Chen Chung-ming and my friend, Eugene Gaughran, for their so gratifying invitation.

I consider of high importance the opportunity that the Ministry of Public Health of the People's Republic of China has given to the main experts and scientists in each branch of production and control of medical products to discuss, here in Beijing, the many problems connected with the utilization of new surgical and medical materials, their sterilization with suitable and proper methodology and their specific security control.

If the most sophisticated devices and methodologies, as heat, gaseous, chemical, ionizing radiation methods of sterilization, and the use of bioindicators for sterility tests are now available for a large variety of medical products, a particular attention has to be also dedicated to the operators, for a better use of these technologies.

For example we have to correlate the problem of nosocomial infections with the correct utilization of the large universe of sterile medical products, which if originally are sterile, their invasive use presents a risk of infection during handling.

In a recent survey on 130 General Hospitals in Italy to evaluate the prevalence of Hospital Acquired Infections, we have found that the overall prevalence rate of hospital infections is 6.8 per 100 in a sample of 34,000 patients studied.

The higher hospital infection rates were in Intensive Care Units (10.5), Geriatrics (9.7), Orthopedics (7.9), Surgery (7.3). The more frequently involved sites were: urinary tract (30.1% of all hospital infections), low, respiratory tract (22.0% and wounds (10.9%). The role of urinary catheterization and endovenous therapy were underlined as the most relevant factors.

I have referred these data for it is important to have available methods to secure sterility of medical products, the final item is the welfare of patients and particular attention must be due to the use of medical products in individual patients.

I want to add that at least more than 50% of these infections may be prevented by a better knowledge of the potential risk of their improper use.

# Perspective On The Use Of Radio-Sterilization In Medicine

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Based on the biocidal effect of ionizing radiation, sterilization by irradiation is widely used in medical practice and medical industry throughout the world.

Due to it, a great amount of new medical products and devices were introduced, especially those made of polymer materials of single use and new suture materials.

Microbiological research (study of the microorganism's radioresistance and choice of sterilizing dose, detection of the initial contamination and sterility monitoring), construction of the potent stationary isotope installations and electron accelerators and development of new dosimetric technology led to the birth of the new branch of medical industry. Much argued rules and codices governed the technology of radiation sterilization of medical products of single use. The products were made already sterilized, ready for use, with high safety guarantee, minimal toxicity and minimal energy loss.

Modern methods of therapy depend upon the principally new devices and equipment, sutures and antiseptic materials and dressings, grafts and bio-preparations which do not endure conventional doses of radiation: 25-35 kGy. After irradiation to such a dose physico-chemical and biological qualities of the product are changed, its general quality and exterior worsened. So a necessity appeared to lower the radiation dose in medicine and in medical industry.

We found it possible to lower the sterilizing dose by modifying the radioresistance of the microorganisms. That was achieved by use of complex radiophysical and radiochemical modulation of the microorganism concerned.

We developed radiothermal, radiomagnetic and radiochemical methods of sterilization, based on the synergistic stimulation of biocidal effects, which allowed us to lower the sterilizing dose by 25-40%.

Data of others and our own testify that hyperthermia is one of the main factors modulating the microorganism's radioresistance. The rise of temperature from 42° to 60°C during irradiation leads to the decrease in the survival of microorganisms independently of their radioresistance. It is reflected by the fall of the  $D_{10}$  value. In most cases this effect is synergistic.

One of the possible mechanisms of the cellular radioresistance and survival is the reparation of the radiation-induced DNA damages. This feature is well-expressed in the strains of Gram positive cocci.

Kitayama and Matsuyama (1978) found that wild type cells of *M. radiourans* irradiated to a dose of 2 kGy could repair at least 80% of single-strand DNA damages in 10 minutes. Also the reparation mechanisms of several DNA damages were shown to be thermosensitive. We found that combining the effects of mild hyperthermia (55°C, 5-11 min) and ionizing radiation one could enhance the occurrence of non-repaired, single-stranded DNA damages and decrease the survival of bacteria.

Radiothermal effect was used for sterilization of catgut, syringes and blood transfusion kits. It made possible the lowering of the sterilization dose to 10 kGy for syringes, to 25 kGy for catgut, to 15 kGy for blood transfusion kits, i.e., by 60, 30 and 40 percent, accordingly.

There is few, if any, data on the magnetic field influence on the microorganisms. There is a work of Schaarschmitt (1977) on the ability of magnetic field to modify the radiosensitivity of yeast.

We tested the influence of changing magnetic field separately and in combination with ionizing radiation upon the survival of bacteria with different radioresistance. It turned out that magnetic field exerted very mild bactericidal effect. The bacteria maintenance in the magnetic field (750 oersteds, 24 hours) decreased the survival of the culture only by 10% compared with control. But the magnetic field with differing tension might influence the radioresistance of bacteria. Combining effect of changing magnetic field and radiation, as maintenance of irradiated bacterial cultures (*E.coli*, *Str. faecium*, *M.radio-proteoliticus*) in magnetic fields with tension 240 or 750 oersteds for 3 hours after irradiation, was shown to synergistically enhance the bactericidal activity of ionizing radiation. The level of postirradiation DNA degradation in bacteria after radiomagnetic treatment was higher compared with control gamma-irradiated cultures. Therefore, the effectiveness of reparation processes in bacteria after combining effects of radiation and magnetic field was decreased. This could be the cause of enhancement of ionizing radiation bactericidal effect after combining treatment. This effect was shown to further increase when the longevity of postirradiation exposition in the magnetic field was lengthened to 18-24 hours.

The results of model study using microorganisms with different radioresistance allowed to make use of combined radiomagnetic treatment to sterilize medical products, e.g., catgut, enzymatic drug renninmesenterine: surgical drapes with immobilized enzymes.

The sterilization of catgut by this method was achieved by maintenance of irradiated catgut in changing magnetic field (240 oersteds, 18 hours), using the dose 15-20 kGy, i.e., the dose that is 1.5-2-fold lower.

Renninmesenterine is used for milk coagulation during the process of chee-semaking and its contamination should not be more than  $10^3$  cells per 1 gram of the matter. The drug is susceptible to temperature: exposure to 40°C for 3 hours leads to its inactivation by 60%. The drug may be sterilized by 50 kGy of irradiation but its activity decreased by 40%. The sterility may be achieved by 25 kGy irradiation combined with exposure to 750 oersteds of magnetic field for 18 hours. The loss of contamination towards the level required is

achieved by irradiation to the dose of 10 kGy. While using combined effect of radiation and magnetic field, the ionizing dose may be lowered two-fold. The latter method used for sterilization of medical dressing materials with immobilized enzymes allows to lower the ionizing dose from 25 to 10 kGy.

The method of radiomagnetic sterilization of blood vessel implants holds special interest. Usually such implants are sterilized using glutaraldehyde, but this method is dangerous, as so sterilized implants are thrombogenic. The dose of 25 kGy could not be used for sterilizing blood vessels because it causes the thrombosis after graft. Taking this into account, a mild method was used based upon combining low doses of gamma-irradiation with changing magnetic field. Irradiated to doses of 10-15 kGy in this way, blood vessels were sterile afterwards. They possessed high durability, were not thrombogenic and were resistant to reinfection.

In order to lower the radiation dose for sterilization, one may use hydrogen peroxide,  $H_2O_2$ . That was proved after treatment of bacteria with different radioresistance (*E. coli*, *S. aureus*, *M. radiodurans*).

The synergistic enhancement of the bactericidal effect of radiation was invariably observed after  $H_2O_2$  treatment. During the combined treatment with  $H_2O_2$  and gamma-irradiation, the number of single-strand DNA damages in bacteria is 1.5-fold higher compared with the action of any of the two effects taken separately. The reparation of DNA damages was also significantly inhibited after combined treatment.

The data observed testify that synergistic enhancement of bactericidal activity after combined effect of irradiation and other physical or chemical factors may be connected with inhibition of the systems that exerted the DNA damages repair. The sterilizing dose of irradiation might be lowered using this method by 25-50%.

We think that future trends of the use of radiosterilization in medicine depends upon the new technology based on the use of combined effect of radiation and other physical and chemical factors.

Such a combined approach permits to broaden the area of radio-sterilization used in medicine and medical industry and may be of economical value due to the decrease of sterilizing doses.

New technology of sterilization should be developed in accordance with the latest results in study of the mechanisms of microorganism's radioresistance and of the ways of its modulation.

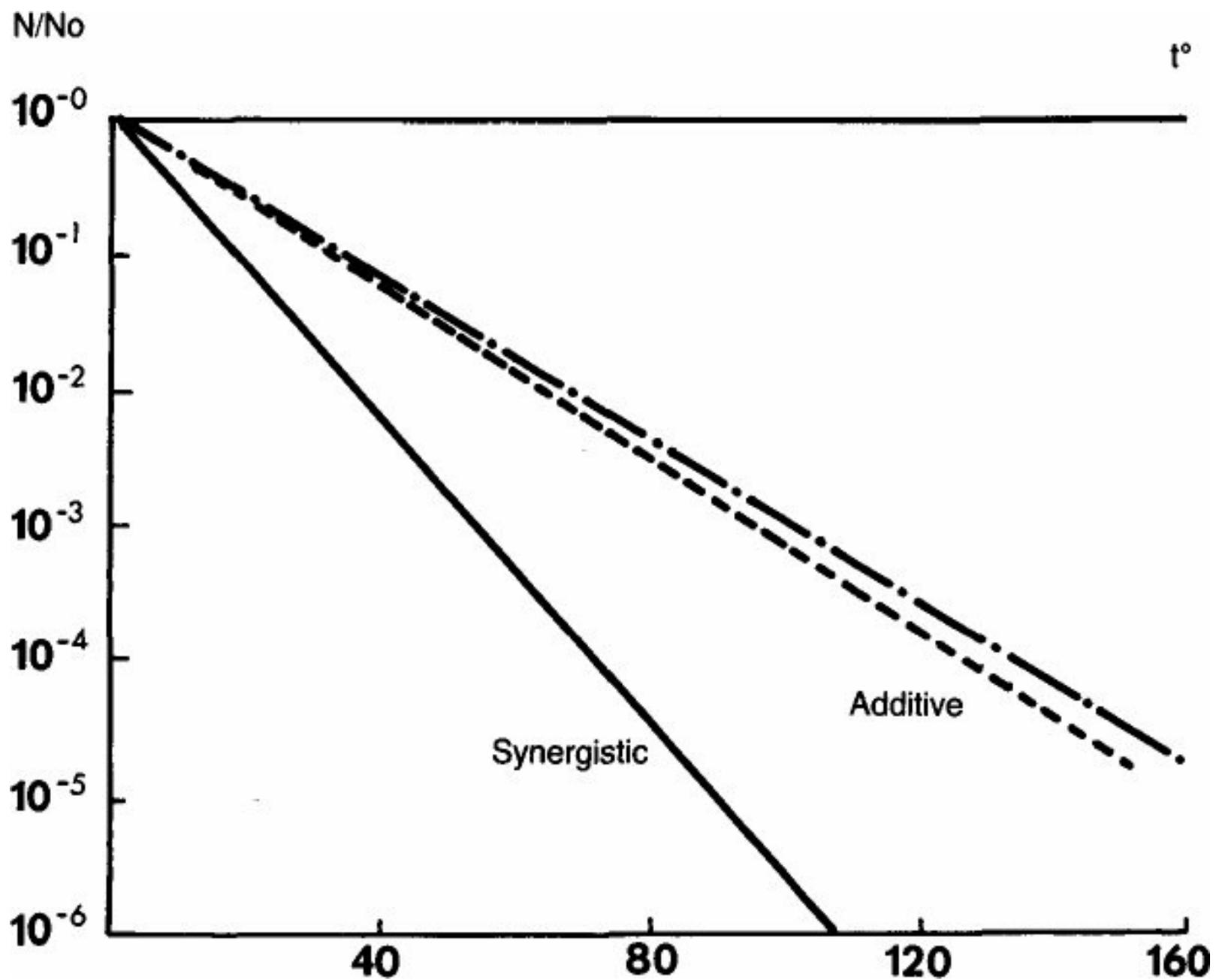


Figure 1. Survival of *B-subtilis* (spores) After Action of the  $t^\circ$  and  $\gamma$ -Rays.

Table I.  $D_{10}$  for the Microorganisms With Different Radioresistance Effect of  $t^\circ$  and Gamma Rays

Microorganisms	10 (kGy)	
	+ $t^\circ$	
<i>Staph. aureus</i> 1737	4.9	0.43
<i>E coli</i> K-12	0.16	0.47
<i>Sar. aurantica</i> 1	0.35	0.16
<i>Sar. lutea</i> 9341 (veg. form)	1.26	0.6

<i>Sar. lutea</i> (spores)	1.45	0.35
<i>Bac. subtilis</i> 7250 (veg. form)	1.63	0.76
<i>Bac. subtilis</i> (spores)	3.8	0.45
<i>Bac. sphaericus</i> C <sub>1</sub> A (spores)	6.79	5.15
<i>Str. faecium</i> A <sub>2</sub> 1	6.0	4.42
<i>M. radioproteoliticus</i> CCM-2703	2.0	1.56

**Table II. Single Strand Damages of DNA High Radioresistance Microorganisma After Radiarion and Thermoradiation**

M-O	Dose (kGy)				
	0.25	0.50	1.00	1.50	2.00
gamma <i>Str.faecium</i> A <sub>2</sub> 1	1.5	2.1	5.4	9.7	12.5
55 gamma	3.7	4.8	7.7	10.8	12.9
gamma <i>M.radiophilus</i> NCTC 10785	1.3	2.2	4.5	8.2	10.3
55 gamma	4.0	5.8	7.8	10.1	11.2
gamma <i>M. radioproteolyticus</i> CCM2703	0.8	1.6	4.0	6.2	9.2
55 gamma	4.1	6.0	8.3	9.8	10.7

**Table III. Combined t° Gamma Sterilization of Syringes**

Tdg	Dose of IR (kGy)				
	30.0	25.0	20.0	17.5	15.0
18°	$\frac{0}{100}$	$\frac{0}{100}$	$\frac{0}{100}$	$\frac{5}{100}$	$\frac{11}{100}$
55°-60°	$\frac{0}{100}$	$\frac{0}{100}$	$\frac{0}{100}$	$\frac{0}{100}$	$\frac{0}{150}$

Above: Number of nonsterile samples.

Below: Total number of samples.

**Table IV. Combined t° Gamma Sterilization of Cat Gut.**

t°	Dose of IR (kGy)				
	35.0	30.0	27.0	25.5	20.0

18°C	$\frac{0}{200}$	$\frac{2}{200}$	$\frac{18}{200}$	$\frac{21}{200}$	$\frac{35}{200}$
55°-60°	$\frac{0}{200}$	$\frac{0}{200}$	$\frac{0}{200}$	$\frac{0}{200}$	$\frac{0}{200}$

Above: Number of nonsterile samples.

Below: Total number of samples.

**Table V. Combined t° Gamma Sterilization of Systems for Blood Transfusion**

t°	Dose of Gamma IR (kGy)						
	35.0	30.0	25.0	20.0	17.5	15.0	10.0
42°-45°	$\frac{0}{50}$	$\frac{0}{50}$	$\frac{0}{100}$	$\frac{2}{100}$	$\frac{2}{100}$	$\frac{9}{100}$	$\frac{13}{100}$
55°-60°	$\frac{0}{50}$	$\frac{0}{50}$	$\frac{0}{50}$	$\frac{0}{200}$	$\frac{0}{500}$	$\frac{0}{200}$	$\frac{5}{100}$

Above: Number of nonsterile samples.

Below: Total number of samples.

**Table VI. Survival *Str. faecium* A<sub>2</sub>I After Three Hours Exposition in Magnetic Field Different Tension**

Condition of Gamma IR	Number of Survival Cells (%) After Dose (kGy)				
	0.1	0.25	0.50	0.75	1.0
Gamma rays rays	$3.85 \cdot 10^{-1}$	$6.81 \cdot 10^{-2}$	$5.10 \cdot 10^3$	$2.0 \cdot 10^4$	$6.23 \cdot 10^6$
Gamma rays MF 240E	$1.71 \cdot 20^{-1}$	$3.0 \cdot 10^{-2}$	$3.88 \cdot 10^4$	$3.98 \cdot 10^5$	$2.88 \cdot 10^6$
Gamma rays MF 750E	—	$2.3 \cdot 10^{-2}$	$2.0 \cdot 10^4$	—	—

**Table VII. Survival *Str. faecium* A<sub>2</sub>I After Three Hours Exposition in Magnetic Field With Different Tension**

Condition of Gamma IR	Number of Survival Cells (%) After kGy		
	2.0	3.0	5.0
Gamma rays	3.0	$1.2 \cdot 10^2$	$5.4 \cdot 10^4$

**Table VIII. Combined MF Gamma Sterilization of Catgut**

Condition of Gamma	35.0	30.0	25.0	20.0	15.0	10.0
Gamma rays	$\frac{0}{30}$	$\frac{4}{30}$	$\frac{5}{30}$	$\frac{7}{30}$	$\frac{8}{30}$	$\frac{11}{30}$
Gamma rays + NF 240E/18H	$\frac{0}{30}$	$\frac{0}{30}$	$\frac{0}{30}$	$\frac{0}{30}$	$\frac{0}{30}$	$\frac{2}{30}$

Above: Number of nonsterile samples.

Below: Total number of samples.

**Table IX. Reduction of Contamination After Combined MF Gamma Treatment and Sterilization of Renninmesenterine**

Condition of Gamma IR	Dose of Gamma IR (kGr)				
	5.0	10.0	20.0	25.1	50.0
Gamma rays	$1.5 \cdot 10^3$	$1. \text{pp} 10^3$	$2 \cdot 10^2$	$7 \cdot 10^1$	0
Gamma rays MF 750E/18h	$7.1 \cdot 10^2$	$6 \cdot 10^3$	$1.6 \cdot 10^2$	0	0

**Table X. Reduction of Contamination After Combined MF Gamma Treatment and Sterilization of Surgical Bandages with Immobilized Enzymes**

Condition of IR	5.0	10.0	15.0	20.0	25.0
Gamma Rays	$\frac{4}{10}$	$\frac{2}{10}$	$\frac{1}{10}$	$\frac{0}{10}$	$\frac{0}{10}$
Gamma rays + MF 750E/18h	$\frac{3}{20}$	$\frac{0}{20}$	$\frac{0}{20}$	$\frac{0}{10}$	$\frac{0}{10}$

Above: Number of sterile samples.

Below: Total number of samples.

# An Overview of Radiation Sterilization Technology\*

Jeffrey A. Beck

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Commercial radiation sterilization marks its 30th anniversary in 1986. Over the past three decades the disposable medical products market has undergone enormous growth, and with it, so too has the use of ionizing radiation as a sterilization method. Currently, 35-40% of disposable medical products manufactured in North America are radiation sterilized. Worldwide, nearly 140 gamma irradiators are being operated for a variety of purposes in over 40 countries, with a combined inventory of over 80 million curies.<sup>1</sup> Several electron accelerators are also being used to sterilize medical products in Europe and the United States, though presently they process only a small fraction of all radiation-sterilized products.

## History

Roentgen's discovery of X-rays in 1895 was the first of a series of revelations that fundamentally changed our view of the physical world and provided the tools for many modern analytic methods and process technologies. The discovery of radioactivity in 1896 and the electron in 1897 were further milestones that heralded the beginning of a 40-year period regarded by many as the 'golden age' of physics.

Although an attempt to investigate the effect of X-rays on microorganisms was made as early as 1896,<sup>2</sup> the lack of adequate sources of the new radiation made extensive studies of the phenomenon difficult, and industrial applications all but impossible. By the 1930's, however, more useful X-ray tubes had been developed, and studies revealed a logarithmic dose-inactivation relationship for microbiological systems exposed to ionizing radiation.

At the same time that the effects of radiation on microorganisms were being quantified, the first large-scale particle accelerators were being invented by Cock-croft and Walton, Van de Graaff, and Lawrence.<sup>3</sup> High-power microwave amplifiers called klystrons were invented in 1939 by the Varian brothers, and a high-energy proton linear accelerator based on design principles developed by R.Wideroe in 1929, was constructed by Luis Alvarez at the University of California, in 1946.

Linear accelerator technology received substantial impetus from radar-related research and development conducted during and after World War II. Ethicon, Inc., a Johnson & Johnson affiliate, began radiation sterilization research in collaboration with MIT in 1949 and pioneered in commercial radiation sterilization with a 2 MeV Van de Graaff electron accelerator in Somerville, New Jersey in 1956, and a 7 MeV microwave linear accelerator (linac) in 1957 (Figure 1).<sup>4</sup> Although it was an effective sterilizer, the Ethicon linac suffered from short klystron lifetimes and other hardware failures which significantly reduced usage and increased operating costs.<sup>5</sup>

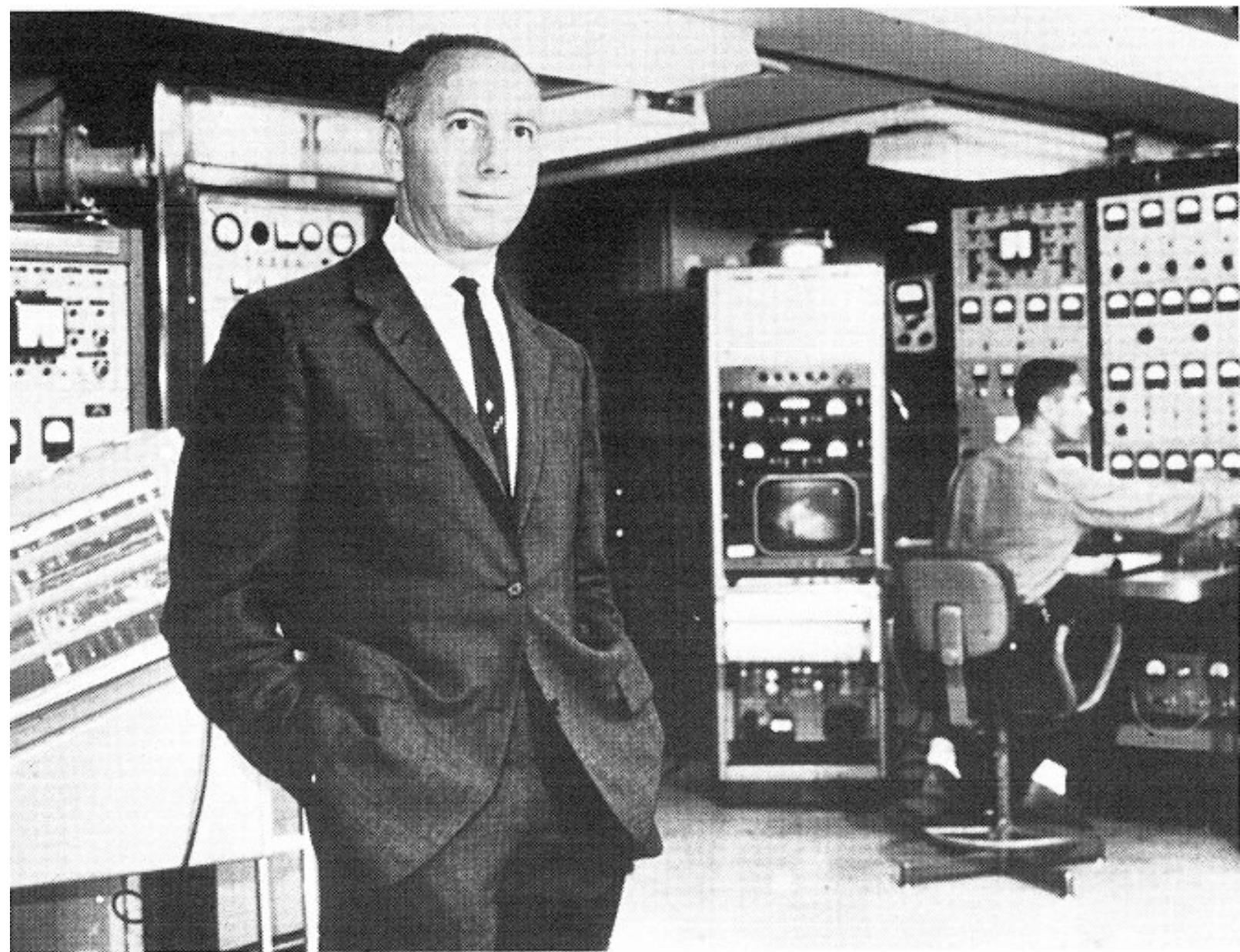


Figure 1. Dr. Charles Artandi in the Ethicon Linear Accelerator Control Room, 1957

The construction of nuclear reactors in the late 1940's and early 1950's made intense neutron sources available for basic research and isotope production. One of the most useful of the isotopes produced in these reactors was cobalt-60, a gamma emitter then used primarily in cancer therapy equipment. By the end of the decade, sufficient quantities of cobalt-60 had become available to permit the construction of large panoramic gamma irradiators for commercial radiation processing. The U.K. Atomic Energy Authority began

sterilization of medical products at Wantage in 1960, the same year that cobalt-60 was first used in Australia for the inactivation of *Bacillus anthracis* in goat hair.

Johnson & Johnson's first gamma irradiator was constructed by H. S. Marsh, Ltd. for Johnson's Ethical Plastics, Ltd. in Slough, England in 1962 (Figure 2).<sup>6</sup> A second Johnson & Johnson irradiator was built by Nuclear Chemical Plant, Ltd. for Ethicon, Ltd. in Edinburgh, Scotland the following year, and both of these plants are still operating full-time today. When Atomic Energy of Canada, Ltd. began producing cobalt-60 in quantities sufficient to support commercial processing in North America, Ethicon, Inc. switched from accelerated electrons to gamma radiation and in 1964 constructed irradiators in Somerville, New Jersey and San Angelo, Texas. Ethicon Sutures, Ltd. (Canada) also built an irradiator in Peterborough, Ontario the same year. Today, Johnson & Johnson operates 13 irradiation facilities in 9 countries, sterilizing over 6 million cubic feet of medical products annually.

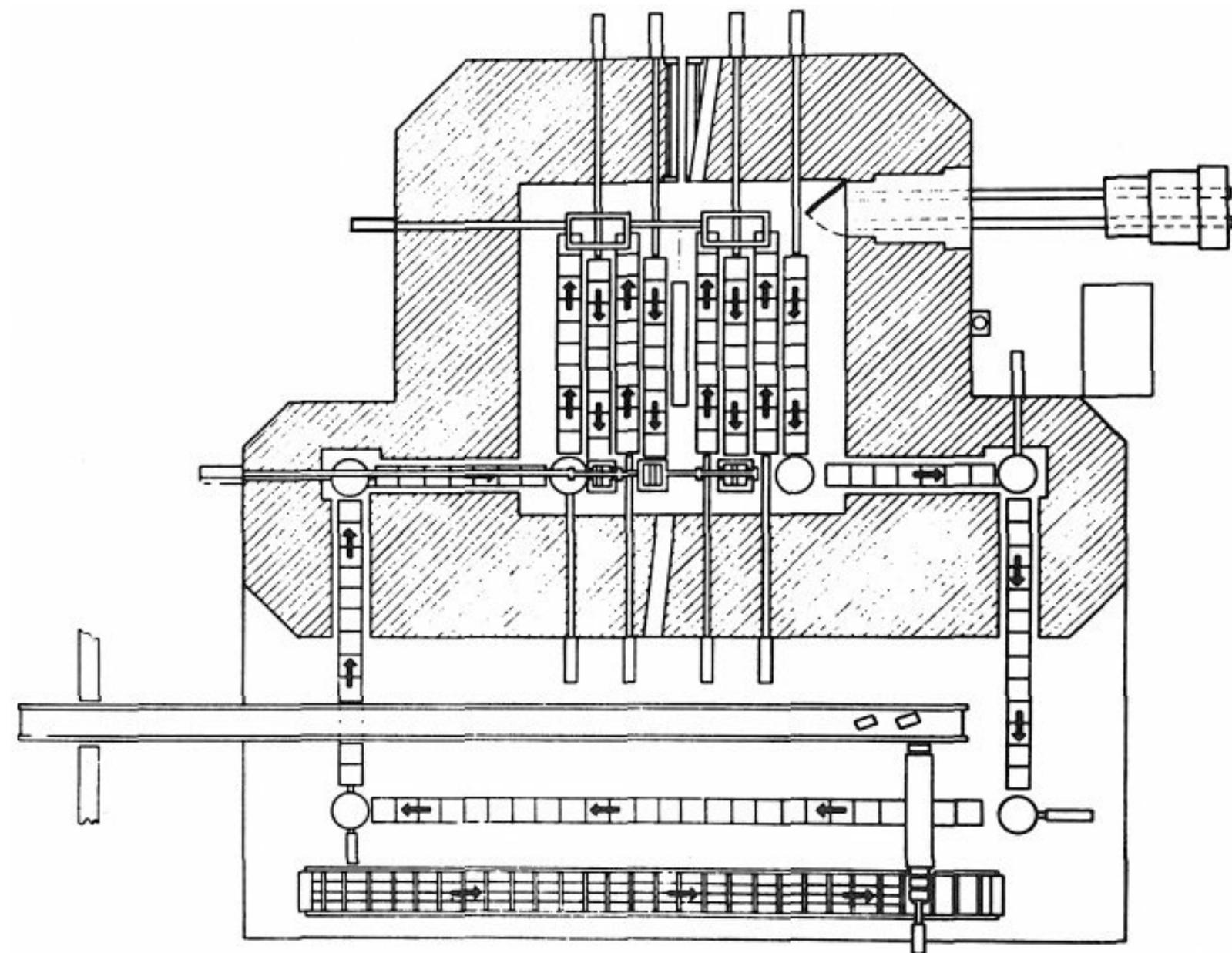


Figure 2. Johnson & Johnson's First Gamma Irradiator, Slough, England

# Types of Ionizing Radiation

Broadly categorized, all ionizing radiations can be classified into two types: electromagnetic and particulate radiation. Ionizing electromagnetic radiations include X and gamma rays, which are distinguished by their mode of origin.<sup>7</sup> Short wavelength ultraviolet radiation, although capable of producing ionization, is usually regarded as occupying a separate category.

X-rays originate either from atomic transitions, from the annihilation of matter in electron-positron collisions or from the deceleration of free electrons as they traverse matter. The latter phenomenon is known as 'bremsstrahlung', the German word for 'braking radiation.' Gamma rays originate from the transitions of atomic nuclei from excited to less energetic states.

There are many particulate radiations, both natural and man-made, including alpha and beta rays, high-energy electrons, positrons, protons, neutrons, ions of elements heavier than hydrogen, and a variety of mesons.<sup>8</sup> Naturally-occurring alpha and beta rays are not penetrating enough to make them useful for radiation processing. Beams of positrons, protons, neutrons, mesons and heavy ions, all man-made, are either too expensive to produce, limited in their ability to penetrate matter, or capable of inducing significant amounts of radioactivity in irradiated materials.

Energies of several million electron-volts (MeV) can be imparted to free electrons in relatively low-cost accelerators, however, and at these energies, electrons provide useful penetration without inducing significant radioactivity.<sup>9</sup> Electron beams of energies up to 12 MeV are currently in use for the sterilization of medical products in the U.S.

## Interactions of Radiation With Matter

Electromagnetic and particulate radiations differ in the way they interact with matter, though the biological effects of the interactions at energies used for sterilization are qualitatively quite similar. Radiation destroys microorganisms by breaking chemical bonds in biologically important molecules such as DNA, and by creating free radicals and reactive molecules which chemically attack the organism.

Ionizing electromagnetic radiation exhibits many particle-like properties. The particles, packets of energy called photons, have no mass or electric charge, and are thus capable of penetrating significant thicknesses of matter before their energy is completely deposited. Although there are 12 processes by which X or gamma rays can interact with matter,<sup>10</sup> only three need to be considered at the energies in use for sterilization: Compton scattering, the photoelectric effect and pair production.

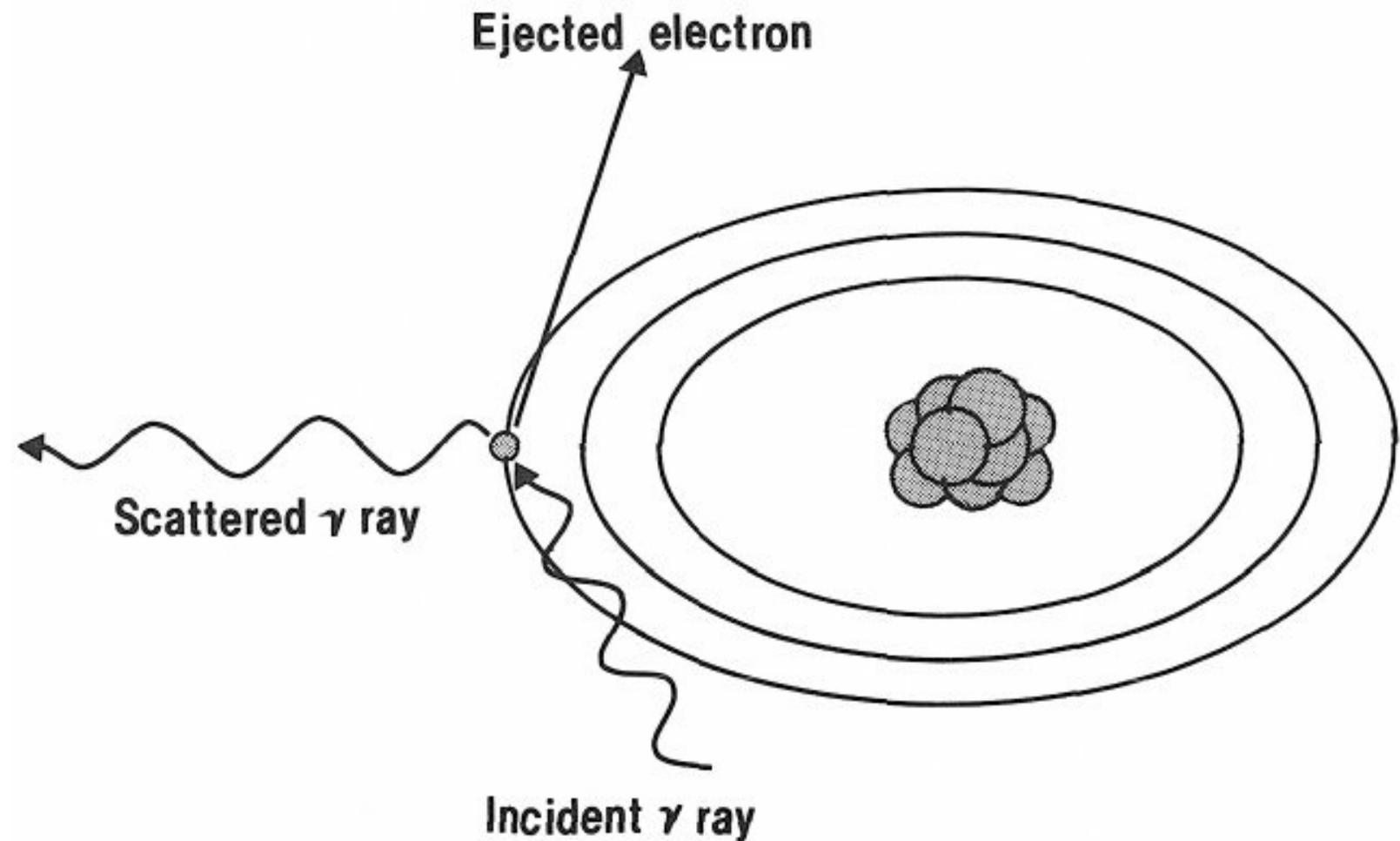


Figure 3. Compton Scattering

In the Compton scattering process (Figure 3), an incoming photon is deflected by a loosely bound atomic electron. The scattered photon and electron move off in different directions with the photon carrying less energy than it initially had. Almost all of the energy lost by the photon is transferred to the electron as energy of motion. Compton scattering is the predominant interaction for photons of energy greater than 0.1 MeV in the polymers and cellulosic materials found in most medical devices.<sup>7</sup>

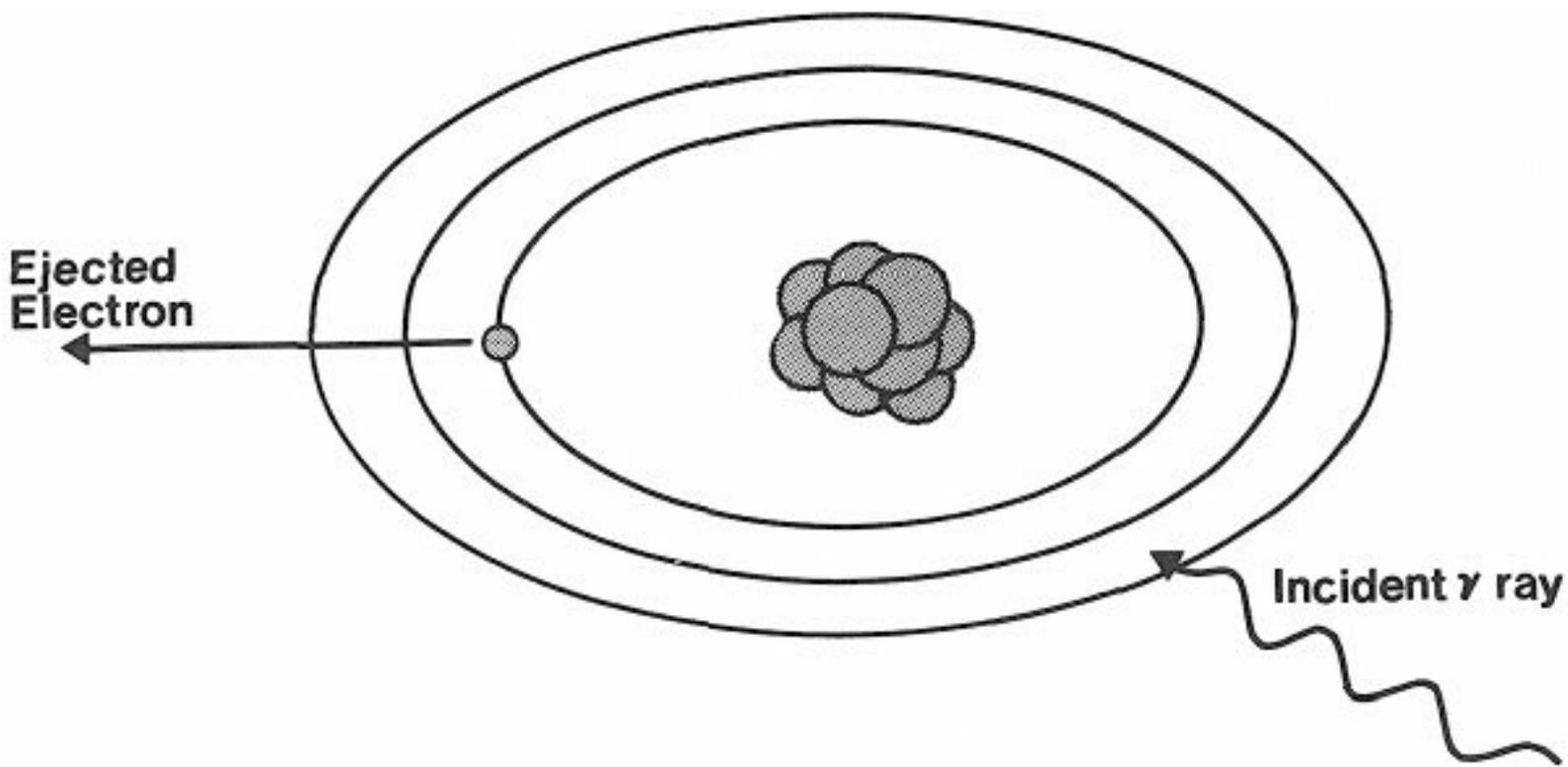


Figure 4. Photoelectric Effect

The photoelectric effect (Figure 4), which begins to become important at energies less than 0.1 MeV, differs from Compton scattering in that the energy of the entire photon is absorbed by an atom. The photon energy is transferred to an inner electron which is ejected from the atom.

Pair production can only occur at energies in excess of 1.02 MeV, and is a rare interaction for the gamma photons in use for radiation sterilization. It involves the complete transformation of a photon into an electron-positron pair.

Both X and gamma rays are attenuated exponentially as they pass through matter. The attenuation coefficient depends on the energy of the photons and the atomic composition and density of the matter through which they pass.

Electrons are small, negatively charged particles with a rest mass of  $9.1 \times 10^{-28}$  grams. Because they are charged, as electrons traverse matter they lose energy nearly continuously through collisions with other electrons, entire atoms, or by the generation of bremsstrahlung. Of these, the collision processes are the most significant, although bremsstrahlung begins to become important in high atomic number elements at energies of about 10 MeV.

The most common type of interaction, collisions with electrons, occurs when an incident electron passes a target atom at a distance on the order of atomic dimensions (Figure 5). The incident and atomic electron repel each other, with the atomic electron being given sufficient energy to eject it from the atom. The incident electron moves on in a slightly different direction carrying less energy than it initially had.

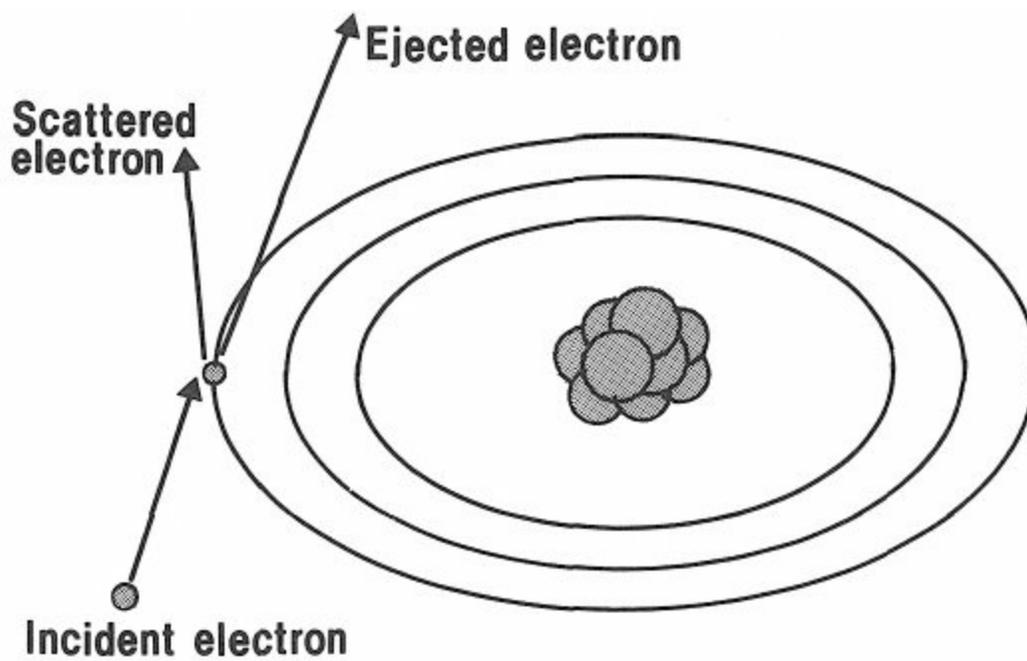


Figure 5. Electron Collisions

Collisions with atoms are very similar to electron collisions, except that the minimum distance between incident electron and target atom is much larger than atomic dimensions. Energy is transferred to the atom as a whole with the result being either excitation or ionization. These interactions are sometimes referred to as 'soft' collisions.

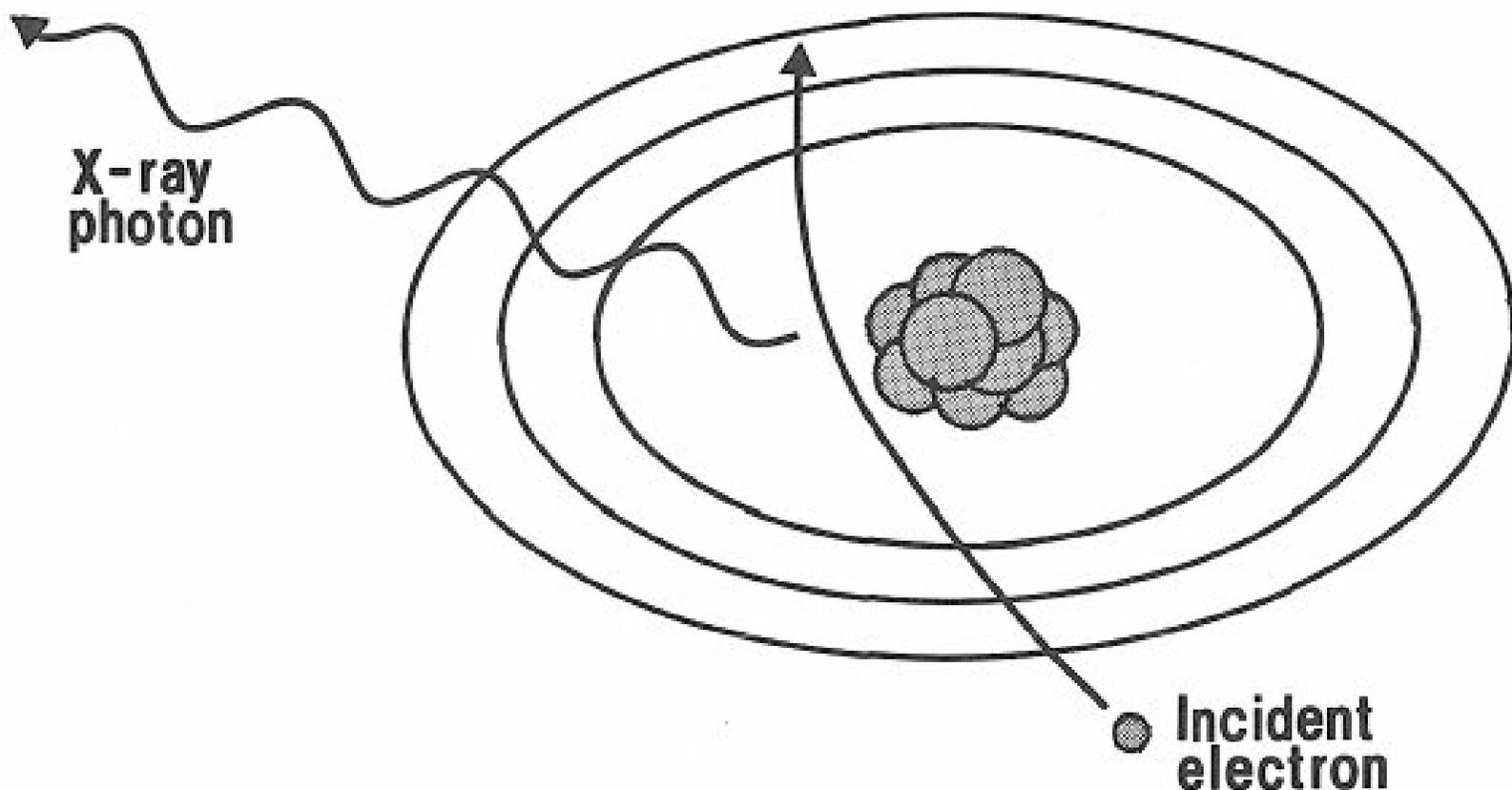


Figure 6. Bremsstrahlung

incident electron passes near an atomic nucleus (Figure 6). The electric field in the vicinity of the nucleus causes the path of the electron to curve, and the particle emits an X-ray photon. Bremsstrahlung accounts for more significant energy dissipation when high-energy electrons pass through heavy elements, because these elements have a correspondingly high nuclear charge.

The penetration of electrons through matter is more restricted than that of electromagnetic radiations of equivalent energy (Figure 7). At energies between 1 and 10 MeV, the maximum range of an electron is nearly proportional to its energy and inversely proportional to the density of the material through which it passes.

In materials irradiated from one side with electrons, the maximum dose is absorbed inside the target at about half the maximum electron range and then diminishes rapidly. In contrast, gamma and X-ray doses build up only slightly near the surface of irradiated materials and fall off gradually at greater depths. These characteristics have practical significance in irradiation facility design as they bear directly on the uniformity with which sterilizing doses can be delivered.

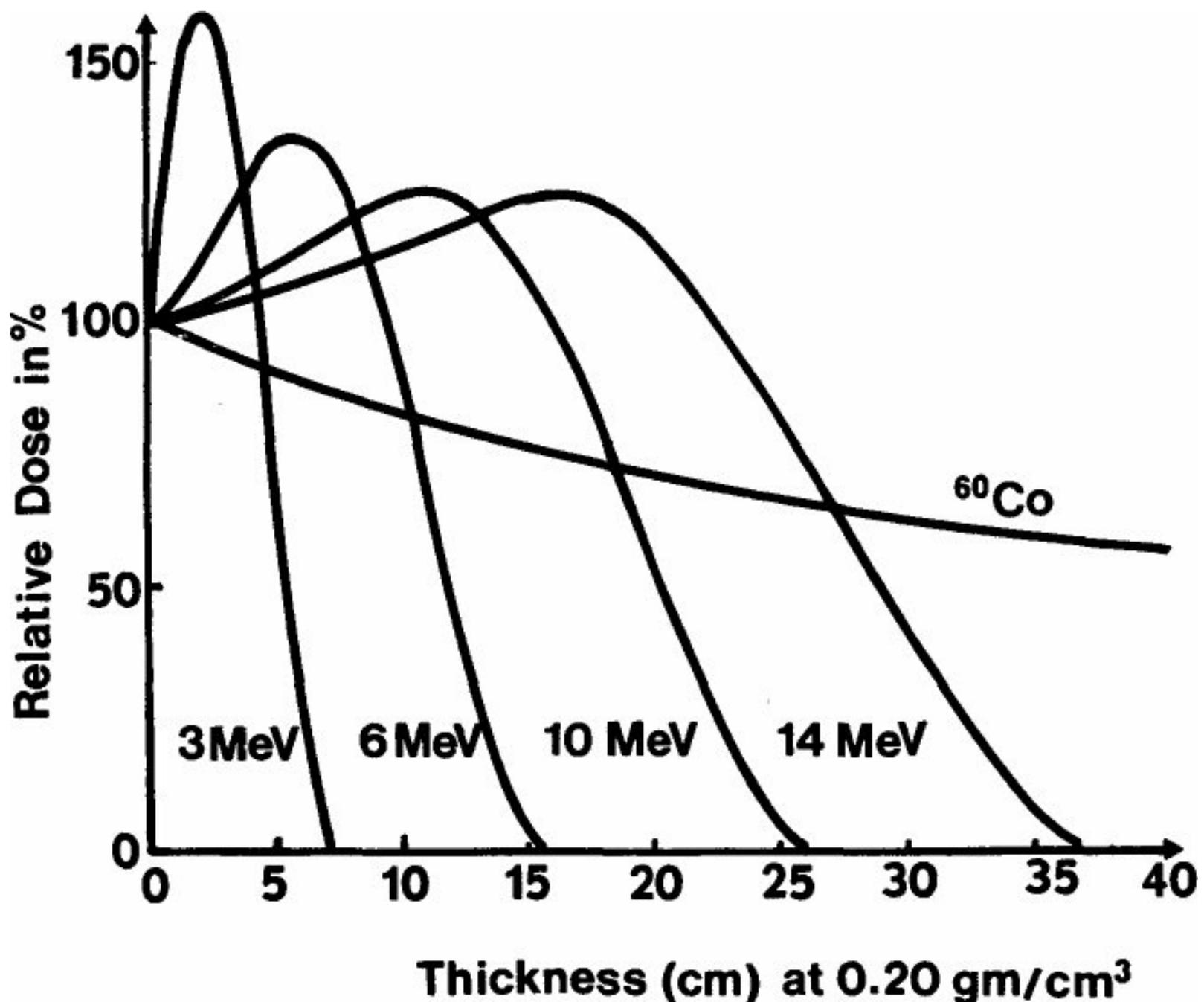


Figure 7. Depth-Dose Distributions: Electrons vs. <sup>60</sup>Co Gamma Radiation

## Irradiation Facilities and Equipment

Though the uses of radiation in a variety of applications have multiplied over the past 30 years,<sup>11</sup> the essential characteristics of irradiation facilities have not changed very much. Naturally, every facility must have a radiation source and a means of conveying material into and out of the radiation field in a controlled and reproducible manner. Each facility must also be equipped with adequate safety and process control systems, and radiation detection and measurement devices (Figure 8). Two types of radiation sources are currently being used for radiation sterilization, radioisotopes and electron accelerators.

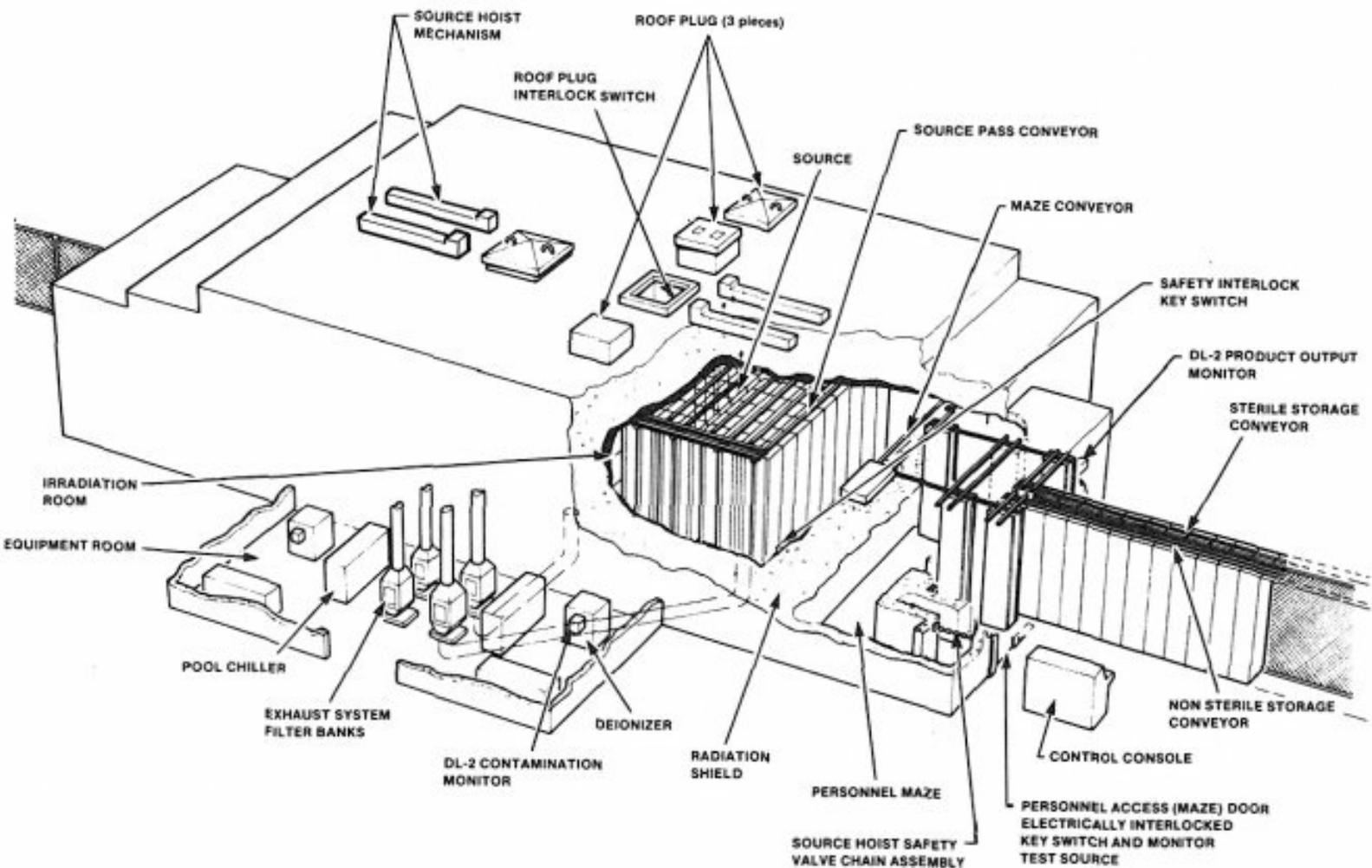


Figure 8. <sup>60</sup>Co Gamma Irradiator (Courtesy Atomic Energy of Canada)

# Isotopes

For an isotope to be useful for commercial radiation processing, it must be available in large quantities, deliver gamma radiation at energies high enough to penetrate product and packaging, and have a half-life long enough to maintain a reasonably steady processing rate for periods of six months to a year or more. Based on these criteria, the isotope of choice in industrial gamma plants is still cobalt-60, although cesium-137 is being used in large-scale irradiation facilities operated by Iotech Inc. in Northglenn, Colorado and by Radiation Sterilizers Inc. in Columbus, Ohio.

Cobalt-60 is a man-made radioisotope produced in nuclear reactors by irradiating cobalt-59 with neutrons. It is a by-product of the nuclear generation of electric power and is relatively inexpensive to produce. Although it is also made in reactors, cesium-137 is produced as a direct result of the fission of reactor fuel. It is therefore found in commercial quantities only in spent reactor fuel rods and must be harvested using expensive and potentially hazardous chemical separation techniques normally employed in reprocessing nuclear waste.

The largest available supplies of cesium-137 are currently held at the Hanford Waste

Encapsulation and Storage Facility (WESF) near Richland, Washington. The 70 million curies stored at Hanford have already been reserved by a number of radiation processing companies, and because long-term plans in the U.S. call for underground storage of spent fuel rather than reprocessing, additional supplies will probably not be forthcoming. For economic reasons, the incentive to reprocess spent fuel from Canadian reactors is very limited, and Canada has no significant stores of cesium-137.

<u>Isotope</u>	<u>Power Output (kW/MCi)</u>	<u>Photon Energy (MeV)</u>	<u>Half-Life (Years)</u>
$^{137}\text{Cs}$	3.34	0.66	30.23
$^{60}\text{Co}$	14.85	1.17, 1.33	5.26

Figure 9. Nuclear Characteristics of  $^{137}\text{Cs}$  and  $^{60}\text{Co}$

Aside from the difficulty of obtaining it, the nuclear characteristics of cesium-137 do not compare favorably with cobalt-60 (Figure 9).<sup>12</sup> Although the energy of its radiation is high enough to allow for sufficient product penetration, its gamma power output is quite low, and this deficiency is compounded by its current encapsulation in WESF capsules which internally absorb a significant portion of their own radiation (Figure 10).

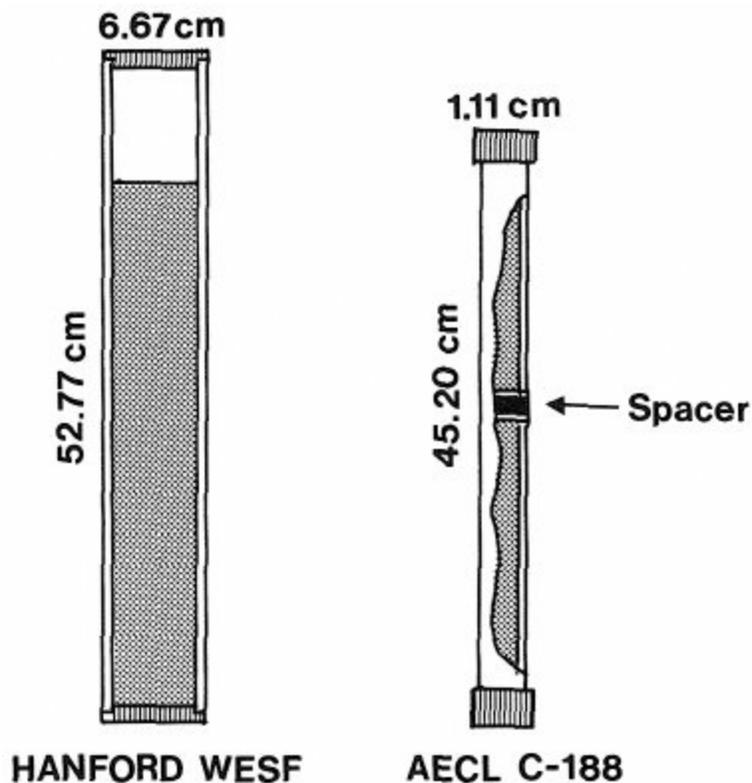


Figure 10. AECL Cobalt and WESF Cesium Encapsulations

A second potential problem has to do with storage of the isotope. Due to the chemical processing needed to separate it from spent reactor fuel, commercial quantities of cesium-137 currently exist only in the form of melt-cast cesium chloride, a compound freely soluble in water. Storage of cesium-137 for extended periods in water-filled pools is therefore somewhat risky, as failure of a source encapsulation could quickly lead to radioactive contamination of the water and internal pool surfaces. On the other hand, the solubility of cesium chloride would facilitate cleanup of contaminated areas, and dry source storage containers are also an option.

## Electron Accelerators

An alternative to the use of radioisotope sources is offered by electron accelerators. The beam of high-energy electrons is usually used to irradiate product directly, but can also generate penetrating X-rays. Accelerators can generally be classed as either pulsed-current or direct-current (dc) machines. Within these categories, however, there are such a variety of designs that a full discussion is clearly outside the scope of this paper.<sup>13</sup> To generate a useful beam of particles in either type of accelerator, free electrons must first be produced in vacuum, usually by a heated cathode. The electrons are then accelerated down an evacuated beam tube by applied electric fields and the beam is magnetically scanned over the width of the treatment area. The method of generating the accelerating electric fields is generally what distinguishes one accelerator design from another.

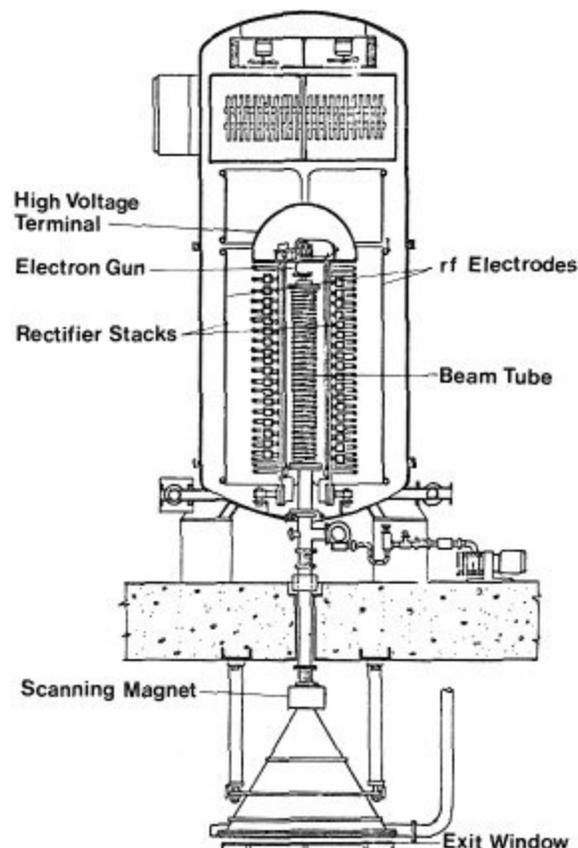


Figure 11. Radiation Dynamics Dynamitron™ Accelerator (Courtesy E-Beam Services)

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In the Dynamitron (Figure 11), a typical direct-current accelerator, energy is transferred

from a radio-frequency (rf) power supply to a high-voltage terminal through a series of rectifier stages capacitively coupled to rf electrodes. The static electric field produced by the high-voltage terminal accelerates the electrons.

Dynamitrons designed for industrial use have so far been limited to a maximum potential of 5 million volts. The chief reason for this is that in the Dynamitron, the full accelerating voltage is present between the high-voltage terminal and ground, and electrical discharges can occur if the voltage is too high.

An advantage of the Dynamitron design is that it is capable of generating beam power in excess of 150 kilowatts (kW), and therefore can provide very high product throughput. However, the need to operate at energies less than 5 MeV restricts applications either to relatively low-density materials or to limited thicknesses of high-density products when the accelerator is used for direct electron irradiation. At typical medical product densities, package thicknesses from 5-15 inches can be treated using two-sided processing.

Pulsed-current accelerators are perhaps best exemplified by microwave linear accelerators. In these machines, energy is transferred to free electrons by microwave radiation propagating down an evacuated beam tube (Figure 12). The microwaves are supplied in short pulses by a high-power klystron, the pulses serving to both bunch and accelerate the electrons. Although these machines operate at peak power levels in the megawatt range, their average power is usually limited to much less than 100 kW, as the time between pulses is very long compared to pulse duration.<sup>14</sup>

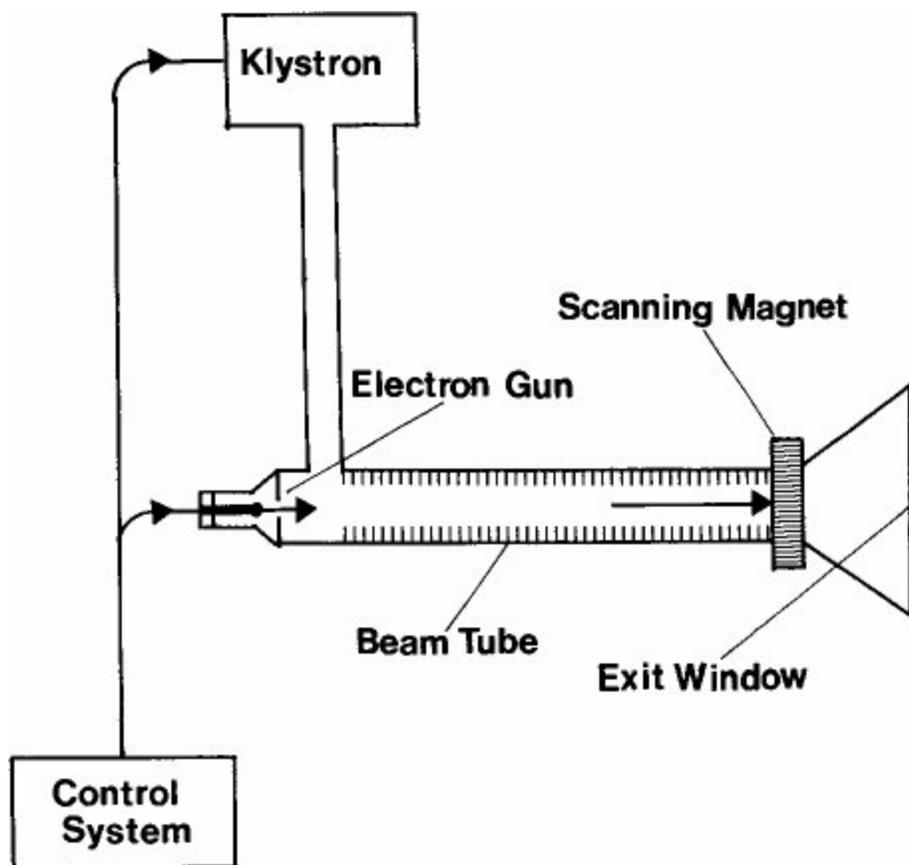


Figure 12. Microwave Linear Accelerator (Adapted from *Particle Accelerators and Their Uses*, Harwood Academic Publishers, New York)

together act as an efficient waveguide at the microwave operating frequency. Because the outside of the beam tube is always at dc ground, electrical discharges cannot occur, and high energies are achievable with very compact machines. The accelerating section of a typical industrial linac operating at 10 MeV, for example, is about 6 feet long.

The high electron energies available through linac technology make it possible to deliver reasonably uniform doses to a range of package thicknesses from 10-30 inches at typical medical product densities. Their application to sterilization is somewhat limited, however, by the relatively high capital cost of currently available machines.

The performance of gamma or electron beam irradiators is highly dependent on the details of the application as well as factors such as the design and operational reliability of associated material handling systems. Technical and economic comparisons of the two systems can be misleading if applied too generally, however, there are some inherent differences in the characteristics of each type of radiation source which can be identified.

Due to the nature of photon interactions and the inherently reliable output of isotopes, gamma irradiators have clear advantages in terms of product penetration and technical simplicity. Electron accelerators, on the other hand, offer considerable flexibility in terms of their ability to process products of widely different densities and dose requirements in rapid succession. Additionally, the high dose rates associated with electron irradiation tend to minimize material effects, and although some costly hardware components require periodic replacement, the full processing capacity of an accelerator is always available, without the need for source replenishment. As cobalt-60 prices have risen over the past five years, the comparative operating costs of electron beam processing have improved dramatically.

## **Process Control**

The fact that there are fewer variables to control in radiation sterilization than in other sterilization processes remains one of the major advantages of the method. Nevertheless, achieving consistent results with radiation depends just as critically on the adoption of adequate process control and validation techniques as it does with other sterilization processes. To varying degrees, the parameters that affect dose delivery in all irradiation facilities are the radiation energy and power output of the source, irradiation geometry, product density and composition, and exposure time. Fundamental differences in the characteristics of gamma irradiators and accelerators require that different systems and procedures be adopted to control these variables in each type of facility.

## **Radiation Energy and Power Output**

One of the chief advantages of radioisotope sources is that they radiate at fixed energies and at power levels that vary with time according to well-known physical laws. Energy production is continuous and unaffected by environmental factors.

In practice, however, the output of industrial sources can be affected by factors such as the physical dimensions of individual source elements, the distribution of activity within each

source element, and the number and distribution of elements within the source rack.<sup>15</sup> For this reason, gamma irradiators are commissioned after installation, both to ensure the adequacy of the biological shield, and to determine the actual throughput or processing capacity of the plant. The establishment of an appropriate exposure time must also be experimentally validated after routine source replenishments, even if other factors influencing dose absorption have not changed. Irradiators are sometimes recommissioned after major changes in the total source activity or after the spatial distribution of source elements is changed significantly.

Unlike gamma sources, the radiation energy and power output of accelerators has to be continuously controlled. In addition, when the beam is scanned over a treatment area, the scan amplitude and frequency must also be monitored. In pulsed-current accelerators, the peak power output can differ from the average power by several orders of magnitude, and additional parameters such as peak rf power, total charge per pulse, and pulse repetition rate should also be monitored.

## **Irradiation Geometry**

In addition to establishing the distribution and intensity of radiant energy in gamma and electron irradiators, it is also necessary to control the relative positions of the radiation source and product during processing in both types of plant. In gamma irradiators this means ensuring that the vertical position of the source rack is reproducible from cycle to cycle, that the dwell positions of irradiation containers (totes or carriers) are fixed, and that product loading patterns are unambiguously defined and adequately constrained within irradiation containers. In most irradiators, the vertical position of the source rack during irradiation is controlled by limit switches on the hoist guide cables. It is important that these switches be accurately repositioned when the cables require replacement.

Loading patterns are normally selected so the positions of product cartons are physically constrained by the sides of the irradiation container. When this is not possible, filler material is usually added to the containers to prevent cartons from shifting during the processing cycle, as this would affect dose absorption.

In electron irradiation facilities, the location of the source, the accelerator, is effectively fixed. Surface dose and depth dose distributions are affected however, by the distance at which the irradiation container (in this case either a single product unit or a shipping carton) passes by the machine's exit window, as the beam suffers small energy losses in passing through air. In some accelerators, positioning of the irradiation container with respect to the length of the scanned treatment area must also be consistently reproduced, as depth-dose distributions are influenced by the incidence of electrons on the product at oblique angles.

Because dose distributions in products irradiated with electrons are significantly affected by local density inhomogeneities, for most products the orientation of irradiation containers has to be specified to ensure consistent dose delivery. The significance of product orientation can usually only be determined by experiment under actual production irradiation conditions.

# Product Density and Composition

The dose absorbed by a sample placed in a known radiation field depends on the exposure rate at the location of the sample, and the density and composition of the absorbing material. For the non-metallic materials that make up many disposable medical products, differences in atomic composition are slight, and their effects on dose absorption can usually be ignored. Monitoring the bulk density of irradiated products, however, is an integral part of process control in all radiation sterilization facilities.

To maximize the absorption of radiant energy in gamma plants, irradiation containers are usually arranged in several rows on either side of the source rack (see Figure 8). Under these conditions, the dose absorbed by the product while it occupies an irradiation container in an outside row is influenced not only by the density of the product itself, but by the densities of other products between it and the source. The impact of shielding on dose delivery can be significant, and should be quantified in each irradiator. Often, maintenance of adequate process control in these plants requires that limits be placed on the range over which the densities of products occupying the irradiator simultaneously are allowed to vary.

Due to the limited penetration of electrons, only single rows of irradiation containers are processed at a given time in accelerator plants, and the scattering of electrons from adjacent products normally has only a slight effect on absorbed dose distributions.

## Exposure Time

The variable that has the greatest effect on absorbed dose is exposure time. In gamma irradiators operating in a shuffle-dwell mode, exposure is controlled by automatically indexing each irradiation container to the next dwell station after a pre-selected time interval has elapsed. An overdose safety timer is usually provided to ensure that product is not over-irradiated through failure of the master timer, and both timers are included in a regular calibration program.

In electron beam processing plants, irradiation containers are carried through the beam either on a continuously-moving conveyor or cart system. Accurate control of the conveyor speed is essential as the total dose in these plants is delivered very rapidly. In some plants, the conveyor speed is 'slaved' to the beam current so fluctuations in the power output of the accelerator can be compensated for by increasing the exposure time.

## Dosimetry

In most countries using radiation to sterilize, the concept of dosimetric release of sterile medical products has gained wide acceptance since its introduction in the mid-1970's. Dosimetric release is the certification of sterility based on measurement of the minimum dose absorbed by the product. Absorbed radiation doses have traditionally been reported in rads (100 ergs/gm), however between 1974 and 1977, a new set of radiation units were recommended by the International Commission on Radiation Units and Measurements and

the International Commission on Radiological Protection, to reconcile radiation measurements with the International System of Units (SI). The new unit of absorbed dose is the gray, which is equal to 100 rads or 1 joule/kg.

Radiation dosimetry is a complex topic that bears directly on the reliability and efficacy of irradiation processes. Full treatment of this topic would require much more space than can be devoted to it here, and has largely been accomplished in several excellent International Atomic Energy Agency publications.<sup>16,17</sup> Three classes of dosimetry systems are generally recognized in industrial processing: absolute, reference, and routine.

Absolute systems are calorimeters or ionization chambers. They measure the energy deposited in a known sample by measuring the associated temperature rise, or they measure the degree of ionization produced in a gas exposed to the radiation field. Calorimeters are of very limited use in gamma irradiators due to the problem of maintaining thermal insulation over the long radiation exposure times. In accelerators they are useful as a calibration device because irradiation times are short enough to prevent significant heat loss.

Reference dosimetry systems include the Fricke, ceric sulphate, and potassium dichromate liquid chemical dosimeters and alanine solid-state dosimeters. Reference systems are those in which dose is measured by the effects of chemical changes in a medium that exhibits a uniform response ( $\pm 3\%$ ) over a range of environmental conditions and dose rates. They are usually of use in commissioning gamma irradiators, but have not been widely employed in accelerator plants due to the availability of calorimeters.



Figure 13. Routine Process-Monitoring Dosimeters

Routine dosimetry systems are so designated because they are easy to use and relatively inexpensive. Examples of these systems are dyed and clear polymethyl methacrylate, and radiochromic dyed films and optical fibers (Figure 13). Routine dosimeters are generally more variable in response ( $\pm 5\%$ ) than reference dosimeters, and

more sensitive to dose rate and temperature variations, however, their ruggedness, availability and low cost make them the systems most often chosen for process monitoring.

## Future Trends

The recent focus on potentially expanding applications of irradiation in the food industry, coupled with temporary shortfalls in cobalt-60 supply, the rising price of the isotope, and public concerns about the transport and proliferation of radioactive materials, are leading current radiation users to consider electron accelerators as an alternative to isotope sources. Economic studies have suggested that for facilities processing more than 1 million cubic feet of products annually, high-energy electrons might be more cost-effective than gamma radiation, assuming the cost of cobalt-60 remains at or above present levels.<sup>18</sup>

Partly because few manufacturers have the need for such high production capacity at a single location, and because accelerators are sophisticated machines that require a higher degree of technical expertise to operate and maintain than do gamma irradiators, few health care companies have thus far been willing to adopt this technology.

Another factor that inhibits the widespread use of accelerators for sterilization is that when electrons are used for bulk processing, the absorption of dose by all parts of a complex product is more sensitive to local variations in density than it is with gamma or X-ray photons. Thus, for all but the most homogeneous products, validation of electron beam sterilization processes requires more careful study of dose distributions than it does in gamma irradiators. Despite this drawback, accelerators still offer the advantages of fast turnaround time, flexibility, and the only industrial source of ionizing radiation as potentially limitless as electric power.

A potential solution to concerns about the dose uniformity achievable with electron irradiation are machines that use the beam to produce high-energy X-rays. Modern cancer therapy clinics use equipment of this type extensively and scale-up to industrially useful machines is not technically difficult.

The major shortcoming of this method is poor efficiency. Using 5 MeV electrons, the theoretical conversion of electron beam to X-ray power is only about 7%.<sup>19</sup> The spectrum of X-rays produced has penetrating characteristics roughly equivalent to cobalt-60 gamma radiation; however, given the best power efficiencies achievable with modern accelerators (about 70%), overall system power conversion is presently too low to give these machines a clear economic advantage over isotopes.

Electron beam to X-ray power conversion improves with increasing electron energy, reaching 16% at 10 MeV, but the production of X-rays with energies in excess of 5 MeV brings with it increased risk of inducing radioactivity in some irradiated products. Although the quantities of induced radioactivity have been shown to be extremely low,<sup>20</sup> the technical, regulatory, and consumer acceptance issues raised by this problem will undoubtedly require years or perhaps even decades to resolve. Nevertheless, a combination of improvements in accelerator technology, and changes in regulatory and consumer outlook might eventually allow higher energies to be used for sterilization and make X-rays a more attractive option

in the 1990's.

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# Sterilization by Filtration

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Filtration, which removes microorganisms physically by sieving or by adsorption on the filter media, is a common method of sterilization. If a fluid is heat labile, it is the only practical way of sterilization.

## Filter Classes

Many kinds of materials have been employed as filter media, and all filter media can be categorized as either depth or screen types. Each offers advantages and meets certain limitations. A depth filter gets its name from the fact that filtration occurs mainly within the depth of the filter matrix itself, by mechanical entrapment or random adsorption. Most filter media belongs to this type.

Screen type filters, on the other hand, are so named because they retain particles or microorganisms on their surface by physically screening them from a liquid or gas. Membrane filters are of this type, and their structure is normally rigid and uniform. The size of pore openings of membranes is determined during the manufacturing process.

**Table I. Typical Filter Media Used for Sterilization Filters.**

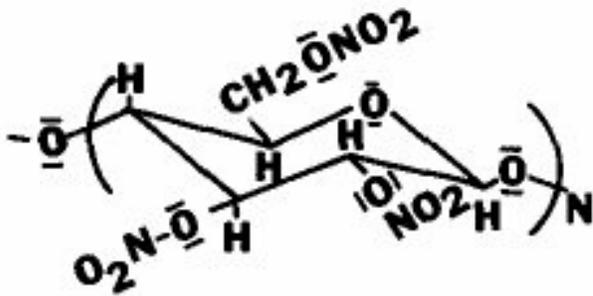
Depth Type		Screen Type	
Name	Materials	Name	Materials
Seitz	Asbestos	Membrane	Cellulose Nitrate
Chamberland	Quartz Sand		Cellulose Acetate
	Kaolin		PVDF
Berkefeld	Celite		PTFE
	Asbestos		Nylon 66
		Polysulfone	

Typical filter media used for sterilizing-grade filters are listed in Table I.

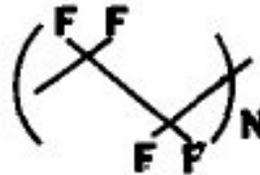
In sterilizing filtration, usually 0.2µm or 0.22µm rated membrane filters are used. Filter media of membrane filters for this purpose include cellulose acetate, cellulose nitrate, polyvinylidene fluoride, polytetrafluoroethylene, nylon 66 and polysulfone (Table II). Before membrane filters came into use, mixtures of quartz and kaolin or fibrous asbestos filters were the main filter elements for sterilization of liquids.

**Table II. Materials of Typical Membrane Filters Used for Sterilization of a Liquid.**

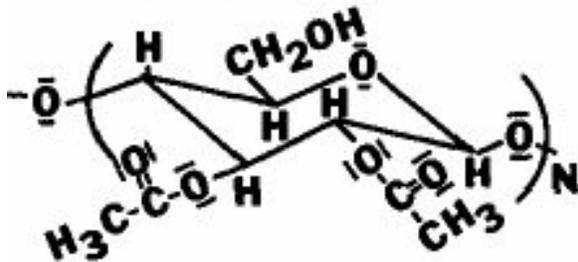
**CELLULOSE NITRATE**



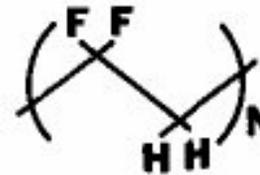
**POLYTETRAFLUORO ETHYLENE (PTFE)**



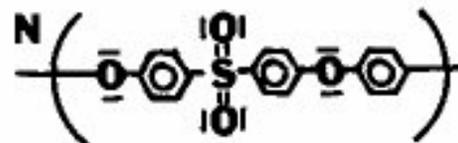
**CELLULOSE ACETATE**



**POLYVINYLIDENE DIFLUORIDE (PVDF)**



**POLYSULFONE**



**Advantages of Membrane Filters**

Due to their random and fragmented construction, depth filters will tend to slough off fragments of medium in the event of hydraulic shock. These can then migrate into the effluent. Because of this, fiber-releasing filters, especially those containing asbestos, are strictly prohibited as a final filter step in the production of injectable drugs intended for human use.<sup>1</sup> Membrane filters are now widely used in parenteral drug application in part because they do not exhibit media shedding and its subsequent migration.

Membrane filter has large open space so that it offers high flow rate. Another advantage of a membrane filter is its very low hold-up volume. Due to its thin film-like structure a minimum amount of product loss will be realized.

One disadvantage of a membrane filter is that it can become quickly clogged if the fluid

being filtered is heavily contaminated. Appropriate prefiltration steps should be implemented in order to avoid this.

## Filter Combinations

A depth filter has high dirt holding capacity, and is particularly efficient at removing large numbers of particles. Therefore it is essential in some applications to place a depth filter upstream of the membrane filter to prolong the life of the final filter.

Relatively coarse prefilters do not provide enough retention efficiency to protect downstream membrane filters. (Fig. 1) A prefilter must remove particles that approximate the pore size rating of the membrane filter. Hence, the retention efficiency requirement of the prefilter is determined by the pore size rating of the downstream membrane filter.

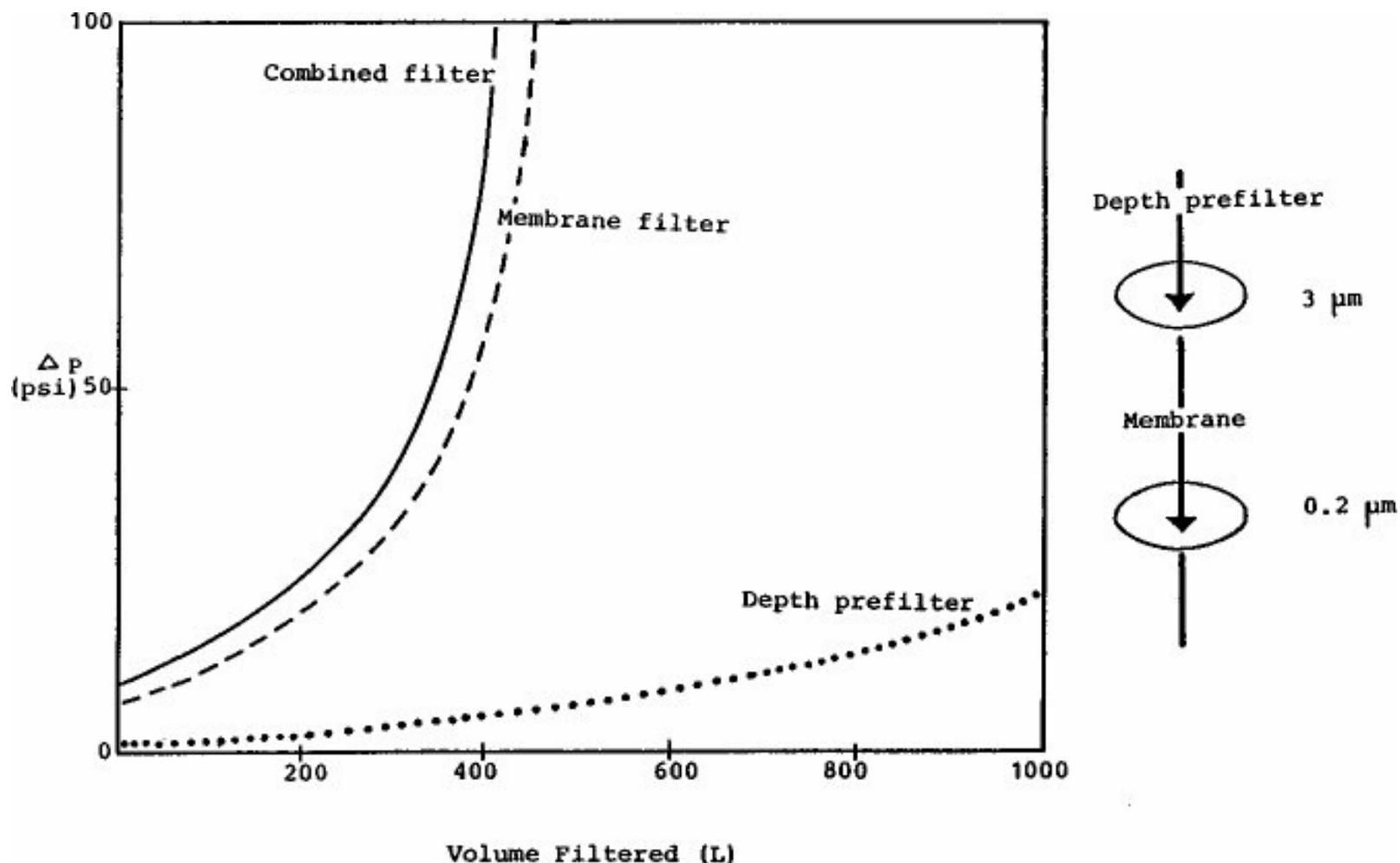


Figure 1. Membrane Filter Protected by Coarse Depth Prefilter.

The retention efficiency of a filter is of primary importance but if adequate surface area is not available high pressures will be required to produce sufficient flow rates. (Fig. 2). Therefore, suitable prefilters must have not only the proper retention efficiencies but also optimum filter areas. (Fig. 3)

In sterile filtration, vacuum filtration is not recommended. Positive pressure is preferred because 1) it prevents leakage into the filtrate, therefore avoiding contamination, 2) it offers higher flow rates and 3) it allows non-destructive testing of filter integrity.<sup>2</sup>

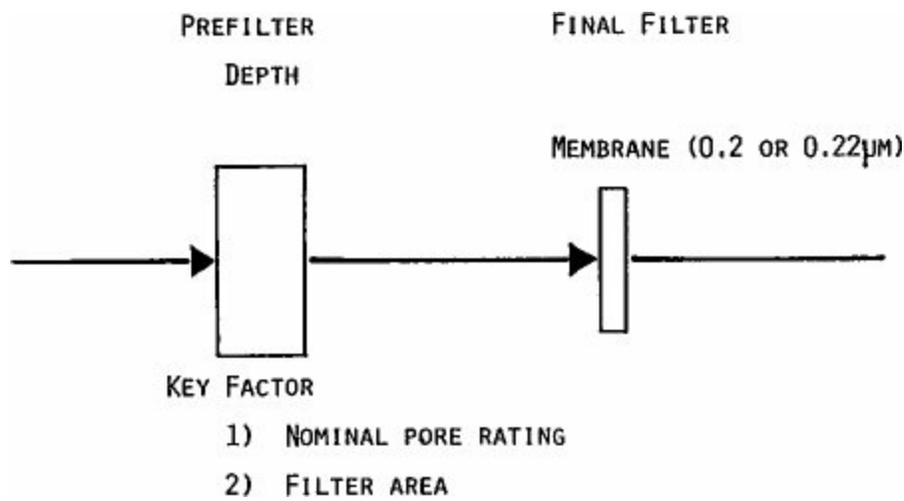


Figure 2. Filter Combination Key Factor.

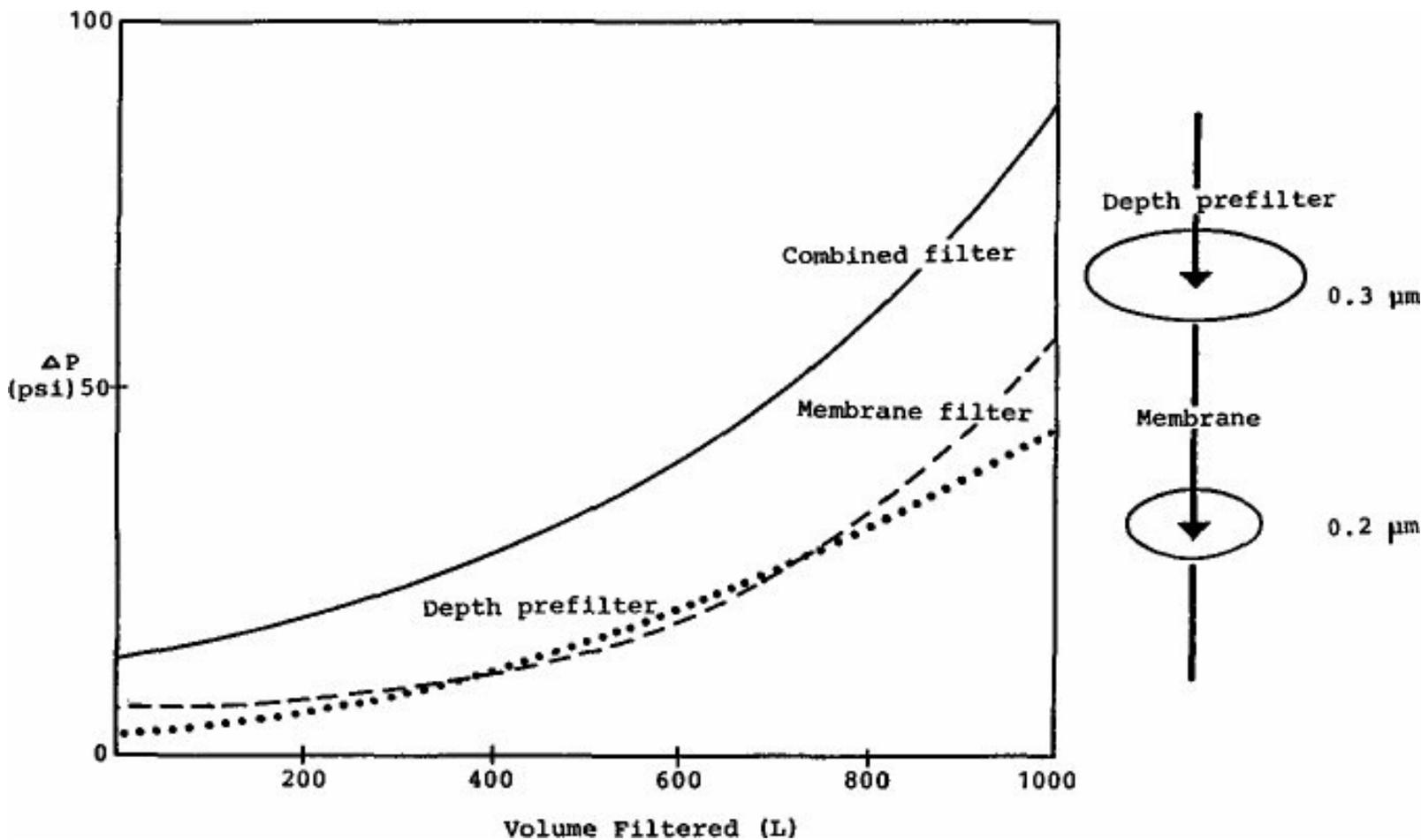


Figure 3. Membrane Filter Protected by Depth Prefilter with Adequate dirt-holding Capacity and Optimum Filter Area.

## Several Factors to affect Filter Performance

The performance of sterile filtration is affected by several factors such as flow rate, trans-membrane pressure drop, pH and viscosity of a fluid, and chemical compatibility with the filter material. (Fig. 4)

The effectiveness of sterile filtration is also influenced by the microbial burden of the solution to be filtered.<sup>3</sup> Therefore, maintaining the number of microorganisms in the solution to be filtered as low as possible is important in assuring sterility of the filtrate.<sup>4</sup>

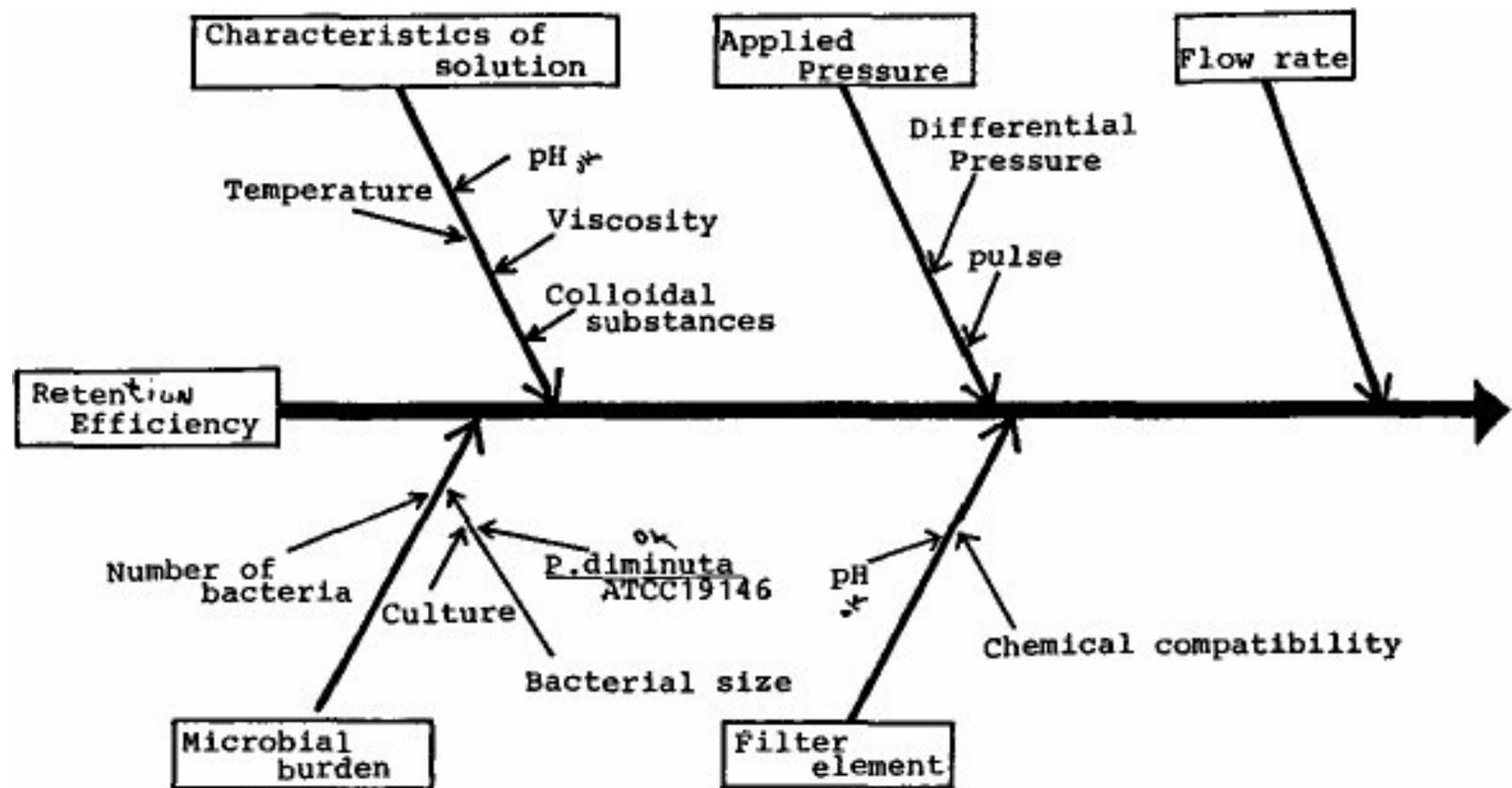


Figure 4. Factors to Affect the Filter Retention Efficiency.

## Bacterial Challenge Test

The ultimate test to validate the microbial retention efficiency of a sterilizing filter is the bacterial challenge test. This test is very sensitive. The level of the sensitivity depends primarily on the choices of test organisms, the method of cultivation and the level of microbial burden.

In the bacterial challenge test, sterilizing-grade membranes are usually challenged with a solution of culture medium containing *Pseudomonas diminuta* ATCC19146 in the range of  $10^7$  per  $\text{cm}^3$ .<sup>5</sup>

Leahy and Sullivan reported some of the important aspects of retention testing with respect to the growth characteristics of *Pseudomonas diminuta*. According to their study, under properly controlled conditions, cells will be arranged in a mono-layered configuration and will be small enough for a severe bacterial challenge test.<sup>6</sup> In their study, test organisms were incubated in a saline lactose broth (SLB) without agitation at  $30^\circ\text{C}$  for 24 hours. SLB is essentially a dilute solution of saline to which are added limiting sources of carbon and nitrogen. (Table III)

This way of cultivation with fewer nutrients results in cells which are single and smaller than those grown in richer tripticase soy broth (TSB). This can be seen in gram stained

preparation and in electron micrographs. A challenge solution of single and small cell organisms is preferred in a microbial validation of a sterilizing grade filter. Multiple-cell clusters may occlude oversized pores producing a false negative result.<sup>4</sup>

**Table III. Saline Lactose Broth Formula.\***

---

Sodium Chloride	7.6 g
Lactose Broth**	30 ml
Water***	970 ml
Final pH 6.9 ± 0.1	

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\* HIMA

\*\* 1.3 g Dehydrated Lactose Broth (BBL 11333) per 100 ml of Water\*\*\*.

\*\*\* Type III Reagent Grade Water, ANSI/ASTM D1193-77

## **Effect of Pressure Drop on Retention Efficiency**

Microbial retention efficiency as function of bubble point and transmembrane pressure drop has been studied by Reti. (Table IV)<sup>7</sup> Reti considers that transmembrane pressure drop is one of the important factors affecting retention efficiencies.

In Table IV, the microbial retention efficiency is expressed by the term of  $\beta$  ratio or the logarithm to the base 10 of  $\beta$  ratio. This term is synonymous with log reduction value (LRV) which is adopted by Health Industry Manufacturers Association (HIMA) as the standard term of expressing microbial retention efficiencies by filters.<sup>5</sup> Both  $\beta$  ratio and LRV are specific number for the used filter element.

**Table IV. Microbial Retention Efficiency as Function of Bubble Point and Transmembrane Pressure Drop.**

Bubble point (Bars)	Transmembrane Pressure Drop (Bar)	Nominal volume Flux ml/cm <sup>2</sup> min	Initial Challenge	Passage	$\beta = \frac{\text{Challenge}}{\text{Passage}}$	Log <sub>10</sub> $\beta$
1.2	0.034	6.0	5.7x10 <sup>10</sup>	2.3x10 <sup>8</sup>	2.5x10 <sup>2</sup>	2.40
1.2	0.034	6.0	6.7x10 <sup>10</sup>	1.1x10 <sup>8</sup>	6.1x10 <sup>2</sup>	2.79
1.3	0.034	6.0	5.7x10 <sup>10</sup>	2.3x10 <sup>8</sup>	2.5x10 <sup>2</sup>	2.40
2.3	0.034	2.0	5.6x10 <sup>10</sup>	2.6x10 <sup>2</sup>	2.2x10 <sup>8</sup>	8.34
2.3	0.034	2.0	4.7x10 <sup>10</sup>	1.0x10 <sup>3</sup>	4.7x10 <sup>7</sup>	7.67
2.3	0.034	2.0	5.4x10 <sup>10</sup>	2.4x10 <sup>3</sup>	2.3x10 <sup>7</sup>	7.36
3.6	0.034	0.80	3.5x10 <sup>10</sup>	0	>10 <sup>10</sup>	>10.54
3.6	0.034	0.80	3.6x10 <sup>10</sup>	0	>10 <sup>10</sup>	>10.56
3.7	0.034	0.80	5.9x10 <sup>10</sup>	0	>10 <sup>10</sup>	>10.77
1.2	3.4	600.0	5.7x10 <sup>10</sup>	3.8x10 <sup>9</sup>	1.5x10 <sup>1</sup>	1.18
1.2	3.4	600.0	6.7x10 <sup>10</sup>	1.4x10 <sup>9</sup>	4.8x10 <sup>1</sup>	1.68
1.3	3.4	600.0	5.7x10 <sup>10</sup>	3.8x10 <sup>9</sup>	1.5x10 <sup>1</sup>	1.18
2.3	3.4	200.0	5.6x10 <sup>10</sup>	5.6x10 <sup>4</sup>	1.0x10 <sup>6</sup>	6.00
2.3	3.4	200.0	4.7x10 <sup>10</sup>	8.8x10 <sup>4</sup>	5.3x10 <sup>5</sup>	5.72
2.3	3.4	200.0	5.4x10 <sup>10</sup>	8.6x10 <sup>4</sup>	6.3x10 <sup>5</sup>	5.80
3.6	3.4	80.0	3.5x10 <sup>10</sup>	0	>10 <sup>10</sup>	>10.54
3.7	3.4	80.0	3.6x10 <sup>10</sup>	0	>10 <sup>10</sup>	>10.56
3.7	3.4	80.0	5.9x10 <sup>10</sup>	0	>10 <sup>10</sup>	>10.77

The dependency of bacteria removal efficiency on transmembrane pressure drop is evidently shown in Fig. 5. The efficiency of retention decreases with increasing transmembrane pressure drop.

## Filter Integrity Test

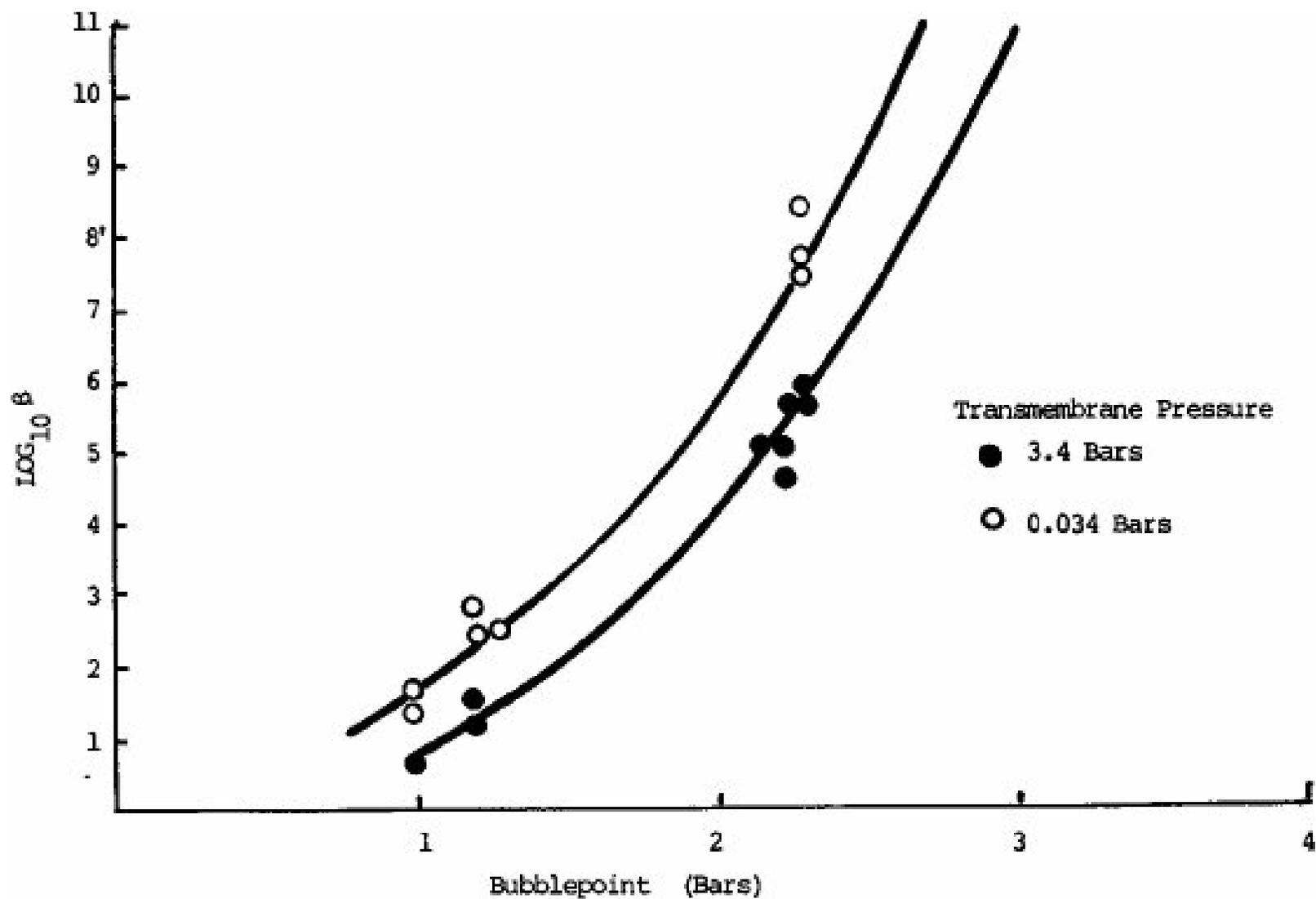


Figure 5. Relationship Between  $\beta$  Ratio and Filter Bubble Point.

Because the bacteria challenge test is destructive, a more practical, non-destructive test is used for confirming the integrity or the validity of sterilizing membrane filters. The ability to be tested for integrity just prior to filtration is a specific advantage for a membraned filter. Typical and most frequently used test of integrity is the bubble point test. The bubble point test is based on the fact that liquid is held in a capillary tube by surface tension and that the minimum pressure required to force liquid out of the tube is in direct measure of tube diameter. When these capillaries are full of liquid, the gas pressure required to force the liquid out must be sufficient to overcome surface tension. Exceeding this pressure will cause air bubbles to emerge from an immersed tube on the downstream side of the filter holder.

The bubble point test which detects minor filter defects and out-of-size pores correlates consistently with the microbial retention test. This strong relationship between the bubble point test and the microbial retention efficiencies by membrane is shown by the study of Reti<sup>7</sup> or Leahy and Sullivan.<sup>6</sup> Here we see that the quantitative removal of bacteria is a function of pore size of the bubble point of the filter used for challenge test. (Table IV) The membrane having the bubble point 3.7 bars shows quantitative microbial retention as high as  $10^{10}$  organisms. This high microbial retention by sterilizing grade membranes has also been confirmed by Leahy and Sullivan.<sup>6</sup>

# Summary

As a result of development of membrane filtration technology, sterilization by filtration has recently attained new levels of skills of filtration. Many advantageous characteristics of membrane filter contribute to establishment of quality control of sterilizing filtration process. For example, non-destructive bubble point test that is specific for membranes offers great opportunity to confirm the integrity of the filtration system before and after use. Many scientists have assured that there exists a strong correlation between the bubble point pressure and the microbial retention efficiency of membrane filter.

In addition, in the sterile filtration by membrane filter, severe bacterial challenge test to assure high degree of microbial retention efficiency has been studied. Those investigations include the work of Leahy and Sullivan who considered some of the important aspect of retention testing, particularly the growth characteristics of test organism in specific medium.

Owing to the development of this technology as well as the development of new type of membrane materials, sterilization by filtration has achieved a high level of confidence on a sterilization of a liquid.

Now, the requirement for the sterilizing grade filter is that it should have at least such levels of microbial retention efficiency that no cells pass through the filter when challenged with a specific microorganism such as *Pseudomonas diminuta* ATTC19146 at a minimum concentration of  $10^7$  organisms per  $\text{cm}^3$ . In addition, the sterilizing grade filter especially those used for parenteral drugs should be of non-fiber releasing. Most sterilizing grade membrane filters available today meet those requirements, however, development of those membranes which are most durable and stable for repeated steam sterilization at more than 130C for 60 min. is expected.

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# Chemical and Biological Monitors of Sterilization Processes

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There is a distinction to be drawn between “sterility tests” i.e. making sure that an article is sterile by testing it after completion of manufacture, and “sterilization assurance” i.e. making sure an article is sterile by the application of appropriate manufacturing procedures. Making sure in either case is, of course, impossible and all one can do is to achieve a level of acceptable probability that if there is any microbial contamination present it is of a very low degree. Any tests for sterility of the finished article, or of sterilization during manufacture must therefore be designed to supply a result which indicates the required probability, i.e. so that the result shows whether or not it has been achieved. It is one of the great advances of sterile article manufacture that the limitation of sterility tests has been realized, and sterilization assurance is many orders of magnitude greater than can be achieved by sterility testing alone. Such assurance depends on a number of factors. These include the actual production and sterilization record of the article or lot of articles showing that the sterilization procedure used has the capability of totally inactivating the established microbial burden (or a more resistant challenge) and that the procedure, as established, has actually been applied. Widely used means of establishing such evidence, as part of the sterilization validation and monitoring procedures, is the use of suitable indicators.

Indicators of sterilization cycle establishment and application can be broadly divided into two classes, chemical and biological. Chemical indicators may be convenient means of rapidly determining the sterilization status (*not* sterility status) of one or a group of articles. However, while they do indicate whether or not the sterilization process has occurred there is generally no indication of the extent of application of such process. Thus for steam sterilization a chemical indicator may show that a particular temperature (and hence corresponding steam pressure) has been reached, but not how long it has been applied. Various materials have been used for chemical indicators such as enzymes which are activated to a degree by the sterilizing agent or chemicals which change color with changes in moisture content. More recent advances are the development of so-called integrators, i.e. a device which indicates the length of application of the sterilizing agent to show both the fact of application and its duration. None of these articles have been sufficiently widely

used, or have been submitted to USP with adequate supporting data, for the Committee of Revision to frame monographs of standards for them. However, manufacturers of pharmaceuticals do use them individually, in adequately validated procedures according to the capabilities they do have for indicating what has occurred. At present chemical indicators must therefore be regarded as possible additional evidence, if used, for sterilization validation or monitoring.

Biological Indicators, on the other hand, have been widely used for part of sterilization procedures and have been of interest for compendial standards for several revision cycles. The historical developments are shown in the accompanying tables. The earlier descriptions of biological indicators have been generally in the informational chapters of the compendia.<sup>1</sup> In USP XVII (1965) and USP XVIII (1970) they were barely mentioned, with the statements that they are items of spores of known microorganisms and that they should exceed the product contamination level. More details were given in USP XIX (1975) and USP XX (1980). Here for each mentioned mode of sterilization the strain and suggested performance characteristics of the specified microbial strains (details of the survival time and kill time and D value) are given.<sup>2</sup> All this information was included in a general informational chapter entitled "Sterilization." It was not until USP XXI (1985) that a separate general chapter, still only informational, entitled "Biological Indicators" was included. This gave many details of the philosophy of use and selection of biological indicators. There were also included several warnings of what could be expected and precautions that should be taken for their use. USP XXI (1985) also, for the first time, included separate monographs of standards for three widely used kinds of biological indicators, paper strips holding particular microbial strains for dry heat, steam, and ethylene oxide gas sterilization.

It has been repeatedly pointed out that the selection of a biological indicator for a particular sterilization mode and cycle is critical. By this is meant that the indicator should provide the relevant information. Sterilization cycles can be broadly divided into "over-kill" approach and "graded" approach. By "over-kill" is meant the application of the sterilizing agent to such an extent that it can be almost certainly expected that any possible microbial contamination of the article, of the order and magnitude usually found, will be removed. The other relates to the kind of article to be sterilized that will not stand too much of the sterilization process. In such a case the procedure is to apply only such as to kill the expected microbial burden of the article, with some margin of safety. This requires considerable calculation on previous observations of the microbial bioburden, and experience in how much can be applied to achieve sterility assurance with an acceptable probability. In either case, biological indicators used must have performance characteristics appropriate to the purpose, and also behave under sterilization conditions predictably, and in accordance with the labeled characteristics. This is the very purpose of setting compendial standards for them.

Unfortunately, it is the case that sterilizing apparatus, dry heat ovens, steam autoclaves or gas sterilization apparatus, differ in their performance even when designed for a particular purpose, e.g. steam sterilization at 121°C for a particular time. Hence biological indicators cannot be expected to behave according to their labeled characteristics in all apparatus indiscriminately. It is clearly the responsibility of the user to determine the characteristics of

the biological indicators to be used, under the particular conditions, and in the particular apparatus for sterilization. This does not mean that labeled performance characteristics are wrong. It is more related to the differences between the user's apparatus and the more sophisticated and elaborate apparatus which manufacturers of biological indicators use to determine the characteristics to put on the label.

Under the circumstances compendial standards for biological indicators include descriptions of the minimum characteristics of the apparatus which should be used for determining label compliance of the articles. At the same time improved methods, based on newer knowledge and experience are being proposed for the compendial monographs. Details of some of the changes are given in the accompanying tables. It can be expected that compendial standards will be developed for more types of biological indicators, e.g. those supplied with accompanying medium for convenience of cultivation. All the monographs presently official deal with paper strips or carriers on which spores of microorganisms concerned are placed. While other carriers may be envisaged, it is more likely that monographs for spore suspensions, which could be added to the article to be sterilized or which may be included in a simulated article, will be forthcoming.

## Footnotes

1. The compendial monographs and general chapters provide mandatory standards for the various articles. Informational chapters, however, give only general information and advice i.e. they are guidelines and do not provide obligatory or mandatory standards.
2. The survival time is that time after which all of the biological indicator strips subjected to the specified sterilization conditions can still be expected to show growth on cultivation in suitable media. After the kill time, none of the strips carrying the spores would be expected to show growth on such cultivation.

**Table I. Biological Indicators.**

USP XVII (1965)	Dry Heat: Thermophilic, Spore-forming, Nonpathogenic Microorganisms.
	Ethylene Oxide: Include items with spores of known microorganisms.
USP XVIII (1970)	Highly resistant to the sterilization process. Inoculum of spores (or vegetative cells) should exceed the product contamination level.

**Table II. USP (1965) Suggested Performance Characteristics.**

Saturated Steam	<i>Bacillus stearothermophilis</i>	ST 5 Min	KT 15 Min
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121 ± 0.5°

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Ethylene Oxide	<i>Bacillus subtilis</i>		
300 ± 0 mg/L, 25 ± 1°, 60 ± 20 RH%		60 Min	360 Min
600 ± 60 mg/L, 4 ± 1°, 60 ± 20 RH%		15 Min	120 Min
1,200 ± 20 mg/L, 54 ± 1°, 60 ± 20 RH%		5 Min	30 Min
Dry Heat	<i>Clostridium tetani</i> (nontoxigenic strain)		
Ionizing Radiation	<i>Bacillus pumilus</i>		

**Table III. USP XX (1980) Typical Performance Characteristics of Some Biological Indicators.**

Ethylene Oxide	<i>Bacillus subtilis</i>		
54°, 50 RH%			
600 mg/L	Approximate Value		3 Min
1,200 mg/L			1.7 Min
Saturated Steam	<i>Bacillus sterothermophilus</i>		
121°			1.5 Min
112° – 121°			3.5 – 0.7 Min
Dry Heat	<i>Bacillus subtilis</i>		
121° – 170°			60 Min – 1 Min
Gamma Radiation	<i>Bacillus pumilus</i>		
	Wet Preparations		0.2 Mrad
	Dry Preparations		

**Table IV. USP XXI (1985) & Supplement 1, Separate Chapter, Monographs.**

Dry Heat	<i>Bacillus subtilis</i> (ATCC 9372) Subsp. <i>niger</i> 160° ± 2° 5 × 10 <sup>5</sup> to 5 × 10 <sup>6</sup> D Value 1.3 to 1.9 Min	ST >3.9	KT <19 Min
Ethylene Oxide	<i>Bacillus subtilis</i> (ATCC 9372) Subsp. <i>niger</i> 600 ± 30 Mg/L		

54 ± 2°;

60 ± 10 RH%

5 × 10<sup>5</sup> to 5 × 10<sup>6</sup>

Value 2.6 to 5.8 Min

ST >7.8

KT <58 Min

Saturated Steam

*Bacillus stearothermophilus*

(ATCC 7953; 12980)

121 ± 0.5°

5 × 10<sup>5</sup> to 5 × 10<sup>6</sup>

D Value 1.3 to 1.9 Min

ST >3.9

KT <19 Min

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**Table V. Biological Indicator for Steam Sterilization, Paper Strip.**



# Sterility Testing and Its Relevance to Sterility

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## Introduction

It has been suggested that if one were to rely on the sterility test to assure sterility then the corollary must be accepted that sterilization could be defined as the process by which microorganisms are killed to the extent that they may not be detected by the sterility test<sup>14</sup>.

For the sterility tester, this is not just an interesting hypothesis, it is the whole basis on which the test is carried out. However sterility is theoretically an absolute condition and must be defined as the state of freedom from all living microorganisms. It is immediately apparent therefore that there is a conflict between this theoretical absolute concept and the sterility testers mathematical concept of the probability of sterility. Since the degree of probability is not absolute, the best state that is possible is that it is acceptably high<sup>13</sup>.

It is then necessary to define what is acceptable in relation to the probability of sterility and to examine to what extent the sterility test can determine that probability.

In this review it is intended to show how a sterility test procedure originally introduced for injectables was gradually applied to other devices through the activities of the Pharmacopoeias. This has led to the value of the sterility test in relation to other large scale processes being questioned.

## Historical

The history of sterilization and the sterility test may be traced back to some extent through various regulations, laws, national formulary and more particularly through the pharmacopoeia when the realization that bacteria could cause clinical disease prompted the need to regulate infusion and parenteral materials through some process that would destroy such microorganisms.

In the Western hemisphere, local or regional Pharmacopoeias were certainly in use in the early sixteenth century under the authority of medical colleges or local medical societies with no national authority (Spanish "Concordia Apotheca Rioum Barchionensium—College of Pharmacists Barcelona 1511). The first Pharmacopoeia regarded as legally binding on medical practitioners and pharmacists was however the "Dispensatorium" by Valerius

Cordus published in Nuremberg in 1546.

In the 19th Century, the first national publication of what may be termed the modern era was the United States Pharmacopoeia when in 1817 a project was submitted to the New York Medical Society which was to culminate in the publication of USP I in 1820. In Europe, Greece followed in 1837 with a publication which was a translation of an earlier Bavarian document and in Britain a national pharmacopoeia (BP 1864) was published implementing the Medical Act of 1858.

At about this time monographs for “injectables” were being published with forms of treatment from gentle heating to boiling for a few minutes being progressively required. When the first German Pharmacopoeia was published in 1882 other forms of sterilization were being considered and just before the turn of the century a pharmacopoeia was published in Greece (Damvergis Pharmacopoeia 1899) which although not of national status referred to various methods of sterilization including dry heat, filtration and autoclaving at 120°C. In the British Pharmacopoeia, however, as late as 1914, injectables were only required to be “recently boiled.”

The subsequent flurry of activity was probably associated to a large extent to the development of many other injectables such as vaccines, antitoxins, sera and antibiotics. The Therapeutic Substances Act published in Britain in 1925 referred to these injectable materials and required for the first time that a Test for Sterility be performed. Under the same Act in 1927, proposed rules for sampling were added.

By 1936 the United States Pharmacopoeia and the US National Formulary both had sophisticated Sterility Tests for sterile injectables although the British Pharmacopoeia only required the test be carried out if sterilization was achieved by filtration. In 1936 the Portuguese Pharmacopoeia on the other hand in its first edition published a Sterility Test and required products other than injectables to conform. Cotton, gauze, bandages, liquid paraffin, talc, silk and injectable solutions were all cited as requiring to be tested.

Other Pharmacopoeias began to include Sterility Tests adding surgical dressings, ophthalmic solutions and device monographs. In 1953 the British Pharmacopoeia also included cat gut as having to comply with the test.

By 1969, volume I of the European Pharmacopoeia had been published and in 1972 volume II became the first multinational publication to prescribe a method of test for sterility.

## Definitions

Before one can embark on a discussion of the value of the sterility test, sterility itself must be defined and an acceptable probability of sterility agreed.

The term sterile when applied in a medical sense may be defined as the state of absolute freedom from microorganisms. In a practical sense, when related to a product that has been sterilized it usually means free from bacteria and fungi since it may only be these microorganisms which the sterilizing process has been validated to kill and the sterility test designed to detect.

Inactivation of such microorganisms typically follows first order kinetics with a consequent probability that a microorganism would survive regardless of the nature of the

sterilizing process. Logarithmic death rates and associated probabilities of survivors have been an integral part of the food canning process since the early 1920s<sup>5</sup>.

In this sense sterility is the probability of existence of a surviving microorganism. But since one is strictly sampling items and only indirectly microbial populations, sterility may also be regarded as the probability of an item being nonsterile or in practical terms the probability of existence of a nonsterile item in a batch.

The debate as to what this probability should be has been further complicated in recent years by the implication that less critical medical needs may be adequately served by “less sterile” product. Whilst this may be a realistic approach it does nothing for the interpretation of sterility as such.

Whatever probability value is accepted for a sterile product it must or should be capable of examination. To this end it has been suggested that less than one item in one million items should be nonsterile<sup>1,14,31</sup>.

Unfortunately where such a mathematical approach has been taken it has often been misinterpreted as being synonymous with sterility rather than a practically achieved minimum level of assurance.

## Statistical Test Criteria

The design and interpretation of sterility testing and the statistical properties of sampling plans have been published by many authors<sup>9,10,15,25,33</sup>.

The principles on which a batch is accepted even though it may have a number of defective items were described in Sample Inspection Tables published in the United States more than forty years ago<sup>20</sup>. If the number of defective items were above an acceptable limit then the batch could be subjected to a 100 percent inspection. Clearly such a scheme is designed for nondestructive testing and cannot be applied to sterility testing.

A direct test for sterility must, by its nature, be destructive and information derived from such a single test relates only to the state of that item. The final analysis is therefore statistical in that all that can be determined is the probability with which the remaining items would pass or fail the test. The probability of rejecting a batch of items as a result of a sterility test depends on the number of sample items taken for test and the frequency with which those items are contaminated<sup>10,13,29</sup>.

$$\text{Probability of rejection} = 1 - (1-p)^n$$

Where  $p$  is the proportion of items contaminated and  $n$  is the number of sample items tested.

In most pharmacopoeia the number of samples to be taken varies with the batch size. In particular the European Pharmacopoeia (EP) requires a maximum of 10 percent and a minimum of 2 percent as a sample rate depending on the size of the batch (Table I).

### Table I. EP Sterility Test Sample Requirement

*Items*

*Sample Items Tested*

100

10% or 4 whichever is the greater

100-500

10%

500

2% or 20 whichever is the lesser

Items

Numbers of items in the batch

The figures relate to batch sizes between 40 and 1,000 items. In industrial practice, however, the extremes are not unlikely. A batch of 4 specialist devices would, using these figures, require a 100 percent sample size whilst a batch of, for example, 250,000 surgical needles would only be required to be tested using a 0.008 percent sample size.

Fortunately the information about the quality of the batch is related to the number of samples tested and not to the batch size and the efficiency of the test will therefore rise with increasing batch size until the maximum of 20 samples are tested. Operating characteristic curves clearly show this relationship (Figure 1).

A sampling plan of 10 items where the batch size was 100 would result in 1 batch in every 3 batches being accepted even if the batch contained 10 percent contaminated items. Conversely a sampling plan of 20 items where the batch size was 10,000 and the contamination rate remained the same would result in 1 batch in every 9 being accepted. In the latter example there is still a 1 in 100 chance of accepting a batch with 20 percent contamination items.

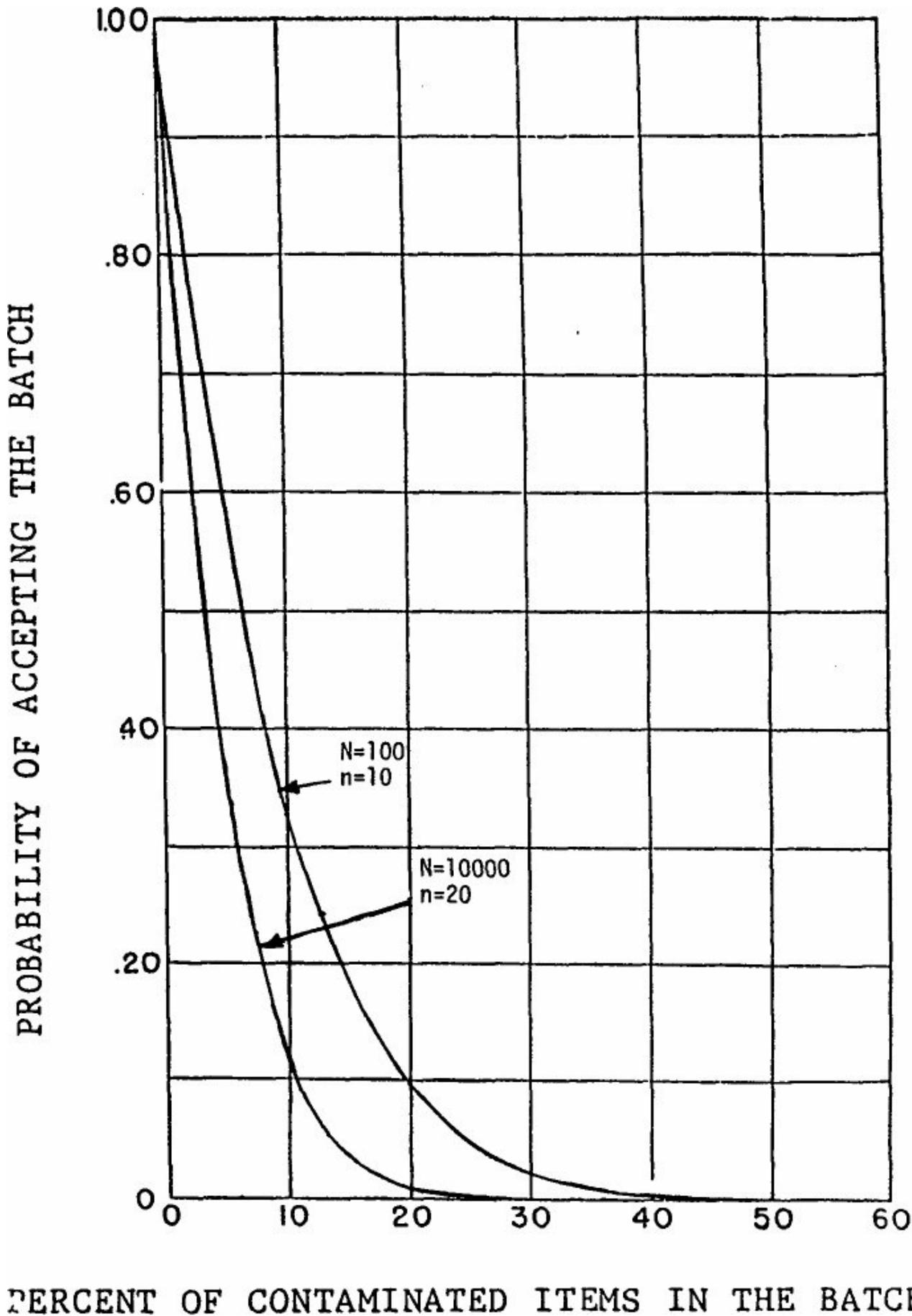
Larger sample sizes improve the situation<sup>9</sup> though the validity of the sterility test must still be in doubt when with a batch size of 1,000 items and a sample size of 500 the probability of accepting a batch with 1.0 percent contaminated items is still 0.01. That is to say such a batch would mistakenly be accepted once in every 100 separate test occasions (Table II).

**Table II. Relationship of Probabilities of Acceptance of Lots of Varying Assumed Degrees of Contamination to Sample Size**

<i>n</i>	% Contaminated					
	0.1	1	5	10	15	20
10	.99	.91	.60	.35	.20	.11
20	.98	.82	.36	.12	.04	.01
50	.95	.61	.08	.007		
100	.91	.37	.01	.00		
300	.74	.05				
500	.61	.01				

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n = Number of samples tested



**PERCENT OF CONTAMINATED ITEMS IN THE BATCH**

Figure 1. Operating Characteristic Curves where (N) is the Number of Items in the Batch and (n) is the Sample size.

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 The inadequacy of the sterility test is further highlighted by the acceptance that

accidental contamination is likely to occur during the test. The test may be repeated if one sample is found to be contaminated. Acceptance is then based on the retest if the results are satisfactory. Indeed the batch may be retested, in all, up to 3 times with the batch being regarded as sterile on any occasion where the test is satisfactory. That is to say where all test samples show no microbial growth. However the batch is rejected if on the second or subsequent test the same microorganism is detected.

This principle has been shown to be fallacious since if the source of contamination is the product and the testing technique is satisfactory then retesting merely serves to double the sample size. This increases the probability of passing the batch even though the contamination rate remains the same<sup>13,15,19</sup>.

Using the earlier example of a batch size of 100 items and a sample rate of 10 items the probability of rejecting a batch with 10 percent contaminated items is 0.653. As may be seen (Table III) with a single retest the probability of rejection decreases to 0.42 or some 23 percent.

**Table III. Probability of Rejecting a Batch by Single and Repeat Sterility Tests**

Items	n	Test	Probability of rejection with % of items in the batch contaminated.			
			0.1	1.0	10.0	20.0
100	10	1	0.0100	0.0960	0.6532	0.893
100	10	2	0.0001	0.0090	0.4200	0.800
1000	20	1	0.0200	0.1800	0.8780	0.988
1000	20	2	0.0004	0.0320	0.7740	0.976

Item = Number of items in the batch

n = Number of samples tested

Test = 1, Initial sterility test; 2, First retest

On the other hand if the batch is sterile and contamination is due to technique, then it is more than likely that the microorganism which has contaminated the initial sterility test will also contaminate the retest. However satisfactory the product, it may be rejected through poor technique and consequent accidental contamination. Indeed it has been postulated that such accidental or adventitious contamination may range from 4 percent when testing a simple surgical dressing to as high as 20 percent for a more complex device<sup>34</sup>.

Another report<sup>4</sup> suggested that it is difficult to fix a value for a certain fixed risk of accidental contamination during the sterility test. However a value equivalent to 1.0 percent was regarded as a reasonable possibility. In industry using appropriate test conditions and skilled staff with experience of the product such accidental contamination rates would not be tolerated. Even so, a 0.1 percent level might be expected where a product was large or

otherwise difficult to manipulate during the test.

It follows that whilst increasing sensitivity, in terms of the probability of rejection of a suspect batch, is obtained by increasing the sample size, the risk of accidental contamination also increases. In the example of a batch of 1,000 items, a sample size of 20 and a contamination rate of 0.1 percent the probability of rejection is 0.98. In other words the batch would be rejected 2 percent of the time due to real product contamination and 2 percent of the time for accidental contamination (i.e. once in every 50 tests each of 20 samples).

In addition, in this example 20 samples per test are little better than 10 where the contamination is as low as 0.1 percent. In fact, in such a case, even when 500 samples are tested the batch would mistakenly be accepted 6 times in ever 10 sterility tests.

These statistics fall far short of the requirement that the batch should contain no more than one nonsterile item in 1 million.

## Nonstatistical Test Criteria

So far the discussion has been related to the probability of detecting contamination in a batch of items. The assumption has been made that microorganisms if present on the items under test will grow if subjected to a particular test regime. This however is probably far from the truth. There are in practice a number of non statistical factors which must be considered when assessing data from a sterility test<sup>14,40</sup>.

The aspect of false positive results from accidental contamination affecting the conclusions drawn has been dealt with earlier. Anomalous results of this nature are not in themselves dangerous unless they are interpreted carelessly since they would usually serve to reject an otherwise satisfactory batch. They are more important from an economic aspect if product is inappropriately rejected by an official test organization. False negatives on the other hand are by their nature undetectable and potentially dangerous to the recipient.

From the first published sterility test to the present time, test results have been influenced by a variety of factors not least associated with the conditions presented for microbial growth. Since no single medium will support the growth of all bacteria, moulds and yeasts, more than one medium must be used if adequate recovery of these microorganisms is to be achieved. But how many media should be employed and what should they be. Although the choice of medium in the pharmacopoeias has changed over the years, there have been and no doubt will continue to be criticism in the literature and reports of microorganisms failing to grow in the recommended media of the time<sup>16,17,21,27</sup>.

The range of microorganisms to be recovered is critical to the choice of media employed. In this respect a fundamental problem exists in the pharmacopoeia where a compromise is necessary in order to recover a broad range of medically important microorganisms. The choice of thioglycolate broth was particularly unfortunate in this respect<sup>7,11,21</sup>.

Incubation temperature and time must also be considered. Although 7 to 14 days is

usually recommended, slow growing microorganisms requiring 21 days incubation have been reported<sup>12</sup>. This phenomenon has been refuted however<sup>6,22</sup> and it is possible that it is an artifact caused either by poor growth promoting properties of the media or by recovery of heat damaged microorganisms in poorly sterilized media.

However, the possibility of recovering microorganisms damaged by the product or the process cannot be overlooked. Such damage may be caused by sterilization, antimicrobial activity in the product or by some other mechanical process such as filtration. Recovery in such instances may be slow and the media used, whilst satisfactory for healthy microorganisms, may be less than appropriate<sup>2,35</sup>.

## Alternatives

If the sterility test has its limitations, what are the limitations? There would seem to be two distinct approaches to this question.

1. What alternative has the manufacturer who wishes to assure sterility?
2. What alternative has the Authority who may need to perform some test to determine the sterility status of the product?

As far as the manufacturer is concerned, the realization that “assurance” is better than “control” has been brought about by technical, economic, legal and in some cases ethical considerations. The control of the manufacturing process, particularly environmental control, was being proposed in the 1890’s<sup>28</sup>. In more recent years Good Manufacturing Practice (GMP) documents have elaborated on the requirement to regulate all aspects of the manufacture of sterile product if a high degree of assurance is to be achieved.

Thorough validation of the sterilization process must be carried out<sup>24</sup> if this assurance is to be maintained. Within this validation the need to determine the bioburden of the product is essential and a rational approach to such testing has been proposed<sup>32</sup>. Undue reliance on bioburden testing may present other problems however. As with sterility testing, economic constraints and the nonuniversal nature of the test media make conclusions as to the quantitative and qualitative nature of the bioburden uncertain. The FDA had the foresight to recognize the problems involved in the collection and interpretation of these data and took a balanced view in relation to this subject<sup>23</sup>.

In practice, whilst it may be necessary during validation to determine with some degree of certainty what the bioburden level of a is, use of the technique accurately to calculate sterility assurance may be unnecessary and should anyway only be regarded as one of a number of parameters. Used on a nonroutine basis bioburden studies do have relevance to the control of the environment and hygiene in manufacture. Although a knowledge of the bioburden is essential therefore in the calculation of a sterility assurance level, a sterilization process that is so marginal as to be affected by normal variations in the microbial population might be regarded as unsound.

Biological monitors may also be used to control and assess the sterilization process, but should be regarded as parameter integrators rather than possessing intrinsic integrity. Of

greater value in some instances in calculating product sterility may be a sub-process dose or challenge dose technique<sup>30,39</sup> where product is subjected to a fraction of the sterilizing exposure to determine the sterility assurance level and thereafter to a set fractional exposure for routine control purposes. A development of this technique has recently been published in relation to processing by radiation sterilization<sup>3</sup>.

For the Authority, the situation is undoubtedly more difficult. Sterility tests may continue to be of value in the control of injectables and some pharmaceutical preparations, particularly where sterilization by filtration has been necessary. Even so results must still be interpreted with caution and developments in technique must continue to be sought. Even with sterilization by filtration, data from properly validated processes may prove of more value in assuring the sterility of a batch than the traditional sterility test.

Modifications of the sterility test technique have been proposed<sup>8,18,26,36,37,38</sup> in recent years which merit further consideration. Shaking of test cultures during incubation, radiometric techniques and filtration methods have all been reported. These methods may serve to improve the sterility test as it is defined in the pharmacopoeias today but the technique is likely to continue to suffer from its inherent statistical and methodological weaknesses.

## Conclusions

It is commonly agreed that the probability of a sterile medical device should be 99.9999 percent. The sterility test on the other hand, using a sample size of 20 items will, 10 percent of the time, accept a batch with up to 10 percent contamination. The implication is that the sterility test will only detect gross contamination and it might more appropriately be termed a "Test for Contamination."

It is suggested that the Authority wishing to assure itself that a batch of products is sterile should first seek appropriate information from the manufacturer as to the manufacturing conditions, sterilization validation procedures and control mechanisms that have been used for that batch. If such documentation is not available or is inadequate a sterility test may be the only possible recourse that the Authority has. If carried, the sterility test results will need careful interpretation.

History tells us that the sterility test was first introduced to determine the microbial status of injectable fluids. In recent years, fatalities have occurred through the use of intravenous fluids that have been inadequately sterilized and improperly controlled. Since growth of contaminant microorganisms in such fluids might be expected to yield gross contamination, it is suggested that the sterility test is best confined to these and similar products.

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# Discussion

## Session I and II

### **Question by Madame Li Rong-fen, Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing.**

As Dr. Outschoorn mentioned, biological and chemical indicators are very useful for sterilization monitoring, and the chemical indicators are very convenient for use. But from my experience, the results of chemical indicators are not always similar to those of biological indicators. I wonder whether some differences between these two kinds of indicators in the same sterilization process might be allowed. If it is the case, what is the range allowable for the differences? How about the prospect for chemical indicators?

### **Answer by Dr. Aubrey Outschoorn, U.S. Pharmacopeia, U.S.A.**

I will try to give a fairly short answer. Chemical indicators have been around for quite some time, but I think that one can safely say that there has been far more interest and experience with biological indicators. Various materials have been used for chemical indicators, such as enzymes which are inactivated to some degree by a sterilization agent or by chemicals which change color with changes in moisture content and these are very ingenious devices. However, they are generally based on the fact that there is a fairly clear end-point only when that the sterilization agent, has in fact, been applied. For instance, that a certain temperature has been reached in steam sterilization or that a certain gas concentration has been achieved for a shorter or longer time in the case of a chemical indicator for gas sterilization. Generally, there is no difference in the indicator when the sterilizing agent has been applied for a longer time or at a greater concentration. That is one of the chief disadvantages of them. We are familiar with the chemical indicators which consist of rods of metal which melt or fuse at different temperatures. So, one or two or three of them melt and bend over and the ones which stand straight indicate the temperature which has been reached. Now that is a very crude form of chemical indicator. There has been more interest recently in being able to find out how long the sterilizing agent

has been applied. None of the information which has been available to the U.S.P. Committee of Revision has warranted their developing requirements or monographs for chemical indicators and I think that it will be safe to say that there is no general set of requirements which would be applicable to a fairly large number or a fairly large group of chemical indicators.

It is different in the case of biological indicators. When the original description of each type of indicator was developed by the author or the originator, people immediately became interested and a tremendous amount of research work has been done. And this has also contributed toward a better understanding of the mechanics, the physics and chemistry, not only the biology of microbial death. I think it would be wrong to assume that any kind of chemical indicator which has particular label characteristics could be compared to a biological indicator which may have similar parameters on the labelling and to imagine that they will be equally reliable. It is not that these are bad products. In the case of chemical indicators or biological indicators, it is merely that they are different and there is, at present, insufficient information to be able to say that if a chemical indicator shows this, a biological indicator will show a correspondence. This is state-of-the-art which I think has not yet been reached and that is why I hope I am not giving the impression that chemical indicators should not be used. Certainly they can be used and they can be used in a variety of situations. But the user must verify exactly how a chemical indicator behaves and the extent of the information which is given. Whereas in the case of biological indicators much more information today is contained in the labelling and it is only a matter of obtaining information on the relationship between the behavior of the indicator as shown in the labelling and in the actual autoclave or the gas sterilization apparatus in the user's establishment.

## **Comments by Dr. Martin Favero, Center for Disease Control, USA**

Two short comments on two papers. Perhaps the authors may wish to comment on my comments. The first one is with Dr. Liu's excellent paper. He had mentioned that one microorganism is of great concern here in China, viral hepatitis type B—the hepatitis type B virus. In the United States for a number of years, we also used the criterion of the inactivation of the hepatitis B surface antigen, the chemical moiety, to the point where it would not react immunologically as the criterion for certain types of physical and chemical agents. Subsequently, we did perform tests using chimpanzees and basically the results showed that the hepatitis B virus is not a resistant virus. It is very susceptible: that the normal types of chemical germicides, for example, 500 parts per million chlorine, 70% isopropyl alcohol, 2% glutaraldehyde, and some other commonly used germicides, in exposures of ten minutes, does indeed inactivate the hepatitis B virus. My point is that I think it would be a mistake to have ultra-conservative procedures.

My second comment is on Mr. Ishii's paper. We have noted many of the things that he pointed out. I would like to comment simply on *Pseudomonas diminuta* as a challenge organism for both depth and skin filters. It has been our observation that many of the

*Pseudomonas* species and other members of gram negative bacteria, which have the capability of growing in distilled water or deionized water, if they colonize the filter during the process, regardless of size, they are able to grow through the filter. In other words, it is quite different than a simple challenge; it is after days of operation the organism will tend to grow through.

## **Reply by Mr. Kuranosuke Ishii, Nihon Millipore, Ltd. Japan**

Thank you for the comment. I understand there are many *Pseudomonas diminuta* and their size is different. However, the challenge organism which I showed here, the *Pseudomonas diminuta* ATCC 19146 was isolated by Frances Bowman of FDA and I understand in their test, before a 0.22 micron filters are used, a 0.45 micron filter was used for three days observation. However, during their test, one sample was contaminated and they studied and isolated the culture and Bowman deposited in the American Type Culture (ATCC) collection *Pseudomonas diminuta* ATCC 19146. And according to Bergey's manual, *Pseudomonas diminuta's* size is, I think, 0.5, at times 0.223, microns. This is a little bit larger than *Serratia marcescens*. However, the ATCC 19146 *Pseudomonas diminuta* is less than that reported in Bergey's manual. The important thing is that the size of microorganisms is changed by the method of cultivation. Then what I wanted to explain here, Leaky and Sullivan used saline lactose broth. That is poor, not rich in nutrients and they cultivated without agitation, i.e., a standing culture. So that, I think the size is reasonable. That means there is a severe challenge. It is like test organisms used for autoclaving validation. What I meant in autoclaving, *Bacillus stearothermophilus* is heat resistant. In filtration, sterilization we have to use very small size organisms.

## **Comments from the floor or report of Dr. Li Zhi-gin**

I want to make several comments on his presentation about sterilization by means of chemicals such as, for instance, acetyl hydroperoxide. We are about to introduce it in China in large amounts. About 37 factories are now producing this kind of disinfectant all over China. And we have this product containing 20% concentration. In his speech, he said that the concentration they use for sterilization is 0.2 to 0.5%, but actually we found that 0.04 to 0.1% is good enough for sterilizing all kinds of micro-organisms.

The second comment is that in Shanghai or somewhere in China, we are producing a drug, NADCC, which is quite useful in many parts of China, you see, and the production is quite all right because we use a new type of catalyst so that this kind of new disinfectant has been quite useful in China.

Now, the third comment is that since we are producing NADCC in China, we use this disinfectant with addition of slow releasing agents and that has increased the effect very much and it is approved by the Shanghai Medical College and some other institutions. They say that this is worthwhile to use for many occasions of disinfection.

# Comments by Madame Cao Zhi-hong, Central Hospital of Yang Pu District, Shanghai

In Dr. Li's report, it is emphasized that the medical instruments such as syringes, needles and dental instruments must be disinfected after using each time for each person, as follows:

Hand-piece is commonly used in dental clinic. It is extremely easy to be contaminated by the HB<sub>s</sub>Ag positive patients' blood or saliva and thus it always leads to iatrogenic cross infection unless all instruments used are strictly disinfected before using.

How to inactivate HB<sub>s</sub>AG on hand-piece which is so complicated in structure that it is rather difficult to be disinfected in common ways, have been given full consideration. Since the hand-piece is heat-resistant but not stainless, we have verified that it is effective and reliable to inactivate HB<sub>s</sub>AG on the hand-piece by oil bath with the sewing machine oil as a medium. The oil bath disinfection method for hand-piece had been studied in the laboratory through a series experiments at various temperatures, in various periods of time, with various titres of HB<sub>s</sub>AG, and with various protein and in simulated clinical trails, Electron microscope observations and five years clinical application had also been made. The results of those showed that the destructive effect on HB<sub>s</sub>AG began to appear when the oil temperature was over 100°C; and the ratio of inactive HB<sub>s</sub>AG would reach 99.9% or even higher by maintaining the oil temperature of 120°C. The oil bath disinfection method for hand-piece to prevent hepatitis B from iatrogenic cross infection has these features: the oil temperature is fairly high, the time needed to maintain is short, it is safe and simple in operation, it is most important that its results are extremely effective.

## Question by Mr. Zhang Han-dong, Chief Engineer, Shanghai Medical Nuclear Instrument Factory, Shanghai

First, regarding the ethylene oxide residue remaining in an article after sterilization. The limitation of the residue from 2 to 25 ppm has been issued in the regulations of different nations. Which level will be more reliable as to safety for the user (patient), more economically reasonable? Are there any test methods which are accepted by most countries?

The mechanism of radiation sterilization is that the components of the nuclei of cells, the chemical bond chains of RNA and DNA will be broke-down during the irradiation. Are there any possibilities that those broke-down chains will be repaired, reconnected by themselves after a certain period?

Which methods and what kind of instruments have been used in the USSR for measuring and monitoring of large absorbed dose inof Co<sup>60</sup> and electron beams of accelerator during irradiation process?

# Answer by Mr. Richard DeRisio, Johnson & Johnson, USA

There have been several levels for ethylene oxide residuals published as guidelines or, in some countries requirements. These may vary from levels as low as one or two parts per million for an implantable device to levels that are as high as 25 or 250 parts per million. It was recognized that in order for these levels to mean something universally, there had to be standard referee test methods. One important development concerns a standard-setting group in the United States, the Association for the Advancement of Medical Instrumentation, that is developing reference methods for residue analysis this will help assure that everyone is using the same measurement technique or an equivalent technique to assess the levels. Also this group is beginning to look at what levels are safe for patient exposure. I think the feeling now is that the levels in the United States, for example, do adequately protect the patient. From the engineering standpoint to possibly use a less severe cycle in order to reduce residue levels. In other words we would optimize the cycle thus reducing the exposure period or the gas concentration or look for better methods of aeration because of the concern over worker exposure. Often the aeration is done in a closed room with frequent changes of air and higher temperatures and optimal humidities to remove the residuals more quickly. Sometimes this is even done inside the sterilizer which not only protects workers in the area, but also gives companies an opportunity to optimize the removal of product sterilizer residues.

The answer for the second question must be held for the general discussion period.

# **Session III**

**Jacques Masse, Ph.D.**

*Sterilization Specialist, Emeritus, Combs la Ville, France*

# Introduction by Session Chairman

Jacques Masse, Ph.D.

First of all, I would like to thank you, Madame Chairman, and the Organizing Committee for the remarkable organization of this International Scientific Conference on the Sterilization of Medical Products, Disinfection and Preservation. I would like also to say how I am honored to chair this morning's session and I thank you, Madame Chairman, for your invitation.

Yesterday we had very interesting presentations on the different ways to sterilize medical devices in the hospital and industry. This morning we will see how to package the products in order to preserve them from contamination on the shelves, which is very important during use. We will also learn how to test if the product is sterile and without any risk for the patient.

Sterility testing is the only way for a consumer to control the product, but the reliability of this test is very poor and the manufacturer can obtain with good manufacturing practices a very high level of probability to achieve sterility. For instance, with the radiation sterilization we can use dosimetry and with ethylene oxide we have to record the different factors like pressure, temperature, humidity, E.O. concentration and use biological indicators.

Everybody recognizes that the sterilisant residues in the devices are dangerous for the patient. But the problem is to fix the allowable amount. The methods used for the dosage of E.O. are very accurate (gas chromatography involving internal or external standard), but the conditions of extraction have to be improved. I ask myself on the need to determine the absolute amount of E.O. contained in the sample and if the use of extraction methods closer to the use of the product could be better (i.e., temperature 37°C, composition of the liquid for extraction...). I don't like at all the manner to present the result as p.p.m. I think it would be better to speak about the amount of toxic substance that the patient will absorb, because for him it is this quantity which is dangerous and not a percentage.

We will have a presentation on the reuse of sterile single-use medical products and we all know that it is a question that the users ask the manufacturers very often.

Finally, we will speak about the peculiar hospital environment in different countries and about the illness due to this environment.

# Pyrogen Testing

Virginia C. Ross

*United States Food and Drug Administration*

Problems with fever producing substances known as pyrogens in distilled water used to prepare injections were first reported in the late 1800s and early 1900s in the medical literature<sup>1</sup>. Further investigations demonstrated that the heat stable substances primarily responsible for pyrogenic reactions in man after exposure to sterile medical products are bacterial endotoxins, cell wall components of gram negative bacteria<sup>2,3</sup>. Endotoxins, known chemically as lipopolysaccharides consist of hydrophilic polysaccharide chain, a core trisaccharide containing 2-keto -3 deoxyoctonic acid, known as KDO, covalently linked to the lipid A structure which is primarily responsible for the molecule's endotoxic effects (see Figure 1)<sup>3,4</sup>. A recently proposed structure for lipid A is shown in Figure 2; A, B and C represent polar substituents and the symbol Ds represents either a long chain fatty acid or hydrogen<sup>4</sup>. Structural variations are common in the O-antigenic polysaccharide chain and depend on bacterial serotype. Somewhat less variation occurs in the core trisaccharide; the least variation is observed in Lipid A. Structural variations are responsible for differences in toxic potency of endotoxins<sup>4</sup>.

Nanogram quantities of endotoxin cause fever in man<sup>5</sup>. The nonspecific activation of the immune system by small amounts of endotoxin may even be beneficial<sup>4</sup>. Larger endotoxin doses overwhelm the body and cause serious destruction effects such as hypotension, and disseminated intravascular coagulation culminating in shock and death<sup>6</sup>. Sensitivity to endotoxin is 1000 times greater through the cerebrospinal fluid area than in other body areas<sup>6</sup>.

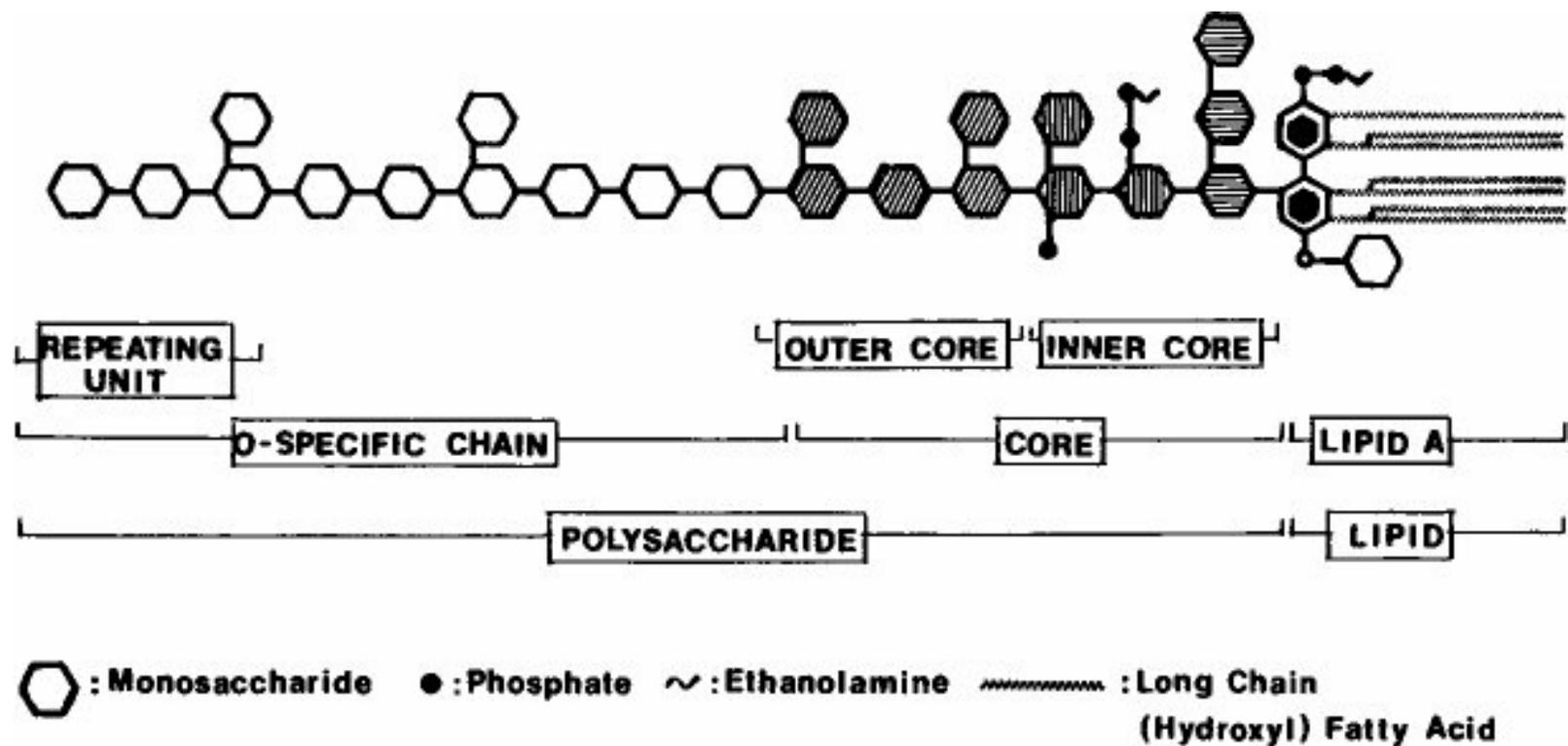


Figure 1. Schematic Representation of the Structure of a *Salmonella* Lipopolysaccharide.

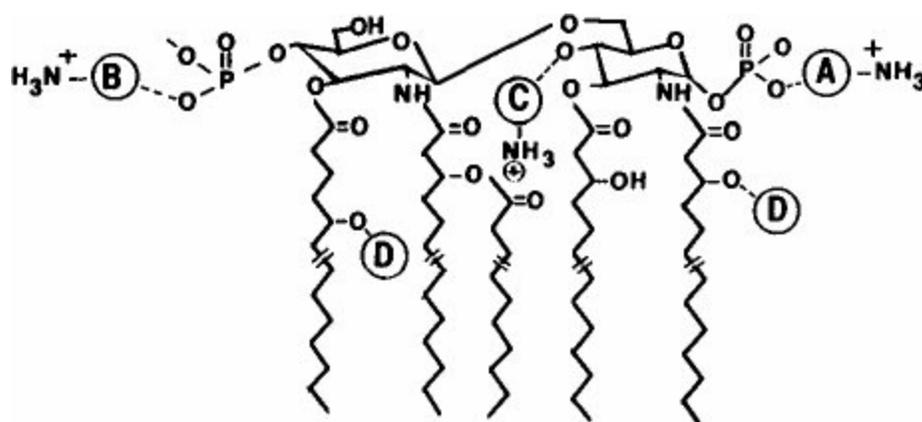


Figure 2. Proposal of a General Structure of Lipid A. Symbols A, B and C Represent Polar Substituents of the Lipid A Backbone or Hydrogen. Symbol D means long chain fatty acid or hydrogen. Backbone-bound 3-hydroxy Fatty Acids May Have 10 to 18 Carbon Atoms. Dotted Lines Indicate Nonstoichiometric Substitution.

The biological activity of endotoxin is not eliminated by sterilization methods other than dry heat or filtration<sup>7</sup>. Depth filtration can be used to reduce, but not eliminate endotoxin activity in processing of liquid products<sup>8</sup>. A recent publication indicates that the effectiveness of molecular filtration as a means for removing endotoxin from liquids depends on the filter's nominal-molecular-weight limit, the state of aggregation of endotoxin, and the molecular size of the product's active ingredients<sup>9</sup>. Since many parenterals and medical devices can not withstand high temperatures, in order to assure nonpyrogenicity of injectable drugs and medical devices, these products must be tested for pyrogens.

A pyrogen test in rabbits was adopted by the United States Pharmacopeia (USP) in

1942<sup>10</sup>. Because of the demonstrated similarity in pyrogenic dose response to endotoxin in rabbits and man, the rabbit test has protected patients from exposure to pyrogenic medical products<sup>5</sup>. The rabbit pyrogen test is well established and is recognized in many national and international pharmacopieas as an official end product test. The purpose of the pyrogen test is to prevent more serious endotoxicity by minimizing febrile response in patients after injections, intravenous administration, or internal contact with medical devices.

The rabbit test is summarized here as it appears in the current United States Pharmacopiea (USP)<sup>11</sup>. Basically, rectal temperature rise is measured over time after intravenous administration of the test solution to rabbits. All glass ware and iluents used must be pyrogen free. Glassware can be depyrogenated by heating at 250°C for 30 minutes. A test dose of 10 ml per kg of body weight is administered into an ear vein of each of three rabbits. Rabbit temperatures are recorded 1, 2, and 3 hours after injection. The product is considered nonpyrogenic if no rabbit shows a temperature rise of 0.6°C or more above its original temperature and the sum of the three maximum rabbit temperature rises is not more than 1.4°C. If any of the individual rabbit temperatures exceed 0.6°C or the sum of the three maximum temperature rises is more than 1.4°C, then 5 additional rabbits are tested. The product is acceptable if no more than three out of all eight rabbits exhibit a temperature increase of 0.6°C or more, and if the total of the eight maximum temperature increases is no more than 3.7°C. A product is considered pyrogenic and is rejected if it does not pass the 8 rabbit test.

For more than forty years, the pyrogen test in rabbits has served well in protecting patients from febrile responses caused by contamination of medical products by endotoxins. One drawback of this animal test is that a product such as a short-lived radiopharmaceutical can not be tested using the rabbit test before it is administered. Also, studies have shown that it has a high coefficient of variation, a high false negative rate, and rabbits develop a tolerance for endotoxin upon repeated exposure<sup>12</sup>. The rabbit pyrogen test is costly, time-consuming, and varies in sensitivity to endotoxin depending on the rabbit colony used.

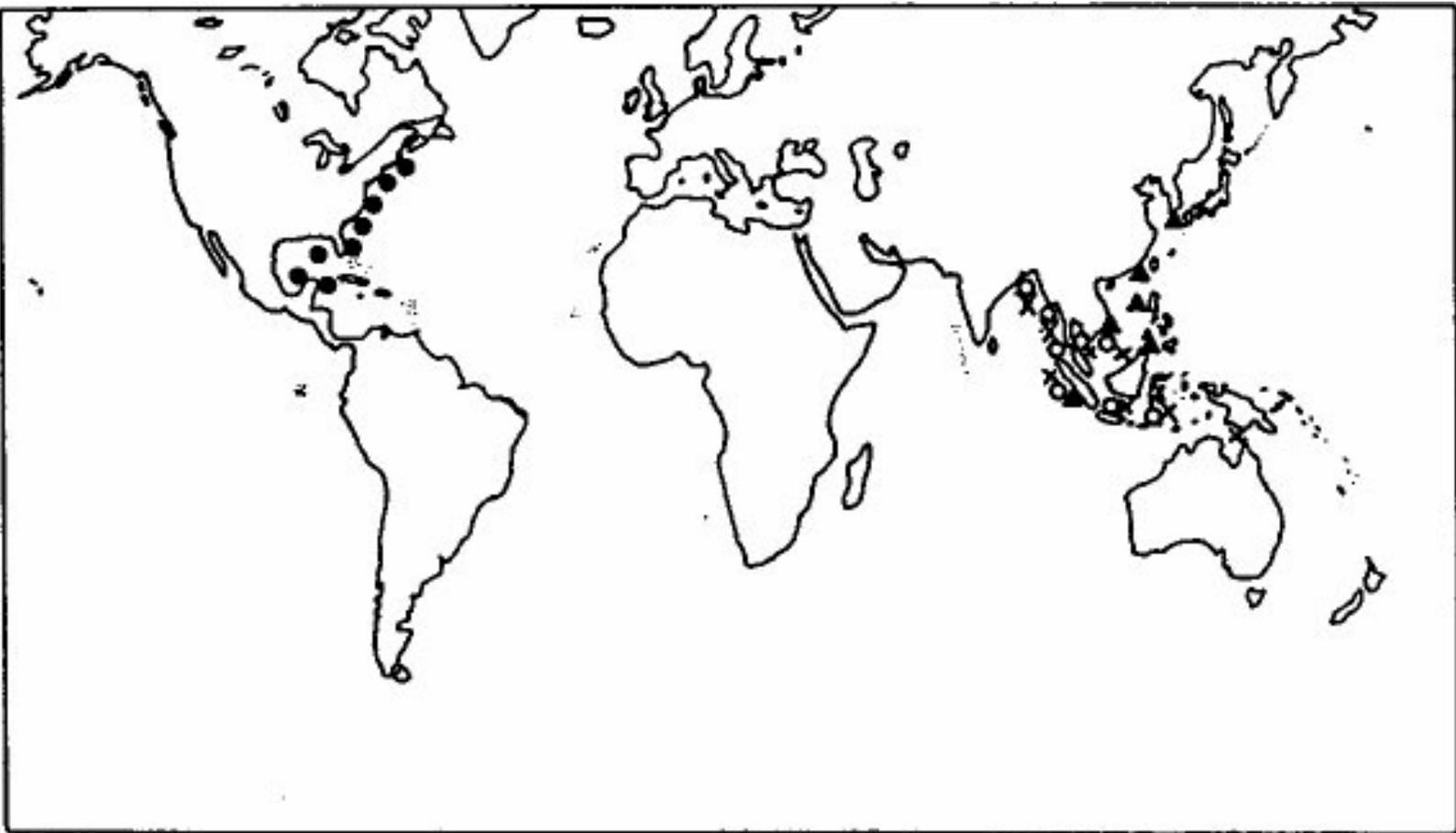


Figure 3. Distribution of the Extant 4 Species of the Horseshoe crabs. *L. polyphemus*: ●, *T. tridentatus*: ▲, *T. gigas*: X, *C. rotundicauda*: ○

Recently a new endotoxin test using a reagent prepared from horseshoe crab blood cells has been developed. Fossils of horseshoe crabs more than 10 million years old have been found. Four species of the horseshoe crab have survived since ancient times. One species, *Limulus polyphemus* is found along the Atlantic coast of North and Central America. The other three species *Tachypleus tridentatus*, *Tachypleus gigas* and *Carcinoscorpius rotundicauda* are native to the Pacific coast of Asia including China (see Figure 3)<sup>13</sup>. It was reported in 1956 that injection of gram negative bacteria into the horseshoe crab resulted in coagulation of its blood<sup>14</sup>. Observation of a gel clot after mixing endotoxin with lysed limulus amebocytes, the horseshoe crab blood cell, was reported in 1968 and was the basis of a new test for endotoxin, the Limulus Amebocyte Lysate (LAL) test<sup>15</sup>. The clotting mechanism, a reaction cascade involving enzyme mediated steps provides an amplification resulting in a test 100 times more sensitive in detecting endotoxin than the rabbit test<sup>16</sup>. In the reaction sequence shown in Figure 4, endotoxin causes activation of factor C which then causes activation of factor B followed by activation of a proclotting enzyme which acts on coagulogen to convert it to coagulen, the observed gel clot. Another activation pathway shown may cause false positives in a small number of test samples.

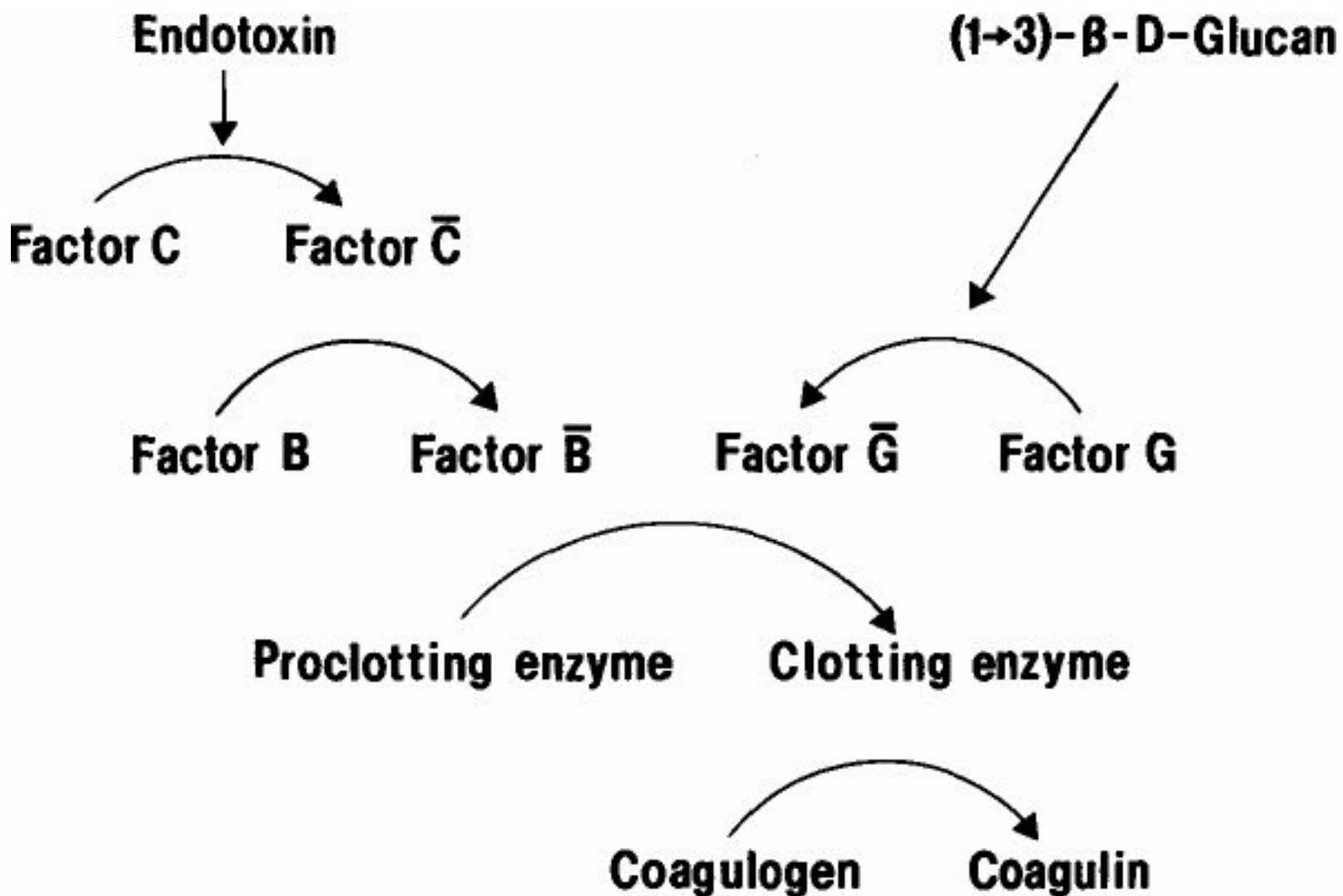


Figure 4. Schematic Representation of Coagulation System Found in Horseshoe Crab Hemocytes. A (1→3)-β-D-glucan-mediated pathway linked with the activation of Proclotting Enzyme is also shown.

The ubiquitous and stable nature of endotoxins compared to other pyrogens permits the substitution of the LAL test for the rabbit test. The LAL test is cheaper, faster, more specific, and sensitive to endotoxin than the rabbit test. Endotoxin tests can now be done in laboratories not maintaining animal colonies.

The United States Food and Drug Administration (USFDA) has permitted substitution of the LAL test for the rabbit pyrogen test since 1977 for medical devices and since 1983 for injectable drugs. USP has published the bacterial Endotoxins Test<sup>11</sup>.

In order to select an appropriate endotoxin limit for testing of medical devices, the device industry conducted a collaborative study to determine the average pyrogenic dose of the HIMA lot of Difco E. coli 055:B5 endotoxin in rabbits<sup>17</sup>. As a result of this investigation, 0.1 ng per ml of this Difco E. coli 055:B5 endotoxin or its equivalent was accepted as the endotoxin limit in medical device rinsings, assuming use of 40 mls rinse solution per device. For devices in contact with cerebrospinal fluid, the limit was set at 0.04 ng per ml.

Later, an official endotoxin standard was developed for determining LAL sensitivity. The United States Food and Drug Administration (USFDA) and the United States Pharmacopeial

Convention jointly developed a purified endotoxin standard from *E. coli* 0113<sup>18</sup>. Its potency is defined in terms of Endotoxin Units; one Endotoxin Unit (EU) is defined as the activity contained in 0.2 nanograms of the United States Reference Standard Lot EC-2, an earlier standard. The threshold pyrogenic dose for humans and rabbits is 5.0 EU per kilogram<sup>19</sup>. For drugs administered intrathecally, the threshold is set at 0.2 EU per kilogram. The potency of other endotoxin preparations in the LAL test can be correlated with the US/USP Standard Endotoxin.

Recent studies have demonstrated similar potency of the Difco *E. coli* 055:B5 endotoxin and the US/USP Standard Endotoxin in rabbits and in the LAL test<sup>20</sup>. In the future, endotoxin limits for medical devices will also be expressed in terms of Endotoxin Units.

Working guidelines for validating and testing medical devices with the LAL test have been available from FDA since 1979 and for drugs since 1983. Before substituting the LAL test for the rabbit pyrogen test for product release, the manufacturer is required to validate the test. Validation of the test involves demonstration of adequate sensitivity and reproducibility when running the test with endotoxin standards and then performing tests to show that the test is not inhibited by the product to be tested.

The sensitivity of the LAL clot test is determined by running a series of two-fold endotoxin dilutions which bracket the expected test endpoint with the selected LAL preparation. Sensitivity of the LAL test must be no less than the endotoxin concentration of the average pyrogenic dose, assuming an injection volume of 10 ml/kg in rabbits. This dose is 0.5 EU per ml for drugs and the equivalent of 0.1 ng per ml of Difco *E. coli* 055:B5 endotoxin for devices.

Rinsings of medical devices are used for inhibition tests and subsequently for product tests. According to the FDA guideline, ten devices per lot are each rinsed with 40 mls of nonpyrogenic fluid which is then combined in a total of 400 mls. This volume was chosen since it is identical to the USP pyrogen test requirement for rinsing intravenous administration sets. For inhibition tests, endotoxin is added to the device rinsings to create the same series of concentrations used in determining lysate sensitivity. If the endotoxin concentration at the clot endpoint is more than one two-fold endotoxin dilution less in sensitivity than the endpoint observed in concurrent sensitivity testing of the standard series, then the test is considered to be inhibited by the product rinse. The gel clot test normally varies by one two-fold dilution of endotoxin. If the product rinse inhibits the LAL test and the inhibition can not be eliminated, then the product rinse should be tested using the rabbit pyrogen test. If inhibition of the LAL test is not observed, the LAL test can be used instead of the rabbit test to routinely assure nonpyrogenicity of the device. If the materials used in or on the device change, then inhibition testing should be repeated. Substances found associated with devices which have been shown to inhibit the LAL test include high salt concentrations, methyl or propyl paraben, and heavy metal ions<sup>21</sup>. Some inhibition may be seen with isotonic saline depending on the lysate used. False positive reactions have been reported with cellulosic materials<sup>22</sup>.

For injectable biological products and human and animal drugs, validation requirements for the LAL test are similar to those for medical devices. In the case of drugs, the concentration of the endotoxin limit is calculated using the formula shown below.

$$\text{Endotoxin limit} = \frac{5 \text{ EU/kg}}{M}$$

M is either the rabbit pyrogen test dose or the maximum human dose that could be administered during one hour. For example, a product having a maximum human dose of 10 mL/kg should contain no more than 0.5 EU per mL. Product inhibition of the LAL test may be relieved by dilution of product with nonpyrogenic water. The maximum dilution permitted for testing may be calculated using the lysate sensitivity, product potency, and endotoxin limit for the product. For drugs and biologics, inhibition may be caused by some antibiotics and blood products such as human serum, plasma, and serum albumin<sup>21</sup>. Other causes of inhibition may include chelators, preservatives, and heavy metal ions<sup>21</sup>. Extremes in pH or viscosity can also effect the LAL test<sup>21</sup>.

In addition to the gel clot LAL test, more quantitative chromogenic and kinetic LAL test methods have been licensed by USFDA. The basis of the chromogenic assay is the use of a chromogenic substrate in the gelation reaction. An activated Limulus proenzyme cleaves substrates having the end configuration glycine-arginine-p-nitroaniline; the amount of p-nitroaniline liberated can be determined spectrophotometrically at 405nm. This assay permits quantitation of endotoxin; levels of 10 picograms endotoxin per ml can be detected using the chromogenic method<sup>23</sup>. The kinetic assay for endotoxin is based on determination of LAL endotoxin reaction kinetics by measuring rate of turbidity development<sup>24</sup>.

The FDA guideline outlines regulatory requirements for changing from the rabbit test to the LAL test for nonpyrogenic products to be sold in the United States. For products not requiring premarket approval, no application to USFDA is necessary if validation is performed according to the USFDA guidelines and documented to assure compatibility of the LAL test with the product. When premarket approval is required by USFDA, then validation data must be submitted to the USFDA in a New Drug Application for injectable drugs or in a Premarket Approval application for medical devices.

In summary, use of the LAL test for bacterial endotoxins provides a less expensive, less time-consuming, and more sensitive method for assuring nonpyrogenicity of medical products. It is anticipated that this test will be more widely used in the future because of its many advantages. The rabbit pyrogen test remains the test for those products which interfere with the LAL test.

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# **Safety of Residual Ethylene Oxide and Ethylene Oxide Concentration in the Working Environment of Sterilization Facilities**

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## **Safety of Residual Ethylene Oxide**

Among plastic materials, plasticized polyvinyl chloride (PVC) harbors the greatest amount of residual ethylene oxide (EO). Blood tubing of artificial kidney is made of PVC of approximately 5m in length. Blood circulates within this tube for 5-6 hours. Hemolysis will occur if residual EO extracted from the tubing were to reach a concentration of 80 ug/ml or more in the circulating blood<sup>1</sup>. As shown in Fig. 1, EO exhibits strong hemolytic activity.

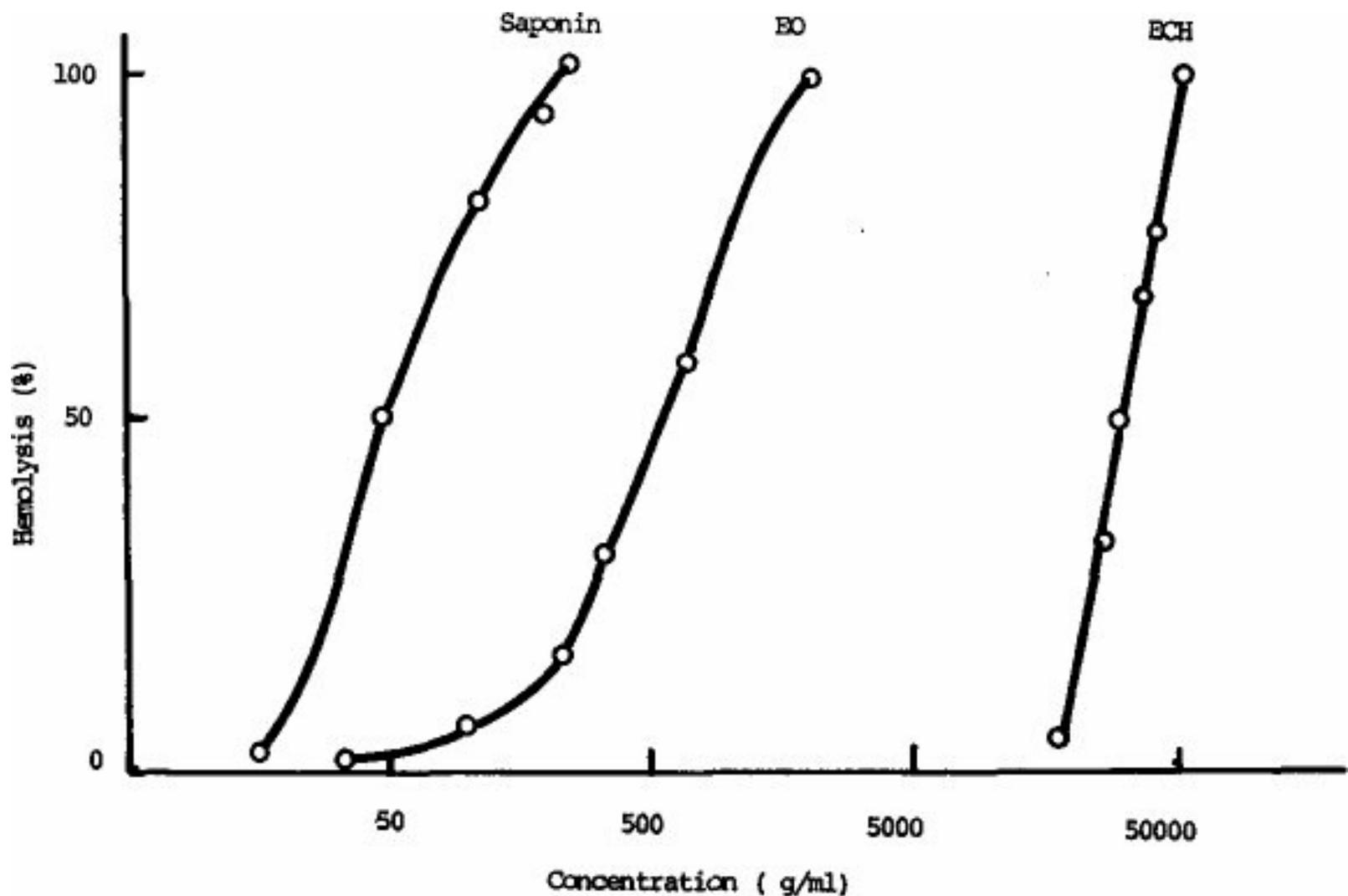


Figure 1. Dose-response Curves of in Vitro hemolytic Activities of EO, ECH and Saponin. The Samples Were Incubated for 24 Hours at 37°C and Each Point Represents the Average of 3 Samples.

This concern prompted us to investigate the relationship between the amount of residual EO in PVC and the amount of EO extracted during blood circulation<sup>2</sup>.

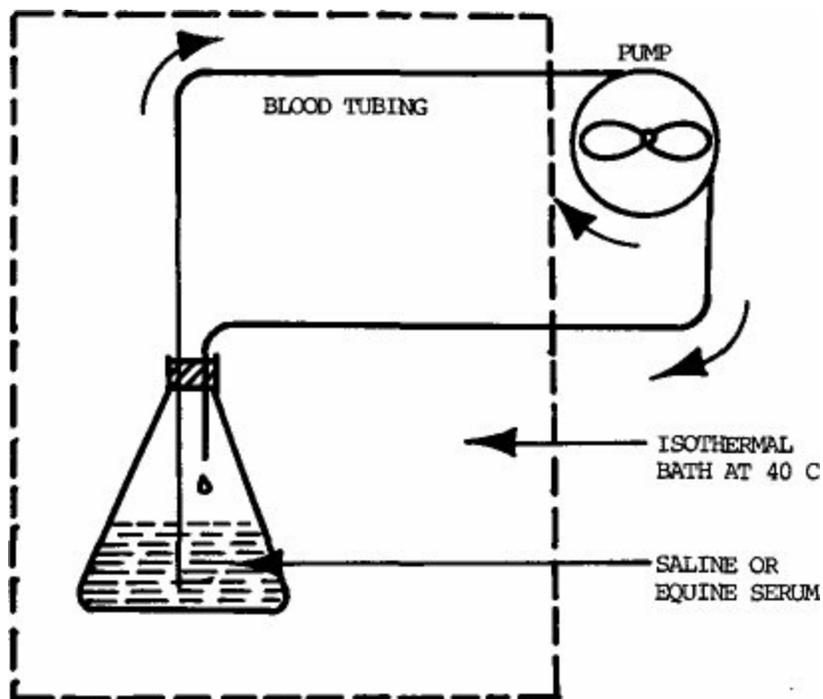


Figure 2. Circulation System.

Prior to setting up a circulation system as shown in Fig. 2, residual EO in blood tubing was measured by head-space GC method. Tubings with about 100ppm of residual EO were used. 500 ml of saline solution was then circulated at 40°C for 10 hrs at the rate of 20 ml/min.

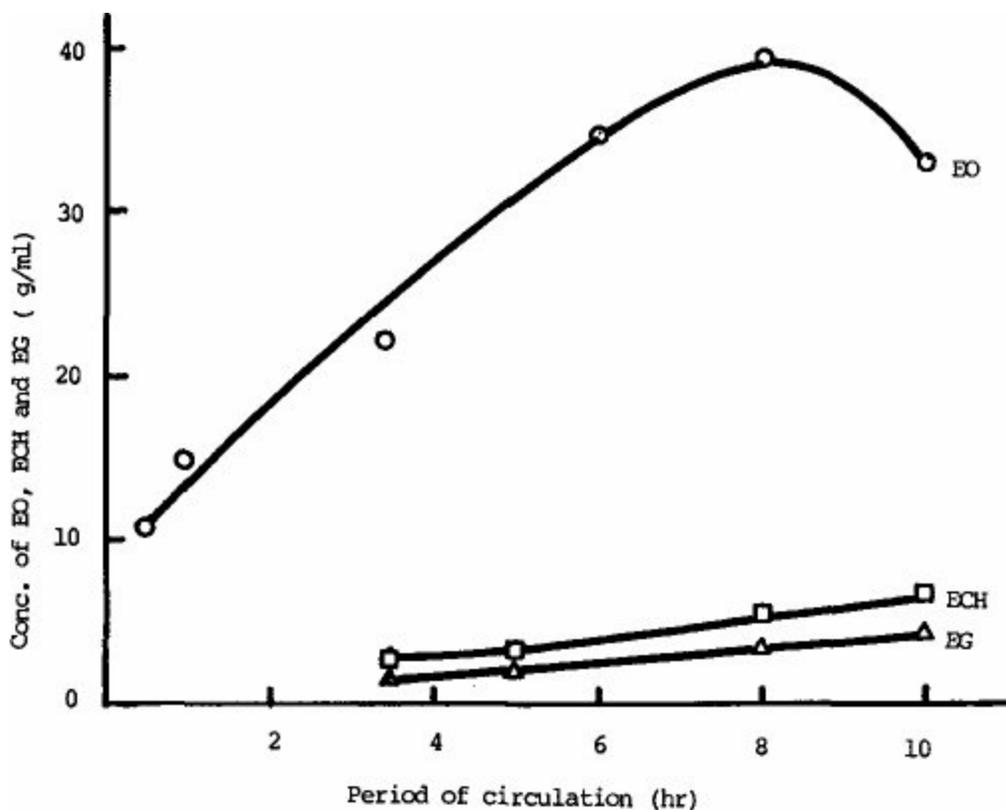


Figure 3. Relation Between Period of Circulation and Extracted Amount of EO, ECH and EG in Saline which was Circulated into Blood Tubing.

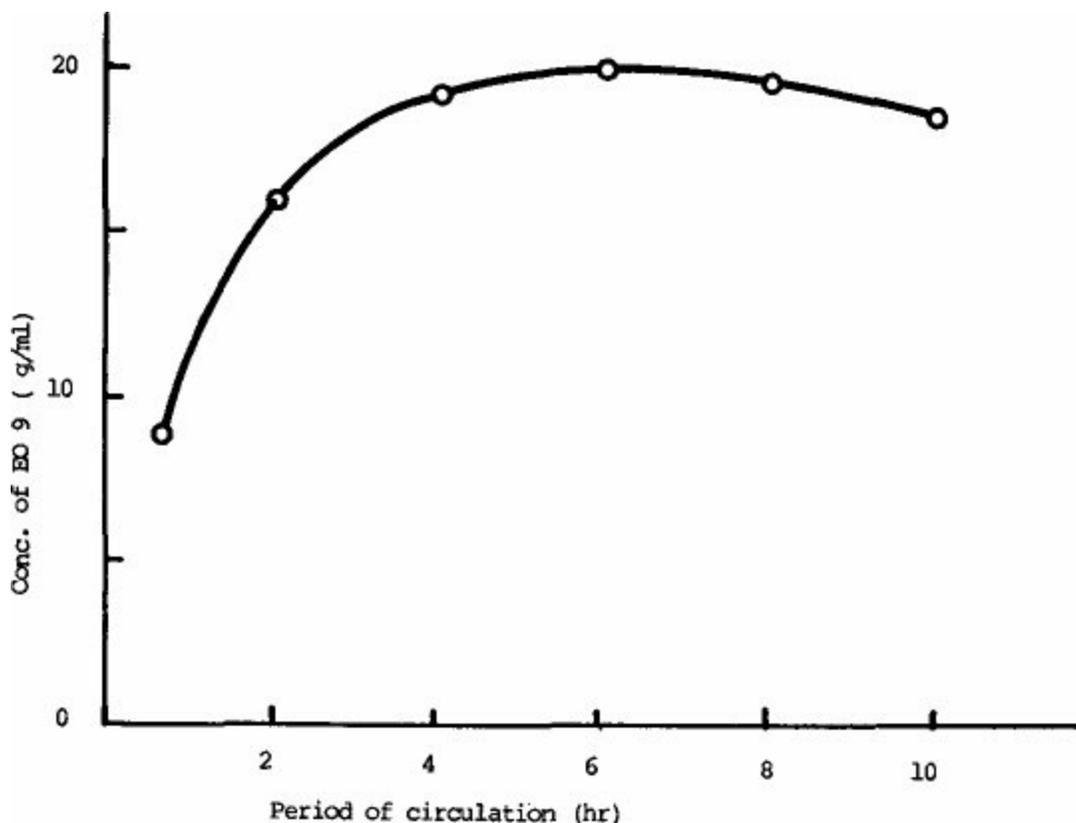


Figure 4. Relation Between Period of Circulation and Extracted Amount of EO in Equine Serum Which was Circulated Into Blood Tubing.

At 1, 3, 6, 8 and 10 hrs after initiating circulation, concentrations of EO, ethylene chlorohydrine (ECH) and ethylene glycol (EG) in 10 ml samples of the circulating saline solution were determined by GC method.

Results are shown in Fig. 3. Concentration of extracted EO was 36 ug/m1 after 6 hrs. Peak concentration of 40 ug/m1 was reached after 8 hrs. Concentration of ECH and EG converted from EO was less than 1/10 that of EO.

**Table I. Results of extracted EO from PVC tube**

Form of tube (weight)	Residual EO of tube (ug/g)	Extracting medium	Extraction condition	Extracted EO after 6 hrs
Commercial blood tubing for articial kidney	150	Saline 500 ml		36 ug/ml
		Closed circulation at 40°C		
	100	Equine serum, 500 ml		20 ug/ml
	40			3.4 ug/ml

This experiment was repeated with equine serum instead of saline solution. Results are shown in Fig. 4. Peak EO concentration of 20 ug/m1 was reached after 6 hrs.

Results of these experiments are again summarized in Table I. Since tubing weighed 200 g, it follows that the amount of EO extracted as a percentage of total residual EO was about 60% in case of saline solution, and about 50% in case of equine serum. Binding of EO to serum protein may account for this 10% difference in free EO. The same difference was measured in other experiments where tubings cut into short fragments were immersed in saline or serum.

From these results, we support the proposed FDA guideline which is 25 ppm maximum residual EO concentration for devices that come into contact with extracorporeal circulation. Table I shows that when saline solution is circulated in a tubing with 150 ppm residual EO, maximum extracted EO concentration is 40 ug/m<sup>1</sup> which is far below 80 ug/m<sup>1</sup>, the concentration required to induce hemolysis. In addition, it was found in another experiment that, when residual EO is 40ppm, extracted EO decreases dramatically to 3.4ug/m<sup>1</sup>.

Also, we propose that only residual EO need be measured to establish safety. When 500 ug/m<sup>1</sup> EO saline solution is incubated at 40°C for 20 hrs, amounts of ECH and EG converted from EO are approximately 10 ug/m<sup>1</sup> and 100ug/m<sup>1</sup>, respectively. Fig. 3 also shows that only very small quantities of ECH and EG are produced. Furthermore, in mutagenicity tests using E. coli, results were negative with up to 30 ppm of EO or 2,000ppm of ECH. Toxicity of EG is even lower than ECH. Therefore, an EO concentration of 25ppm or lower is sufficient to guarantee safety.

## **Ethylene Oxide Concentration in the Working Environment of Serilization Facilities**

Eight hours time weighted average (TWA) of EO to which workers are exposed in the working environment of EO sterilization facilities is limited to 50 ppm in Japan and 5ppm in the U.K. However, there was significant occurrence of primary brain neoplasm when rats were exposed to 30ppm of EO in a large-scale inhalation study conducted in the U.S.A. Thus, in 1984, the Occupational Safety and Health Administration<sup>3</sup> changed the limitation on environmental EO concentration from 50ppm, which is possibly carcinogenic, to 1 ppm.

We have measured the environmental EO concentration and the EO concentration to which workers are exposed in a medical device sterilization facility using 3M-3551 passive monitors.

The study<sup>4</sup> was conducted in a sterilization room of 3 m in height with a floor space of 6 m × 8 m. Within this room, there were two sterilization chambers and one ventilation fan in a wall. Each chamber was manned with a worker who worked eight hours from 9:30 a.m. to 5:30 p.m.

At 10:00 a.m. and at 4:30 p.m., the chambers were aerated twice, after which the workers emptied the chambers and filled them with a new set of unsterilized boxes of goods. During the 10:00 a.m. cycle, the workers emptied the chambers immediately after aerating the chambers. During the 4:30 p.m. cycle, the chamber door was left open for five minutes before emptying the chambers.

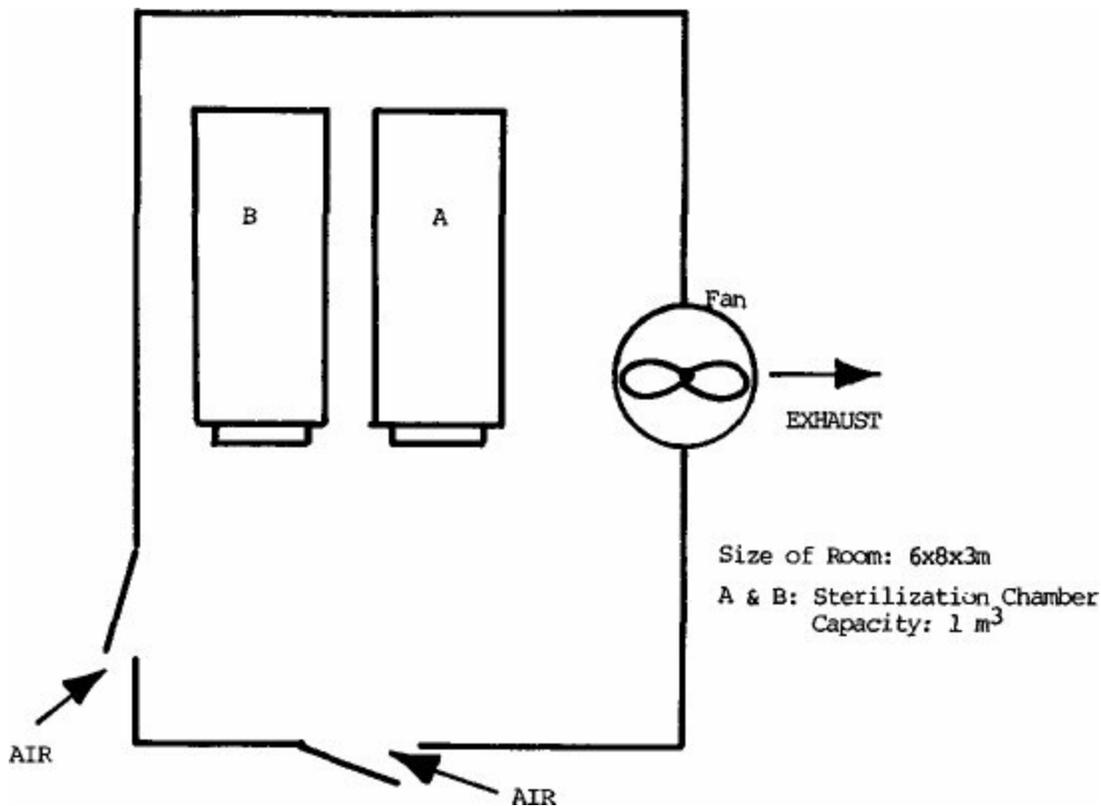


Figure 5. Sketch of Sterilization Room.

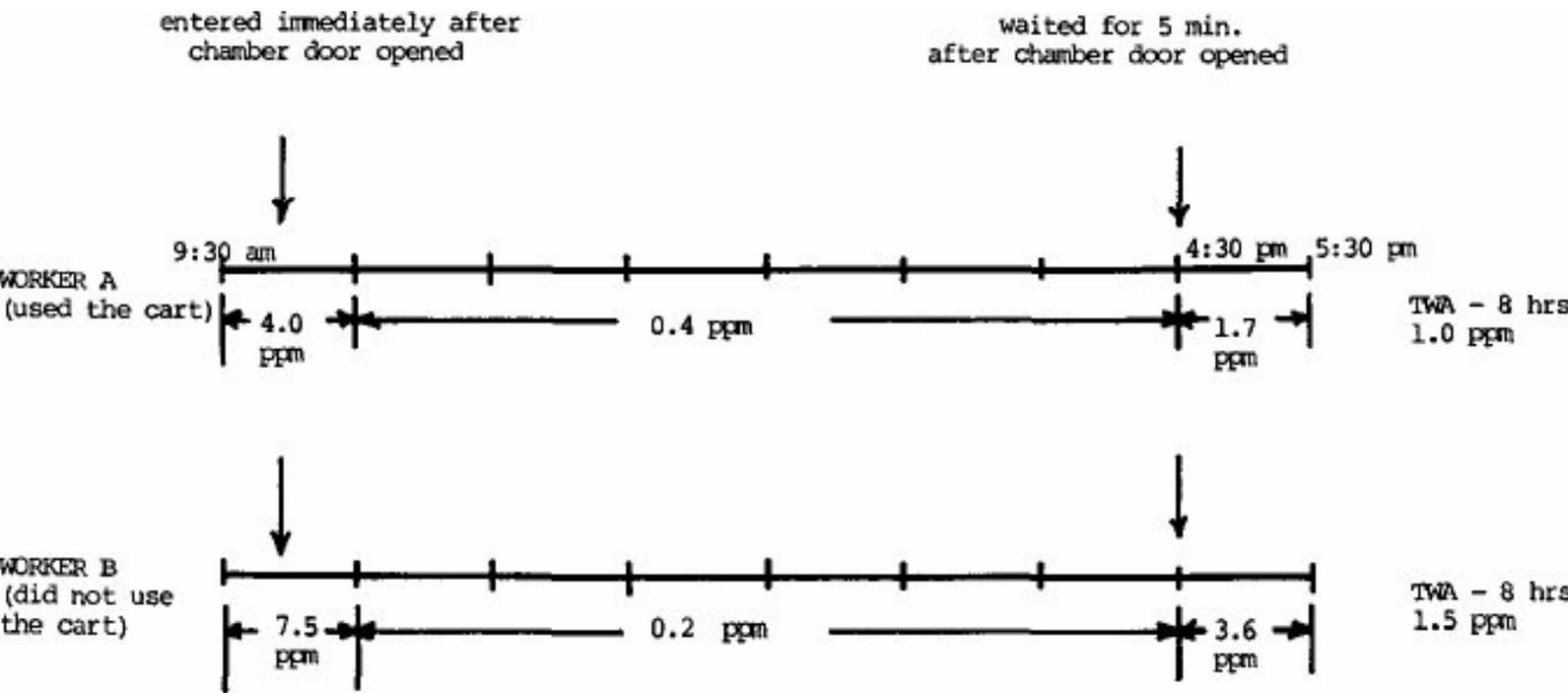


Figure 6. Time Table of Workers and EO Concentration Exposed.

One chamber was equipped with a rolling cart on which boxes were loaded. The cart slid in and out so that the worker (A) did not have to enter the chamber. The other chamber was not equipped with a cart; the worker (B) had to enter the chamber and work inside for approximately five minutes.

Results of this study were as follows:

1. Worker B who worked inside the chamber was exposed to twice as much EO as worker A who used the cart.
2. When workers left the chamber door open for five minutes before initiating work, they were exposed to one-half as much EO as when they started working immediately after opening the door.
3. Under these conditions TWA for 8 hours were 1.0ppm and 1.5ppm for worker A and worker B respectively.

At present, we are making efforts to further reduce the 8-hour TWA to less than 1ppm by ventilating the chambers through a hood which is installed above the chamber door, or leaving the door open for ten minutes or more before initiating work.

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# **Sterile Packaging in the Hospital and Medical Products Industry**

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## **Introduction**

There are two principle criteria for a satisfactory package containing a sterile medical device or product. The first is that the package allow the sterilization process to take place. This means that the package should not prevent the sterilization agent from reaching every surface of the device to be sterilized. With dry heat and radiation this may not be generally a problem, but with steam and gases such as ethylene oxide it is critical that the package allow penetration of the steam or gas. It follows, of course, that the package must allow the escape of the steam or gas following sterilization.

The second criteria is that the package maintain the sterility of the product or device until the moment of its use. Obviously all the efforts of hospitals and industry to design and validate reliable and efficient sterilization cycles are destroyed if the package is unable to withstand the environmental conditions and the handling that will occur during the interval between sterilization and use.

The importance of these two packaging criteria is put in perspective if one realizes that in the United States alone it is said that approximately 200 billion sterile packaged items and preparations are used annually in health care<sup>1</sup>.

These two primary criteria for sterile packaging apply equally to products packaged and sterilized in the hospital and those supplied by industry. However, they may be carried out differently because there are different conditions and restraints. For example industry must design its packages to withstand shipping and transporting over long distances, whereas hospital packages are generally used sooner within the same institution. Also, with radiation sterilization, industry must ensure that the package (and the device) will resist the degrading effects of the rays. Hospitals, on the other hand, do not generally use Gamma or Beta rays for sterilization.

The discussion below will briefly cover some historical comments and the identification of packaging types. Then it will cover four factors involved in the provision of efficient and cost effective sterile packaging: (1) selection of packaging materials, (2) validating packaging design, (3) process control during manufacture, and (4) environmental resistance.

# Historical Comments

Sterile barrier closures and test methods developed by eminent bacteriologists who founded this science provide an interesting prelude to our 20th century practices. The names of famous early scientists such as Pasteur, Appert, Mitscherlich, Schulze, Koch, and Spallanzani are among those whose findings and apparatus relate or serve as background, even today, to some aspect of sterile packaging technology. (Za,b) This is especially true with regard to the two packaging criteria of allowing and maintaining sterility.

- Spallanzani (1729-1799)—discovered that when organic solutions were placed in hermetically sealed and evacuated flasks and heated sterility could be maintained.
- Appert (1750-1841)—using Spallanzani's results answered a challenge of the French Government and devised a commercial method for packaging and preserving food and wine.
- Mitscherlich (1841)—while engaged in fermentation experiments may have devised the first apparatus for testing the microbial barrier properties of filter paper.
- Schulze (1815-1873)—devised an apparatus to show that air did not have to be excluded from a container in order to maintain sterility.
- Schroder, von Dusch, Pouchet, and others in the mid-1800's devised various apparatus to show how sterile fluids and environments could be maintained within various containers and packages.
- Pasteur (1822-1895)—devised various apparatus which proved that a tortuous path closure could be used to maintain sterility.
- Finally, the names of Chamberland (1851-1908), and Nordtmeyer (Berkefeld filter) call to mind the contributions of these early scientists with apparatus to remove microorganisms from fluids by filtration.

Thus, some basic packaging precepts, in the sense of barriers which allow and maintain sterility, were established by the forefathers of microbiology as they made discoveries that established our science. At the turn of the last century we already knew what conditions were needed to achieve and maintain sterility, including filtration principles, and various closure mechanisms.

## The Nature of Sterile Packaging

To return to the 20th century, we should begin by first mentioning the nature of modern-day medical device packaging. The materials used in industry to package sterile medical devices include films, foils, laminates, and webs.<sup>3a,b</sup> In addition, hospitals also use various types of cotton and synthetic wrapping materials in making, for example, sterile put-ups. Paper-based webs are by far the most widely used for industrially produced sterile product packages but synthetic materials are seeing increased use. The materials used by industry

almost always carry inked-printing and are frequently coated or laminated in various ways for various reasons. Hospital produced sterile packages are labeled in various ways but it is usual to see handmade, dated labels.

The configuration of sterile product packages varies widely in size, shape and function. Usually one or more of the types of packaging materials are used together with a suitable closure system to seal the device within the package. Closure systems utilize a variety of seal mechanisms including synthetic polymers, casein and animal glues and heat induced seals.<sup>4</sup> Packages frequently are designed with “peel-apart” opening features to facilitate use of the device without contamination occurring. Some devices are designed to be self-contained and still others may combine the self-contained features with a tortuous path mechanism to maintain sterility. In rare instances double sterile packages are used for staging devices into a sterile operating field or into a sterile environment occupied by an immunosuppressed patient. In some packages, attention is given to the need to avoid lint or fiber fall-out that could contaminate a surgical operating field. Finally, some packages also fill the function of maintaining the proper orientation of the enclosed item or of presenting enclosed items in the desired sequence.

## **Selection of Barrier Materials**

The selection of the barrier material to be used for a sterile package is an all-important first step. Initially the aim is usually to establish a preliminary specification for the purchase of the material. Obviously the sterilization method to be used in part determines the material requirements. For example, the characteristics of the barrier materials may derive from tests done by the manufacturer of the material (the vendor) or by the purchaser. Microbiological tests are usually done by the purchaser.

Many types of tests can be done in attempting to establish the material specification and these include:

- identification of composition
- weight per unit area
- permeation tests (particles or bacteria)
- porosity tests (air)
- infrared analyses
- microbiological tests (starch, etc.)

For the purposes of this discussion I will discuss briefly those tests and characteristics relating directly to barrier properties.

### *Microbial Penetration Tests*

In addition to the types of aerosol apparatus used in basic research with barrier materials a number of simpler test apparatus have been devised for evaluating industrial

packaging materials. The principle is simple; cause an aerosol of known concentration to be in a stream of air passing through the test material and then measure the downstream aerosol that penetrates the material. The results are generally expressed in terms of per cent penetration.

A number of such testing apparatus have been designed for both single and multiple sample testing<sup>5a,b,c</sup> and some are described in official specifications<sup>6a,b,c</sup>.

In the U.S. the Sterility Research Center of the Food and Drug Administration has been developing a small chamber in which bacterial spores that penetrate a 15 cm<sup>2</sup> disc of the packaging material are collected on a filter membrane<sup>7</sup>. In the FDA apparatus the flow rate through the material is 2.832 liters/min. (0.1 ft<sup>3</sup>/min) and the exposure time is 15 minutes.

The critical questions to ask about penetration testing relate to what are reasonable challenge conditions that would likely duplicate how the package is actually challenged when in use. To date there are no universally accepted standards as to size or type of aerosol challenge, flow rate, length of the test and other variables that could be included. It has been stated, however, that in use typical sterile packages can experience pressure differences of up to 2 cm of water pressure<sup>8</sup> and we believe that sterile packages are rarely exposed to environments with greater than 10 to 100 viable airborne particles per liter. Generally, however, we expect the exposure concentrations to be less than 10 particles per liter. As packaging technology advances, it is important that careful consideration be given to the setting of these parameters because setting them too stringent only adds to the cost of sterile disposable goods with no benefits.

## *Air Permeability Tests*

In the U.S. the Gurley densometer test is often used to determine the amount of time, in seconds, to pass a specific amount of air through a material at a controlled pressure<sup>4</sup>. In the U.K. the Bendtsen test is used which determines m1 of air per minute under a standard set of conditions. Both are good screening tests for candidate porous packaging materials and manufacturers generally establish an average acceptable range for such materials. For example Gurley densometer test values in excess of 600 seconds would suggest slow penetration of gaseous sterilants, while very low values would suggest possible higher aerosol penetrations.

## *Agar Contact Challenge Tests*

Typically these tests are designed to give some relative measure of the penetration of microorganisms when the material is in contact with a moist surface or a liquid.<sup>4</sup> In one version, the test material is placed on a sterile agar plate and wetted with an aqueous suspension of *Serratia marcescens*. Growth on the plate indicates penetration. In another test the packaging material is placed on an inoculated agar surface and penetration is detected by sampling the upper side of the material. The major utility of these tests is that the relative degree of penetration of several candidate materials can be evaluated. They also give some indication of resistivity to moisture during storage. Several other tests have

used flasks or jars of inoculated or uninoculated media to achieve similar comparisons<sup>4</sup>.

With regard to the testing of packaging materials to determine the barrier properties, the aerosol penetration test appears to provide the most valuable information. However, other permeability or microbial penetration tests under moist or wet conditions provide relevant information.

## Validation of Package Design

In addition to selecting an adequate barrier material, the design of the finished package must be validated. While many physical, chemical and other characteristics must be established and defined, the important microbiological evaluations can be called “finished package validation tests.” For convenience I will divide the tests that have been used into 4 types:

- Storage Tests
- Mechanical Agitation With Challenge
- Package Immersion
- Aerosol Challenge

### *Storage Tests*

This consists of sterility testing packages following (1) storage under in-use conditions, (2) a designated shipping routine, or (3) exposure to stressing conditions of vibration, temperature and relative humidity. Such testing in the U.S. has been used to show that properly designed packages maintain sterility under normal storage and transportation conditions over long periods. From such studies industry has concluded that the maintenance of sterility of a proper sterile package is event, not time, related.<sup>9a,b</sup> This directly relates to whether or not one should label sterile packages with sterilization expiration dates.

While actual storage tests do serve a purpose as just illustrated, they are “after the fact” and may not alone be the most important for package validation.

### *Mechanical Agitation With Challenge*

A typical test of this type might utilize a sterile packaged device placed into an outer wrap or bag containing a dry spore-talc suspension<sup>4</sup>. The outer wrap is sealed and placed in an agitation chamber for a specified time. Another version avoids the overwrap and places the primary packages in a tumbling chamber containing very small charcoal particles. In both types, entrance of the tracer organism or charcoal indicates package failure. In some instances these tests have utility but one must remember that they probably are not simulating real world conditions.

## *Package Immersion*

In tests of this type a primary package may contain a medical device or be filled with culture media and then sealed and sterilized. The test consists of immersing the package in a liquid suspension of challenge microorganisms and then determining if penetration occurred.<sup>4</sup> Obviously the use of this test is limited to packages that will withstand liquid immersion.

## *Aerosol Challenge*

Microbial aerosol challenge tests of finished intact packages have ranged from rather crude apparatus using arbitrary aerosol challenge conditions to more sophisticated instruments that attempt to duplicate real world conditions of exposure of the packaged sterile item.

Generally intact packages are placed in a closed chamber and a slight vacuum is applied. Then a microbial aerosol is generated within the chamber. Microbial penetration is indicated by a positive sterility test of the contents of the package.<sup>4</sup> Traditionally tests of these types have had two types of problems, one technique related and the other related to setting test parameters. From the technique point of view positive controls were needed to demonstrate the sensitivity of the test and negative controls were required to eliminate false positives due to inadequate decontamination before sterility testing.

As illustrated by a recent investigation the technique problems may have been largely overcome<sup>10</sup>: the use of high intensity ultraviolet light adequately decontaminates the outside of the challenged packages before sterility testing and a device capable of putting very small holes of consistent size in packages has provided the positive control and a measure of test sensitivity.

Of the general approaches to validating the package design and evaluating intact packages, aerosol challenge methods seem to be the most useful. Also, most importantly, the aerosol challenge can be combined with or applied after other treatments to validate environmental resistance during and after shipping and handling. This is not to say, however, that storage, package immersion and other tests should not be done as appropriate for certain purposes or for certain types of packages.

A final comment on the aerosol challenge method relates to the test parameters selected. It was earlier indicated that intact sterile packages are generally not expected to see pressure differentials greater than 2 inches of water. With regard to expected concentrations of airborne microorganisms to which sterile packages might be exposed, a recent study<sup>11</sup> quoted an average hospital ambient microbial aerol level of 125 per m<sup>3</sup> (0.0125 per liter or 0.375 per ft<sup>3</sup>). Such figures should be kept in mind when setting challenge levels for intact package aerosol challenge tests.

## **Process Control During Manufacture**

When a satisfactory barrier material for a sterile package has been selected and the package design shown to be valid, the next consideration relates to whether the manufacturing process or processes will daily produce thousands of the package sterile items in a consistent manner. Historically, the theme of quality control programs during the manufacture, packaging and sterilization of medical products has been toward the *detection* of defective products or packages. Stated another way, quality control by the traditional method seeks to detect component, product, package or process defects by testing and inspection in order to separate satisfactory product from product that must be reworked, repackaged, resterilized or scrapped. The problems of the detection approach are many but two principal ones are that (1) the control is after-the-fact (e.g.: the product is already manufactured) and (2) the system is inefficient and does not detect and eliminate all defects.

Modern process control during manufacture, packaging and sterilization is turning away from the *detection* approach in favor of what is called the *prevention* approach. Thus defect *prevention* is the monitoring of the process, for example the packaging process, to determine in real time or near-real time (1) when adjustments are needed to maintain process stability and (2) when changes to the process are necessary to reduce inherent variability. The overall objective is the application of statistical process control to allow corrective action to be taken *before* a defective product or package is produced. Furthermore this *prevention* approach to process control concentrates on the customer or user of the sterile packaged device by:

- Assuring satisfaction in use
- Meeting customer needs and expectations
- Providing the proper product life cycle
- Providing product at an acceptable cost

This prevention quality theme is being applied in most industries worldwide today and it applies to sterile product packaging. For package defects, the objective and the concentration of effort should be on defect *prevention*, not defect *detection*. While routine control measures and control tests during and after manufacture serve as a safeguard against defects already produced, true process control using robotics, computers, servo-feedback mechanisms and aiming at maximum package defect levels in parts per million instead of parts per hundred are the most needed ingredients for better sterile product packaging.

To illustrate this, it has been my observation that most problems with sterile packages in meeting the two criteria mentioned in the beginning are due to the variability of the process when high volume production takes place. The problems are *not* primarily due to selection of the wrong barrier material, nor to an untested or inadequate design, nor to degradation of sterility during handling or over time. Most are due, in fact, to uncontrolled variation in the day to day, hour to hour sterile package production process. Failure to maintain critical process parameters (such as sealing temperature or pressure) or even the failure to identify which parameters are critical, are examples of why industry must employ statistical

engineering tools for process diagnosis and then use remedial measures that eliminate process variability. This point is further illustrated by the fact that in the USA most recalls of packaged sterile medical devices are not due to failure to properly sterilize the devices. Instead most recalls of this nature follow the discovery in the field of open packages, largely due to lack of process control during manufacture.

It is clear, I believe, that the most important contribution we can make toward further improving sterile product packaging is to design our processes for defect prevention and to achieve absolute process control.

## Conclusions

A satisfactory package for a sterile medical device must (1) allow the sterilization process to take place and (2) maintain the sterility of the device until the moment of use. The history of microbiology and public health saw the development of many of the microbiological basics that apply to modern-day packaging. Advances in modern packaging technology are a logical outgrowth of the tremendous increase in the use of sterile medical devices (especially disposables), which in turn results from continual advances in medical and surgical procedures.

Industry and hospitals have combined microbiological expertise, materials experts, manufacturing process controls, and various standardized tests to insure a high level of confidence that packages are efficient and cost effective. In broad terms, the development and use of sterile medical device packaging requires attention to four factors:

- Selection and testing of the barrier materials
- Adequately validated package design
- Process control during manufacture
- Environmental resistance during shipping and handling

Of the microbiological methods available for initial testing of barrier materials, aerosol penetration tests appear to provide the most information although others are certainly useful. The testing of complete packages is done both to validate packaging designs and to demonstrate adequate environmental resistance during and after shipping and handling. Of the tests that have been used, controlled aerosol challenge of intact packages, using both positive and negative controls, is often the method of choice. Aerosol challenge tests, however, are not suitable for routine quality control checking. For both, however, it is essential that the challenge parameters be set carefully to reasonably simulate real world conditions.

Of all of the factors related to production of sterile product packages, the need for improvements in process control is the most pressing. The application of statistical engineering tools to identify critical and varying manufacturing process steps and the use of improved procedural, mechanical and micro-electronic controls should be used in advancing our packaging objective of producing and maintaining product sterility.

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# The Hospital Environment - Nosocomial Infections

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## Introduction

What is the purpose of the industrial production of sterile single-use medical equipment, as well as of the sterilization and disinfection performed by the hospitals and by the primary health care systems? Obviously, it is to supply the public health and medical services with safe and efficient products. Due to that aim, the end use of the products should be kept in mind during the entire manufacturing process. The function and quality of the products should be considered in the context of their practical use. The requirement as to sterility, disinfection and packaging should agree with the quality needed for the practical use. If the requirements are too low, the results of the medical measures are jeopardized. On the other hand, should the requirements be too high, there is a waste of material and economical resources encroaching on such resources better needed for other purposes within the medical services. The equilibrium in the reasonable levels of quality may be difficult to assess, as the products intended for world trade need a uniform quality although they may be used under very different conditions. Double standards should be avoided for several reasons. It is often impossible to know the final use of a certain product. It may be used under very sophisticated conditions during a heart transplantation or for vaccination in a jungle village or in a camp for refugees or during conditions of war. Also, for legal reasons, the quality requirements need to be uniform whether national laws and regulations are concerned or in the event of civil suits for damages.

The relationship between the risks of using a certain product in the hospital environment and other risks for hospital infections should be considered when assessing a reasonable safety level for the product. To elucidate that, I shall first point to some characteristics of the hospital environment and causes of hospital infections, then make an attempt to summarize the current states of the art in the field of hospital injections; types of infections, incidences, trends, costs and counteractions. Finally, I shall discuss the implications in terms of needs for sterilization and disinfection of medical equipment and environment with examples of critical medical procedures, critical products and the basic levels of essential requirements.

# Definition

First of all, however, we need to define the concept of nosocomial infections (Table I). Initially, nosocomial infection was used for hospital-acquired infection mainly for cross-infection meaning infection acquired in hospital from other people, either patients or staff, or from inanimate objects within the hospital. All these infections of a person occurring from exogenous sources during hospitalization are called exogenous infections. The exogenous hospital infections dominated as a recognized problem in western medicine from the time of Semmelweis, Florence Nightingale and Lister up to the 1960s when the main interest gradually shifted from the cross-infections caused by streptococci or staphylococci to infections in patients with decreased resistance to infections due to some severe underlying disease, advanced age or advanced surgery. These infections are often caused by microorganisms that originate from endogenous sources, as the indigenous commensal flora carried by the patient. The skin, the mouth, the bowels and so on contain a normal microbial flora with a large variety of microorganisms; several hundred different types of bacteria and microscopic fungi, especially anaerobic bacteria. The numbers of these microorganisms are very high; for instance, the colon of a health person contains about  $10^{11}$  bacterial cells per gram of bowel content. Infections due to this commensal flora are called endogenous infections. Debilitated patients may succumb not only to infections caused by their own normal flora, but only to free-living organisms with other habitat than the human body such as bacteria originating from the water system of the hospital, eg., *Pseudomonas* or *Legionella* bacteria, or from the food. Infections with organisms that do not usually harm the healthy person are called opportunistic infections.

**Table I. Some Common Concepts**

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Nosocomial Infection	Exogenous Infection
Hospital-Acquired Infection	Endogenous Infection
Cross-Infection	Opportunistic Infection

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However, with increased understanding of the problems, the definition of nosocomial infection has been widened. Particularly after the spread of hepatitis B during the 1960s among hospital personnel in renal transplant surgery, in hemodialysis units and in the laboratory service from handling of blood specimens, it was obvious that the occupational hazard of infections in the hospital workers had to be considered part of the nosocomial infection complex. The staff of a hospital is an integral part of the intra-hospital epidemiological system. The staff members can both contract infections and participate in the further spreading within the hospital to patients or to other members of the staff, for instance of airborne infections as tuberculosis.

Another aspect concerns the relation between hospital and community acquired infections. A hospital is not a closed community. The infections spread in a hospital may reflect the infections prevailing in the community in addition to the more specific hospital

infections. However, due to the special environmental circumstances in a hospital with a crowd of patients especially vulnerable due to their age or severe diseases, community-acquired infections may spread more readily and be more dangerous to the lives of the patients in a hospital than in the private homes, the schools or places of work. Influenza may be fatal to the elderly patients, diarrhoeal diseases or measles to the pediatric patients.

The next step in the development of the understanding of the nosocomial infection problem is to broaden the concept to the out-patient and primary health care system and stop over-emphasizing the role of the hospital buildings themselves. Patients are referred to the hospitals from the primary health care bringing infections with them when admitted and are returning to the primary health care after hospital treatment, maybe with new, hospital-acquired infections that may not have been recognized during a short hospital stay for an acute operation. Hospital infections in the new-borns are often not recognized until the mother and baby are back home from the obstetric clinic or the delivery station. It is very short-sighted therefore, not to include the out-patient and primary health care in the surveillance and prevention of nosocomial infections.

The analysis of the problems just discussed brings us to the collected and up to date definition of nosocomial infections (Table II):

### **Table II. Definition of Nosocomial Infection**

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Any Clinically Recognizable Infection Disease in

- PATIENTS as consequence being admitted to hospital or treated in out-patient care whether or not agent originates from patient or symptoms appear while in hospital.
  - STAFF as consequence of the occupation
- 

Nosocomial infections are defined as any clinically recognizable infectious disease that effect:

- The patients as a consequence of being admitted to hospital or treated in out-patient care, whether or not the causative agent originates from the patient and whether or not the symptoms of the disease appear while the effected person is in the hospital or
- The staff as a consequence of the occupation.

### **Characteristics of the Hospital Environment**

I have already indicated some of the conditions that make the hospital environment hazardous from the epidemiological point of view (Table III).

- Gathering of persons with diminished resistance to infections due to their diseases, injuries, advanced age or prematurity.

### **Table III. Some Reasons for the Appearance of Hospital Infections**

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- Gathering of people with diminished resistance to infections due to disease and age
- “The price of success”, advanced surgery, intensive care, invasive procedures, immunosuppressive therapy

- Appearance and spread of antibiotic resistance
- Use of equipment posing new risks (endoprosthesis, respirators, dialysis)
- Overcrowding of patients
- Lack of trained personnel
- Rationalization of medical and auxiliary services (E.. central operation theaters and I.C.U., ambulatory teams for laboratory analysis, for I.V. supplies, physiotherapy, for taking specimens, central auxiliary services for cleaning and bedmaking, dishwashing)

- “The price of success” diminished resistance to infections due to medical progress in the treatment of underlying otherwise fatal disease of the patients. Such progress may be advanced surgery, intensive care with artificial respiration, intravenous lines for alimentation, urethral catheters, dialysis, invasive diagnostic procedures (i.e. with arterial catheters), immunosuppressive chemotherapy used in transplantation or cancer therapy, radiation therapy.
- Providing an environment that favors the appearance as well as the spreading of antibiotic resistant bacteria
- Use of technical equipment that increases the risk of infections such as endoprosthesis in neurosurgery, thoracic and orthopedic surgery, respirators and other sets of instruments or mechanical appliances, e.g. for dialysis.

Unfortunately, in most countries, the hospitals are also characterized by overcrowding and lack of trained personnel. It seems to be a universal phenomenon that the demand for hospital care is bigger than the economical and physical resources, even in the richest countries. The disproportion between supply and demand has been coped with increased rationalization in many countries, e.g. connecting all operative activity to a central operation department, all intensive care to one I.C.U. (intensive care unit), creation of special teams for intravenous supplies, for physiotherapy, for drawing blood specimens for laboratory analysis. Central auxiliary services have been arranged, e.g. for cleaning, for bedmaking, for preparation of food and for dishwashing. All these services increase the opportunities for infectious agents to be spread between different wards and clinics.

## Types of Hospital Infections

In the big US so called “SENIC project” (Study of the Efficacy of Nosocomial Infection Control) urinary tract infections (UTI) was reported in 42%, postoperative wound infections in 24%, pneumonia in 10% and bacteremia in 5% (Table IV) (Haley, R. W., et al, 1985).

**Table IV. Senic-Project**

	Percent
Urinary Tract Infection	42
Surgical Wound Infection	24

Pneumonia	10
Bacteraemia	5
Other Infections	19

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The types and proportions vary for different countries. In tropical areas and in areas with poor sanitation, diarrhoeal diseases are responsible for a considerable proportion, especially in the pediatric clinics. Thus, nosocomial spreading of bacillary dysentery, enteropathogenic coli infections, salmonellosis and rota virus infections are known to pose nosocomial problems in many countries.

In addition, the causative agents differ between the countries and also with the time. *Streptococcus pyogenes* (-hemolytic streptococci group A) that constituted a major nosocomial problem during World War II and *Staphylococcus aureus* that caused the main problems during the 1950s and 1960s have been replaced by Gram-negative bacilli in many western countries and in Japan though *S. aureus* still constitutes the biggest threat in a big part of the world. Besides *S. aureus* has returned as a cause of serious hospital outbreaks to some western countries during recent years, now as methicillin resistant.

With respect to the object of the present conference, I would like to give two important examples of infectious diseases where contaminated needles, syringes and blood transfusions play an important role for the spreading of the disease, namely hepatitis B and acquired immune deficiency syndrome (AIDS). Hepatitis B is very common in this part of the world as we heard from Professor Li yesterday with a virus carrier rate around 10%. Due to the high virus titers hepatitis B is highly contagious through blood transfusions, injections, needle sticks, acupuncture, ritual scarring, circumcision, tattooing and other practices when the skin or mucous membranes are penetrated.

AIDS-virus is now rapidly spreading in Central Africa, North American and Europe. In addition, there are many cases in Western Pacific, Central and South America. However, in Asia there are, in October 1985, only a few cases known—so far. As the disease is fatal and there is, at present, neither a vaccine nor an effective care, it is important to try to prevent further spreading. Similar to hepatitis B-virus, AIDS-virus is transmitted horizontally through blood and sexual contacts and vertically from mother to child. Both diseases have a long incubation period and carrier state. In AIDS, the incubation period may be at least five years and the carrier state life-long. In Central Africa, AIDS-virus infections seem already to be common in the general population. Serological markers for virus carriers have been demonstrated between 4-10% in blood donors, pregnant women and the population at large in studies from various urban African areas and much higher in risk groups as female prostitutes in the big cities. The major part (about 80-90%) of the sero-positive adults seem to have contracted the infection by heterosexual contacts. Homosexuality or intravenous drug addiction are not known in these countries. The remaining 10-20% infections of the adult persons are thought to be caused by blood transfusions or injections. In the sero-positive children, 50% are considered to be due to vertical transmission and the other 50% due to blood transfusions or injections. It seems to be a widely used practice to give

transfusions and injections on very weak indications and without proper hygienic precautions. The same syringe and needle are frequently used for several patients without cleaning and sterilization. In addition, syringes and needles for single use are reused after boiling, making many of the products deformed with bad functioning. An especially alarming feature in African AIDS is the high proportion of sero-positive women in child-bearing age. The male to female ratio is 50-50 and the infected persons are younger than in North-America and Europe. Therefore, the vertical transmission from the mother to the fetus or infant may constitute a serious threat for the future for millions of human beings. As one of the steps to counteract AIDS in Africa, it was recommended at a WHO meeting in Africa last week to pay particular attention to the sterilization of syringes and needles and to control the blood donors.

## **Incidences and Costs of Nosocomial Infections**

Based on the results of the above mentioned SENIC-project, it is estimated that 2.1 million nosocomial infections occurred in the acute-care hospitals in the United States in 1976. The total number of admissions was about 38 million that year and the infection rate 5.7 nosocomial infections per 100 admissions. That means at least 7.5 million extra hospital days and over one billion dollars of extra costs. The estimated number of hospital infections rises from 2.1 to 3.6-4 million when the longterm care hospitals are included, increasing the costs accordingly.

The annual costs for nosocomial infections in Germany were estimated to be 500 million to 1 billion German marks (DM) (Daschner, 1984). In another US study, the costs for different nosocomial infections in various hospital departments were compared (Pinner et al, 1982). Some of the results are summarized in the next slide (Table V). The infections are ranked according to costs. Surgical patients with lower respiratory infections were especially expensive.

An Israeli group estimated the effect of nosocomial infections on the length of hospitalization in a prospective study. The increased mean hospital stay was about 5 days for UTI, 12 days for surgical wound infections and 25 days for patients with more than one surgical infection (Green, M.S., et al, 1982).

In France, the costs of nosocomial infection in a neonatal unit were compared with matched, uninfected controls. The increase in length of hospital stay was 23% and in the total hospitalization costs 32% equal to 1250 US dollars for each case of infection (Girard et al, 1983). In a European multicenter study, a survey of the incidence of bacteraemia and the use of intravenous (IV) devices among about 11,000 surgical patients was performed in eight countries, among them the Scandinavian countries, the Netherlands, West Germany and England (Nystrom et al, 1983). Sixty-three percent of the patients had had an IV device inserted some time during their hospital stay. The incidence of device-related thrombophlebitis was about 10%. Among the surgical patients not given IV therapy, 0.05% had a hospital acquired bacteraemia. The corresponding figure for patients with a peripheral but not central IV device was about 4% and for patients with a central venous catheter as high as 45% (Table VI).

**Table V.**

Site (N = 183 Patients)	Average Costs (USD) Attributable to Nosocomial Infection
Lower Respiratory Tract	1,255
Injury	1,221
Bacteraemia	903
Surgical Wound Infection	886

In another European multicenter study on UTI and bacteraemia of some 3,900 patients in about 170 wards, again in the eight countries, the prevalence of UTI and bacteraemia was 12.6 and 1.6, respectively (Table VII) (Jepsen et al, 1982). One-half of the infections were acquired after the admission. The association between nosocomial UTI and the presence of an indwelling catheter was statistically confirmed. An indwelling catheter was present in 57% of the infected patients and in 6% of those without UTI.

**Table VI. Incidence of Hospital Acquired Bacteraemia in 10,616 Patients.**

	Percent
Patients with IV-Device	63
Device-Related Thrombophlebitis	10
— No Device	0.05
Bacteraemia	
— Peripheral IV Device	3.7
— Central IV Device	44.8

**Table VII. Prevalence of UTI Infection and Bacteraemia in 3,899 Patients.**

	Percent
UTI	12.6
Bacteraemia	1.6

Indwellin cather present in 56.7% of infected patients vs 6.3% of those without UTI. Nosocomial bacteraemia 5 times higher in patients with UTI. Significant part of UTI and bacteraemia device-related.

## Current Trends

It is suggested that the incidence of nosocomial infections will increase in spite of various

intervention programs due to the progress of medical treatment that will tend to prolong the survival of persons at advanced age or with severe injuries or diseases, thereby creating a patient population more prone to contract hospital infections. The immunosuppressive chemotherapy in cancer and transplantation will be used more widely and the problem of antibiotic resistance will be still larger. Emerging new pathogens as the AIDS-virus will worsen the situation by creating more immunodeficient patients needing advanced nursing and occupying medical services that are already too short.

All this calls for more efficient measures to control the nosocomial infections and antibiotic usage. In addition, there are activities in many countries to reduce the costs by discontinuing, for instance, unnecessary disinfection procedures as formaldehyde fogging, floor disinfection and disinfection mats by employing more cost effective environmental control procedures (i.e. no routine environmental cultures, no UV lights, provided there is an effective ventilation system) and by re-sterilizing expensive products as well as saving money by more rational antibiotic treatment and prophylaxis (Table VIII) (Kallings, 1981 and Daschner, 1984).

**Table VIII. Save Money by Avoiding Ineffective Practices.**

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Unnecessary disinfection procedures	— Fogging
	— Floor disinfection
	— Disinfection mats
No UV lights in operating theatres	
No routine environmental culture	
No plastic shoe covers	
Restricted antibiotic usage	

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Programs for Control of Hospital Infections

The programs include, i.e., (McGowan, 1985), (Table IX)

- Training
- Surveillance of infection rate and performance
- Proven procedures as handwashing, closed systems for urinary drainage, handling of IV devices, cleaning of equipment for respiration, isolation and dressing techniques
- Monitoring of antibiotic prophylaxis for certain surgical procedures
- Essential disinfection practices
- Sterilization
- Eliminate practices that do not work

The mere recording of surgical wound infection has been found to reduce the frequency of infections by about 30% both in the US SENIC study and in multicenter studies in Sweden during several years. Several studies from different countries have verified the cost-effectiveness of the programs listed.

## Table IX. Priorities for Control of Nosocomial Infection

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- Training
  - Surveillance of infection rate and performance
  - Proven procedures as handwashing, closed systems for urinary drainage, handling of I.V. devices, cleaning of equipment for respiration, isolation and dressing techniques.
  - Monitoring of antibiotic prophylaxis for certain surgical procedures
  - Essential disinfection practices
  - Sterilization
  - Eliminate practices that do not work
- 

## Critical Procedures

Nosocomial infections have no single or predominant cause. Each infection has a multifactor background. Most factors are either unknown or not evaluated in terms of figures. Thus, the infectious agent is not the sole factor that determines if an infection will occur. Risk factors connected to medical procedures and deficiencies in the patient's defense to infections depending on age, disease and so on are equally important. The relative importance of these various determinants can be measured by the aid of multivariate analysis (Table X) (Simchen et al, 1981). In a large prospective Israelic study, the variable most highly associated with nosocomial infection was more than one operation during a single episode of hospitalization with an odds ratio, up to 9.8. Prolonged operation time resulted in an increased odds ratio, up to 3.5. If the recommended protocol for prophylaxis in surgery was not followed, the odds ratio rose up to 8.6.

Certain risk factors are unalterable such as age and underlying disease. Obviously, we cannot influence these given factors. The major alterable factors are those connected to specific medical procedures or environmental conditions. In a study from Boston, USA, the presence of an endotracheal tube was associated with an increased risk of acquiring a hospital infection equivalent to an odds ratio of 10.6 (Kass, 1980). The odds associated with the presence of an urethral catheter was 5.9 and with an intravenous catheter 3.4. The corresponding odds associated with some unalterable factors were 3.4 for acute admission and 2.9 for age over 65 years.

## Table X. Risks Associated with Various Determinants

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	Odds Ratio
More than one operation	9.8
Increase length of operation	3.5
Prophyl, protocol not followed	8.6
Presence of endotracheal tube	10.6
Presence of urethral catheter	5.9

Acute admission	3.4
Age > 65 years	2.9

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## Critical Products

The sterility of some products are clearly more critical than that of others. The sterility of endoprosthesis, i.e., used for total hip replacement or heart surgery, of surgical sutures, instruments, gloves of intravascular device and infusion fluids, or blood donor sets is more important than that of the surgical masks and caps or the plastic bag for collection of urine or that of the emesis (kidney) basin or of the rectal tube.

In principle, the procedure may be separated in groups of relation to the clinical requirement, i.e., (Table XI).

- I. Products packed by the piece, sterile (less than  $10^{-6}$  viable microorganisms per unit), pyrogen free, low content of particles. The packaging maintains sterility during transportation and storing and permits sterile handling in the hospital.
- II. Products packed collected for use on a single occasion, sterile as above.
- III. Clean products in dust-proof packaging (100 viable microorganisms per unit).
- IV. Products without specific medical requirements (household degree of cleanliness).

### Table XI. Categories of Medical Products

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- I. Products packed by the piece, sterile ( $< 10^{-6}$  viable microorganisms per unit), pyrogen free, low content of particles. The packaging maintains sterility during transportation and storing, and permits sterile handling in the hospital.
  - II. Products packed collected for use on a single occasion, sterile as above.
  - III. Clean products in dust-proof packaging ( $< 100$  viable microorganisms per unit).
  - IV. Products without specific medical requirements (household degree of cleanliness).
- 

## Conclusion

The consistent idea in my presentation is to communicate the understanding that nosocomial infections are caused by interactions of multiple biological variables simultaneously and that some of them are unalterable. However, a series of risk factors and critical procedures now have been identified where it has been proven in controlled, prospective studies that nosocomial infections can be prevented to a considerable extent by intervention through surveillance programs and adherence to guidelines and protocols concerning medical devices, therapeutic agents and performance.

It should be strongly emphasized that, first of all, a basic level of hospital hygiene has to be observed pertaining to sanitation and cleaning. These baseline requirements have been summarized by Simpson in 1982 (Table XII) (Simpson, 1984). Priorities for hospital

cleaning, disinfection, sterilization and control of infection comprise safe water supply, disposal of waste and sewage, sterilization of instruments and dressings, cleaning the hospital, care in the kitchen, laundry, disinfection. I would like to stress the importance of sterilization and disinfection in the primary health care. There is a need for inexpensive, reusable plastic syringes that do not break like glass syringes and that can be disinfected or sterilized under primitive conditions by simple procedures as boiling.

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## **Table XII. Priorities for Hospital Cleaning, Disinfection, Sterilization and Control of Infection.**

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Safe, clean water supply  
Waste disposal and sewage  
Sterilization of Instruments and dressings  
Cleaning the hospital  
Care in the kitchen  
Laundry  
Disinfection

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Simpson, R., Brit. Med. J. 289, 1898-1900, 1984.

In the industrial production of pharmaceuticals and medical equipment, the environmental factors and processes can be more easily controlled. The variables are fewer and should be known according to the rules of Good Manufacturing Practice (GMP). Certain medical procedures permit attempts to control the nosocomial infections in strict ways as in a factory, for instance, in orthopedic surgery where it has been possible to reduce the frequency of postoperative infections after total hip replacement from some 10% to 1-2% or less.

I would like to finish my presentation by pointing to the fact that sterilization and disinfection belong to the essential requirements for the baseline hospital hygiene as well as for the advanced procedures. In the advanced procedures, many nosocomial infections are device-related, which calls for a mutual understanding between the manufacturers and the medical profession concerning the design, quality and performance of the products. A mutual understanding is equally important to coordinate the essential hygienic needs of the primary health care in rural areas with the actual practical conditions, in many countries of the world characterized by deficient supply and lack of disinfectants and proper equipment for sterilization.

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# Microbiological Environmental Control Of Air In Hospitals And The Medical Products Industry

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## Summary

Patient and hospital environmental conditions should be considered as two entities between both should be established barriers. As these two entities are intrinsic bioburden carriers and, therefore, continuous sources of microbial contamination; these barriers must be identified, established and continuously monitored by developing a formal Hospital Sanitation Program.

Regarding the Entity Hospital Environment, the most common sources of particle and microbes contaminants are the following:

AIR: Not as a growth-promoting medium, but as a dangerous carrier of  
1st contamination in the form of dust and droplets which may be laden with microbes,  
(a) constantly introduced into work places by our own respiratory tracts and/or by  
dusts.

2nd  
(a) WATER: is a primary barrier medium.

(b) Identified Barriers are cleaning equipment and treatment systems.

3rd SURFACES: are primary sources of spreading particles and microbial  
(a) contamination.

Identified Barriers are effective cleaning and sanitizing procedures to remove and  
(b) destroy organisms. Physical sanitizing agents or Barriers are immersing, pumping, steam, dry heat, ultraviolet radiation and gamma radiation.

The chemical sanitizing agents or Barriers are classified disinfectants as: phenol or phenolic substances chlorine and its components, iodophors, alcohols and quaternary ammonium compounds (Brazilian legislation no. 196, from 6/26/83).

4th PEOPLE: are the most common source of contamination.

(a)

Identified Barriers are good sanitation practices which should comprise personal

(b) training programs, emphasizing a hospital and/or plant sanitation programs consisting of:

- Good personal hygienic procedures
- Written cleaning and sanitation procedures
- Documented work procedures
- Reports on equipment or areas which may cause contamination
- Routine checks on air and water systems, filters, drains and others
- Proper removal of trash and waste materials
- Validated antiseptics, as mercurials, chlorine compounds, iodophors, chlorhexidine compounds

The above mentioned and identified Barriers must be constantly monitored and challenged by written routine control and assurance procedures.

If the entity hospital plant is an identified contamination source, the entity patient product is also identified as a continuous contamination source.

Again, adequate Barriers must be identified by not permitting that these contamination sources break into the hospital plant environments. In the medical products industry, the same Barrier must be identified and continuously monitored. Medical devices can be primary contamination sources by spreading microbes into body cavities, injured skin, open wounds and others.

A brief list of sources of contamination carried by people or products into the hospital environment:

- Human hair or skin, humans shed approximately 10,000 microbes per minute from skin and by breathing, coughing and perspiring.
- Perspiring produces droplets of moisture containing particulate and microbial contaminants.
- Body liquids and injured skin/open wound.

## Final Consideration

The abovementioned summary briefly describes the purpose of this paper, in which detailed Barrier Systems and specific procedures will be presented and discussed during the Conference. This paper will also inform the Conference attendants on historical data and current levels of hospital infection regarding international standards and specific policies and procedures identified and applied by the Medical Product Industry to control

environment and bioburden levels.

After summarizing the main purposes of my presentation, I wish to present a brief summary on the current Brazilian hospital situation, and also some historical and statistical data regarding hospital environment controls and hospital infection indexes.

Currently, the Brazilian population is being served by 5,240 hospitals, and regarding specifically to hospital infection (HI) indexes, these vary from 3.2 to approximately 25%, which permit approximately 700.000 new cases of HI in 7,000,000 internations every year, with approximately 35,000 fatal cases every year.

Based on government statistics, the main sources of HI are: Hospital materials, inadequate disinfecting techniques and antisepsis techniques and sterilization methods and human beings.

The main causes of HI are:

- Urinary contamination
- Open wounds
- Microorganisms from the own patient
- Medical devices (catheters, etc.)

In 1968-69, our Microbiology Department started participating actively in Workshop Seminars and Congresses, with the objective to discuss and to evaluate the present condition in hospitals regarding sterilization, disinfection and antisepsis. During these years, we developed and distributed a booklet entitled, “Modern Concepts of Sterilization, Disinfection and Antisepsis” which focused on new techniques as ETO, Glutaraldehyde and others, and which also focused on the classification of all available chemical compounds and physical/chemical techniques regarding specifically to:

- Class of compounds
- Concentration for use
- Activity against bacterias and spores
- Activity against lipophilic and nonlipophilic virus
- Classification regarding antimicrobial activity and classification regarding their use in hospitals

In June, 1983, the Brazilian Health Ministry published a law no. 196/83, with the purpose of regulating measures to control and prevent hospital infection, establishing to be compulsory to all hospitals to develop and maintain an internal Commission for Controlling Hospital Infection (CCHI).

The law also established that this commission should be composed by:

- One nursery representative
- One medical doctor representative
- One clinical pathology lab representative
- One resident doctor representative
- One hospital pharmacy representative
- One hospital administration representative

The basic responsibilities and technical activities of this commission are:

- To establish epidemiological statistics
- To train people
- To develop and introduce technical procedures for prevention of HI and reduction use of antimicrobial agents
- To participate in the technical investigation to identify how patients acquired HI and also eventual transmission cases

Currently in Brazil, not all existing hospitals have their own CCHI; The Ministry of Health is administrating technical courses to develop hospital techniques with the objective of introducing in each hospital an internal CCHI. The main problems for the hospitals to better control and prevent hospital infection are:

1st Lack of sufficient Microbiologists

2nd High cost of maintenance of hospital laboratories and analysis

Lack of control of in and outside materials used in hospitals:

- 3rd
- Not validated sterilization cycles (steam, chemicals, etc.)
  - Lack of Quality Control and Microbiological Control of Antiseptics, Disinfectants and chemical sterilants

4th Use of low qualified people in all paramedical hospitals

5th Lack of qualified technicians specifically in the areas of HI control

6th High cost of acquired HI patients

7th High incidence of hospital visitors

In specific seminars, congresses, workshops and courses, many suggestions were discussed with the objective to better control HI, such as:

- To establish central microbiology laboratories to perform specific analyses and to attend different hospitals
- To establish central sterilization centers
- To validate and certify specific laboratories to manufacture antiseptics, disinfectants and chemical sterilizers
- To develop specific training courses
- To control hospital visits
- To emphasize and control specific prevention barriers

Currently in Brazil, the law 196 decreed in June 24, 1983, basically established that each hospital will have to maintain a commission for HI control; the major objectives of this commission are:

To implement a system for epidemiologic vigilance (which comprises collection analysis

and divulcation of all significant information) and technical training.

- A. — To suggest and implant measures to prevent and/or reduce and to monitor hospital infection
- To control the use of antimicrobials
- To participate in the investigation of all notified HI cases

Criteria for the identification of HI

For the characterization of HI the adopted criterias are:

- B. — The communitary infection which is not an institutional or a hospital infection; is the evident infection at the admission date;
- HI is an infection process acquired after the patient admission date or during the internation period or even after;

Criteria for the Diagnosis of HI

- When a recently admitted patient with a communitary infection presents clinical symptoms of an infection in a different body site, even if it is the same infectious agent, these cases are classified as HI cases.
- C. — If in the same site where an initial infection focus was detected during the patient admission, is isolated a different infectious agent, aggravating the clinical condition of this patient, these cases are also considered a HI case.
- When no evident clinical symptoms are presented during the patient admission period and any manifestation of an infection picture after 72 hours of admission is also a case of HI.

Following the law 196/83, we wish to point out the importance given, in respect to:

1. Policy for selection of germicides
  - Selection of germicides in adequate concentration
  - Non-use of “germicidal materials” which may vehiculate infective agents in hospital environments
  - Use only approved formulation
2. Classification of germicides based on their use
  - a. Sterilizing solution
  - b. Disinfectant detergent (surfactant) solution
  - c. Sanitizers
  - d. Antiseptics

These formulations are used for chemical sterilization of high risk medical devices not thermo-resistant and which are used in contact with subcutaneous tissues and vascular tissues. These sterilant solutions must be capable to destroy spore bearers, bacteria, mycobacteria, fungi and virus.

A. Sterilizant solution considered for hospital use: Sterilizant solution considered for hospital use:

1. Glutaraldehyde 2% aqueous solution (\*)

2. Formaldehyde alcohol 8% solution (\*)

3. 10% Formaldehyde aqueous solution with glycerin or propilenglycol (\*)

4. ETO in accordance to the medical device specification

## B. Disinfectant/Detergent

These formulations are used for cleaning, disinfecting and deodorizing static surfaces (floors, walls, large equipments, etc.) of critical and semi-critical areas. The selected disinfectant formulations must destroy within 30 minutes: bacteria, microbacteria, fungi, lipophylic viruses.

These formulations must be used also for semi-critical medical devices (which are in contact with noninjured mucous surfaces) and for non-critical devices (which are in contact with non-injured skin) or (which are not in direct contact to patients)

— phenolic solution at 0.3% (3,000 ppm) associated to soaps/anionic surfactants, EDTA and antioxidants

## C. Sanitizers/Detergents

— To be applied for cleaning, disinfection and deodorizing static surfaces (floors, walls, etc.), large equipment and in food processing areas: quaternary association (min. conc. 0.2% associated to detergent)

— To be used in high-risk contamination areas: (hydrophilic viruses, hepatitis, polio, etc.)

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(\*) The sterilization period varies from 30 minutes to 18 hours.

Na hypochlorite formulations:

— pediatric devices 125 ppm conc.

— food utensils 250 ppm conc.

— virus contaminated devices 10.000 ppm conc.

— dialysis devices 1.000 ppm conc.

## D. Antiseptics

To be applied on skin (injured) and mucous surfaces, due to their low toxicity, (hypoallergenic), are considered and recommended alcoholic or aqueous formulations, as follows:

— PVP Iodine (polyvinylpyrrolidone – I)

— Iodine iodate K

— Chlorhexidine

— Hexachlorophene – surfactant

Are considered inadequate for hospital use:

— quaternary ammonium formulation or solution (benzalconium chloride, cetylpyridinium bromide or chloride)

— organic mercurials

— acetone, ether, chloroform

As adequate antiseptic-surfactant formulation for skin antiseptic and debris removal, are recommended:

— PVP-I at 10% (1% active iodine) for hand degerming in critical areas and for

**presurgical skin antisepsis;**

- Four percent chlorhexidine in 4% ethanol aqueous solution (to avoid *Proteus sp* and *Pseudomonas sp* contamination) for hand degerming in critical areas and for iodine allergic users
- Hexachlorophene 1-2%—surfactant solution (containing 0.3% of chlorcresol to avoid *Pseudomonas sp* and other gram (–) germs; indicated for presurgical body asepsis and specifically for skin antiseptics when *Staphylococcus aureus* contaminants are suspected
- Aqueous PVP-I at 10% (1% active iodine) are recommended for mouth and oral antiseptics, intraocular, intestinal and vaginal antiseptics, also recommended for open wounds and burned skin.

For skin preparatory antiseptics (for rapid action) are recommended the alcoholic solutions and for residual activity are recommended PVP-I and chlorhexidine solutions.

Case study (230 beds hospital, located in S. Paulo). Currently this hospital which is working with well-defined validated and monitored Environmental Sanitation Program established by their Internal Hospital

Infection Control Commission, presents currently 3 to 4% of cases of HI.

In this hospital, the basic validation, control and monitoring procedures were established by the Hospital Infection Control Commission; the daily controls and statistics are performed by one nurse (full-time) and one Infectologist (medical doctor part-time).

This daily information is monitored manually or by computer to focus critical peaks; in these cases the specific barriers will be evaluated, rechecked, and revalidated if necessary, always in conformity with pre-established policies and procedures.

Referring specifically to the validated and monitored barriers of this hospital, the identified contamination sources are:

- Five percent are attributed to static surfaces as: walls, floors, furnishings, large equipment, wash basins, etc.).
- Ninety-five percent are attributed to: people (doctors, nurses, attendants, patients) and devices (surgical dressing, hospital bed cloth, catheters, gloves, cytosopes, etc.).

To control and monitor these contamination sources, hospital barriers and activities are classified as follows:

1. Critical
2. Non-critical; specific barriers are established and required for each hospital area and activity as follows:

Critical barriers

Material sterilization:

for thermolabile materials:

- a. Sterilization by moist heat: is the most widely used technique 121°C, 30', 15 psi. For heavily contaminated materials, derived from critical situation, presterilization techniques are being used as: This heavily contaminated material is submitted to 3 min at 200-240°C, then cleaned, packaged and submitted to 121°C, 30', 15 psi.
- b. Sterilization by dry heat and ETO: Normally not being used, only for cases due to inadequate penetration of steam (talcum powder, oils, fats), usually 160°C for 1-2

hours.

c. Chemical Sterilization

Formaldehyde — alcohol sol. at 8%

Glutaraldehyde — 2% solution. These are the selected sterilized in accordance with the decret 196/83.

d. Disinfection procedures (for clean grease-free surfaces) “in risk areas”

— Syntetic phenolics (ortho or buthyl benzyl parachlorophenol, ortho penylphenol 0.3% (3,000 ppm) associated to soaps or anionic detergents EDTA and antioxidants.

These disinfectants are not used in nurseries and first aid attending rooms (with exception when occurs infection focuses) but used routinely in surgery rooms, (fumigation of formaldehyde is not used anymore) in “high risk areas”

Treatment areas for hepatitis, AIDS, Shigella and Salmonella sp infections, enterobacterial infections and in dialysis centers are high risk areas.

In these areas, the environmental disinfecting solutions are Na Hypochlorite sol. 1.0% (10,000 ppm). Equipment and devices in “high risk” areas are disinfected with glutaraldehyde 2% sol. and 25% Ethanol solution.

e. Antiseptic procedures

Ten percent PVP — I (1% active iodine) and chlorhexidine (0.5% alcoholic solution) formulations are used for operatory skin area antisepsis and for hand degermation.

Hexachlorophene formulations are not permitted to be used in newborn areas.

Specific barriers control and audits procedures were developed by the Internal Prevention Committee and established as recommended Policies and Procedures; important is to point out that each single procedure was statistically evaluated, validated and regularly monitored.

Barrier Control 1

All commercial or prepared by the hospital pharmacy germicidal formulations have to be microbiologically controlled not only by the hospital laboratory but also must be analyzed and certified by the Adolfo Lutz Institute (S. Paulo State Public Health Institute).

All chemical sterilizers are evaluated against the test microorganism described in the law 196/83.

All disinfectants and antiseptics are evaluted by MIC test method (with and without body fluids) — (Minimum Inhibition Concentration)

Barrier Control 2

Air cleaners (laminar flow) are calibrated and monitored in pre-established periods (6 months) by DOP tests (dioctylphtalate gas and particle retention tests) (HEPA) (High efficiency particulate Air) 99.97% levels of particle removing efficacy (for viable and non-viable particles).

Barrier Control 3

Surface residual activity are monitored by using recommended inactivators to avoid false results.

Barrier Control 4

Visual wall labels in toilets, washrooms, surgery areas on hand cleaning and

antissepsis.

#### Barrier Control 5

Strong control over the use of antibiotics to avoid build up of resistants (antibiotic residuals into the hospital air, excessive use of presurgery preventive antibiotic therapy which can permit subdosis, and hospital antibiotic garbage).

#### Barrier Control 6

Surgery areas, settling plates are not used (only in cases of an epidemiological suite), disinfectants + surfactants (phenolics) are used after each surgery (formaldehyde fumigation is not used anymore); visual wall labels stressing how to use gowns and gloves.

The same barrier control are used in ambulatories.

#### HI Monitoring

Daily patient bulletins are statistically evaluated within 24, 48 and 72 hours after the patient internation to detect the presence of acquired HI.

## Final Conclusions

As presented in this paper and specifically the case study which presents currently HI rate of 3 to 3.5%, we wish to point out that if barriers are identified and correctly validated and monitored, these levels of HI recommended by WHO (3 to 5%) can be attained, even working in inappropriate circumstances. Referring specifically to Environmental control barriers in the Medical Device Industry between the two entities product/Manufacturing Environment, are used normally the same procedures as in hospitals. Barrier controls as settling plates, air controllers and swab techniques for bioburden controls are heavily used.

In Medical Device Industries, all specifications for each simple operation must be validated and routinely monitored. Barriers must exist between:

- Product/manufacturing devices and environment
- Operator/product
- Product/environment (recontamination)

Currently, all these barriers and respective results are statistically controlled and monitored by computers, permitting immediate responses to critical peaks regarding predetermined limits.

# Reuse Of Sterile Single-Use Medical Products in Hospital

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## Preface

According to Hill's report (1983) on cost containment and quality assurance issue in health care in the United States in the 1960s and 1970s, hospital physical plants, programs, personnel and financing have continuously been expanding to meet the need of physicians who are armed with dramatically new technologies. As well as other countries, increases in health care costs in the last two decades have been attributed to many different factors such as technology, labor costs, inflation, increased demands for newer service and aging of the population. In the USA in 1980s, the annual increase in health care expense is continuing to climb at a rate that exceeds the gross national product (GNP); the fact that the health care cost was 10.5% of GNP in 1982 was particularly noteworthy.

In 1983, Perry wrote his paper titled "Quality Health Care and Cost Containment—Are They Necessarily Incompatible?" and stated in this paper that there are expensive technologies that raise difficult national issues in research and development, safety and efficacy, reimbursement and economic, ethics and distributive justice and law. There is a great clamor to reduce health care costs while there are potentially life-saving but expensive technologies that are developed from year to year. It is natural that any patient wants best possible care from whatever dysfunction or disease he suffers. Every medical profession wishes to give his patient as high quality care as possible.

The physicians play a critical role in cost containment, who are estimated to control between 70 and 90 percent of health care expense. Perry wrote that we would be well-advised to take steps to foster the program with provisions to assure quality, reducing health care cost without compromising quality. There are many underlying problems to be solved in the area of medical and administrative matters. The most effective measure to reduce the cost should be taken; for this purpose good strategies must be induced from trend analysis of revenues and expenditures at the medical institutes, especially from existing data resources. Reduction of expenditure for manpower and materials should

contribute to suppress the growing medical cost to a great extent.

In recent years, practice of reusing sterile medical devices once used has increasingly been prevailing, and as a result, medical expenditures are beginning to show downward tendency. However, there is a difference in approaching the concept of sterility between the manufacturers who are under the Good Manufacturing Practice (GMP), and the central processing departments of hospitals. The former takes a number of complicated and sophisticated procedures when they proceed with quality assurance programs; they try to insure the sterility to the probability of remaining micro-organism,  $10^{-6}$ . These include determination of microbial burden, appropriate physical and biological indicator systems which validate overkill approach systems and end-product testings.

Concerning the interpretation of quality control tests, the United States Pharmacopoeia (USP) XXI (1985) mentioned that overall responsibility for the operation of test unit and the interpretation of test results in relation to acceptance or rejection of those who have appropriate formal training in microbiology, and have the statistical concept involved in sampling. Those individuals should have a good knowledge of environmental control program for hazardous substances. It should be recognized that a referee sterility test might not detect microbial contamination if present in only a small percentage of the finished article in the lot, because the specified number of unit to be taken imposes a significant statistical limitation on the utility of the test results. In the biological test (plastic), transfusion and infusion assemblies test, and the pyrogen tests, we take statistical analysis of obtained data, from viewpoints of not only the binomial distribution, but also the Poisson distributions. Therefore, these tests should be carried out by experts. As such degree of assurance cannot be realized in hospitals, any decision to reuse items once used must be made only at the highest administrative level and only after careful study of necessary research to establish proper standard by expertized personnel.

## **Viewpoint of Medical Devices and Materials Along With Health Insurance Renumeration Scheme in Japan**

A history of the Japanese health insurance system started in 1922. In the beginning, the system was intended mainly for company employees, and its tariff was inexpensive. Gradually, its scale has been enlarged, and a number of insurance payees have extended. The system has been in operation as the current state since 1958. Almost all of the Japanese population is under this health insurance scheme, which means that the medical treatment system in Japan is regulated and supported by the National Health Insurance Scheme.

There are problems that the Japanese health insurance system is encountering, one of which is tight funds to operate the system, mainly because of the coverage of reimbursement for expensive drugs and highly technical examinations. At present, the tariff system is slightly favored to practitioners. For example, hospitalization fees per patient per day is 6,800, which includes costs for meals and nursing services. The reimbursement for high technology is not fully covered by the insurance, although it is beginning to be improved

in recent years.

In spite of the difficult financial conditions, note should be taken of a remarkable record of the Japanese health sector. Life expectancy at birth rose from 63.6 years for males and 67.7 years for females in 1955 to 74.5 and 80.2 years in 1984, respectively. The infant mortality rate decreased from 39.8 to 3.7 deaths per 1000 live births during the corresponding period. However, ratio of the national medical expenditure to GNP still remains low, although there was a rise from 3.7% to 5.1-5.5%. This percentage is about a half or one-third of that in most western countries.

**Table I. Revenues (in Yen) at the Osaka University Hospital, Surgical Center, 1966-1984.**

Year	No. of cases	Surgery revenues (¥)	Total length of stay in OR (hr)	Total anesthesia revenue (¥)	Monitoring & laboratory test revenues (¥)	Others (¥)	Total (¥)
1966	4,323	29,859,758	9,271	1,455,115	595,800	4,688	31,915,361
1967	4,161	36,659,420	10,068	19,207,582	4,647,980	38,360	60,553,342
1968	4,315	75,220,654	11,127	27,711,854	7,149,548	24,533,325	134,615,381
1969	3,624	65,323,435	10,070	25,258,355	8,935,440	26,698,807	126,216,037
1970	3,475	63,513,250	10,304	29,257,763	9,597,996	30,371,497	132,740,506
1971	3,619	67,171,640	11,021	31,052,236	9,493,470	29,311,879	137,029,225
1972	4,151	101,351,250	12,192	40,257,203	13,682,700	47,853,518	203,144,671
1973	4,213	104,485,580	12,011	40,282,207	17,821,850	51,302,431	213,892,068
1974	4,198	126,060,230	11,784	45,455,388	22,395,560	59,317,255	253,228,433
1975	4,372	189,722,805	12,008	61,186,010	34,377,626	77,878,233	363,164,674
1976	4,398	207,946,790	12,300	67,166,125	37,094,570	91,064,518	403,272,003
1977	4,299	215,164,150	11,825	66,974,070	36,507,060	105,856,670	424,501,950
1978	4,275	294,277,750	12,286	91,178,050	40,888,590	132,219,830	558,564,220
1979	4,368	310,087,530	13,247	99,830,740	50,284,930	154,324,260	614,527,460
1980	4,298	315,044,950	13,824	99,491,830	53,237,250	163,351,290	631,125,320
1981	4,403	374,463,430	14,298	118,933,710	36,589,300	179,346,020	709,332,460
1982	4,593	425,523,050	15,276	135,773,950	29,792,250	200,038,070	791,127,320
1983	4,648	434,701,830	16,390	139,235,330	29,242,770	268,391,940	871,571,870
1984	5,025	449,196,950	16,929	141,896,050	30,187,550	312,222,640	933,503,190
<b>Total</b>	<b>80,758</b>	<b>3,885,774,452</b>	<b>236,231</b>	<b>1,281,603,568</b>	<b>472,522,240</b>	<b>1,954,125,231</b>	<b>7,594,025,491</b>

**Table II. Monthly Revenues at the Surgical Center, Osaka University Hospital in 1984. (Yen)**

Month	Total operation charges	Total anesthesia revenues	General anesthesia revenues	Monitoring & laboratory test revenues	Special authorized material	Others	Monthly revenues
01	33,943,360 (n=363)	10,415,240	10,091,640 (n=187)	2,402,200 (n=194)	18,645,730 (n=218)	6,013,630	71,420,160 (n=363)
02	39,575,040 (n=429)	11,632,670	11,222,070 (n=218)	2,532,750 (n=220)	21,530,630 (n=254)	6,783,920	82,055,010 (n=429)
03	35,998,320 (n=451)	11,465,640	11,075,040 (n=224)	2,352,600 (n=227)	13,438,580 (n=248)	5,590,680	69,236,420 (n=451)
04	37,761,900 (n=383)	11,230,870	10,903,070 (n=209)	2,296,400 (n=214)	17,946,310 (n=244)	6,146,490	75,381,970 (n=383)
05	38,767,690 (n=402)	12,217,000	11,919,400 (n=223)	2,601,450 (n=225)	26,132,090 (n=243)	5,180,670	84,898,900 (n=402)
06	38,418,530 (n=403)	11,592,630	11,263,590 (n=219)	2,710,500 (n=223)	19,860,210 (n=259)	8,065,190	80,647,060 (n=403)
07	37,664,440 (n=446)	12,025,070	11,664,070 (n=231)	2,593,500 (n=234)	17,924,700 (n=257)	6,525,850	76,733,560 (n=446)
08	43,327,260 (n=487)	14,874,290	14,498,290 (n=279)	2,941,050 (n=283)	21,875,720 (n=291)	9,348,800	92,367,120 (n=487)
09	30,933,390 (n=378)	10,168,010	9,876,610 (n=192)	2,201,000 (n=194)	16,306,360 (n=228)	7,427,550	67,036,310 (n=378)
10	39,130,210 (n=471)	13,098,680	12,663,880 (n=253)	2,784,700 (n=262)	16,202,160 (n=298)	8,299,750	79,515,500 (n=471)
11	40,737,480 (n=440)	12,429,230	12,035,830 (n=235)	2,531,700 (n=236)	19,658,780 (n=293)	9,289,410	84,646,600 (n=440)
12	32,939,330 (n=372)	10,719,720	10,438,620 (n=195)	2,239,700 (n=198)	15,750,270 (n=236)	7,915,560	69,564,580 (n=372)
<b>Total</b>	<b>449,196,950</b> (n=5,025)	<b>141,869,050</b>	<b>137,652,110</b> (n=2,665)	<b>30,187,550</b> (n=2,710)	<b>225,271,540</b> (n=3,069)	<b>86,587,500</b>	<b>933,503,190</b> (n=5,052)
<b>Mean</b>	<b>89,392</b>		<b>51,651</b>	<b>11,139</b>	<b>73,402</b>		<b>184,778</b>

Recently, the usage of expensive drugs has begun to be reconsidered in Japan, and the people concerned have been making every effort to improve the technical remuneration tariff and economize hospital expenses. First of all, medical service and instrumentation are focused in order to enforce the quality of medical procedures carried out by experienced technicians under supervision of physicians. This can apply to the surgical center where the author belongs. Secondly, the current trend analysis of revenues at each medical institute is taken to establish effective strategies for the future. The annual revenues from 1966 to 1984, and the monthly revenues at the surgical center of Osaka University Medical School are shown in Table I and II, respectively. Further, Table III indicates an example of the amount claimed on one patient who was recently operated at the center. Thirdly, reconsiderations are taken regarding labor charge including salaries, employees' fringe benefits, contracted services and technical fee for specialists, which are threatening factors in the medical expenditure. Efforts are being made to seek feasibility of automated medical instrumentations, such as fully automated ultrasonic cleansing and conveying apparatus instead of manual washing of soiled medical instruments, and the computerized monitoring information networks with medical and administrative information instead of handwritten records.

On the other hand, practicality of reusing sterile medical products once used is widely investigated in many countries in order to economize medical expenditure. As far as

sterilization on thermo-sensitive and/or moisture sensitive medical products are concerned, the Japanese has been behind the world until recently. Development of technology on utilization of nuclear energy and EO in Japan has caught up with the advanced countries long after World War II.

Due to the tight condition of the national health insurance fund, applications of the scheme to 'single-use' medical devices are still limited. The advantage secured with polymeric medical devices are both economical and functional. The most important benefit is to improve technology in the manufacture of resin for medical devices to lower unit cost if automated machinery is further progressed. Similarly, a wide range of films, tubes and laminates are becoming available. Such a variety of characteristic devices readily explains the widespread interest in polymers and the enormous growth in production of the materials. The awareness of necessity for sterilized polymeric single-use medical devices is greatly increasing every year. After long-term negotiations between the governmental health authority and medical institutes, newly-developed products and devices have been adopted by the medical tariff system. Many Japanese medical institutes are trying to save manpower and labor expenses by taking advantage of the single-use medical devices.

In the Japanese health insurance system, costs of materials and expenses for depreciable medical devices used during medical procedures are not reimbursable, because these matters are considered to be components of procedures in cost counting. However, the Minister for the Health and Welfare authorizes major single-use materials and devices to be reimbursed to the medical institutes as 'specially authorized materials' (Tokutei-Zairyo).

**Table III. An Example of the Amount Claimed**

(Diagnosis of the patient)

- (1) Double outlet of the right ventricle (DOPV)
- (2) Tricuspid regurgitation (TR)
- (3) Aortic regurgitation (AR)
- (4) Ventricular septal defect (VSD)
- (5) Atrial septal defect (ASD)

**List of Items**

<b>(1) Operation Charges</b>	570,000
(1) Double valve replacement	400,000
(2) Cardio-pulmonary bypass	170,000
<b>(2) Anesthesia Charges</b>	257,000
(1) General anesthesia (850 min) + Hypothermia	

<b>(3) Specially Authorized Materials</b>	2,140,340
(1) Balloon catheter	450
(2) Hemofilter for blood transfusion	1,750
(3) Silascone tube (No. 7)	1,950
(4) Aortic canula × 2	8,500
(5) Surgical suture (Ethybond MB994G) × 6	19,400
(6) Surgical suture (Ethybond MB927G) × 6	19,400
(7) Pacemaker wire × 3	9,600
(8) Venting	21,500
(9) Venous canula	28,500
(10) Reservoir	38,000
(11) Oxygenator (CP 3. 3H)	153,000
(12) Cardio-pulmonary bypass circuit (membrane M2)	201,270
(13) Prosthetic valve (SJM)	808,000
(14) Prosthetic valve (BS)	828,000
(15) Intravenous administration tubing	300
(16) Plastic disposable indwelling catheter × 3	720
4. Drugs	<u>120,990</u>
TOTAL	3,088,330

Prices of most important items are decided by the Minister for the Health and Welfare, as shown in the list of Table IV. Concerning other authorized items, the price is determined according to price negotiation institute by institute (according to local government).

**Table IV Specially Authorized Materials in Japanese Health Insurance Remuneration Scheme March, 1985**

**(1) Artificial Kidney Assemblies**

(1) Dialyzer: Coil	6,000
Hollowfiber (1.5 m <sup>2</sup> )	7,500
Hollowfiber (1.5-2.0 m <sup>2</sup> )	7,900
Hollowfiber (2.0 m <sup>2</sup> )	8,100

Kiil dialyzer	7,000
Parallel Plate (specific)	12,500
(2) Hemofilter	12,000

**(2) Other Authorized Materials** (Purchased Price at the medical facilities)

- (1) Elastic bandage for fixation of head, neck and trunk
- (2) Materials for reconstructive surgery of bones and joints, and implant for defect repair
  - Artificial bone cap
  - Interposition membrane for joint
  - Artificial acetabulum
  - Artificial bone head, including hip tendoprosthesis, tibial component, patella dome
  - Artificial bone graft, including Kiel bone graft
  - Compression nail and screw
  - Artificial hip joint
  - Artificial knee joint
  - Artificial finger joint
  - Fixing nail
  - Intramedullary nail
  - Fixing screw
  - Fixing screw
  - Fixing wire
  - Fixing metal pin
  - Angle plate
- (3) Artificial skin graft (PVF sponge)
- (4) Prosthesis for maxilla, including cleft palate
- (5) Teflon tube for post-tracheotomy
- (6) Skin graft (temporary use), including artificial skin porcine skin
- (7) Nonadhesive silicone gauze
- (8) Splint, including Kramel's splint, cast with heel and cast boot
- (9) Halo-pelvic traction apparatus
- (10) Halo vest
- (11) Continuous infusion, drainage and aspiration devices to be inserted
  - Resin tube
  - Disposable ventricular canula, ventriculo-arterial shunt, ventriculo-peritoneal shunt, Pudent's shunt canula
  - Latex tube, thoracic catheter, mediastinal catheter, lacrymal catheter
  - Disposable balloon catheter
  - Trocar catheter
  - Tracheotomy tube

- Endotracheal tube
- Continuous suction catheter
- Infusion devices
- (12) Intraperitoneal dialysis devices
- (13) Intraperitoneal dialysis catheter
- (14) Shunt valve
- (15) Absorbent tube for cycling dialyzer system
- (16) Disposable plasma separator for plasmapheresis
- (17) Disposable plasma component separator
- (18) Blood detoxifier cartridge
- (19) Reinfusion device of filtered and concentrated ascitic fluid
- (20) Arterial graft
- (21) Esophageal prosthesis
- (22) Artificial larynx
- (23) Heart valve, cardiac valve
- (24) Artificial heart-lung circuit
- (25) Artificial dura mater
- (26) Lyophilized dura mater
- (27) Artificial mesh prosthesis for tissue
- (28) Artificial lung (disposable sheet type)
- (29) Arterio-venous shunt
- (30) Hemofilter for blood transfusion
- (31) Cardiac pacemaker
- (32) Catheter electrode for pacemaker
- (33) Cardiac wire for pacemaker
- (34) Catheter for cardiac surgery
- (35) Catheter for ambulatory peritoneal dialysis
- (36) Connecting tube for intraperitoneal dialysis
- (37) Angioplastic catheter
- (38) Catheter for removal of gall stone
- (39) Specially authorized surgical suture materials
  - Polyester surgical suture for cardiovascular surgery
  - Absorbable polyglycolic acid and polyglacin 910 surgical suture for use in closure of mucous membrane, and subcutaneous tissues
  - Nonabsorbable monofilament surgical suture for use in plastic surgery for face and neck, and surgical suture for trachea
  - Monofilament suture for use in cataracta, glaucoma, and cornea transplantation
- (40) Balloon catheter for intra-aortic balloon pumping
- (41) Cerebral aneurysm clip
- (42) Cartridge for the automatic suturing devices, when used in followings.
  - Reconstructive procedure for esophago-cardiac cancer
  - Ligation of esophageal varices

- Low anterior resection for lower colonic cancer (without colostomy)
- Radical operation for Hirshsprung's disease

(43) Reconstructive implant materials

**(3) Cornea**

**(4) Materials to be Used in Specific Tests**

- (1) Catheter for blood pressure monitoring
- (2) Catheter for blood sampling from organs
- (3) Thermodilution catheter

**(5) Materials for Specific Image Processing**

- (1) Catheter for angiography
- (2) Guidewire for angiography

**(6) Materials to be Used in Specific Drug Administration**

- (1) Intravenous hyperalimentation assemblies
- (2) Sterile disposable syringe and needle when used in the followings
  - Insulin syringe
  - Administration of growth hormone
  - Administration of anti-hemophilic globulin derivatives
  - Administration of complex for coagulation factor IX deficiency (lyophilized)
- (3) Automatic mobile continuous peritoneal dialysis

**(7) Intravenous Administration Assemblies**

- |     |   |     |
|-----|---|-----|
| (1) | Accurate continuous drip infusion tubings       | 400 |
| (2) | Intravenous administration tubings              | 100 |
| (3) | Plastic disposable flexible indwelling catheter | 240 |
| (4) | Butterfly needle                                | 70  |
- 

Before a medical institute purchases medical products, they confirm whether the proposed products are registered or not. Further, they examine if the products satisfy the national standard (next Chapter 3).

If the purchased items are reprocessed and resterilized in the medical institutes, expense for such procedures is not reimbursed from the health insurance fund.

## **Japanese Manufacturing Standards of Medical Devices Fixed by the Pharmaceutical Affairs Law**

In Japan, the pharmaceutical affairs law determines manufacturing standards on major medical devices to ensure safety and efficacy. Article 42, Paragraph 2 of this law describes

as follows:

“The Minister for the Health and Welfare, when it is indispensable for the prevention of hazards to the public health and sanitation, fix the necessary standards relating to the properties, quality, efficiency, etc. of medical devices, after hearing the opinion of the Central Pharmaceutical Affairs Investigation Council.”

Accordingly, listed medical devices are requested to have its respective characteristics to keep satisfactory status in clinical use. The following devices are standardized at present:

- (1) Disposable hypodermic needle (1970)
- (2) Hypodermic needle (1961)
- (3) Disposable syringe (1970)
- (4) Syringe (1961)
- (5) Disposable transfusion and administration set (1970)
- (6) Disposable blood sampling set (1970)
- (7) Blood sampling and blood transfusion device (1960, rev. 1965)
- (8) Polyvinyl chloride resin blood transfusion set (1965; rev. 1967)
- (9) Disposable artificial lung for use in cardio-pulmonary bypass (1977)
- (10) Arterial graft (1970)
- (11) Medical adhesive (1970)
- (12) Contact lens (1970)
- (13) Plastic surgical suture (1970)
- (14) Latex condom (1961)
- (15) Pessary (1961)
- (16) Tampon for menses (1970; rev. 1974)
- (17) Materials treating menses (1966)
- (18) Heart valve (1972)
- (19) Cardiac Pacemaker (1976)
- (20) Hemodialyzer devices (1983)

## **Reuse of Single-use Medical Devices, Focusing on Hemodialyzers**

<sup>31</sup>“Reuse,” “resterilization” and “reprocessing” are defined in various ways, and usually

subjectively. The author agrees in opinion with Dr. Favero's definition (1983):

*Resterilization:* When a sterile procedure tray or pack containing sterile items is

(1) subsequently opened, these items would be reprocessed either by steam sterilization or EO sterilization.

*Reprocessing:* It has two connotations. One term is used frequently when a sterile

(2) device's packaging is opened in preparation (e.g., for use during surgery), or it is opened by mistake but not used. Reprocessing is also used synonymously with reuse.

(3) *Reuse:* Procedures of rinsing, cleaning and disinfecting or sterilizing an item or medical device that has been used at least once for a patient before.

When items are autoclavable, procedures are easily performed. On the other hand, when items are thermo- sensitive and/or moisture-sensitive, the process conditions vary greatly.

In approaching the problem of the appropriate reuse of sterile single-use polymeric medical devices, it becomes obvious that we are dealing with an extraordinary complex subject. Ever expanding family of polymeric materials in the medical area have shown many variations with respect to their range of physical and chemical properties. Not only can different properties be obtained by alterations in the molecular weight and geometry of a polymer, but the addition of plasticizer, fillers, lubricants and other chemical substances can have an additional modifying effect on the ultimate devices. In addition to this, the existence of oligomer and/or monomer should be taken into consideration, because the percentage of these substances is often difficult for staff in the medical institutes to know.

In 1981, the Center for Disease Control (CDC) published guidelines for the prevention and control of nosocomial infections concerning cleaning, disinfection and sterilization of hospital equipment. This article describes that contaminated patient-care supplies or equipment are most likely objects of the inanimate environment to cause infection, and that ironically contaminated antiseptics and low-level disinfectants themselves are associated with infections.

Objects potentially contaminated with virulent organisms require high-level disinfection. Hospitals should perform most cleaning, disinfection and sterilization of reusable, patient-care objects to maintain high levels of quality control. These procedures should be carefully and respectively standardized by authorities.

As a good example, the AAMI's Recommended Practice for Reuse of Hemodialyzer (draft) March, 1985) is worthwhile for evaluation. This draft issue includes considerations of personnel and patient matters, records, equipment, physical plant and environmental safety, reprocessing materials, patient identification and hemodialyzer and its reuse. According to the issue, manufacturers are responsible for providing safe and effective devices with appropriate recommendations for initial use, but attending physicians are requested to take responsibilities if the product is reused. When formaldehyde is used as a sole disinfecting agent, the CDC recommends a concentration of 4 percent in both blood and dialysate compartment should be used with minimum contact time of 24 hours at a temperature of

10-20°C. The dialyzer should be repeatedly filled with the germicide solution.

The AAMI's Recommended Practice gives careful consideration for patient's benefit, and also indications of reuse procedures for medical professions; for example, reprocessing of hemodialyzers should be performed with care if any of the following is seen:

- (1) A patient has a 'first-use' syndrome during dialysis with new hemodialyzer, e.g., chest or back pain, or respiratory distress with or without wheezing (though rarely occurs), and sometimes chills followed by fever.
- (2) The quality of dialysis is maintained or enhanced as the result of the cost saving arising from reprocessing hemodialyzers.

The same issue of the Practice also indicates the contraindications for reuse:

- (1) Hepatitis B surface antigen positively
- (2) Unexplained abnormal liver function tests indicative of viral hepatitis
- (3) AIDS
- (4) Septicemia
- (5) Sensitivity of the patient to materials used in hemodialyzer processing

These indicate that processed hemodialyzer must be used for the same patient. Therefore, the labeling must be identifiable enough to tell who is using the dialyzer. Note should be taken that the proposed draft is currently under review of the relevant committee, and that the final standard will come out in due course. Early introduction of the standard is hoped in clinical practice.

On the other hand, attention is paid to toxic materials such as formaldehyde. Harmful effects of this substance on workers who handle it must be avoided. Frazier, et al, reported on "use of computer-generated maps in occupational hazard and mortality surveillance" in 1984, concerning the usage of this map as a surveillance technique for monitoring work-related hazards and mortality. They mentioned in this article that the geographic patterns for causes of deaths that may be related to occupational risks can be displayed in the map using standardized county-level, cause-specific mortality rates, and they introduced an exemplification map showing mortality rates for nasal cancer by county (thought to be related to formaldehyde exposure). According to the American Conference of Governmental Industrial Hygienists (ACGIH), threshold limit value for formaldehyde is 2 ppm, and the Japan Industrial Hygiene Association decides the permissible exposure limit of formaldehyde as 2 ppm (2.5 mg/m<sup>3</sup>).

It is essential that a method for testing small, invisible particles remaining inside, which may have an influence on patients on later days, be added to the draft mentioned above.

Conversely speaking, a very important question on reuse procedures is raised. According to Smith (1983), when an automated machine is handled, the total consolidated per-treatment cost ranges from \$21.08 for two treatments to \$11.28 for ten treatments by

reusing the same dialyzer. By manual procedures, it was from \$9.86 to \$14.12 per treatment. Smith commented that the three clinics investigated were able to take advantage of lower per item supply costs because they purchased used supplies in bulk quantities on behalf of a large group of dialysis clinics and their supply costs are therefore probably below those of minor operations, smaller groups of clinics, or free-standing clinics. The cost of reuse would vary depending on location of clinics and possible affiliations with other dialysis clinics which might share the costs of overhead and supplies.

When initial cost is discussed, hospital location and amount of purchase must be considered. Thus, the author investigated current status of hemodialyzer manufacturing and importing in Japan (Table V). In Table VI, the author indicates present status along with dialyzers (1983) so as to compare with the data obtained in foreign countries.

**Table V. Status Along with Dialyzers in Japan (1983)**

**1. Statistics of hemodialysis for patients with chronic renal failure**

(1) Number of institutions using hemodialyzer	1,442
(2) Number of devices equipped	24,474
(3) Number of patients requiring repeated hemodialysis	54,017
(4) Number of patients newly starting hemodialysis	11,348
(5) Number of patients dead	4,538
(6) Number of patients having been treated by dialyzer for more than 10 years	3,283
(7) Number of patients suffering from acute renal failure	3,529

**2. Actual numbers of dialyzers manufactured in Japan (1983)**

Dialyzer	No. of Products	Manufacturer's Cost (estimate) (Yen)	Amount of Products (Yen)
Coil	612,489	4,438.86	2,718,753,000.00
Hollowfiber	7,716,072	4,556.61	35,930,796,000.00
Parallel Plate	942,736	5,421.89	5,111,408,000.00
<b>Total</b>	<b>9,271,297</b>	<b>4,720.05</b>	<b>43,760,957,000.00</b>

**3. Monthly production and imported dialyzer in Japan (May 1984) including domestic consumption and export — Unit: 1,000 yen)**

Dialyzer	No. of Items	Amount of Products	Imported	Domestic Consumption	Exported
Coil	7	37,622	—	46,649	7,215
Hollowfiber	39	3,836,949	543,764	3,176,999	1,063,129
Parallel Plate	3	427,617	1,425	92,938	337,920
Total	49	4,302,188	545,189	3,319,586	1,408,264

**Table VI. Status Along With Treatment on Chronic Renal Failure (Sonoda)**

<b>1) No. of patients treated by hemodialyzer (1982)</b>	
Europe	65,386
America	65,270
Japan	47,978
<b>2) No. of patients treated by renal transplantatio (until 1982)</b>	
Europe	ca 40,000
America	ca 35,000
Japan	2,457
<b>3) No. of patients having renal transplantation (1984)</b>	
U.K.	1,160
U.S.A.	5,600
Japan	393

Fundamentally, treatment for chronic renal failure in Japan greatly differs from other western countries. For various reasons, renal transplantation is not widely performed in Japan. Almost all patients are treated by hemodialyzers. As shown in Table V.2., manufacturer's cost of a hollow-fiber was 4,556 in 1983, which recently comes down to 4,000 (from \$19 to \$16 if converted to US currency). The dialyzers are accepted by the national standard. The author considers that we should find a ceiling price of reuse procedures.

Although treatment of the disease is necessary, efforts to prevent is are far more

important. When a urine test is given to primary school children, abnormal findings are seen in one percent. Among this 1%, renal diseases are found in 0.1-0.2%, while chronic renal diseases are seen in 0.01-0.02%. If signs of renal disease are found earlier, they could be treated early enough to be cured so as to lessen the number of renal deficiency patients in the middle age group. In any case, prevention is always better than treatment. No expense must be grudged for early treatment; otherwise heavy duty will be burdened in the future. The approach will be the best way to reduce total medical expenditure. The Japanese health insurance scheme guarantees all necessary requirements for this purpose with no limitation on reimbursement.

## **Sterilization of single-use devices by EO Gas Process in Hospitals**

In 1949, Phillip and Kaye published a monumental series of four articles on “the sterilizing action of gaseous ethylene oxide.” The use of EO has since become universal to sterilize thermo-sensitive medical materials in hospitals. Its highly diffusive nature and permeability make it possible to sterilize through sealed wrapping materials. Thus, it is a desirable process for sterilizing sterile disposable (single-use) medical devices in the industries.

Microbiological control is very important to the manufacturers of sterile medical devices. For all sterilizing processes, the spore survivor curve shows the logarithmic death of micro-organism. A plot of such kinetics down to microbial survivor levels expressed as negative logarithmus reveals that the sterilization process is a probability function. However, pharmacopeia of many countries describe the sterility test, on the assay of a small sample of sterilized product as the way to insure process effectiveness. This method corresponds to an approximate probability of survivor of  $10^{-1}$ . Country-wise, the biological indicators can estimate survival levels of  $10^{-6}$  or less.

Consequently, when the sterilization process is to be established in the hospitals, the following subject should be taken into consideration.

- (1) Pharmacopeial testing
- (2) Biological indicators (process challenge)
- (3) Microbial burden (bioburden)
- (4) Good Manufacturing Process (Good Hospital Practice)
- (5) Process control and validation

The decimal reduction values (D-value) derived from the spore survivor curve of BIs are one of the most important factors as mentioned before. As BIs, *Bacillus subtilis* ATCC 9372 is mainly adopted in EO sterilization. By analyzing spore survivor curve, necessary time is estimated to obtain the probability of spore remaining  $10^{-6}$ .

However, the design and capacity of hospital EO sterilizer is different from that used in the industries. Not many hospitals have such trained technicians as the industries. When hospitals need EO sterilization, necessary instruments must be equipped according to the standard. The author refers to the CSA standard published in 1977 by the Canadian Standard Association on the EO sterilizer and effective EO sterilization in hospitals (CSA Standard Z314.1-M1977 & Z314.2-M1977).

In order to establish efficient and practical procedures for the hospital EO sterilization, the author has examined under simulated practical conditions, various commonly-used wrapping materials and their effectiveness. As wrapping materials, double thickness muslin, medical grade paper, high density polyethylene, low density polyethylene, polypropylene, and Nylon 6 were tested. Employing AMSCO's Spordex Scale (*Bacillus subtilis* ATCC 9372) as the graded BIs, experiments were carried out repeatedly to measure each survival and kill time of BIs in the materials in each package. In this study, the author was unable to sort out the ideal lineality, as seen in BIER vessel of decimal reduction because the experimental sterilizer had complex structure. However, the survival and kill times of spore were significant and it was possible to apply the results to establish actual sterilization processes concurring to U.S.P.XIX. Thus, graded BIs can easily demonstrate effectiveness. Further, observations of twilight time (partial kill or partial survival) suggests that single sterility test would mislead results to hasty conclusions.

When the abovementioned procedure is carried out by the hospital staff, the items can easily be resterilized by EO process. It is necessary to keep environmental EO exposure under action level of 0.5 ppm and check the level by taking batch monitor system.

## Conclusions

The reuse of sterile medical devices once used is a matter of great interest with expectation of cost containment in medical expenditure. As indicated in many literatures, development of methodology is now underway, and more information is awaited so as to establish the standard for ordinary hospital use. As mentioned in AAMI's Recommended Practice for Reuse of Hemodialyzers, reuse of medical devices once used must be handled by physicians responsible for reprocessing items. After accumulating data on medical and economical information, the policy of reuse should be determined. Unless the standard is established, the reuse of such products must be limited to those who have enough knowledge to ensure safety for patients and workers.

# Session IV

**Chairman: Anna Skopek, Ph.D.**

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# Introduction by Session Chairman

Anna Skopek, Ph.D.

In this session, we will address the subjects of liquid chemical sterilants in the hospital, the application of sodium dichloroisocyanurate as a disinfectant, the role of barrier materials in preventing infection and the particulates in parenterals and on medical devices. Let me briefly introduce the topics we will discuss.

The increasing incidence of hospital-acquired infections has been the subject of extensive investigations and has resulted in growing interest in the liquid chemical sterilizing agents. The incidence of infection can be decreased if an effective, liquid chemical agent is used to rapidly sterilize materials and instruments that cannot be sterilized by other conventional methods.

The ideal, liquid chemical sterilant used in hospitals has to:

- Be rapidly acting
- Possess effective bactericidal, fungicidal, viricidal and sporicidal properties
- Have no deleterious effect on the materials and instruments
- Be of a low toxicity to the patient and to the user
- Retain its activity for a reasonable period of time and in the presence of organic soiling matter
- Be suitable for a practical, easy use in hospitals as well as in small surgery rooms

In reviewing the destruction of microorganisms by liquid chemical sterilants, the many factors and variables associated with the sterilizing action are to be considered. Selectivity must be exercised and only those chemical compounds which exhibit rapid sporicidal activity should be employed as hospital sterilants.

While a sterilization procedure involves destruction of all forms of microbial life, a disinfection procedure does not. Chemical disinfection differs from sterilization by its lack of sporicidal power.

In the proper use of chemosterilizers, time, concentration, temperature, pH, numbers and types of micro-organisms to be sterilized, surface tension, the nature of chemicals employed, the type of surface to be sterilized and the limitations associated with the chemical agent are the important factors.

Although a chemosterilizer may be sporicidal in its action, it cannot be effective unless it

penetrates to the spore site. Other typical limitations associated with some chemical preparations include their inactivation in presence of organic soiling matter and absence of wetting agents that would lower the surface tension and enhance penetration.

Any system of chemical sterilization requires meticulous handling by personnel and strict adherence to the recommended protocol. Based on long-term hospital and medical practical experience, it is now evident that chemical sterilization can be carried out routinely without jeopardizing the safety of the patient.

Bactericidal capacity of sodium dichloroisocyanurate (NaDCC) is widely utilized in disinfection for general hygiene purposes in hospitals, public premises and domestic situations. Sodium dichloroisocyanurate is also used in food industry, water industry and for some specific purposes, such as disinfection of infant feeding utensils.

Results of numerous studies indicated two main factors which determine the efficacy of chlorine-releasing compounds: the rate of diffusion of undissociated hypochlorite (HOCl) molecules into the cell and the degree of interaction with cell components. These two factors depend on concentration of H<sup>+</sup> ions (pH), which effects the concentration of undissociated hypochlorite molecules.

NaDCC may offer advantages in disinfection in presence of organic matter, while retaining rapid bactericidal activity. Other advantages include easy preparation, low cost, long stability and, consequently, longer shelf life.

The role of the barrier materials used in operating rooms has received much attention in the surgical literature. The incidence of post-operative wound infections remains a major problem leading to significant morbidity and mortality as well as to increased health costs. The factors that have been implicated in wound infections include the operating room environment, defective gloves, masks and drapes, the location of the incision, misuse of antibiotics, the presence of devitalized tissue, and the type of barrier material.

Surgical barrier materials are used to create an aseptic field and to eliminate transfer of bacteria. It is suggested that their use in operating theatre drapes is advantageous in efforts to minimize the incidence of wound infections. Presence of moisture is unavoidable in many surgical procedures. Recommendation is made for the use of fabrics which, as opposed to linen, are impermeable to bacteria in the presence of moisture. Current availability of single-use, disposable gown and drape materials provides a new potential for prevention of wound infections.

Numerous studies have been undertaken to determine the amount and the nature of microbial penetration through surgical gown materials under in-use conditions and to evaluate practical considerations such as comfort and cost.

Absence of particulate matter in parenteral penetrations and on some medical devices is one of the major concerns to industry and regulatory agencies in the product safety evaluation. These products must therefore be manufactured, assembled or filled and sealed in special areas from which sources of contamination are excluded. These special areas are known as clean rooms or work stations. Since airborne particles can arise from a number of sources, the control of particulate contamination must include adoption of certain measures, as dictated by the finished product requirement. The measures may involve controlling of the particulate content of incoming air, adjusting manufacturing procedures,

cleaning all materials before entry to production areas, controlling the movement and clothing of personnel, covering sensitive materials as much as possible and rigorous cleaning of the room surface.

The products and processes requiring controlled environmental conditions are numerous and varied. Owing to continuous development of new products, it is difficult to lay down hard rules relating the product and process requirements to a particular class of environment, and to particular environmental conditions to be achieved. It is up to personnel responsible for the finished product to ensure that the conditions best suited for the type of products and the process are achieved and maintained.

# Liquid Chemical Sterilants In The Hospital

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## Introduction

In the United States, the use of liquid chemical germicides in health care institutions such as hospitals, hemodialysis units, and long-term care facilities is exceeded only by the use of heat in the form of steam autoclaving, pasteurization or dry heat. The effective use of chemical germicides formulated as antiseptics or disinfectants or sterilants is important in preventing hospital infections. In recent years, there has been a significant increase in the number of germicidal products available in hospitals in the United States. In 1973, the American Society for Microbiology Ad Hoc Committee on Microbiological Standards of Disinfection in Hospitals surveyed 16 U.S. hospitals with a combined bed capacity of more than 9,000. The Survey showed that the average number of different formulations used per hospital was 14.5, with a range of 8 to 22. A total of 224 products was used in the 16 hospitals, and 125 of them were proprietary products.

The choice of liquid chemical germicides to be used for hospital environmental sanitation and antisepsis depends on a variety of factors, and no single agent or procedure is adequate for all purposes. Factors to be considered in selecting procedures include the degree of microbial killing required, the nature of the item to be treated, and the cost and ease of using the available agents. This paper discusses each of these factors and practical methods for evaluating the effectiveness of the various agents and procedures.

## Regulation of Chemical Germicides in the United States

Chemical germicides that are formulated as disinfectants are regulated and registered by the Environmental Protection Agency. The Environmental Protection Agency requires manufacturers of chemical germicides formulated as general disinfectants, hospital disinfectants, and disinfectants applied in other environments, such as the food industry, to test these formulations by using specific protocols for microbicidal efficiency, stability and

toxicity to humans. The decision to register a disinfectant is based on data provided to the Environmental Protection Agency by the manufacturer.

Chemical germicides that are formulated as antiseptics, preservatives, drugs, to be used on or in the human body or formulated as preparations used to inhibit or kill organisms on skin are regulated by the Food and Drug Administration (FDA). The FDA has an advisory review panel on nonprescription, antimicrobial drug products. Manufacturers of such formulations voluntarily submit data to the panel, which in turn categorizes them for their intended use, i.e., antimicrobial soaps, health-care personnel handwashes, patient preoperative skin preparations, skin antiseptics, skin wound cleansers, skin wound protectants, and surgical hand scrubs. Generic chemical germicides for each use category are further divided: Category I (safe and efficacious); Category II (not safe and/or efficacious); Category III (insufficient data to categorize) (5, 26).

## Antimicrobial Effectiveness

Although the definitions of sterilization, disinfection and antisepsis<sup>13</sup> have been generally accepted, it is common to see all three terms misused. The precise definitions of the three terms and the basic knowledge of how to achieve and monitor each state are extremely important if long-known principles are to be effectively applied.

1. *Sterilization* is defined as the use of a physical or chemical procedure to destroy all microbial life, including highly resistant bacterial endospores. In the hospital, this particularly pertains to those organisms that may exist on inanimate objects. Moist heat under pressure (steam autoclaving) and ethylene oxide gas are the major sterilizing agents used in hospitals. However, when used appropriately, some chemical germicides normally considered disinfectants, can also be used for sterilization.

2. *Disinfection* is generally less lethal than sterilization. A disinfection procedure inactivates virtually all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial endospores) on inanimate objects. As can be seen by this definition, disinfection lacks the margin of safety achieved by sterilization.

The effectiveness of a disinfection procedure depends on a number of factors: (a) nature and number of contaminating microorganisms (especially the presence of bacterial endospores), (b) concentration of the chemical, (c) length of exposure to the chemical, (d) amount of organic matter (soil, feces, blood, etc.) present, (3) type and condition of the material to be disinfected, and (f) temperature.

Thus, disinfection is a procedure which reduces the level of microbial contamination, but there is a broad range of activity which extends from sterility at one extreme to a minimal reduction in the number of contaminating micro-organisms at the other.

Absolute sterility is difficult to prove and, as a result, it is common to define sterility in terms of the probability that a contaminating microorganism will survive treatment. For example, sterilizing processes are usually challenged and verified with  $10^6 - 10^9$  dried bacterial endospores, and sterilization is defined as the state in which the probability of any one spore surviving is  $10^{-6}$  or lower. This rationale has been used to establish cycles for

steam sterilizers and ethylene oxide gas sterilizers, and it produces a degree of overkill as well as a quantitative assurance of true sterilization. It is difficult to evaluate liquid chemical disinfection processes by using these criteria, however, and disinfection processes cannot be assumed to have the same reliability as sterilization.

3. An *antiseptic* is a substance that is used on or in living tissue to inhibit or destroy microorganisms. Quite often, the distinction between an antiseptic and a disinfectant is not made; a disinfectant is a chemical germicide that is used solely for destroying microorganisms on inanimate objects; an antiseptic is one that is used on or in living tissue. Some chemical agents, iodophors for example, are used as active agents in chemical germicides that are formulated as disinfectants as well as antiseptics. However, the precise formulations are usually significantly different depending on intended use and microbial efficacy differs substantially. Thus, disinfectants should never be used as antiseptics and vice versa.

## Disinfectant Activity

Classifying chemical germicides in their respective concentrations according to their levels of microbicidal potency is important but arbitrary. I have decided to retain the system originally proposed by Spaulding<sup>22</sup> rather than the system used by the EPA<sup>5</sup>. For the purposes of this chapter, three levels of disinfection are defined: high, intermediate and low (Table I). In the EPA classification<sup>5</sup>, chemical germicides that are registered as sporicides would be equivalent to high-level disinfectants, depending on the specific label claims. For example, some chemical germicide formulations are claimed to be efficacious against *Mycobacterium tuberculosis*. By Spaulding's system, such a formulation would probably be classified as an intermediate-level disinfectant. However, chemical germicide formulations with specific label claims for *Salmonella choleraesuis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, could fall into intermediate- or low-level disinfectant categories. These are the challenge organisms required for EPA classification as a "hospital disinfectant."

Some high-level disinfectants can kill large numbers of resistant bacterial endospores under severe test conditions but may require as long as 24 hr. to do so. However, most disinfectants cannot achieve this level of antimicrobial activity. In practical terms, high-level disinfection procedures, if done properly, can be considered almost equivalent to sterilization without the added insurance of overkill: since a number of critical patient-care items are damaged by high temperatures and cannot be heat sterilized, they must be disinfected with chemical germicides. High-level disinfectants are used fairly often to process medical and surgical materials. In the absence of bacterial spores, these germicides are rapidly effective; however, the absence of spores cannot usually be assured. Although the number of spores will generally be small<sup>23</sup>, sporicidal capacity is nevertheless an essential property of high-level disinfection, and the sporicidal activity depends both on the agent and how it is used.

A good example of this is 2% aqueous glutaraldehyde, which is capable of sterilizing, but

only after extended contact time in the absence of extraneous organic material. Some medical devices are not physically able to withstand immersion in fluid for 6-10 hr. Even if prolonged contact were possible, the treated materials would have to be rinsed thoroughly with sterile water, dried in a special cabinet with sterile air, and stored in a sterile container to assure that the material remained sterile. Thus, glutaraldehyde-based chemical germicides are capable of sterilization but only under a strict set of circumstances (i.e., precleaned items, 6-10 hr. contact time, room temperature).

Intermediate-level disinfectants are ones that do not necessarily kill bacterial endospores but do inactivate the tubercle bacillus, which is significantly more resistant to aqueous germicides than are ordinary vegetative bacteria. These disinfectants are also effective against fungi (asexual spores, but not necessarily dried chlamyospores or sexual spores) as well as lipid and nonlipid, and medium- and small-sized viruses (Table I). The tubercle bacillus, lipid and nonlipid viruses and other microbial types in Table I are used as indicator organisms having varying degrees of resistance to chemical germicides and not necessarily because of their importance in causing nosocomial infections. For example, cells of *M. tuberculosis* are among the most resistant vegetative microorganism known, and after bacterial endospores, constitute a most severe challenge to a chemical germicide. This type of chemical germicide may be used as a high or intermediate level disinfectant targeted to many types of nosocomial pathogens but not specifically to control respiratory tuberculosis, which in the United States, is no longer a major infection control problem in hospitals.

**Table I. Levels of Germicidal action**

	Bacteria				Viruses	
	Vegetative Bacteria	Tubercule Bacillus	Spores	Fungi <sup>1</sup>	Lipid & Medium-sized	Nonlipid & Small-sized
High	+ <sup>2</sup>	+	+ <sup>3</sup>	+	+	+
Intermediate	+	+	± <sup>4</sup>	+	+	± <sup>5</sup>
Low	+	-	-	±	-	-

<sup>1</sup> Includes asexual spores but not necessarily chlamyospores or sexual spores.

<sup>2</sup> Plus sign indicates that a killing effect can be expected when the normal use-concentrations of chemical disinfectants or pasteurization are properly employed; a negative sign indicates little or no killing effect.

<sup>3</sup> Only with extended exposure times are high-level disinfectant chemicals capable of actual sterilization.

<sup>4</sup> Some intermediate-level disinfectants, e.g., iodophors, formaldehyde, tincture of iodine or chlorine compounds, can be expected to exhibit some sporicidal action.

<sup>5</sup> Some intermediate-level disinfectants, e.g., alcohols, phenolics, may have limited virucidal activity.

Although intermediate-level disinfectants are considered effective against viruses, there are some exceptions. Klein and Deforest<sup>19</sup> have shown that the resistance of viruses to chemical disinfectants can vary significantly. They reported that small, nonlipid viruses were significantly more resistant than medium-sized viruses with lipid in their protein coats. Some of the most widely used chemical germicides failed to destroy picornaviruses, among which are the enterovirus group and the rhinoviruses of the common cold. Consequently, it is not necessarily true that intermediate-level disinfectants that have good tuberculocidal activity also destroy all viruses. The human hepatitis viruses (A, B, and non-A, non-B) have been difficult to study because some (Type B and non-A, non-B) have not yet been cultured in the laboratory. However, there is no evidence that any of these viruses are unusually resistant to physical or chemical agents<sup>8,20</sup> and the hepatitis B virus has been shown to be inactivated by several intermediate to high-level disinfectants including two glutaraldehyde-based formulations, 500 ppm free chlorine, an iodophor disinfectant, and 70% isopropanol<sup>7</sup>.

Low-level disinfectants are those that cannot be relied on to destroy bacterial spores, tubercle bacilli, or small nonlipid viruses within a practical time period. These disinfectants may be useful in actual practice because they can rapidly kill vegetative forms of bacteria and fungi as well as medium-sized lipid containing viruses. Examples of low-level germicides, formulated as disinfectants or antiseptics are: aqueous quaternary ammonium compounds, hexachlorophene, chlorhexidine, and (PCMX). Relatively high concentrations of mercurials are required to achieve significant bactericidal activity. They are fairly low-level disinfectants, are not considered efficient, and are of very little use in modern disinfection strategies in hospitals.

## Factors Influencing Germicidal Procedures

Microorganisms vary widely in their responses to physical and chemical stresses, but it is generally agreed that few, if any, approach the resistance of bacterial endospores<sup>13</sup>. Because of this, bacterial spores are used as biological indicators for sterilization cycles. In a broad descending order of relative resistance, considerably below that of bacterial spores, are the tubercle bacilli, nontuberculous mycobacteria, fungal spores, small or nonlipid viruses, vegetative fungi, medium-sized or lipid viruses, and vegetative bacterial cells.

The differences in chemical resistance exhibited by various vegetative bacteria are relatively minor except for tubercle bacilli and some nontuberculous mycobacteria<sup>10</sup>, which, presumably because of their hydrophobic cell surfaces, are comparatively resistant to a variety of chemical germicides. Among the ordinary vegetative bacteria, staphylococci and enterococci are somewhat more resistant than most other gram-positive bacteria. Antibiotic-resistant "hospital" strains of *Staphylococcus* are not discernibly more resistant to germicides than are susceptible strains. A number of gram-negative bacteria such as

*Pseudomonas*, *Klebsiella*, *Enterobacter*, and *Serratia* may also show comparative resistance to some disinfectants and antiseptics. This is noteworthy since these species are among the emerging pathogens responsible for outbreaks of hospital infection.

Gram-negative water bacteria that can grow well and achieve levels of  $10^3$  to  $10^7$  per ml in distilled, deionized, or reverse osmosis water have been shown to be significantly more resistant to a variety of disinfectants isolated and grown in pure culture in water without subculturing on laboratory media as compared to cells subcultured in the normal fashion<sup>16,18</sup>. The same phenomenon has been shown for nontuberculous mycobacteria<sup>10</sup>. These differences in resistance become important when low-level disinfectants are used, particularly at marginal or dilute concentrations. In addition, naturally occurring spores are significantly more resistant to dry heat than are those that are subcultured<sup>6</sup>. Some gram-negative water bacteria can attach to and colonize surfaces and may form a film or glycocalyx<sup>11</sup>. Microbial cells can thus be shielded and survive for significant periods in the presence of chemical germicides that ordinarily inactivate these organisms rapidly<sup>3,14,15</sup>. These phenomena emphasize the importance of actual “use” tests when evaluating chemical germicides for application in specific environments, as well as the importance of precleaning items before disinfection.

Under a given set of circumstances, the higher the level of microbial contamination, the longer the exposure to the inactivating agent must be. Consequently, the lack of good physical cleaning of an item before subjecting it to disinfection or sterilization may easily cause the process to fall far short of its intended goal. Feces, blood, mucous, or soil may also contribute to the failure of a given disinfection or sterilization process. Organic soil may occlude microorganisms and prevent penetration of physical and chemical agents, or the soil may directly inactivate certain germicidal chemicals. Cleaning is particularly important in disinfection that does not include the overkill factor of sterilization. Indeed, in one report, a flexible fiberoptic endoscope was implicated in an outbreak of *Serratia* septicemia in a hospital<sup>25</sup>. This instrument had been “sterilized” with ethylene oxide gas but had not been properly cleaned before the procedure. Thus, even a rigorous sterilization cycle capable of killing exposed bacterial spores may not even kill relatively delicate vegetative bacterial cells that are protected by organic material.

Generally speaking, with all other variables constant, the higher the concentration of chemical agent or the longer a process is continued, the greater is its effectiveness. For temperature-based procedures, an increase in temperature during exposure will usually significantly increase the efficacy of the chemical germicide.

## **Directions for Using Commercially Available Chemical Germicides**

Another important factor that should be kept in mind when formulating a procedure for sterilization, disinfection or antisepsis is the necessity to read and follow the manufacturer’s directions for use. For disinfectants and antiseptics, general guidelines for use and contraindications, as well as a listing of the active ingredients, are found on the label, and in the

literature supplied with a particular product. When these directions are not taken into consideration, significant errors can be made in use dilutions as well as in applying certain chemical germicides. A good example is iodophors.

Iodophors are the combination of iodine and a solubilizing agent or carrier in which the resulting complex or combination acts as a reservoir of iodine and liberates small amounts of free iodine when diluted with water. Examples of carriers are quaternary ammonium compounds, detergents, polyvinylpyrrolidone (PVP or povidone). Formulations containing iodophors usually list certain percentages of available iodine and do not specify the amount of free iodine which is the chemical form responsible for killing microorganisms. Available iodine content often is used as an indicator of germicidal potency, but this approach is incorrect. Many aspects related to the physical and organic chemistry of iodine complexes are not fully understood. For example, a povidone-iodine germicide formulated as an antiseptic may contain 10% povidone-iodine and 1% available iodine. The terms "available" when used with iodine refers to the amount of iodine that is titratable with sodium thiosulfate. When a solution contains 1% available iodine, almost all of it is in a complexed form, and very little exists as free iodine; but during the chemical assay with sodium thiosulfate, both the complexed and free iodine titrate. The amount of free iodine in these types of solutions is approximately 1 ppm and is controlled significantly by the amount of potassium iodide present as well as by the amount of water. Concentrated solutions of iodophor contains less free iodine in undiluted solutions than those diluted up to a specific point. It is virtually impossible to chemically assay free iodine in the presence of complexed iodine without resorting to an extraction technique using solvent. Thus, one can readily appreciate that the manufacturer's label direction for an iodophor disinfectant that calls for a 1 to 213 aqueous dilution of a concentrated formulation is designed to give the maximum degree of microbicidal efficiency that correlates with the amount of free iodine present.

The amount of available iodine noted on the label of a chemical germicide formulated as an antiseptic may be very similar to that listed on one formulated as a disinfectant. This does not alter the rationale for classifying disinfectants as having intermediate-level activity, but it does present a problem in defining the appropriate use concentration. Since it is complicated to assay for free iodine in the presence of iodophor solutions and since it is the current practice of manufacturers to include the amount of available iodine on product labels as an implication of potency, I have elected to retain the amount of available iodine to denote strength (Table II). With iodophors the manufacturer's directions are much more critical with respect to actual use dilutions with water than most other disinfectants, and care should be taken to follow label directions closely. Further, iodophors formulated as antiseptics contains significantly less free iodine than those formulated as disinfectants, so that iodophor antiseptics should not be used as disinfectants and vice versa<sup>3,13,14</sup>.

## **Intended Uses of Germicidal Agents**

Patient-care equipment can be categorized into critical, semi-critical and non-critical<sup>13</sup> (Table III). **Critical items include instruments or objects, e.g., scalpels, cardiac catheters,**

implants, hemodialyzers, that are introduced directly into the body—either into the bloodstream or into normally sterile areas. In this instance, sterility is required, and all contaminating microorganisms must be destroyed.

**Table II. Methods of Sterilization or Disinfection<sup>1</sup> Sterilization**

<i>Class</i>	<i>Concentration/Level</i> (See manufacturer's recommendations for exposure times and conditions)	
<i>Heat</i>	250°F (121°C) or above;	
Moist heat (steam under pressure)	pressure-pre-vacuum cycle 271°F (132°C)	
Dry heat	170°C—1 hr 160°C—2 hr 121°C—16 hr or longer	
<i>Gas</i> <sup>1</sup>	<i>Ethylene oxide</i> 450-500 mg/L-55°-60°C <sup>2</sup>	
<i>Liquid</i> <sup>1</sup>		
Glutaraldehyde, aqueous	Variable <sup>3</sup>	
Hydrogen peroxide, stabilized	6%-25%	
Formaldehyde, aqueous	6%-8%	

### DISINFECTION

<i>Class</i>	<i>Concentration/Level</i> (exposure times 10-30 min)	<i>Activity Level</i>
<i>Heat</i>		
Moist heat <sup>4</sup>	75°-100°C	High
<i>Liquid</i>		
Glutaraldehyde, aqueous <sup>3</sup>	2%	High
Hydrogen peroxide stabilized	3%-6%	High
Formaldehyde, aqueous <sup>5</sup>	1%-8 %	High-to- intermediate
Iodophors <sup>6</sup>	30-50 mg/L free iodine; 70-150 mg/L available iodine	Intermediate
Chlorine compounds <sup>7</sup>	500-5,000 mg/L free available chlorine	Intermediate
Alcohols (ethyl; isopropyl) <sup>8</sup>	70%	Intermediate
Iodine and alcohol	0.5% + 70%	Intermediate
Phenol compounds, aqueous	0.5% – 3%	Intermediate- to-low
Quaternary ammonium com- pounds, aqueous	0.1% – 0.2%	Low

## ANTISEPTICS<sup>9</sup>

<i>Class</i>	<i>Concentration</i>	<i>Activity Level</i>
Alcohols (ethanol; iso- propanol)	70%	Intermediate
Iodophors	1-2 mg/L free iodine 1-2% available iodine	Intermediate- to-low
Chlorhexidine	.75%-4.0%	Low
Parchlorometa xylenol (PCMX)	– 0.5%-4%	Low
Mercurial compounds	0.1%-0.2%	Low

- <sup>1</sup> This list of chemical germicides contains generic formulations. Other commercially available formulations can also be considered for use. Users should ensure that the formulations are registered with the EPA or categorized by the FDA. Information in the scientific literature or presented at symposia or scientific meetings can also be considered in determining the suitability of certain formulations.
- <sup>2</sup> In humidified sterilizer designed for ethylene oxide at 55°-60°C.
- <sup>3</sup> There are several glutaraldehyde-based proprietary formulations on the U.S. market, i.e., low, neutral or high-pH formulations recommended for use at normal temperatures with or without ultrasonic energy and also a formulation containing glutaraldehyde 2% and phenol 7%. Manufacturer's instructions regarding use as a sterilant or disinfectant or anticipated dilution during use should be closely followed.
- <sup>4</sup> Includes hot water pasteurization.
- <sup>5</sup> Because of the ongoing controversy of the role of formaldehyde as a potential occupational carcinogen, the use of formaldehyde is recommended only in limited circumstances under carefully controlled conditions, i.e., disinfection of certain hemodialysis equipment.
- <sup>6</sup> Only those iodophors registered with the EPA as hard surface disinfectants should be used, and manufacturer's instructions regarding proper use dilution and product stability should be closely followed. Antiseptic iodophors are not suitable for use as disinfectants.
- <sup>7</sup> There currently is a formulation registered with EPA as a sterilant and disinfectant depending on contact time and whose active ingredient is chlorine dioxide. Manufacturer's instructions regarding use as a sterilant or disinfectant or anticipated dilution during use should be closely followed.
- <sup>8</sup> With volatile products such as alcohols, careful attention should be given to proper contact time during a disinfecting protocol.

<sup>9</sup> This list includes those antiseptics which are commonly used in hospitals; the list is not complete in that all formulations categorized the FDA are not mentioned. For more detail, the reader is referred to the paper by Zanowiak and Jacobs<sup>26</sup>.

*Comment:* Adequate precleaning of surfaces is the first prerequisite for any disinfecting or sterilizing procedure. The longer the exposure to a physical or chemical agent, the more likely it is that all pertinent microorganisms will be eliminated. Ten minutes exposure may not be adequate to disinfect many objects, especially those that are difficult to clean because of narrow channels or other areas that can harbor organic material as well as microorganisms; thus, longer exposure times, ie., 20-30 min, may be necessary. This is especially true when high-level disinfection is to be achieved.

Semi-critical items come into contact with mucous membranes, but they do not ordinarily enter normally sterile tissues. Local host-defense mechanisms can be expected to protect against challenges from small numbers of exogenous microorganisms, but for safety these items should not be contaminated with vegetative bacteria. Although sterilizing these items is desirable and quite often the cheapest and fastest procedure available, it is not absolutely essential. For semi-critical items that do not tolerate heat or cannot withstand long period of immersion in chemical germicides or exposure to ethylene oxide gas, it is reasonable to use a high-level disinfection process, a procedure designed to destroy ordinary vegetative bacteria, most fungal spores, tubercle bacilli and small nonlipid viruses.

Non-critical items offer little risk of transmitting infectious agents. Such items include face masks, carafes, electrocardiogram electrodes, walls, floors, furniture and other environmental surfaces that do not ordinarily come into contact with mucous membranes. Many persons rely on hot water or detergent cleansing, but some use low-level disinfectants either alone or in addition to the washing.

**Table III. Methods of Assuring Adequate Processing and Safe Use of Medical Devices**

Object and Classification	Example	Method	Comment
<b>PATIENT-CARE OBJECTS</b>			
	<i>Critical</i>	1. Thoroughly clean objects and wrap or package for sterilization 2. Follow manufacturer's instructions for use of each sterilizer or	

Sterilized in the hospital

Surgical instruments and devices; trays and sets

- use recommended protocol
- 3. Monitor time-temperature charts
- 4. Use commercial spore preparations to monitor sterilizer.
- 5. Inspect package for integrity and for exposure of sterility indicator before use.
- 6. use before maximum safe time has expired.

Sterilization processes are designed to have a wide margin of safety. if spores are not killed, the sterilizer should be checked for proper use and function; if spore tests remain positive, discontinue use of the sterilizer until properly serviced. maximum viced. maximum safe storage time of items processed in the hospital varies according to type of package or wrapping material(s) used; follow manufacturer's instructions for use and storage times.

Purchased as sterile

Intravenous fluids; irrigation fluids; normal saline; trays and sets.

- 1. Store in safe, clean area.
- 2. Inspect package for integrity before use.
- 3. Use before expiration date, if one is given.

Notify the U.S. Food and Drug Administration if intrinsic contamination is suspected.

*Semi-critical*

Should be

Respiratory

- 1. Sterilize if possible; if not, follow a protocol for high-level

free of vegetative bacteria. May be subjected to high-level disinfection rather than sterilization.

therapy equipment and instruments that will touch mucous membranes

disinfection.  
2. Bag and store in safe, clean area.  
3. Conduct quality control monitoring after any important changes in the disinfection process.

Bacterial spores may survive after high-level disinfection, but these usually are not pathogenic. Microbiologic sampling can verify that a high-level disinfection process has resulted in destruction of vegetative bacteria.

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## Evaluating Actual Germicidal Effectiveness Microbiologic assays

A hospital can make a rational choice from the various sterilizing, disinfecting and antiseptic processes that are available by considering the intended uses for the product (is sterility required, or may high, intermediate, or low-level disinfection be adequate?) and by understanding the factors that influence germicidal effectiveness discussed above. It is not practical for hospital laboratories to test the antimicrobial effectiveness of commercially available chemical germicides unless such testing is part of a well-designed research project. In the United States, hospitals rely on scientific literature, scientific meetings, and scientific data provided by manufacturers in addition to Environmental Protection Agency registration and Food and Drug Administration categorization. Testing of antiseptics<sup>9,21,24</sup> and disinfectants<sup>4,12,13</sup> is a complex and expensive process, and few clinical microbiology laboratories will devote their resources to such testing.

The actual effectiveness of a germicide is influenced only in part by the nature of the agent. Of equal, or perhaps greater, importance is the way it is used in the hospital. Many disinfectants, especially low and intermediate-level disinfectants, have little margin of safety; misuse by hospital personnel may lead to germicidal failure. Thus, a hospital's infection control program may decide to use microbiologic cultures in a limited program to monitor the effectiveness of disinfection and sterilization.

Routine or widespread environmental culturing is generally discouraged, because it offers few data of use to an infection control program. Moreover, an environmental monitoring program must be well designed with a specific objective in mind. It makes little sense, for example, to evaluate items or areas that are unlikely to play a role in disease transmission. Thus, floors, furniture, or other non-critical items should *not* be tested even to evaluate the effectiveness of hospital housekeeping personnel. Environmental assays, to the extent that they are used, should be limited to high-risk (critical or semi-critical) items. Even then, they should not take the place of scrupulous attention to the actual performance

of the sterilization or disinfection procedures. With these cautions in mind, the following guidelines are offered for the microbiologic monitoring of selected high-risk procedures.

## **Respiratory therapy breathing circuits and anesthesia equipment**

Proper cleaning and disinfection procedures are the most important part of an environmental control program to reduce infections transmitted by respiratory therapy and anesthesia equipment breathing circuits. Many items may be sterilized with steam or ethylene oxide, but if they are subjected to chemical disinfection, they may be spot checked every few months or when disinfection or usage procedures change. Routine or scheduled microbiologic assays are not required. There is no adequate microbiologic guideline for this supported by epidemiologic studies. The most widely used criterion of acceptability is the absence of vegetative bacteria on components of the breathing circuits after disinfection.

## **Hemodialysis systems**

Gram-negative water bacteria can multiply relatively fast in fluids associated with hemodialysis systems such as distilled, softened, deionized, and reverse osmosis water, as well as in the dialysis fluid itself. Although these fluids do not need to be sterile, excessive levels of gram-negative bacterial contamination pose a risk of pyrogenic reactions and septicemia. An epidemiologic-based, quantitative microbiologic guideline for levels of contamination has been proposed<sup>1,17</sup>. It is suggested that dialysis fluid and water used to prepare dialysis fluids be checked microbiologically at least once a month. Microbiologic guidelines for these procedures include sampling the water used to prepare dialysis fluid at the point where it is mixed with concentrated dialysis fluid. The level of bacterial contamination should not exceed 200 per ml. Dialysis fluid should be sampled at the end of a dialysis treatment, and the level of bacterial contamination should not exceed 2,000 per ml. In both instances, routine standard plate counts can be done by pour or surfaces plating or membrane-filter procedures with appropriate culture media such as tryptic soy or standard methods agar.

## **Arterial pressure transducers**

Arterial pressure transducers have been incriminated in disease transmission and the best means of control are adequate cleaning and sterilizing as well as proper placement. Disposable domes should not be reused. Scheduled microbiologic sampling is not required. If a microbiologic assay is performed, the criterion of acceptability is sterility.

## **Miscellaneous procedures and equipment**

There are numerous items of patient-care equipment that pose varying degrees of risk of infection. They make direct contact with skin or mucous membranes or body orifices but not with deep tissue. Items in this category are flexible fiberoptics, antiseptic solutions, soaps, hydrotherapy equipment, and nonsterile solutions prepared in the hospitals. However, with most of these, the most important element in environment control is not microbiologic sampling, but rather adherence to tested protocols for their cleaning, preparation, disinfection or sterilization, length of use and maintenance. Even spot checking these items and procedures in most instances is not recommended because of the absence of meaningful microbiologic guidelines supported by epidemiologic criteria.

## **Reuse of Disposable Medical Devices**

In the 1970's, many medical devices that were reusable and thus could be cleaned and resterilized were replaced by disposable medical devices. This was thought to be cost effective, because personnel and operational costs of cleaning, sterilizing and repackaging reusable devices could be eliminated. However, in recent efforts to save money, some hospitals are reusing disposable medical devices. The number and types of disposable medical devices are varied and range from critical medical devices to non-critical ones. The question arises: Can medical devices labelled "single-use, sterile and disposable" be safely reprocessed and reused in a hospital? Currently, no reported studies have shown that specific disposable medical devices can be reprocessed, sterilized or disinfected and safely function as originally intended. A notable exception is the disposable hollowfiber hemodialyzer. Approximately 43% of dialysis centers in the United States process and reuse disposable hemodialyzers on the same patient<sup>2</sup>. When standardized protocols and adequate sterilization procedures are used, these procedures do not appear to be associated with problems of disease transmission. However, dialysis centers that do reuse dialyzers use standardized cleaning and sterilization protocols and quality control measures that determine the efficacy of the dialyzer. With other medical devices, there basically are no data available that show that the device after reprocessing and sterilization still functions safely and does not retain toxic levels of chemical germicides used in the reprocessing procedure.

Hospitals should be aware that if they reuse disposable medical devices, the liability associated with those devices shifts from the manufacturer to the user. Further, there are some medical devices that can be sterilized but can be discernibly damaged and the function of the device can be effected, which in turn affects patients' safety. Unless there are overriding cost considerations, disposable critical and semi-critical medical devices meant for one-time use probably should not be reused. If they are reused, it should be determined that they can be cleaned, sterilized or disinfected without altering their function, and that problems of residual toxicity and overall safety will not be involved with their reuse.

## **Unnecessary Microbiological Assays**

There are a number of items and procedures in the hospital environment for which microbiologic sampling either on a scheduled or periodic basis is not cost effective or rational. These include sterile intravenous solutions, injectables, disposable syringes, disposable blood lines, artificial kidneys and all other items that are received in a sterile state. Equipment and solutions sterilized in the hospitals do not need to be sampled microbiologically. Rather, quality assurance testing associated with sterilization procedures as described above should be used to verify that the sterilization process *per se* is performing to specifications and that the associated procedures are being performed correctly.

Although it is recognized that inanimate surfaces and air associated with critical areas such as surgical suites and intensive-care areas may contain reservoirs of microorganisms, the chance for disease transmission in environments that are adequately cleaned and maintained is remote. Environmental control procedures associated with housekeeping and engineering services should use and adhere to tested cleaning, disinfection and maintenance protocols. Consequently, microbiologic sampling should not be necessary for intramural air or inanimate environmental surfaces. However, in an outbreak of hospital-acquired disease, if a certain part of the environment, such as the air ventilation system, appears to be associated with disease transmission, appropriate sampling should be initiated.

## **Environmental Microbiologic Sampling During Outbreaks of Infections**

The strategy that should be used during an outbreak of infection with respect to environmental microbiologic sampling depends on several things. First, the epidemiologist must determine whether certain procedures, equipment, instruments, or other parts of the environment may be playing a direct or indirect role in the outbreak. The occurrence of an outbreak of nosocomial infections does not automatically mean that environmental microbiologic sampling at any level is required. Second, if environmental microbiologic sampling is believed to be necessary, the microbiologist and epidemiologist should coordinate the sampling strategy and determine the implicated procedures, items, or parts of the environment requiring microbiologic assay.

A microbiologic guideline applied in this context differs from scheduled or periodic sampling. During the investigation of an outbreak of nosocomial infections, environmental sampling is usually directed toward a specific pathogen. Consequently, if the outbreak is due to *Pseudomonas aeruginosa*, this organism and perhaps specific serotypes are sought in the various environmental items that are sampled. In this respect, the guideline tends to be more qualitative than quantitative, although in some instances one must rely on established guidelines. For example, if water or ice in a hospital is incriminated in an outbreak of nosocomial salmonellosis, procedures should be used to determine fecal coliform bacteria and the total number of microorganisms, in addition to searching for *Salmonella*. The guideline here, then, is flexible and basically is determined by the nature of

the outbreak and the results of the epidemiologic investigation.

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# A Study on the Disinfectant Effect and Toxicity of Sodium Dichloroisocyanurate

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Sodium dichloroisocyanurate (NaDCC), as a disinfectant, has been used in China since 1972. The synthetic processes require that, in the first step, urea and ammonium chlorite are heated to produce isocyanuric acid, then, after adding sodium hydroxide and chlorine, dichloroisocyanuric acid is obtained, finally sodium carbonate is added to form an end product—sodium dichloroisocyanurate (NaDCC), in which the amount of available chlorine is usually between 62 and 64%.<sup>1</sup> In addition, varieties in combination products, e.g., Utensil Clean-333, Xinxiaojing etc., have been produced. In this study, an attempt is being made to determine the disinfectant effect and toxicity of sodium dichloroisocyanurate based on the evidence of laboratory tests and field trials.

## Effect of Disinfectant on Vegetative Bacteria and Spores

Table I. The Effect of Bactericidal and Sporicidal of NaDCC in Vitro at 15-25°C

	Spores		
	<i>E.coli</i>	<i>B.cerus</i>	<i>B.anthraxis</i>
Ten min critical bactericidal	15 ppm	350 ppm	60 ppm

concentration

Quantitative bactericidal  
test

250 ppm  
5 min

60 ppm  
5 min

A series of dilutions was mixed with the cultures of *E. coli*, spores of *B. cereus* and *B. anthracis* contained initially  $10^8$  ml. After ten minutes exposure to disinfectant, each sample was immediately neutralized by the addition of equimolar amount of sodium thiosulfate. Each sample (0.5 ml) of five tubes were subcultured into nutrient broth which was incubated and examined for growth.

Critical bactericidal concentration refers to the endpoint of the test, which is the highest dilution of NaDCC capable of killing a particular organism within ten minutes. Meanwhile, a series of ten-fold dilutions prepared for pour plates to do a viable count after varying times and the time for a four log reduction calculated, as a quantitative bactericidal test.

Table I presents the effect of NaDCC on bactericidal and sporicidal results.

## Factors Affecting NaDCC

(1) Temperature:

The organisms of *E. coli* ( $10^8$ /ml) and spores of *B. cereus* ( $10^8$ /ml) were exposed with NaDCC containing 300 ppm of available chlorine at 15, 25 and 35°C respectively. The rate of 5 log (99.999%) killing time between higher and lower temperature groups was calculated. The temperature coefficient of *E. coli* ( $Q_{10}$ ) was equal to, and the temperature coefficient of *B. cereus* spores was 1 to 1.25. The results demonstrated that, unlike other disinfectants, bactericidal or sporicidal action of NaDCC had not been markedly influenced by the usual room temperature.<sup>2</sup>

(2) Organic Material:

The effect of bactericidal action of NaDCC was detected in the presence of 5-20% bovine serum, the action was not destroyed when the low concentration of serum was existing. The results are shown in Table II. NaDCC has reduced germicidal nature in the presence of organic material.<sup>2,7</sup>

**Table II. The Killing Time of *E. coli* and Spores of *B. cereus* Under the Protection of Varies Concentration of Serum**

Organism	The concentration of NaDCC (ppm of Available Chlorine	0	5	10	20
<i>E. coli</i>	250	2*	2	30	60
<i>B. cereus</i>	800	6	10	10	10

### (1) Inactivation of HB<sub>s</sub>Ag antigenicity

To draw a calibrated curve, a series of reference HB<sub>s</sub>Ag dilutions were detected by RIA or ELISA methods. The values of cpm or optical density were then transferred to probit units to plot a log-regression linear line. After each sterilizing procedure, the residual HB<sub>s</sub>Ag was studied by RIA or ELISA.

The rate of destruction could be calculated by substitution to the formula, 2.1 times of the negative control mean was used as a cutoff value. As shown in Table III.<sup>3</sup>

**Table III. The Time Required (Min) for Destroying Antigenicity of HB<sub>s</sub>Ag Under NaDCC Containing Various ppm of Available Chlorine**

Methods		ppm of Available Chlorine					
		2000	1000	500	250	125	60
RIA	HB <sub>s</sub> AG (4.6 μg/ml)	—	2	5	10	30	—
	HB <sub>s</sub> aG + BSA (2 mg/ml)	—	—	—	10	—	—
	HB <sub>s</sub> Ag + BSA (5 mg/ml)	—	10	—	—	—	—
ELISA	HB <sub>s</sub> AG (11 μg/ml)	2	2	2	2	5	10
	HB <sub>s</sub> Ag + BSA (2 mg/ml)	10	—	—	—	—	—
	HB <sub>s</sub> Ag + BSA (5 mg/ml)	10	—	—	—	—	—

The sensitivity of RIA was 0.2 ng/ml, of ELISA, 20 ng/ml.

According to the difference of sensitivity in RIA or ELISA method, the inactivation of HB<sub>s</sub>AG antigenicity after an exposure time of two minutes required the concentration of NaDCC containing 250-1000 ppm of available chlorine. If the exposure time extended to five minutes or more, the requirement of available chlorine was reduced to 125-500 ppm. In contrast, at the presence of 5 mg/ml bovine serum albumin in the test system, the efficiency of NaDCC was substantially reduced. HB<sub>s</sub>AG could not be detected after a ten minute incubation only in the high concentration of available chlorine (4000 ppm).<sup>3,4</sup> (2) Effect of NaDCC on hepatitis B virus-associated DNA polymerase. Serum containing DNA polymerase activity was centrifuged in a MSE-75 ultracentrifuge 110,000 × g for four hours. The titre of DNAP was 1:32. The pellet was resuspended with phosphate-buffered saline in 1/20 of the initial serum volume. The activity of DNAP was determined by measuring the incorporation of 3H-thymidine by a method described previously.<sup>5</sup> A resuspended pellet from HB<sub>s</sub>Ag nonreactive

serum was used as the negative control.

Equal volume of disinfectant and resuspended pellet was mixed and incubated for a certain time then neutralized by sodium thiosulfate. No residual DNAP activity was detectable after five minutes exposure with NaDCC containing 500 ppm of available chlorine or ten minutes with 250 ppm of available chlorine. The results are shown in Figure 1.

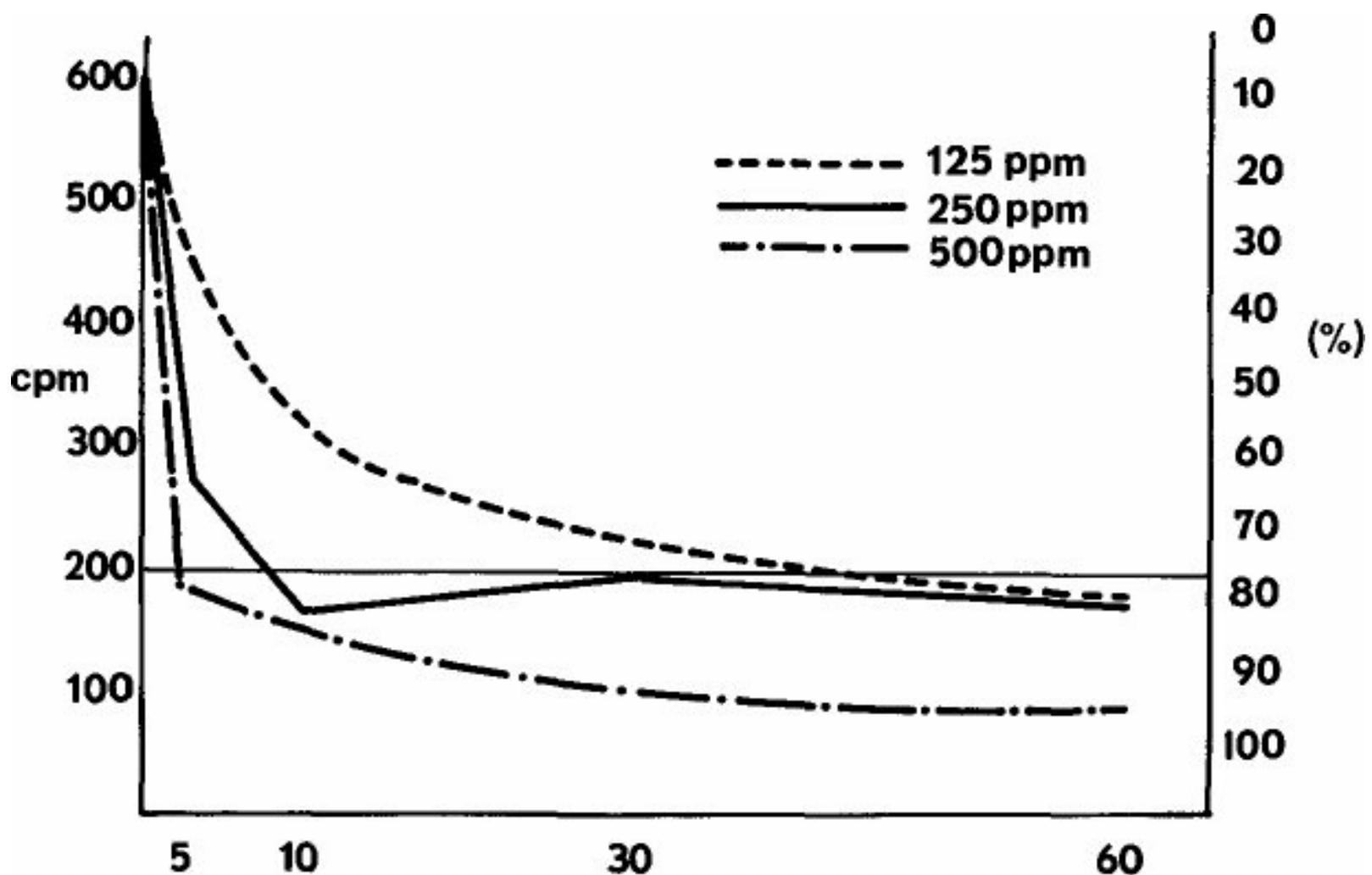


Figure 1. Effect of NaDCC on DNAP at Room Temperature. Samples of DNAP Rich Pellets Were Tested With Different Concentrations of NaDCC.

### (3) The effect of NaDCC on HB<sub>s</sub>Ag immunological reactivity.

The purified HB<sub>s</sub>Ag was exposed to 60 or 250 ppm of available chlorine for five minutes following neutralization with sodium thiosulfate, then absorbed by aluminum hydroxide. Mice were immunized with HB<sub>s</sub>Ag twice intraperitoneally at ten day intervals, no anti-HBs response had occurred, however, 83% of the mice of the control group revealed anti-HBs response. The results demonstrated that the loss of immunological reactivity was in accordance with the extent of degradation of HB<sub>s</sub>Ag particles.<sup>6</sup>

### (4) Morphological change of HB<sub>s</sub>Ag after NaDCC treatment.

Twenty-two HB<sub>s</sub>Ag particles were purified by a rate-zonal ultracentrifugation from pool sera of chronic HB<sub>s</sub>Ag carriers. The purified particles were added to different concentrations of

NaDCC. the results showed that, on electron microscopic grids, the particles became morphological alterations and de-round with rough membrane after two minutes exposure with 125 ppm of available chlorine. The particles intended to be aggregated at the concentration of 250-500 ppm. The particles of HB<sub>s</sub>Ag was rarely found following treatment with NaDCC containing 1000 ppm of available chlorine for 30 minutes.<sup>6</sup>

(5) The stability of NaDCC solution.

NaDCC was examined for free available chlorine by sodium thiosulfate titration. The powder of NaDCC was very stable. After storing in two and a half years, it still maintained in 62% free available chlorine. NaDCC solution containing 500 ppm of available chlorine, after 72 hours in storage, was declined in its concentration to 415 ppm (83%). The stability of NaDCC solution was associated with starting concentration and room temperature. The average decrease rate of available chlorine per day was about 2-5%.<sup>1,6</sup>

## **Disinfection of Drinking Water<sup>1</sup>**

(1) Laboratory tests

Water was contaminated with cultures of *E. coli* (10<sup>6</sup>/ml) and mixed with NaDCC solution containing 2 ppm of available chlorine. The samples were incubated for 10 min and prepared for pour plates. Mean reduction of viable counts within pH range 5.0 to 7.1 was 99.99%. In contrast, when the pH value reached 8.0-9.0, there were a great number of bacterial colonies growing in the dishes. The results indicated that the bactericidal activity against *E. coli* decreased with high pH, specially above pH 8.0.

(2) Field trial

A comparative study was carried out in the effect of NaDCC on 4 kinds of water, e.g., deep well water, shallow well water, river water and canal water. In this test, a large inoculum of *E. coli* (238, 000/ml) was added to different water sources (13-17C, pH 8.0-8.8). The most probable numbers of *E. coli* per 1000 ml water was less than 4 after treatment with NaDCC containing 4 ppm of available chlorine for 20 minutes.

## **The Disinfection of Cooking Utensils**

NaDCC has been widely used for cooking utensils or chopsticks disinfection. *E. coli* was killed in NaDCC solution containing 300 ppm available chlorine within two minutes. The viable counts were 0.08-0.12 per cm<sup>2</sup>. If clean utensils were immersed into 500 ppm of available chlorine solution for 4 min, the total number of bacteria would decrease 99.8%. The exhaustive rate of available chlorine in the solution of NaDCC is correlated with the washing process and the residual food in the utensils.<sup>8,1</sup>

## **Toxicity of Sodium Dichloroisocyanurate**

(1) Acute toxicity test

Mice and rats were randomized allocation. Ten mice or five rats were used in each group. NaDCC was given by the administration of oral route. The number of deaths was observed within three days. Fifty percent lethal dose and 95% confidence limits were calculated by

probit unit or Karber methods. The results indicated that NaDCC was a low toxicity substance. (Table IV)

**Table IV. Acute Toxicity Test of NaDCC**

Animal	Sex	LD <sub>50</sub> (mg/Kg)	95% C.I. (mg/Kg)
Mouse	Male	719-1050	645-1174
	Female	880	749-1011
Rat	Male	604-1470	

### (2) Cumulative toxicity test

Twenty mice or rats were selected for cumulative toxicity test. At the beginning of each trial, each mouse or rat was given 0.1 LC<sub>50</sub> dose of NaDCC, then increased the dose 1.5 times at four-day intervals, up to the total dosage reached to 9 LC<sub>50</sub> dose. The mortality of animal was 15-20% within 25 days. The cumulative coefficient was equal to or more than 9. The results showed that cumulative toxicity of NaDCC to both mouse and rat was very weak. Thirty-two rats fed with water containing 200, 200, 400 ppm of available chlorine for 60 days. There was no significant difference between tested group and control group concerning food intake, weights, hemaglobulin and white cell counts.<sup>6,9</sup>

### (3) Ames test

To determine whether NaDCC has potential mutagenesis or not, TA97, TA98, TA100, TA102 four tester strains of *S. typhimurium* were used in Ames test. The mutagenic rate is measured as the rate of reversion of auxotrophic mutants to prototrophy. The results showed that the numbers of reverse colonies on the plates which contained NaDCC 1 ng, 10 ng and 100 ng, respectively, with or without the detoxifying enzymes of liver microsomes s-9 complex, were not induced reversions two times more than the control plates. Furthermore, when NaDCC was neutralized by sodium thiosulfate, even the concentration of NaDCC increased to 5000 ng per dish, no mutagenic results appeared. The results demonstrated that potential mutagenic could not be existed in NaDCC per se or neutralized.<sup>9</sup>

### (4) Micronucleus test

Male mice were used, each group comprised seven mice. Three doses of NaDCC 20 and 200 mg/Kg were given, animals were killed 6 hr after the last dose. The sternum was removed from each animal and bone-marrow cells were examined. One-thousand polychromatic erythrocytes per mouse were examined for the presence of micronuclei at high magnification. The frequency of micronucleated erythrocytes in groups was 1.4%, 2.5% and 2.7% respectively, even the maximal frequency of MNEs was in the normal variation (4%). The results obtained showed that NaDCC was not activated in the mouse and induced chromosomal alterations in bone-marrow.<sup>9</sup>

### (5) The chromosomal aberration of testis cell

Eighteen male mice were divided into three dose groups, which was given NaDCC 20, 100,

200 mg/Kg consecutive daily dose for five days. Before killing the mice, each mouse was injected with colchicine at the dose of 4 mg/Kg. The aberration rate of spermatogonial stem cells was 1-6.8%, actually it was within the normal range (0-10%), and also no dose-response reaction could be observed. It seems that NaDCC is not a potential mutagen of male post-meiotic germ cell.<sup>9</sup>

#### (6) Seven-day feeding test

Sixty rats were divided into five groups, each group fed various doses of NaDCC, e.g., 125, 500, 2000 and 8000 ppm, respectively, except the control group. The weight was detected periodically. The ratio between visceral organs and body weight and the usage rate were calculated. The results showed that the body weight of animals fed with 500 ppm of available chlorine was increased slowly. The food usage rate in rat, which fed 8000 ppm or more, was significantly decreased. The results demonstrated that the subacute toxicity of NaDCC was also fairly low, 500 ppm NaDCC seem to be a minimum effective dose.<sup>9</sup>

## Summary

The effects of sodium dichloroisocyanurate on bactericidal and virucidal activities both in laboratory practice and field application were evaluated.

The results indicated that NaDCC is a stable, highly effective and low-toxic disinfectant. Concentration of NaDCC containing 15 ppm of available chlorine were found to be effective in destroying *E. coli* after an exposure time of ten minutes. For the presence of *B. cereus*, the sporicidal concentration of ten minute contact time was 350 ppm. The inactivation of HB<sub>s</sub>Ag (5-11 µg/ml) could have occurred within 5 min at the concentration of 500 ppm of available chlorine. The DNA polymerase activity was also completely destroyed. Although the morphological forms characteristic of HB<sub>s</sub>Ag particle were still distinguishable, negative staining visualized they appeared to be aggregated.

Laboratory analysis further confirmed that no evidence of cumulative toxicity or mutagenesis of NaDCC was obtained by the use of various tests (Ames, micronucleus, chromosomal aberration in testis cell, feeding test, etc.)

It has been suggested that the appropriate concentration of NaDCC for treatment food utensils or cookery is 500 ppm of available chlorine for five minutes.

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# Role of Barrier Materials In Preventing Infection

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For over a century, we have recognized the role of bacteria in causing postoperative wound infections. We have been able to eliminate bacteria on the instruments and supplies delivered to the operative field. While we can reduce or eliminate bacteria on inanimate objects, we have much more difficulty in controlling bacteria from human sources. Every effort is made to reduce the bacterial count on the patient's skin through utilization of disinfectant skin preparation. This preparation depends on the removal and destruction of surface bacteria and any residual cidal effect on organisms that surface following the surgical preparation. But we cannot eliminate all resident organisms.

Bacteria residing on the skin of the operating team is much more difficult to control. Toward the end of the 19th century, the team began using sterilized drapes and gowns. This innovation along with implementation of the principles of sterilization and disinfection brought the infection rate down from almost 100% to 16% by the 1940's.

But even the use of antibiotics, thought to be a panacea, failed to eliminate post-operative infections.

Then we learned that the materials being used to isolate the sterile field were ineffective. In 1952, Dr. William Beck and Thomas Collette<sup>1</sup> demonstrated the ineffectiveness of 140 thread count muslin in preventing bacterial contamination. Their work proved that the wet cloth did not provide a barrier, but instead became a wick to transport the bacteria.

In 1963, Beck and Carlson<sup>2</sup> reported, "Not only are most of the usual barriers employed in surgical practice pervious to water, but because of their absorptive properties, they diffuse liquid media over a wide area. This wicking action will diffuse aqueous fluids such as blood, serum, or amniotic fluid over wide areas, and if these fluids are contaminated, the contained microorganisms will be transported to all of the moistened areas."

In that same presentation, Dr. Beck defined an aseptic barrier as "A material placed between an aseptic area, such as an operative incision and areas which harbor microorganisms with the purpose of preventing the spread of bacteria into the sterile zone."

Since then, multiple studies have confirmed the ineffectiveness of 140 thread count muslin. In 1969, Charnley and Eftekhar<sup>3</sup> reported penetration of balloon cloth by organisms in "near sterile air." They had reduced their infection rate from 9% to 1% through the use of clean air, but the 1% infection rate persisted. In an effort to determine the source of the

organisms causing the remaining 1%, they cultured the exterior surface of the surgeon's gown obtaining positive cultures in almost 50% of the samples.

Their assumption was that operations that are unusually difficult technically and require unusual physical effort, tend more often to be followed by infection than do simple operations. They postulated that unusual exertion by the surgeon might result in direct contamination of the wound from the surface of his gown even in the presence of air recorded as sterile by settling plates.

In 1975, Laufman and associates<sup>4</sup> described a test to correlate the stress of stretching surgical gown and drape material with moist strike-through. He demonstrated that not all woven and non-woven surgical gown and drape materials are impermeable to moist contamination for equal periods of time.

Manufacturers and concerned investigators may be interested in G. M. Olderman's<sup>5</sup> article on liquid repellency and barrier properties.

The same year, Moylan and co-workers evaluated intraoperative bacterial transmission in 100 general surgical operations. This study compared cloth gowns to single-use gowns. Five post-operative wound infections occurred in this series. "In four of those infections, organisms were traced from the scrub suit pre-operatively to the external surface of the gown post-operatively and then cultured from the wound, indicating gown penetration as one of the primary pathways of wound infection during operation."

In 1980, Laufman utilized scanning electron microscopy to demonstrate the process of moist bacterial strike-through of woven and non-woven surgical materials. Three woven and three non-woven materials were challenged with an aqueous solution of *Serratia Marcescens*. These studies confirmed that relatively new, less than 100 cycles of washing and sterilizing, 270 thread count Quarpel treated pima cotton prevents moist bacterial penetration. After more than 75 to 100 cycles, the same material allowed bacterial penetration.

Non-woven materials prevented penetration only when they were impregnated with plastic or reinforced with a plastic film. Prevention of moist bacterial strike-through of surgical materials, whether they be woven or non-woven, is dependent upon the effectiveness of their waterproof quality.

In 1980, Schwartz and Saunders reported results of a study on four types of materials used in surgical gowns. Testing was done in 90 operations, selected at random. These were divided into three groups of 30 each. Distribution by time interval and type of procedure were equal. In each group, a test gown was worn by one surgeon and a 140 thread count muslin gown worn by the second surgeon.

Cultures of the chest and upper abdominal areas of the scrub suit were taken from both surgeons. Similar to Moylan's protocol, patches of polyethylene were sealed onto the upper abdominal areas of all gowns prior to sterilization. In use the 140 thread count muslin showed evidence of penetration within the first five minutes in 15% of 90 gowns. After two hours, 51% of 90 gowns were penetrated. The same organisms that had been removed from the scrub suit and the outside of the gown were also isolated from the forearms of the gown of the same surgeon in 25 of 51 instances, and from the forearms of the gown of the other surgeon whose gown was not penetrated, or from drapes or both in 12 of 51

instances. This distribution of organisms about the operative field occurred within the first five minutes after donning the gown. Organisms cultured from beneath the polyethylene patch could not have come from the operative field and this demonstrated actual penetration of the gown.

Of the three test gowns, spunbound olefin was an effective barrier in use, but had defects which reduced its barrier capabilities. (It is no longer commercially available as barrier material in the United States.) Spunlaced wood pulp-polyester fabric and treated 270 plus pima cotton were found to be effective barriers in both laboratories and in-use testing.

Dr. Laufman and colleagues<sup>9</sup> also evaluated non-woven fabrics in his study in 1979. Non-woven fabrics evaluated were spunlace, scrim reinforced tissue, spunbound olefin and fiber or scrim reinforced tissue. Single layered materials tended to be unevenly permeable to moist contamination. Those reinforced with a polyethylene film were totally impermeable.

He also discovered that seams, whether stitched or glued, were permeable to moist contamination regardless of the barrier effect on the material.

Based on this work, he recommended that non-woven disposable gowns may be considered suitable for lengthy wet operations provided their front and sleeves are reinforced with polyethylene film and provided the seams are properly placed. Dr. Laufman also cautioned readers of the wicking ability of the stockinette cuff which allows immediate wet bacterial penetration.

The data conclusively demonstrates the inability of 140 thread count muslin to perform as a barrier material.

There are materials, both non-woven and woven, available that serve as effective barriers.

Acceptable woven fabric is composed of a very tightly woven cloth, 270 thread per inch, that can be chemically treated to render it nonwicking. The best known of these materials is "quarapel," originally designed by the U.S.

Army Quartermaster Corps. This material will retain these qualities throughout an average of at least 75 hospital launderings. Laufman reported, "None of the tightly woven pima cloth gowns or drapes which were new or uncycled, or had gone through fewer than 75 washings—sterilizing cycles, were permeable to bacterial solution in 30 minutes provided they were Quarapel-treated." A new fabric sandwiches an air pervious water resistant film between textiles resulting in a synthetic reusable.

Recognizing the importance of barrier materials, the Association of Operating Room Nurses published the "Recommended Practices for Draping and Gowning Materials" in 1975. Shortly thereafter, the American College of Surgeon's Committee on the Operating Room Environment acknowledged AORN's statement and in doing so, charged the entire textile industry with responsibility for developing performance standards for gowning and draping materials. These were revised in 1983 and became a separate recommended practice: "Recommended Practices for Aseptic Barrier Materials for Surgical Drapes and Recommended Practices for Aseptic Barrier Materials for Surgical Gowns. Recommended Practices for Aseptic Barrier Materials for Surgical Drapes".<sup>10</sup>

- I. Surgical drapes should be made either of a single-use or reusable material that establishes an effective barrier minimizing the passage of microorganisms between non-sterile and sterile areas.
  1. Materials should be resistant to blood and liquid penetration.
  2. Reusable fabrics should withstand multiple laundering. A system should be provided to determine the number of times laundered.
  3. Reusable fabrics should withstand multiple sterilization.
  4. Unused single-use items should not be resterilized unless the manufacturer provides written instructions for processing.
  5. Materials should be resistant to tears and punctures.
  6. Materials should maintain integrity over the expected life of the drape as claimed by the manufacturer and/or recommended within the institution's policies.
  7. Documentation should be available to show that materials provide an effective barrier to microbes. This includes quantitative assessment of barrier fabrics.

The Recommended Practices for Aseptic Barrier Materials for Surgical Gowns are identical to the drape recommended practice up to this point. Then it goes on to state that the quantitative assessment should have special reference to cuffs and seams.

8. Gloves worn by the surgical team should cover the stockinette cuffs completely to prevent blood and fluid penetration.

Gowns and drapes are not the only uses for barrier materials in the operating room. Any surface that contains sterile instruments or supplies for use during a surgical procedure must be protected by barrier materials. The Mayo or overbed instrument tray that holds the instruments ready for immediate use by the surgeon must be protected. This area is not only subjected to wet objects constantly, but sustains consistent wear from heavy and sharp instruments being continuously placed upon it and removed. This tray must be protected by an impermeable barrier of sufficient strength to sustain such constant abuse.

All other tables used to hold instruments and supplies must be protected by barrier materials as they are subjected to moisture and are abused, but usually to a lesser degree than the work tray.

The patient population continues to become more susceptible to infection. The patients are older, prosthetic materials are implanted more frequently, and the incidence of immunosuppressed individuals requiring surgery is increasing. Post-operative wound infections are devastating for these patients.

It is essential that all health care professionals recognize the significance of the data that has been amassed and base their surgical practice on the utilization of all reasonable efforts to reduce the possibility of post-operative wound infections. Bacteria cause wound infections. Barrier materials can reduce the number or dosage of bacteria in the surgical field.

While our primary concern is the welfare of the surgical patient, it is important to consider the cost of nosocomial infections. It is estimated that in 1981, nosocomial infections in the United States added two billion dollars to the cost of health care.

A recent article published in the "Annals of Surgery" by Dr. Francis Moore of Harvard Medical School states that when the patient's course is marked by surgical complications, the economic impact is massive. An example of one such complication is advent of infection. Dr. Moore estimates that ordinary surgical costs are increased 8-20 times if a serious complication arises.

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# Particulates In Parenterals And On Devices

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I would like to thank the organizing committee of this conference, the China Ministry of Public Health, the China National Centre for Preventive Medicine, and Johnson & Johnson for giving me this opportunity to meet with you. Today I will be talking about the subject of particulate matter in parenterals and on devices.

Particulate matter consists of extraneous, mobile, undissolved, and generally undesirable substances that unintentionally cling to, fall off of, are found floating in, or otherwise contaminate a product or device or its use.

Particulate matter has been observed in or resulting from the use of many different medical products. Examples include:

Infusions

IV administration sets

Syringes

Catheters

Needles

Intraocular lenses

Blood oxygenators

Blood filters

Surgical drapes, gowns, and gloves

Hemoperfusion devices

Orthopedic joints

Typical types of particulate matter often found in the products just mentioned include metal, rubber, plastic, bone fragments, glass and carbon particles, lint fibers, starch and talc granules, fat emboli, bacteria, fungi and microbubbles of air.

Today I will not discuss fungi, bacteria or other microbiological contaminants, but will limit my comments to non-biological contamination and particulate matter that results from using medical products.

Of those medical products now known to generate particles, parenterals for infusion have yielded the largest amount of data and have received the most attention.

Therefore, in talking about particulate matter, I will first discuss particulates in

parenterals—their history, the standards that have evolved, and methods currently used to count particles—and then talk about particulates on devices.

From 1949 to 1963, many articles appeared describing the adverse effects of the vascular and neurological systems of animals and humans following the injection of fluids excessively contaminated with particulate matter.<sup>1-7</sup> The authors, many of whom were pathologists, reached conflicting conclusions, about the possible medical hazards associated with these fluids.

However, this problem did not gain worldwide attention until two Australians, Garvan and Gunner, a pathologist and anesthesiologist, quantified and classified the particles present in intravenous solutions then available in Australia in the early 1960's.

In their first paper<sup>8</sup> in 1963, Garvan and Gunner examined approximately 200 bottles of fluids from five Australian manufacturers. They found 500 ml glass bottles of saline and dextrose fluids to contain particles of rubber, cotton, bast-cellulose fibers and inorganic impurities in the size range of 1 to 100 microns. They theorized that most contamination originated during the manufacturing process and from the rubber closures. They also described a simple way to detect particles in the fluids, which they called the “dark-ground illumination method”. Here a beam of light was passed through the fluid and observed for particles in it. They also filtered the solutions and examined the deposits microscopically.

Their followup paper<sup>9</sup> in 1964, reported on their observations of the medical hazards of particles in intravenous fluids, based on animal experiments and the examination of the lungs of patients who had received large quantities of infusion solutions. They found granulomas containing bast fiber debris, a pathological finding for which they suggested the term “vascular bastosis”. They were the first to recommend that an official standard be set for particulate matter in solutions for injection and infusion. Until that time, health authorities had not considered such contamination, they only demanded that parenteral solutions be sterile, pyrogen-free and free from visible impurities.

Garvan and Gunner's work was so significant that in 1966, the top health authority in the United States, the Food and Drug Administration sponsored a national symposium on the safety of large volume parenterals.<sup>10</sup> However, it was not until seven years later in 1973, that the British Pharmacopeia (B.P.) became the first to include a limit test for particulates in such solution.<sup>11</sup>

In the same year, Turco and Davis<sup>12</sup> reported on the clinical significance of all types of particulate matter except wear debris. They summarized volumes of alarming statistics about damage observed in animals and humans exposed to or deliberately injected with particles and fibers. They pointed out the serious damage observed when particles greater than 12 microns progressed to the lungs or to organs with end-arteries, such as the brain, eyes or kidneys. They said, however, that the literature did not provide clinical proof that foreign bodies introduced intravenously actually caused serious damage or death. Although the biological effects of particulates injected into humans were still undefined, they concluded that the undesirability of particulates in parenterals was clear.

A year later, in 1974, Thomas and Lee in New Zealand published a review on particulate matter in intravenous systems.<sup>13</sup> They agreed with Turco and Davis' findings and concluded

that, while no proof existed that particle contamination had killed any patient, considerable evidence implicated injected particles in causing untoward pathological reactions.

In 1975, two years after the British established a limit for particulate matter, the United States Pharmacopeia (XIX) also set standards for large volume infusion solutions. In addition, in 1977 and 1979, the U.S. National Coordinating Committee on Large Volume Parenterals (NCCLVP) published recommendations for the standards of practice, policies and procedures for intravenous therapy.<sup>15,16</sup>

Standards for small volume infusion solutions (less than 99 ml), first proposed by the United States Pharmacopeia in 1983, were published in United States Pharmacopeia twenty-first edition in 1985.<sup>17</sup> They still are undergoing procedural changes, however, and thus will not become effective until January 1, 1986.

Presently, no standards for particulate matter exist in countries other than the United States and the United Kingdom, although the International Pharmacopeia is considering the subject for inclusion in their next edition.

With this background on the history of particulates in parenterals for infusion, I would like to now discuss the current official standards in more depth and comment on the instrumentation and methods used to detect and identify particulate matter.

The 1973 British Pharmacopeia limit test for particulate matter is still the official standard today in the United Kingdom. The test applies to certain large-volume parenteral injections such that each container does not exceed 1000 particles per ml greater than 2 microns and does not exceed 100 particles per ml greater than 5 microns.

The limits set were based on empirical rather than clinical data and represent the technically attainable degree of purity at that time in producing such solutions.

The 1975 United States Pharmacopeia (USP) limits for particulate matter in large volume parenterals which are still in effect today, were also developed empirically. A large volume parenteral passes the test if it does not exceed 50 particles per ml greater than 10 microns and does not exceed 5 particles per ml greater than 25 microns.

The limits for particulate matter in small-volume parenterals are unique in that they are 1. the first limits set for small volume parenterals, 2. "patient-oriented" standards based on "patient-particle loading", and 3. use an electronic counter.

The decision to establish standards by the USP for small volume parenterals was based on the decade of success with large volume parenterals standards. These standards clearly had improved the quality of LVPs: In ten years, there had been no adverse clinical reports concerning particulates in LVPs. Also, the pharmaceutical industry had accepted these standards and reported few problems in using them. Thus it now seemed time to set limits for particulate matter in SVP. Initially, the standards would apply to SVPs that are administered intravenously, intra-arterially or intrathecally which are given continuously or repeatedly over a course of treatment. Eliminated from the standards were intramuscular and subcutaneous injections since they would result when given in insignificant numbers of particles going into the bloodstream. Also, drugs intended for acute emergency use, for diagnostic procedures, for anti-cancer therapy, or for one-time use were excluded. With these exceptions, the standards apply only to approximately 100 out of 610 small volume parenterals listed in the current USP.

In setting limits for particulate matter in SVPs, the USP LVP limits could not apply because IV additives contribute far more particulates than the large volume IV solution itself. Therefore, it was arbitrarily decided that a patient who undergoes IV therapy for a long period of time should not receive more than twice the number of particulates irrespective of the number of drugs (or IV additives) added to the IV solution—in effect, the large particulate limit was doubled for SVPs.

Although it is impossible to determine the exact number of additives that go into each IV bottle, the Parenteral Subcommittee did have a good idea of the upper limits and proposed the “1/5th rule”. That is, the addition of up to five additives to a single IV for a specific patient should not exceed twice the number of particles allowed for one liter of large volume injection.

By using this concept, the USP limit for small volume injections would not be based on the number of milliliters in the container, or on the size of the plug of a lyophilized product, or on the grams of drug material in a container. The particle limit for any small volume injection would be the same whether the container contained 1 ml or 99 mls. (It is the total content of each SVP that is important.)

Thus, the small-volume injection meets the test requirements if it contains no more than 10,000 particles per container greater than 10 microns and 1,000 particles per container greater than 25 microns.

The three main methods of counting particulate contamination in solutions are:

1. Membrane—Microscopic method
2. Electrical resistance method
3. Light scattering and blockage method

As seen in Table I, all three methods are used in either the USP or BP for counting particles in large and small volume parenterals. Also seen in Table I is the different working principals, expressions of particle size, advantages and disadvantages for the three methods for counting particles.

1. *Membrane-Microscope Method*: This method involves filtering a portion of the fluid and counting the particles using a microscope. This is the method used in the USP for large volume parenterals and has advantages in that it is directed so that particles may be seen and identified. On the other hand, it is also time-consuming and expensive in operation time.

2. *Electrical Resistance Method*: As a particle passes between two electrodes, it distorts the electric field and is counted. The disadvantage is that it cannot be used with nonelectrolyte solutions. This method involves the use of the Coulter Counter in the B.P. for large volume parenterals.

3. *Light Scattering and the Blockage Method*: The working principle of both methods is similar but not identical. Light scattering method is based upon the generation of (Fraunhofer) diffraction patterns from particles illuminated in laser light. The technique assumes spherical particles and a particle density low enough to not cause multiple scatterings. Light blockage method—measures particle size and number by the reduction in light intensity rather than by examining the degree of light diffraction. Technique based on

light blockage in a window by particles flowing between a light source and a photo-detector. Particles in the sample fluid passing through a sensing zone block a portion of the light beam resulting in a momentary reduction in the photo tube output signal proportional to the particle size. This is the method used in the USP for small volume parenterals. Advantage of these instruments is they do not require electrolyte, can count the particles in a pre-set volume to insure sufficient counting accuracy, and are ideally suited for "on-line" usage. The major difficulty with both methods is the calibration of the machines.

**TABLE I. Methods Used to Count Particles**

	Membrane- Microscopic	Electrical Resistance	Light Scattering and Blockade
Instrument	Microscope	Coulter counter	HIAC or ROYCO
Pharmacopeia- Parenterals	USP—LVP	V.P.—LVP	USP—SVP
Working Principle	Particles filtered & counted on filter membrane	Particle passes between two electrodes & distorts elec- tric field	L.B.-by reduc- tion in light intensity  L.S.-By degree  of light dif- fraction
Expression of Particle Size	Linear dimen- sion	Dimension of a sphere of equivalent volume	Diameter of a sphere of equivalent area
Advantages Particles can	Electronic  be seen and identified; cheap instru- ment	Electronic  counter	counting pre- set volume can be used to in- sure accuracy; "on-line" usage
Disadvantages	Time consuming; operator time is costly	Calibration of instrument; electrolyte in solution	Calibration of instrument

It should be noted that the three methods size particles differently. The microscope method expresses size as longest linear dimension. The optical blockage method expresses size as the diameter of a circle of equivalent area and the Coulter Counter as the diameter of a sphere of equivalent volume. Some work has been done on the above three methods to establish their rough equivalence but it has not been confirmed or adopted.

## Particulate Matter on Devices

In addition to the problem of particulates in parenterals and infusions, many other medical devices have been implicated as a serious source of particulate matter. The devices previously mentioned fall into five major groups: 1. injection administration systems, including sets, syringes, catheters and needles. 2. cardiopulmonary bypass systems, 3. surgical gowns, drapes and gloves, 4. dialysis systems and 5. orthopedic devices. Although reports of particulate matter on devices have been published since the late 1940s, health officials have made no significant policy changes based on this information.

With reference to injection administration systems, in 1968 Ernerot and Sandell<sup>18</sup> studied particle shedding from injected syringes and reported that a large number of glass particles are shed continuously during the use of glass syringes and that plastic syringes shed particles at a much lower rate. In 1976, the Health Industry Manufacturers Association (HIMA) compiled a bibliography related to the quantity of particulate matter reported to be present in IV devices.<sup>19</sup> The studies showed that there was no valid evidence of particulate matter in or on the IV devices.

Referring to particle generation in cardiopulmonary bypass systems, studies have shown that devices such as blood filters, oxygenators and pump tubing continue to release particles from component surfaces long after startup.<sup>20</sup> Of specific concern in this group are gas emboli generated in bubble oxygenators and the great number and type of particles resulting from the use of these cardiotomy devices.<sup>21-25</sup> The FDA's Cardiovascular Devices Classification Panel<sup>26</sup> concluded in 1977 that insufficient information existed for both these problems and that they should undergo further research.

With reference to surgical gowns, drapes and gloves, studies of cases of intestinal blockage and peritonitis attributable to severe foreign body reactions following open abdominal surgery, date back to the late 1940s. The literature refers to iatrogenic patient care problems associated with airborne particles, the infiltration of lint or particles from gown and drapes into surgical wounds,<sup>27</sup> and particles such as starch from gloves, sponges and syringes. A review article by Williamson<sup>28</sup> in 1979 reported that starch particles behaved as foreign substances while being absorbed causing starch granulomas in the body.

The source of particulate matter in dialysis equipment closely parallels the sources in both IV sets and cardiopulmonary bypass systems. In 1980, Keshavia<sup>29</sup> reported on the shedding of and hazards resulting from particles from hemoperfusion devices, sediment filters, concentrated dialysate and from AV fistula needle sets during hemodialysis. Specifically mentioned was the problem of plastic shavings from reinserting the penetration

needle into the plastic catheter.

Since 1968, many articles have reported significant corrosion products and wear debris from orthopedic devices, seen at autopsy and upon corrective surgery. These particles have been observed in tissues immediately surrounding implants<sup>30,33</sup> and in more distant tissues such as regional lymph nodes<sup>34</sup> and alveolar walls in the lungs.<sup>35</sup> Some evidence also links the development and degree of corrosion to the formation of infection sites that necessitate removing the prosthesis.

The above citations are just a small sample of the articles published on this subject. Although they illustrate the hazards of particulate contamination of medical devices, to date no new standards have been proposed. This seems especially illogical for medical devices for intravenous use. It would seem that if we now have stringent limits on particulates for infusions, this level of quality should not be substantially lower when the infusion passes through an IV administration set.

The Health Protection Branch of the Canadian Government recently proposed that manufacturers and distributors of medical devices for intravenous use—including IV administration sets use a possible test method involving rinsing the sets with ultra-clean water and then apply the USP test limits for LVP.<sup>36</sup> Some responses to this proposal have been as follows, some say particle limits will result in substantially higher costs, others say adhering to government and well-established GMP and QC requirements, for visual inspection and acceptance of products, is sufficient, and still others recommend in-line filtration to reduce the patient particle burden.

A comment should be made here about in-line filtration or final filters. Until recently, no study had proved that final filters improve the medical condition of the patient. However, this year Falchuk<sup>37</sup> reported that using a final filter helped lower the phlebitis rate in patients in a double blind prospective study. However, it should be noted that final filters increase the cost of each administration, may bind certain drugs to the filter and may interfere with flow rates as the filter's surface becomes loaded.

In conclusion, particulate matter that contaminates parenteral solutions and many types of medical devices has been a concern of health regulatory agencies since 1963. A significant amount of animal and post-autopsy human evidence exists to show that particles larger than 12 microns introduced into the body have detrimental clinical effects. However, this has been a controversial issue for many years. There have been several proposals to study the medical hazards involved when such substances are introduced into humans, but none have been approved. Such studies are costly, and the results are difficult to quantify and identify. Although the biological effects of particulates introduced into humans remain undefined, the undesirability of particulates is generally agreed upon.

The USP Standards for particulate matter in small volume parenterals that will become effective in 1986 should further improve the problem of particles in parenterals. In addition, plans are underway by the USP's Parenteral Subcommittee to develop particle test methods and limits for IV administration sets, and to evaluate and recommend newer electronic counters including those that would allow for inspection of intact transparent containers.

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Until this occurs, particulate matter that originates in IV sets, blood oxygenators,

cardiopulmonary bypass systems and other direct cardiovascular devices may be easiest to control through good manufacturing practices and performance requirements on their maximum permissible levels.

Particle matter from items such as disposable drapes, gowns and gloves is much more difficult to control because how they are constructed is directly related to the source of their particulate problem. Wear debris from moveable joints presents a special problem, since the very material chosen to improve the frictional characteristics of the joint is the source of the wear products. These products are being studied by several device manufacturers.

In summary, although standards for particulate matter have been established for parenterals, none exist for medical devices. There is an urgent and critical need to set particulate matter standards for all medical devices that are introduced into humans, especially now with the increased use of implantable pumps and other devices for intravenous use.

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# Discussion

## Sessions III and IV

Invited comment by Virginia Ross

*USA Food and Drug Administration.*

I would like to share with you the proposed limits for ethylene oxide residue levels which were published by the FDA on June 23, 1978. This document is a proposed rule and the final levels are at present undergoing further review. However, these levels in the 1978 document are currently being used in the U.S. as a guideline for levels which should be achieved in manufacturing of medical devices and for drugs. I would like to read to you these limits now and I would be happy to send a copy of this to Madame Chen, or several copies, so it would be easier for you to have the information that way. I will just read the limits for ethylene oxide since there are so many figures here. After I do that, if you would like me to read the ones for ethylene chlorohydrin and ethylene glycol, I can do that also.

To start, actually it turns out I only have the limit for medical devices ones here, I will have to send you the ones for drugs. All of these figures are expressed in parts per million (ppm). For small implants of less than 10 grams, 250 parts per million; for (ppm) medium-sized implants, 10-100 grams, 100 ppm; for large implants, greater than 100 grams, 25 ppm; for intra-uterine devices, 5 ppm; for intra-ocular lenses, 25 ppm; for devices contacting mucosa, 250 ppm; devices contacting blood, ex-vivo, meaning dialyzers, 25 parts per million; devices contacting skin, 250 ppm. Surgical scrub sponges, 25 ppm.

Ethylene chlorohydrin for small implants 250 ppm, medium 100 ppm, large 25 ppm. Intra-uterine device, 10 ppm, intra-ocular lenses 25 ppm, devices contacting mucosa 250 ppm, devices contacting blood 25 ppm, devices contacting skin 250 ppm, surgical scrub sponges 250 ppm.

Now I will read the recommended ethylene glycol limits for implants. Implants small 5000 ppm, medium 2000 ppm, large 500 ppm. Intra-uterine device 10 ppm, intra-ocular lenses 500 ppm, devices contacting mucosa 5000 ppm, devices contacting blood 250 ppm, devices contacting skin 5000 ppm. Surgical scrub sponges, 500 ppm. I apologize for not being able to tell you the limits established in this document for drugs, but as you can see, there are a lot of numbers and I really can't remember them without having the document in front of me, but I will send a copy of the complete document including the devices and drugs proposed limits to Madame Chen for distribution of the information.

## **Question: by Dr. Gavin Hildick-Smith, Johnson & Johnson, USA**

I would like to ask Professor Kallings if he could give us some thoughts on approaches in the hospital in relation to the prevention of transmission of hepatitis and AIDS. What sort of precautions does he see, for instance, for hospital personnel taking care of such patients and other practices which he thinks may be indicated in light of the new knowledge of these two rather sinister diseases?

## **Answer: by Dr. Lars Kallings, National Bacteriological Laboratory, Stockholm, Sweden.**

You mean for the care of the personnel in the hospital? It is a long experience how to prevent spreading to the personnel concerning hepatitis B because it was very predominant during the 1960s when there were many cases in the startup of the dialysis units, blood banks in the chemical laboratories. That problem has almost been extinct. We have seen no cases anymore since we started very simple procedures. The most important thing is to avoid direct skin contact with blood so the staff nowadays are using gloves when they are in contact with blood from patients suspected to be carriers of the hepatitis B virus. Now I understand in a country like China with, I saw it in the morning paper here, 18 million cases of hepatitis B—almost every patient has to be considered to be a risk patient for the personnel. I do not know the practices here and the possibility to use gloves, but there are, of course, certain procedures which all aim at avoiding contact with blood and the virus titer as I mentioned is very high in the blood. As discussed with Dr. Favero yesterday, I think that the virus titer in the blood of a hepatitis B carrier is about in the order of  $10^8$ . So, actually a small amount of blood is needed to infect a person. In studies when hospital staff has received needlesticks, as much as 27% have been reported to be infected. There are other studies which are somewhat lower, but say around 20%—that is a very high infectivity. Concerning the HTLV III/LAV virus, AIDS virus, there is now a considerable amount of experience regarding hospital personnel at risk. There are only three to four cases known in the whole world of hospital staff that has been serum-positive after exposure. Actually, there was only one that could be proven to have serum—converted after a needlestick or accidental injection. The most obvious case is one case from England. Perhaps people here from the United Kingdom can explain that better, but according to what I have been told, it was a nurse who was carrying a syringe, with needle and blood from an AIDS patient, she stumbled and got a slight injection of blood. From the U.S., there are three cases reported, but there was no blood serum taken before the accident so it is very difficult to prove that these cases actually contracted the serum positivity through needlesticks. It is always very difficult to rule out that they simultaneously belong to a risk group. So for the AIDS virus, it does not seem to be any danger for hospital personnel. There is a study now of I think 500-600 accidents with needlesticks and I have just said there are only three or four cases known. There is a bigger CDC study that we could ask Dr. Martin Favero to relate. I could

also say that the practice in our country with AIDS patients is to avoid too many recommendations and thereby stick to the same precautions as for hepatitis B, although they are a little exaggerated. As the virus titer in the HLV III infected persons, as we discussed maybe  $10^2$ , it is a much lower titer than for hepatitis B and much larger volume of blood is needed to infect a person. Now, the reason why injection is thought that important in children and adults in Africa is that many receive injections without cleaning the syringes and the needles. There are figures for children that have received 50 injections, for instance, so if you think of the denominator, that could offer an explanation why frequent injections transmit HCLV III virus.

**Question: by Dr. Ge Yun-san, Deputy Chief Engineer, the Fourth Pharmaceutical Works, Shanghai.**

What is the current prospects of removing virus or even removing pyrogens by the filtration methods in the pharmaceutical industry?

**Answer: by Mr. Kuranosuke Ishii, Nihon Millipore, Ltd., Japan**

This is very good question but is one difficult to answer. As far as I know, virus is thought unable to filter by membrane filter. It is generally believed that the microfiltration using membrane filter is to remove the contaminants over the size of 0.05 micro meter and removing the substances under this size come into the category of ultrafiltration. By using ultrafiltration, virus is retained on the surface of the membrane. There are some cases in some Japanese pharmaceutical companies that the ultrafiltration is used for virus filtration but it is used not to filter out virus but to concentrate it and the batch is not so large. As to pyrogen, it is also thought to be very difficult to filter out by membrane filter. There seems to be some companies which are using zeta plus charged membrane to filter out low level of pyrogen of water. We have an abundant literature on both subjects I can send later.

**Question: by Dr. Gavin Hildick-Smith, Johnson & Johnson, USA.**

You must have reviewed the literature very carefully with regard to your materials and bacteria passing through them. I was wondering if anybody has done any research on the bacterial contamination of surgical instruments used during clean operations? Because if you think about it, they are contaminating the surfaces around and about a wound and that might be an end-marker or indicator of contaminants in a wound. Is there any data on surgical instruments during an operative procedure and/or in relation to various materials?

**Answer: Madame Koch answered by a negative gesture.**

**Comment by Dr. Gaughran, Johnson & Johnson, USA.**

There was one excellent piece of work done by a graduate student of Dr. Velvyl Greene at the University of Minnesota on the contamination of surgical instruments at the beginning of surgery and through the course of surgery. These were all, with few exceptions, cardiovascular operations in which they discovered that the surgical instruments immediately after opening the packs in the operating room were contaminated at the extent of 1.7%. This increased very rapidly in the first few hours of surgery. It was done by a microbiologist in the operating room. When the surgeon took an instrument, the microbiologist took a companion instrument at the same time and cultured it in the operating room. This you will find summarized in the Australian monograph that we published just recently. It is a very detailed study. It is the only one of which I know.

**Question: by Dr. Shen De-lin, Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing.**

1. The diseases caused by nosocomial infection vary from time to time. What is the predominant diseases acquired in hospitals recently in Europe and America?
2. As Prof. Kallings mentioned, ultraviolet radiation is not the most suitable measure used for air disinfection in the operating room, especially with the high intensity of radiation and for long duration. Then, what means would be more practical for air disinfection in the operating room without air conditioning with filtered air?
3. As we know, comprehensive measures are necessary for control of the nosocomial infection, I would like to know what are the key measures among them.

**Answer: by Dr. Lars Kallings, National Bacteriological Laboratory, Stockholm, Sweden**

Concerning the use of UV light. It was a very good question, because if you have no ventilation, then UV light may be of some use. But in many cases in western hospitals, they have a good ventilation in addition to UV light. What UV light does is just to add a little to the effect of the ventilation to get rid of the microorganisms. As you well know, the effect of UV light is very limited to one hundred centimeters or so from the UV lamps. So anyhow you have to circulate the air to get close to the UV lamp itself. Perhaps if you use it the whole night, then you could have some affect on the distance but only where the beams are directly hitting the microorganisms. So from many points of view, we think that it is a waste to use UV lights. If you do not have artificial ventilation, even ordinary ventilation through

windows would help a lot. But I could think of situations where you could use UV lights. But generally throughout the world, I think there is an overuse of UV lights.

Concerning the most important matter to counteract cross-infection. What is the single most important measure? I think that is handwashing. There are several studies proving that handwashing is important. One has to provide, of course, the possibility to wash the hands and not only for nurses but also for doctors. We know it is very difficult to get the doctors to wash their hands. It is particularly hard for the nurses to understand. The trainers have difficulties in explaining why the nurses have to wash their hands and not the doctors when going from one patient to another. So that is an educational problem.

There was a question about which infections that are most common. The urinary tract infections are the most common. The respiratory tract infections are very common too. I think that wound infection comes as number three. To give some figures, urinary tract infection used to be about 40% of all hospital-acquired infections. Now there are also geographical differences. In some countries, the diarrheal diseases may be the most common. Particularly in pediatric wards. We have some experience from Southeast Asia. There the diarrheal diseases, like shigellosis or rotaviruses or *Campylobacter*, is very common in the pediatric wards. I think one has to analyze which type of specialty, which branch of medicine, which country, which conditions.

## **Question: by Dr. Shen De-lin, Academy of Medical Sciences, Beijing.**

In Dr. Favero's report, a variety of disinfectants were considered, except one which is widely used in China. This one is acetyl hydroperoxide—peracetic acid. Would Dr. Favero make an evaluation if it?

## **Answer: by Dr. Martin Favero, Center for Disease Control, USA**

The question concerning peracetic acid is a very good one. I had a note on my paper to mention that. But I did not, so it is a very timely question. Peracetic acid is not used extensively in the United States. It is in other parts of the world. There is one commercially available product that is a mixture of peracetic acid and hydrogen peroxide that is used to sterilize the hemodialyzers when they are reused. From the data I have seen, it has excellent efficacy and the scientific literature contains a great number of publications on peracetic acid. So I think, without question, it is a good germicide. There are some problems with peracetic acid in terms of its stability and in terms of its compatibility with certain materials and exposure to humans. Now if those problems can be solved, and apparently they have been here, I think it is a good germicide. Some of the concentrations I have heard at this conference are in the range of what I would consider sanitization, which would be fine if that is the purpose of the procedure. I would caution, however, that if

peracetic acid solutions are going to be used for sterilization that the criterion of those concentrations and temperatures at use dilutions be sporicidal and that is, of course, very easy to determine. So in summary, I believe that peracetic acid is an excellent germicide if used properly.

## **Comment by Dr. Wang Shi-heng, Peking Union Medical College Hospital, Beijing**

I am a physician. I am very pleased to hear from the presentation of our distinguished experts during these two days that great emphasis has been laid on the safety of the pharmaceuticals and the medical devices for the patients. This is what we clinicians care about most.

Since time for discussion is short, I am not going to ask more questions, although I am also much concerned with the problem of the prevention of HBV and AIDS virus infections. Now I would like to make a suggestion. I think we clinical people should be considered as to be more qualified to assess the safety, the effectiveness, and the practicability of operability of the products. But in this conference, I notice we have very few representatives from the clinical side. So I suggest, if we are going to hold another similar conference, next time we should invite more representatives of clinical personnel to participate in order to advance mutual understanding between the manufacturers and the clinical medical professionals concerning the design, the quality and the performance of the products, just as Dr. Lars O. Kallings pertinently pointed out in his presentation this morning.

## **Response: by Mr. Paul Harbord, Johnson & Johnson, England**

Just a brief comment since the Chairman looked at me. I think we would all agree that it is vitally important that industry and the medical profession to whom our industry is directing their products should get together and I cannot believe that there is anyone in this conference who is presenting papers who would not believe that to be the case.

I think that we are trying to do here is to show the level of which industry is interested in producing products which are acceptable to the customer and we are doing this in the fullest possible way by validating processes because we believe that validation of the manufacturer of the device, or whatever product it is, the packaging of that device, or whatever product, is extremely important and is the way in which we can produce sterile product rather than trying to control the product after the event. In my small way just talking about the sterility side of the product, it is not only very difficult, I would suggest it is virtually impossible, to control sterility after the event of sterilization. One has to look at all the aspects of the manufacture of that sterile product or device. Again it is the environmental methods and we have heard about environment here in this conference, validation of the sterilization cycle and so on.

## **Question: by Dr. Dai Jing-ling, Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing**

The clothes made of closely woven fabrics have been used in the operation room and pharmaceutical plants in our country to prevent the dispersal of bacteria from human body surface for several years, but few surgeons and workers are willing to work in them, because they are very muggy. Some authors have suggested including a little ventilator and a filter in the garment for overcoming above drawbacks, but it is too complicated to be applicable to all situations. Are there any other new materials or measures, which cannot only prevent the dispersal of bacteria but also make the user comfortable with simple operation and low cost which have been developed or are being researched?

## **Answer: by Mrs. Fran Koch, Spohn Hospital, USA**

Well, let me say we have had the same problem in the United States—that of pleasing the surgeon and that was very difficult. Comfort is important. There is no doubt about that. However, there is a product that is a spun-laced woodpulp polyester fiber that is a very comfortable product. It has breathability and may be reinforced with the polyethelene film to maintain the barrier qualities that you desire when you use the product. So I think if you look at that it might meet both of your needs—comfort and barrier qualities at the same time.

## **Comment: by Dr. Kennard Morgenstern, Medical Sterilization, Inc., USA**

There is another new material out, I am not familiar with it, perhaps you are. It is a foamed porous teflon, I think it is made by the Gore Company, Gortex, which is supposed to have excellent barrier properties and is breathable.

## **Comment: by Mr. Mika Reinikainen, Pfizer Hospital Products, England**

I have a comment on what one of the participants said earlier about cooperation between doctors and industry.

My company makes sophisticated implantable products. What happens typically in this case, is that when products are designed, they are designed with the cooperation of a particular surgeon to the extent that, in fact, sometimes they acquire the name of that surgeon. This seems to be a very typical pattern of product design. For other products that are not so typical but which are more for mass general use, I think that you would find that a lot of product development as a consequence of complaints received from hospitals. So I

would certainly encourage you, if you use products imported from outside China, to write to the manufacturer if you have a problem with a particular product and I think you will find that most manufacturers will be very happy to reply to your questions about the possible deficiencies in the product. They will possibly even take steps to improve if they find that there is adequate reason to do it. After all, we do compete in a market and usually the one who has the best product will sell more, so we are very happy about these sort of comments.

## **Comment: by Dr. Lars Kallings, National Bacteriological Laboratory, Stockholm, Sweden**

I have a comment to Dr. Harbord's very excellent paper on sterility testing. I think it is pertinent now to comment on the widening of the concept of sterility testing that we have to do due to the very rapid development in biotechnology and the new knowledge about retroviruses and oncogens, for instance. I think that we must include also in the testing of certain products freedom of viruses and even oncogens.

There is great awareness now of the necessity to have regulations for freedom of, for instance, retroviruses causing tumors, central nervous system diseases or AIDS and other agents causing Kuru, Creutzfeldt-Jakob Syndrome and others. And these agents may be transmitted from pharmaceuticals for injection originating from human origin. Like growth hormone from human pituitary glands, these products have been withdrawn from the market in certain countries. They will be produced by the recombinant DNA technique. In China, if you have growth hormone that has been extracted from pituitary glands of humans, then you may have such a problem.

Another problem is that we are using more and more continuous cultures for producing vaccines. For instance, polio vaccine continuous cultures are derived either from monkeys or from human origin. Therefore, there is now discussion in the World Health Organization of requirements for freedom of these retroviruses and also a very low degree of presence of cellular DNA. For instance, in polio vaccine it should be less than ten picogram of cellular DNA. Now when we have this experience with AIDS and similar diseases, it should be disastrous really if we inject our children with vaccines or other products that contain such agents. Going back to the recombinant DNA technique, when products intended for humans are produced on bacteria or yeast, you do not have these problems. But if you use mammalian cells for the recombinant technique, then this will again be a problem. Another modern problem is the use of monoclonals for human beings

## **Answer: by Mr. Paul Harbord, Johnson & Johnson, England**

Well, I think that was very much more a comment than a question but to the extent that I can answer the latter part of it which Dr. Kallings mentioned, I do not have direct knowledge of the pharmacopeial discussions at this time. Perhaps the United States pharmacopeia representative, if he is in the audience, could answer that question. But as to the other

aspects, certainly the necessity to carry out a sterility test is very much related to the value of the test. And I was hoping to suggest that the clinician or the authority would look very carefully at the value and ascertain that there is a need to carry out a sterility test, as in the case of a fluid, parenteral or ophthalmic solution. Anywhere where there is likely to be a reasonable degree of contamination then it obviously has a value and has a place. The suggestion that I made was that where you are looking at sterile product and your contamination rate is less than say 0.1%, then I would suggest the test is not very good or not very useful. But if you have a potential of contamination that is greater than that or if you have a potential where any contamination is life-threatening or a situation that Dr. Kallings was commenting upon, then I think it is very much up to the clinician or the authority to be advised by the medical profession as to what is or is not the best thing to do. I think one goes back to this idea that you should really look at the sterility test very critically before you do it, than try to look at the sterility test results critically after you have done it.

## **Comment: by Dr. Gavin Hildick-Smith, Johnson & Johnson, USA**

I would like to make a comment on Dr. Kallings issue that he brought up about technical products. We recently marketed in the States a monoclonal product made from a mouse and as far as I know, government regulations required that the company do tests for viruses and look for DNA and confirm sterility. The point, of course, that this happens to be a mouse monoclonal which in itself has problems relating to allergic response to it if used repeatedly in therapy in man.

## **Question: by Dr. Zhu Jin-gui, Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, Beijing**

Now the viral hepatitis B is a very important infectious disease all over the world. Substantial evidence showed that viral hepatitis B is also an occupational hazard to medical personnel. Many authors have done a lot of research on it, but there are still some problems unsolved. For example, tissue culture of HBV, susceptible animals other than chimpanzees. At present, in the experiments on HBV, which of the tests for evaluation of inactivating effect of the virus are practical and reliable? In the near future, what research works about the problem are planned to be conducted by the specialists in the United States?

## **Answer: by Dr. Martin Favero, Center for Disease Control, USA**

Two questions. The first one dealt with what is perceived as the best chemical germicides for the hepatitis B virus in the United States. The answer to that is the chemical germicides that are labeled and approved by the Environmental Protection Agency as sterilants and as chemical germicides for hospital use that also have a tuberculocidal claim. What that really means is that the unusual high level germicides that are used in hospitals are considered effective against the hepatitis B virus. This would range from glutaraldehyde, formaldehyde, hypochlorite solutions of at least 500 parts per million, iodophors, disinfectants, alcohol – 70% ethyl alcohol, 70% isopropyl alcohol. Hydrogen peroxide and peracetic acid and phenolic disinfectants have not been tested. However, I think that it would be the consensus among the public health officials who make those recommendations that these also would be effective. In my personal opinion, I would consider them effective. I would like to add that any sterilization procedure, be it a steam autoclave or ethylene oxide sterilization or heat pasteurization, does not have to be modified or lengthened of a real or potential suspicion of hepatitis B virus contamination. All that I have just said I would say as well as for the AIDS virus.

The second question dealt with a more suitable method to test for the safety of the hepatitis B vaccine that now uses a chimpanzee model. To my knowledge, humans, the chimpanzee, and in some cases, some other primates, are the only ones that are susceptible to hepatitis B viral infection. However, I think that the second and third generation hepatitis B vaccines, for example, the vaccine that will be marketed very soon, that is genetically engineered in *Saccharomyces cerevisiae*. I do not believe that the requirement for that vaccine will be the same as for the original vaccine with respect to chimpanzee testing. I would make the same prediction for the subunit vaccines that are several years off.

Madame Chairman, I was told that I misunderstood the second part of the question. The second question dealt with testing germicides against the hepatitis B virus. The hepatitis B virus is a virus that cannot be cultured in tissue culture. One of the ways is to test for infectivity in a chimpanzee. The question was if we do not have chimpanzees, then what about all the other ways? As you saw from a previous paper, there are a number of approaches. One can see hepatitis B surface antigen and determine if the chemical agent or the physical agent destroys the immunologic detection of the antigen. This is a very severe test in my opinion. Certainly if a chemical or physical agent inactivated hepatitis B antigen, it certainly would destroy infectivity of the virus. But that is a severe test. Also there are some agents, such as formaldehyde which tend to fix HB<sub>s</sub>Ag at concentrations that are sporicidal. We assumed that the virus would be inactivated. So it is not a perfect system and it is a severe test yielding overkills. The DNA polymerase system, I think, is overly sensitive. The problem is there are not sufficient data to compare DNA polymerase with viral inactivation as measured by infectivity. There is the system of using HB<sub>s</sub>Ag physical alternation as observed by electron microscopy as a criterion of inaction. I think this test too is a very severe test resulting in overkills. And then there is the infectivity test using chimpanzees where the problem is cost in the United States. A chimpanzee is a \$70,000 test tube; so it is not practical.

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Finally it is very useful to obtain the kind of information that we heard this afternoon on

the four systems that I talked about. From a practical situation, however, I would suggest that the criteria to use in determining whether a germicide is effective against the hepatitis B virus is: if the germicide is sporicidal, it will also be effective against the hepatitis B virus; if the germicide is tuberculocidal, it is effective against the hepatitis B virus.

## **Question: by Dr. Xue Guang-bo, Department of Epidemiology, Second Military Medical College, Shanghai**

Just now, Dr. Martin Favero has introduced us to the chemical sterilants used in hospitals now and we all think highly of his report. Now, I would like to ask him two questions and also give my views on the problems.

First, beta-propiolactone is a highly efficient disinfectant and much more effective against microorganisms than ethylene oxide, an extensively used sterilant today. But it was found in early studies to have induced cancer in experimental animals, so that its use in disinfection was limited. However, as yet we have not seen epidemiological evidence that this compound causes cancer in man. The way of metabolism for a chemical compound in man differs from that in animal, and the compounds causing cancer in animals do not necessarily cause cancer in the human body. Therefore, I think that we can cautiously apply it in disinfection and sterilization before its cancerogenic effects in man are established and make an epidemiological evaluation of the problem during the course. Will Dr. Martin Favero kindly give his comments on the soundness of the idea?

Second, in 1949, C. R. Phillips, etc. found that ethylene imine possesses most killing effects among ethylene oxide compounds series, but we have not found reports of its use as a disinfectant or sterilant. Will Dr. Martin Favero be so kind as to explain it to us?

## **Answer: by Dr. Martin Favero, Center for Disease Control, USA**

The question deals with beta-propiolactone as used a sterilizing gas and presumably as a procedure disinfectant. Perhaps some of the other panelists may want to comment. In my opinion, there is a long history and good data on the effectiveness of beta-propiolactone. It is an excellent sterilizing gas but there are problems in handling the gas. But to the question is it effective as a sterilant, the answer is "yes." It would be effective against some of the agents that we talked about here such as the hepatitis B virus. It would not be practical to use as a disinfectant.

# Session V

**Chairman: Robert F. Morrissey, Ph.D.**

*Director, Johnson & Johnson  
Sterilization Sciences Group  
Somerville, U.S.A.*

# Introduction by Session Chairman

Robert F. Morrissey, Ph.D.

*Johnson & Johnson  
Sterilization Sciences Group  
U.S.A.*

On behalf of the Conference Organizing Committee, welcome to our third and final day dedicated to the exchange of information on the control and inactivation of microorganisms.

This morning's session will address clean room design, the use of ultraviolet radiation for disinfection, rapid methods and automation in the microbiology laboratory, preservative systems, and pharmaceutical water systems. Although somewhat diversified, these subjects fall under the general category of environmental microbiology. Certainly, the application of ultra-violet radiation, clean rooms, and preservative systems are designed to reduce or remove microorganisms from a specified environment. The efficacy of all these techniques is based on qualitative and quantitative procedures designed to characterize the microbial population. The ability to characterize microorganisms in pure culture would be impossible without the development and application of sterilization methodologies.

In fact, the single most significant event in the field of microbiology and ultimately in the control of human disease was the evolution of the concept of sterility. It all started with the controversy over spontaneous generation of life.

## Another View of Sterilization

To most of us, the concept of sterility centers around a procedure—a procedure for the destruction or elimination of microorganisms on medical items to prevent infection. Certainly an attainable notion! But there was a time when the development of microbiology as a science, and the quest for the elimination of contagious disease was based on an intellectual and highly theoretical pursuit of “sterility”.

The development of microbiology is frequently linked with the evolution of the microscope. However, it was the application of sterilization methods that caused microbiology to take a quantum leap forward—away from the medieval doctrine of spontaneous generation and into the study of pure bacterial cultures, so necessary for the elucidation of human infectious disease.

The relationship between the theories of spontaneous generation, putrefication,

fermentation, and contagious disease was a complex one.

Proponents of spontaneous generation believed that animal or vegetable matter contained a "vital or vegetative force" capable of converting matter into new and different forms of life. Opponents to spontaneous generation had an extremely difficult task. They were trying to prove a negative: that spontaneous generation does not occur! Because of this, a single positive culture, easily attributed to poor technique, was all that was necessary to damage their case.

On a macroscopic scale, spontaneous generation was given a fatal blow about 300 years ago when Redi (1665) demonstrated that maggots did not arise from meat when a barrier in the form of a piece of gauze prevented flies from depositing their eggs. Things were quite different on the unseen microscopic scale.

Putrefaction and fermentation were difficult subjects to study. Putrefaction, the decay of meat or vegetable matter, followed a process of decomposition, odor production, and finally the appearance of life forms (protozoa). Fruit juices containing sugars fermented, releasing gas accompanied by alcohol; yeasts were then observed. But one thing was never clear: which came first, the putrefaction/fermentation or the life forms?

Spallanzani, in 1799, using a differential heating procedure was able to classify organisms according to their heat resistance. He went on to conduct experiments, the results of which strongly indicated that the organisms that grew in his flasks entered via the air. His opponents claimed that air only helped the organisms grow if they were already present.

Thirty-eight years later (1837) Theodore Schwann confirmed Spallanzani's work while conducting studies on fermentation. He went on to demonstrate the appearance of alcohol and CO<sub>2</sub> during fermentation, and that yeast cells were always associated with these events. His conclusion was simple: yeasts are living organisms capable of excreting alcohol via fermentation.

Unfortunately, there were powerful proponents of spontaneous generation, and Schwann's work was discarded for several decades due to the influence of the German chemist Liebig. This was a major setback for the early development of microbiology.

In 1857 Pasteur entered into a battle with Professor Liebig by showing that lactic acid fermentation was caused by a living organism. He used microscopic observations to follow the growth of yeasts.

Three years later, Pasteur published a paper which clarified once and for all speculation about the nature of alcoholic fermentation. He introduced quantitative methods along with a chemically defined medium consisting only of trace elements, ammonium salts, and sugar.

Fermentation was always accompanied by the development of yeast. As yeast protein increased, nitrogen in the medium decreased. Pasteur concluded that there was a correlation between growth and chemical changes, and that these changes were mediated by microorganisms.

In 1861 the supporters of spontaneous generation received a crushing defeat when Pasteur, in a series of simple yet monumental experiments, showed that organisms arising from previously heated culture media came from atmospheric air. Using his famous "swan neck" flask, he demonstrated how to sterilize a liquid and maintain sterility indefinitely. He

had perfected a series of procedures to achieve sterility—but he had accomplished more.

Aside from clarifying the theory of spontaneous generation, Pasteur's sterilization techniques paved the way for the culturing of one organism in the complete absence of other organisms. Pure cultures would be used later by Koch to characterize the conditions for bacterial growth and reproduction.

Further advances in sterilization science were made by Ferdinand Cohn when he studied the resistance of bacteria to boiling using hay infusion enrichment culture techniques. He discovered the heat resistance properties of bacterial spores, and developed various combinations of temperature and time treatments to achieve sterility. His discovery of heat-resistant spores provided the answer as to why variable results were often obtained by workers who attempted to sterilize materials using boiling water.

It was Lister, however, 11 years after his work on antiseptic surgery, who perfected the first method for the isolation of a pure culture (by what we know of today as the most probable number technique). He showed that a pure culture caused a specific change in a liquid. Lister reasoned that a specific organism might cause a specific change in a human being which might lead to disease and death.

By 1877 Koch had provided the first proof that a specific microorganism could cause a characteristic disease in an animal. The disease was anthrax. He characterized the development of spores from vegetable cells, confirmed Cohn's work, and demonstrated the medical importance of spore-forming organisms. He even considered the idea of speciation; i.e., separate species based on morphological characteristics.

The application of pure culture studies as a direct result of reliable sterilization methods led to the isolation and characterization of most major bacterial diseases in only a 20-year time period (1881 to 1901).

Lister put it all into perspective when he said, "A few years ago it would have seemed very improbable that the souring of milk should have any bearing upon human disease; but all will now be ready to admit that the study of fermentative changes deservedly occupies a prominent place in the minds of pathologists."

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# Clean Room Design and Operation

Guy Van Gestel

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First of all, I would like to thank the organizing committee for the opportunity of coming to this conference in Beijing. I will present to you what we consider now as the most important factors that contribute to the production of high-quality injectables.

Nowadays, the design of parenterals plant is, in more than one way, very much different from other pharmaceutical production facilities. Current Good Manufacturing Practice guidelines for non-sterile products call already for adherence to high hygienic standards, the use of detailed preparation instructions and manufacturing areas which must be easy to clean, be in good repair and kept orderly.

Nevertheless, the GMP-guidelines for the preparation of injectables are in this regard many times more stringent. Extreme care has to be taken to prevent the contamination of injectables by microorganisms and particles. The battle against these intruders must start as early as possible and, therefore, the design of a sterile plant is studied and planned very carefully. Our goal is to create an environment that guarantees us the biggest chance of producing germ-free, as well as particle-free, injectables.

As you can see on the following slide, modern pharmaceutical technology tries to achieve this goal by:

- (A) Working out a proper basic design
- (B) Using the proper building materials
- (C) Placing the technical areas in completely separated locations
- (D) Creating the possibility of a proper product flow
- (E) Establishing a progression in cleanliness
- (F) Installing the necessary protection against insects and rodents

Before discussing these six items in more detail, I want you to realize that all these requirements result in very high construction costs and, later on, in very high maintenance expenses. Nevertheless, there is no doubt that it is necessary to make this kind of expenses to create the utmost certainty for the patient that he runs no risk at all when treated with these parenterals.

# A. Basic Design

Three considerations are crucial when selecting the layout of a new, state-of-the-art sterile manufacturing building.

(a) easy cleaning, disinfection and maintenance, (b) the efficiency of the component, personnel and product flow, (c) operational flexibility.

Easy cleaning and disinfection implies that walls, ceilings and floors must have smooth surfaces, that all corners must be covered to prevent dust accumulation and that the filling and preparation areas are equipped with lighting, windows and HEPA-filters which are flush-mounted and completely sealed. Furthermore, recessed zones, columns and all places where dust can deposit must not be present in the sterile areas. Finally, inside the rooms the piping is kept to a minimum. The piping is as much as possible built into the walls or is running in separated, technical areas.

The second point, the efficiency of the component, personnel and product flow will be handled later on in my presentation.

The third consideration when choosing the layout of a sterile manufacturing layout is the operational flexibility. To prevent cross-contamination and to create the possibility of preparing more than one formulation at the same time, it is advisable to install a modular system in the building. By using this modular system, the different preparation and filling areas can be completely separated from each other, thus allowing the simultaneous production of several parenterals and excluding all risks of cross-contamination.

Here you see such a module as it is installed in Janssen, our factory in Belgium.

Here you have the complete layout of the factory with, as you can see, four different modules.

A second important factor to consider in the design-phase is the selection of the:

# B. Proper Building Materials

As you all know, the combination of humidity, nutrients and heat creates the ideal environment for bacterial growth. So to avoid the occurrence of this combination as much as possible, modern pharmaceutical technology will use non-classical building materials.

The presence of humidity can be minimized by eliminating all porous materials for the finishing of the rooms, like cement, wood, cardboard or joints between tiles. Instead of these materials we nowadays use stainless steel, glass or polymeric substances. When cement cannot be avoided, it must be covered with an epoxy lining. Cracks and crevices which can also withhold moisture are precluded by using elastic paints on the walls and ceiling and by using monolithic floors.

Also, all materials which can act as a nutrient, in fact all sources of carbon, are banned from the sterile production and filling areas. So no wooden, cardboard, plywood or other packaging materials that may shed particles, can be used in modern sterile facilities. Here you can see a few examples of objects which are made of unusual materials:

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— aluminum pallet instead of wooden pallet

- plastic boxes instead of cardboard boxes
- plastic containers for raw materials instead of paper bags or wooden containers

The third growth factor, heat, cannot be eliminated through the choice of proper building materials for obvious reasons. Nevertheless, the air-conditioning system must be able to prevent temperatures raising above 22°C. The next factor in design is the location of the technical areas.

## C. Technical Areas Location

The technical areas are now completely separated from the production areas. They are located and designed so that they facilitate maintenance and that we have great flexibility by allowing easy modification of the utilities distribution and/or connection. Another advantage of this way of building is that the main heat load is located outside the production areas so that the cooling of the air-conditioning system needs not be heavily loaded.

The technical areas must also be designed in such a way that they can contain all technical equipment and piping which are not absolutely necessary in the production areas, e.g., compressors, filters, air-filters, pumps, heat exchangers, etc.

The next point is the creation of the possibility of a proper product flow within the sterile building.

## D. Creating a Proper Product Flow

The circuits of materials and the circulation of persons must be studied thoroughly because both are important to maintain the cleanliness at the required level and to prevent mix-up. We can distinguish three main types of circulation:

### (1) Persons

- Production personnel
- Maintenance personnel Visitors

### (2) Clean Items

- Raw materials
- Packaging materials
- Clean equipment
- Clean tools and garments
- Finished products

### (3) Contaminated Items

- Contaminated but reused
  - + Contaminated equipment
  - + Contaminated tools, garments, autoclave trays

- Contaminated and rejected
  - + Disposable material
  - + Waste
  - + Rejected finished goods

For each of these types of circulation, we need to design a flow through the production areas so that they are:

- As simple as possible
- Preferably one way
- That no crossing between clean and contaminated items can occur

Now we come to item 5, which is the factor that makes a sterile facility so very special among other production departments:

## **E. Establishing a Progression in Cleanliness**

A modern facility must be designed so that within the building itself, there are dedicated areas with different cleanliness classes. It must be possible to go gradually from the most contaminated zone, let us say the areas with street contamination levels, towards the sterile core where virtually all particles and bacteria must be absent.

To protect the sterile core of a sterile manufacturing unit, it is necessary to create zones with different cleanliness classes.

- (A) What we call “black area”, with the street contamination level. This is the area where no precautions against microbiological and particulate matter contamination are taken.
- (B) A “dark-grey” area which guarantees a cleanliness class of 100,000 particles per cubic foot.
- (C) A “light-grey” area with maximum 10,000 particles per cubic foot.
- (D) A “white” sterile zone where only 100 particles per cubic foot are tolerated.

Each zone is pressurized at a different level. The sterile zone has the highest overpressure, going down by steps of 15 Pas to the black area where there is no overpressure at all. By doing this, the area with the higher cleanliness is protected from being contaminated by particles coming from areas with a lower cleanliness.

To go from one zone to the other, all personnel has to pass through a locker or a changing room. Each time when passing through these changing rooms or lockers, specific gowning and disinfection procedures have to be followed to reduce the number of particles and microorganisms as much as possible.

Last but not least, we need to install the necessary protections against insects and rodents.

## **F. Installing the Necessary Protection Against Insects and Rodents**

To protect the sterile production area against insects, we must build in the following features:

- (a) Tightness of ancillary buildings, such as warehouses, mechanical shop, technical areas
- (b) All air inlets and outlets must have appropriate screens
- (c) Insect killers need to be placed near all entrances

The means we have against rodents are the following:

- (a) First of all, pay special attention to the tightness of the building, e.g., doors, roof openings, sewer and stairs
- (b) High-frequency devices
- (c) Grills within sewers

It is, of course, clear that the design of the building alone cannot lead to the production of high-quality injectables. Only the use in this building of proper production equipment, utilities together with a thorough training of all clean-room personnel and a well-organized quality assurance system will finally guarantee the proper quality of injectables.

## **A. Production Equipment**

Production equipment must be designed in a way that dust deposition cannot occur and that disinfection and cleaning can be done easily. It is advisable to use cleaning and sterilizing in place systems because they give an excellent protection against contamination. To create a proper product flow, we have to install as much as possible equipment that allows a uni-directional product flow, e.g., two-door autoclaves, sterilization tunnels, etc.

## **B. Utilities**

The utilities nowadays used in a sterile manufacturing facility must comply with very high quality standards. As you can see on this slide, we have to provide the facility with quite a lot of different gases or fluids:

- warm pyrogen-free water
- cold pyrogen-free water
- compressed air
- air
- propane, butane, ...
- nitrogen
- oxygen
- steam

To give you an idea of the requirements for these utilities, I will go quickly through the specifications we use in the Janssen Pharmaceutica sterile operation.

- (1) Pyrogen-free water
  - pyrogen-free
  - sterile
  - conductivity: 1 S/cm
  - temperature: warm pyrogen-free water: 85°C cold pyrogen-free water: 15-20°C
  - purity: complies with USP/EP requirements
    - number of particles: maximum 50 particles 5 m/ml, no particles 50 m/ml
- (2) Compressed air
  - oil-free
  - particle-free
  - moisture-free
- (3) Air
  - HEPA-filtered
  - overpressure: 15 Pas
  - number of air changes: 20 times/h
  - decontamination time: 20 minutes
  - microbiological purity: class 100,000 500 CFU/m<sup>3</sup>
    - class 10,000 10 CFU/m<sup>3</sup>
    - class 100 absent
  - particle counts: comply with the specifications of the different air cleanliness classes
  - air-stream pattern: continuous decontamination with flux of clean air
- (4) Propane, Butane
  - particle-free
- (5) Nitrogen
  - Complies with USP requirements
  - oil-free
  - carbohydrates-free
  - moisture-free
  - particle-free
  - sterile
- (6) Oxygen
  - particle-free
- (7) Steam
  - condensate: complies with requirements for pyrogen-free water of USP/EP
  - particles: maximum 50 particles 5 m/ml no particles 50 m/ml
  - pyrogen-free

When we ask for utilities with this kind of specifications, there is, of course, a need for controlling these specifications on a routine basis. That is the reason why we implemented a thorough system of microbiological, physical and chemical controls.

- |  |  |  |
|--|--|--|
| - Pyrogen test of raw materials  | - Sterility test on water for injection                    | - Integrity testing of all sterile air filters |
| - Microbial count of water for injection                                 | - Microbiological monitoring of all surfaces and equipment | - Medical control of all personnel             |
| - Bioburden of the product before billing                                |  | - Complete analysis of clean steam             |
| - Testing of product filter before & after sterile filtration of product |  | - Complete analysis of nitrogen                |
|  |  | - Particle analysis of oxygen and propane      |
| - Microbiological air monitoring   |  |  |
| - Pyrogen testing on all injectables                                     |  |  |
| - Sterility testing on all injectables                                   |  |  |
| - Microbial count of all raw materials                                   |  |  |
| - Microbial count of all packaging materials                             |  |  |
- 

From the different chemical, physical and microbiological control activities performed by QA, I will show to you, just as an example, what kind of environmental controls we have in our factory.

## **C. Quality Assurance—Environmental Controls**

### *Controls of the Air*

Every day the microbiological bioburden of the air is measured at various control points in the sterile areas and particle counts are performed on a monthly basis. Interesting to know is that we concluded, from our experience, that if you have the correct design and all your personnel is following the GMP's, the quality of the air normally presents no problem and can comply easily with the preset specifications of the different air cleanliness classes.

### *Hygiene of Personnel*

For the medical control of our clean-room personnel, we implemented the following program:

— Contact cultures of the hands and clothes are made every fortnight, together with a

throat smear test.

- Twice a year we have a general health examination.
- All personnel has to report all wounds or infections immediately and if a person demonstrates signs of an illness or open wound, he will not be allowed into the sterile manufacturing areas.

## *Control of Surfaces*

The cleanliness of walls, ceilings and floors is microbiologically monitored with contact plates and the cleanliness of the equipment is controlled with swabs.

## *Media Fill*

Ultimately, every month we run a one-hour media fill test on every filling machine for injectables. After incubation of the filled ampoules, we check whether the ampoules show microbial growth or not. Even when we detect only one positive ampoule, we will start an investigation to see if it is possible to improve the filling and/or cleaning conditions.

Finally I want to spend some time on the training of clean-room personnel because I think that above all, this personnel holds the key position in producing high quality injectables.

## **D. Training of Clean-Room Personnel**

Most of the employees working in a sterile facility do not have the proper school education to correctly understand what is so special about making parenterals. Therefore, great importance should be attached to careful selection of the employees and to the in-house training of these people. I shall now describe how we handle this training at Janssen Pharmaceutica.

### *(1) Basic Training*

All new personnel, not only operational but also cleaning and maintenance staff, receive a training session which includes the basic principles of hygiene. This basic training consists of lectures, films and also microbiological demonstrations.

### *(2) Training Specific to the Workplace*

Each new employee is trained by an experienced operator for one month. In this period, he is closely followed and corrected if necessary. The methods of production are discussed in detail, together with the necessary hygiene guidelines.

### *(3) In-depth Training*

In-depth training is given periodically to all operators, technicians and IPC-controllers. Each lesson lasts about four hours and in total the training sessions consist of eight lessons.

— Items that are treated are:

— Basic microbiology

- Presence and significance of bacteria
- Detection methods
- Personal hygiene
- Factory hygiene
- Cleaning and disinfection
- Aseptic work methods
- Sterilization and depyrogenization
- etc.

The participants of these courses must actively participate through discussions and microbiological experiments, e.g.,

- Detection of microbes through cultures, e.g., fingerprints on Petri dishes before and after work or disinfection
- Human hair on an agar plate
- Microscopic demonstration of bacteria

At the end of each session, a small test is built in to see whether the employees understand the content of the training or not.

#### (4) *Discipline*

Regardless of how good training courses are, it is still necessary to motivate all employees constantly and to keep discipline at a high level. This is realized at Janssen by a quality expert for the parenterals plant. This highly qualified person organizes all training sessions and controls the proper application of hygiene rules, GMP regulations and manufacturing directions. He has made a very good working relationship with the head of the parenterals department as well as with all other employees. He is not a kind of policeman who punishes people when he sees GMP violations, but he tries to explain to the people through discussions why it was wrong what they did and how they should proceed in the future.

I hope that I was able to give you an impression of the efforts that the modern pharmaceutical industry is making to assure the medical profession and the patients that they can fully trust the quality of the parenterals produced.

I thank you very much for your attention.

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# The Application and Monitoring of Disinfection Ultraviolet

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Since the discovery of germicidal action of sunlight by Down and Blunt (1877) in England, investigators began to research the methods of disinfection with ultraviolet (UV) irradiation. The successful development of low pressure mercury vapor lamp provided a favorable condition for the extensive application of UV irradiation in disinfection. Research on disinfection with UV irradiation have been carried out in many countries and brought about steady progress. In China, research on UV disinfection and its application were reinforced after liberation in 1949. Now various types of germicidal lamps can be manufactured and are used extensively in disinfection of air, water and other surface in medical units and industries. In addition, for monitoring the process of UV disinfection, the radiometer and chemical indicator were developed recently.

## Application of UV Irradiation in Disinfection

### Disinfection of Air

Although there are different opinions upon disinfection of air by UV light in practice, it is still used popularly in Chinese hospitals. In hospitals, there are three methods to perform UV disinfection of air:

(1) Irradiation with fixed UV lamp. For non-occupied room, the lamp with or without the aluminum downward reflector is usually hung on the ceiling 2.5 m above the floor. For occupied room, to irradiate its upper part, the reflector is turned upward by which the

reduction in bacterial count of the air of the whole room can be achieved through air convection.

- (2) Irradiation with mobile UV lamp. It is usually used for local air disinfection on wards. The stand fitted with 3-4 UV lamps without reflector is umbrella-like.
- (3) Irradiation in ventilation tunnel. The air is forced to flow through a ventilation tunnel equipped with UV lamps and thus disinfected.

In experiment, the bacterial aerosol was produced by XPQ-84 atomizer in an aerosol chamber of 20 m<sup>3</sup> capacity (85% of the particles had a diameter less than 5 µm). After one hour, when the number of bacteria suspended in air became to be stable, then the air was irradiated by a UV lamp without reflector in the center of the chamber 2.5 m above the floor. The samples of air were collected with a TWL rotating impinger at a place 1.2 m under the lamp. The results showed that after 15 min irradiation, the killing rate of *Staphylococcus albus* in air was 99.9% and that of spores of *Bacillus subtilis var. niger* was 99.0% (Table I).

**Table I. The Germicidal Effect of UV Irradiation Against the Bacteria in Room Air**

Irradiation Time (min)	Average Killing Rate (%)	
	<i>S. Albus</i>	Spore of <i>B. subtilis var. niger</i>
15	99.89	99.05
30	100.00	99.80
45	100.00	99.96
60	—	100.0

**Notes:**

- (1) Intensity of UV light of the lamps was 120 W/cm<sup>2</sup>.
- (2) Bacterial count in air before treatment was 31-77/L.

Another experiment was reported by Institute of Epidemiology and Microbiology of the Chinese Academy of Medical Sciences (1973). Forty UV lamps (30 W) were fitted in a ventilation tunnel (21 m in length and 36 cm in diameter).

**Table II. The Germicidal Effect of UV Irradiation Against the Bacteria in Air Passing Through Ventilation Tunnel**

Bacteria	Killing Rate (%) by Different Number of UV Lamps		
	8	24	32
<i>M. albus</i>	71.56	99.69	100.00
<i>S. aureus</i>	92.00	100.00	100.00
<i>E. coli</i>	100.00	100.00	100.00

## Notes:

- (1) Air temperature 30°C, RH 40-50%.
- (2) Bacterial count in air before treatment was 143-539/L.

The lamps were divided into five groups, eight lamps each were arranged evenly against the inner wall of the tunnel. The bacteria was atomized into the chamber (90% of the particles had a diameter less than 10 m) and the contaminated air was then forced through the tunnel. When the velocity of the air flow was 200 m/min, the number of the lamps needed to kill 100% of *Micrococcus albus*, *S. aureus* and *Escherichia coli* were 32, 24 and 8 respectively (Table II).

## Disinfection of Water

In recent years, there were some reports on UV disinfection of plasma, wine, fruit juice and even sewage. But owing to the inhibition of penetration of UV ray by suspended organic matter and dissolved salts in water, the devices used for disinfection of such fluids were quite complicated and expensive and its germicidal efficiency was unstable. Therefore, they were not commonly used in China.

In the experiment of water disinfection, a 30 W UV lamp (130 W/cm<sup>2</sup>) put at 3 cm above water surface was used to irradiate the tap water (contaminated artificially with *E. coli*) 2 cm deep. *E. coli* with a concentration of about 1700/L of water could be suppressed to less than 3/L by UV irradiation for more than 60 sec (Table III).

**Table III. The Germicidal Effect of UV Irradiation Against *E. Coli* in Water**

Irradiation Time (sec)	Bacteria Count per Liter	
	Before Treatment	After Treatment
30	1720	4
60	1680	1
180	1710	0

Now there are various types of UV water disinfection equipment available in China. The rates of treatment range from 600 L/hr to 60,000 L/hr. If the turbidity index of water is less than 5 and color index is less than 15, the total bacterial count of water can be reduced from 20,000/ml to less than 100/ml and the *E. coli* count from 800/L to 0/L after treatment in the above equipment.

## Disinfection of Solid Surfaces

For disinfection of solid surface, because of the lack of germicidal effect on the shaded

area, UV irradiation is usually used to disinfect the surface of laboratory bench. In some hospitals, UV irradiation is used for disinfection of medical documents on the infectious disease wards, sometimes also for disinfection of the surface of small medical instruments in a specially designed disinfection chamber. The UV lamps are installed on the top and the bottom of the chamber and the articles to be disinfected are usually put on wire meshes. Since the parts in contact with the wire cannot be reached by UV ray, the position of the articles must be changed from time to time to ensure thorough irradiation.

For disinfection of solid surfaces, irradiation for 30-60 min is needed. In an experiment when the glass slide was used as bacteria carrier and irradiated at 1 m under UV lamp for 30-60 min over 99.9% of the bacteria on its surfaces were killed. The spore of *B. subtilis* var. *niger* is the most resistant to UV irradiation. *Pseudomonas aeruginosa* the intermediate, while *S. albus* and *E. coli* the least resistant (Table IV).

**Table IV. The Germicidal Effect of UV Irradiation Against the Bacteria on Glass**

Bacteria	Dose for 99.9% Killing Rate (W. sec/cm <sup>2</sup> )
<i>S. albus</i>	4,200
<i>E. coli</i>	3,900
<i>P. aeruginosa</i>	139,200
<i>B. subtilis</i> var. <i>niger</i> (spore)	208,800

The germicidal effect of UV irradiation on the bacteria on aluminum surface is better than on glass (Table V).

**Table V. The Germicidal Effect of UV Irradiation Against the Spore of *B. subtilis* var. *niger* on Different Surfaces**

Bacteria Carrier	Average Bacterial Count		
	Control Group	Test Group	Killing Rate (%)
Glass slide	160,150	2087	98.69
Aluminum plate	153,000	241	99.84

*Note:*

(1) Uv irradiation dose is 50,400 W.sec/cm<sup>2</sup>.

## Monitoring of UV Disinfection

In China, hospital staffs often raised some questions about UV disinfection, e.g., are the lamps used qualified? When should the UV tube be replaced? Is the lamp installed correctly?, etc. Such questions can only be answered by routine monitoring process.

Microbiological examination is the best monitoring method, but the test is too complicated and can be conducted only by specially trained personnel. So it is very difficult to popularize the examination.

In order to simplify the monitoring method, a radiometer and a chemical indicator were developed in our laboratory.

## **Radiometer of UV Irradiation**

The radiometer developed is small in size ( $10 \times 8 \times 5.5$  cm) and light in weight (0.5 kg). It can be used to measure the intensity of the UV ray with wave length ranging from 250 nm to 350 nm. The instrument consists of three parts: light receptor, circuit for transmission and amplification of electric signal and an amperemeter used as indicator.

The light receptor is the most important part and consists of: (1) a light filter no. 1, (2) frequency transformation membrane, (3) a light filter no. 2, and (4) photosensitive resistance. It works as follows:

- (1) When the light passes through the filter no. 1, only the germicidal UV ray can penetrate it, while the visible light is absorbed by the filter (Figure 1).
- (2) The UV ray which passed the filter irradiates the frequency transformation membrane and a visible light of 520-530 nm is produced.
- (3) The visible light produced by the membrane passes through the filter no. 2 which absorbs the light other than the one of 520-530 nm and the light is thus purified.

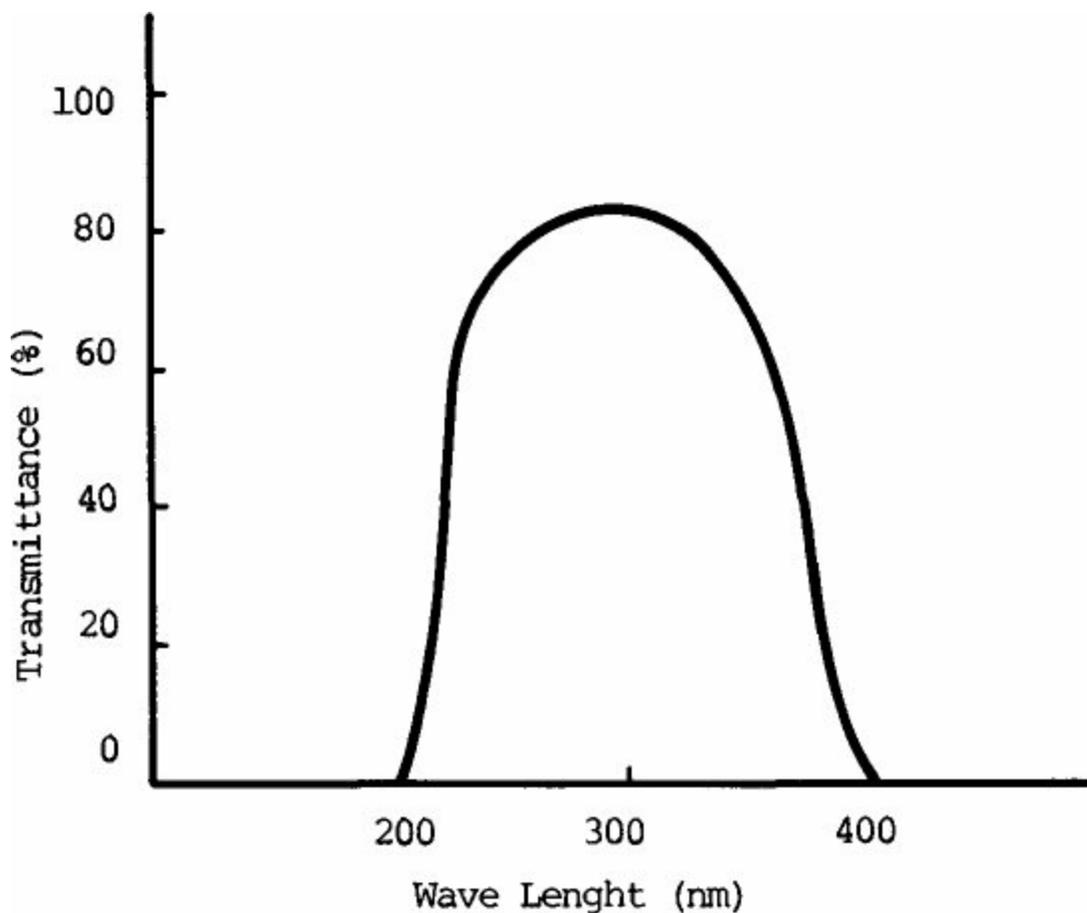


Figure 1. Transmittance Curve of UV Ray Through Filter NO.1.

(4) The light purified by filter no. 2 acts on photosensitive resistance made of semiconductor of cadmium sulfide (CdS) which is only sensitive to the light with the wavelength ranged from 520-530 nm. The light changes the amperage of the current produced by dry cell and produces corresponding current changes which can be shown by amperemeter. The intensity of the current flowing.

The accuracy of the radiometer was checked by Chinese Academy of Meterology. It is compared in parallel tests with UV radiometer type UVR-254 (Topcon, Japan). In that type of radiometer, the electric signal is produced by a photosensitive tube. The differences of readings between these two radiometers ( $y - x$ ) show a normal distribution (Figure 2). The average difference is  $1.07 \text{ W/cm}^2$ , the rate of equality is 58.82%. The regression equation is:  $y = 1.021 x - 1.258$ . The correlation coefficient (4) = 0.996,  $p < 0.01$ . They are closely correlated.

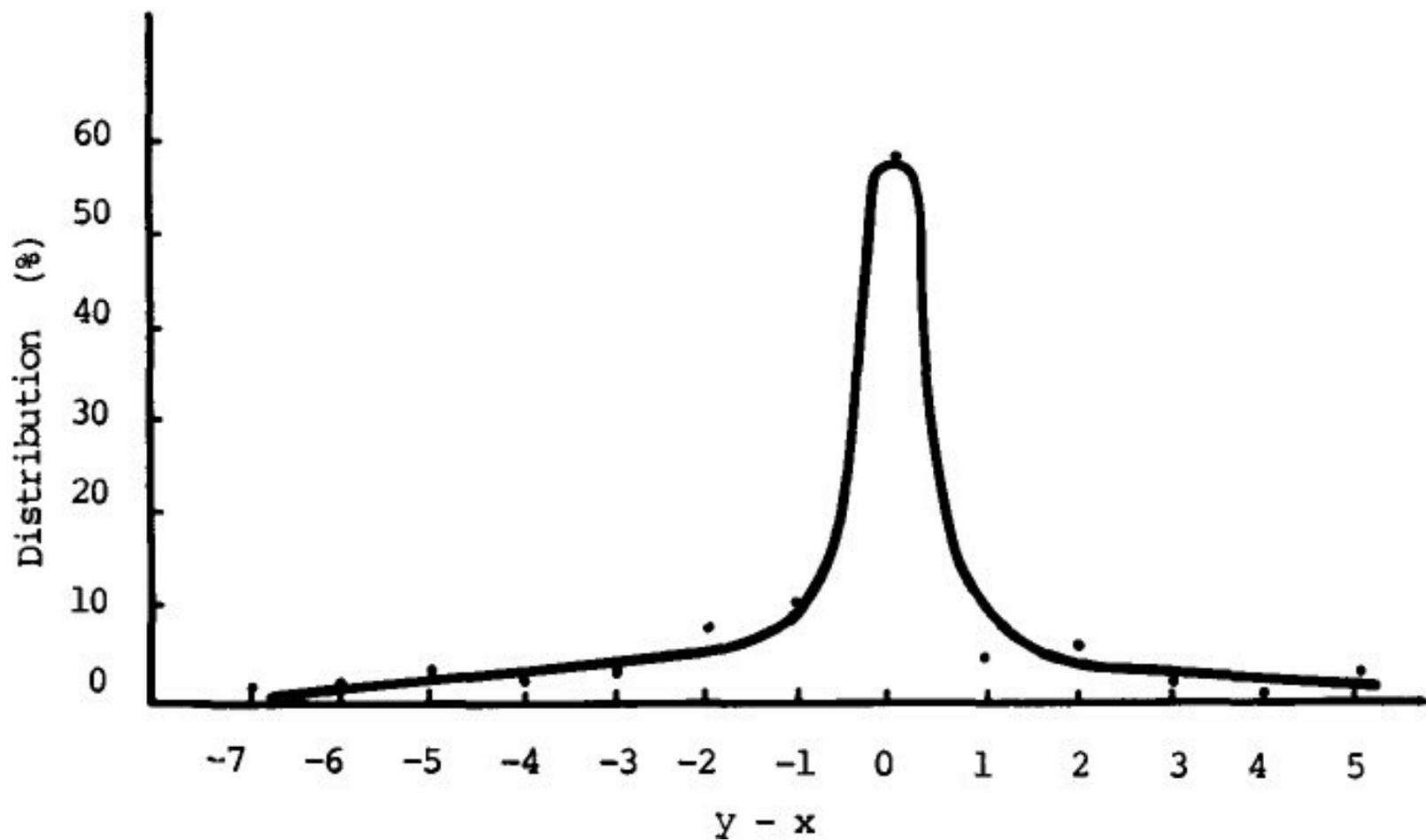


Figure 2. Distribution Curve  $y-x$

## Chemical Indicator

Some of the photosensitive dyes irradiated by UV ray show color changes of various intensity which is directly proportional to the dose of irradiation. According to this principle, a chemical indicator was developed in our laboratory. The indicator can be used not only to determine whether the radiation intensity of the UV tube is up to the standard, but also to assess if the radiation dose meets the demand of disinfection. Figure 3 shows the arrangement of standard color columns printed on the indicator cards.

The key point for development of the indicator is to select an ideal photosensitive point which can indicate the dose of UV irradiation precisely. In the experiments, a photo-densitometer (type PDA-65) was used to estimate the photo-densitometric value (PV) of the color (instrumental error is 0.02). The colors of various chemicals irradiated under different conditions such as light sources, distances between the light source and the card, temperature and relative humidity were compared. An optimal paint combination was selected by screening more than a hundred combinations. The paint combination selected is white in color originally and changes to violet after UV irradiation. The paint was smeared on a card coated with polyvinyl and tested under various conditions.

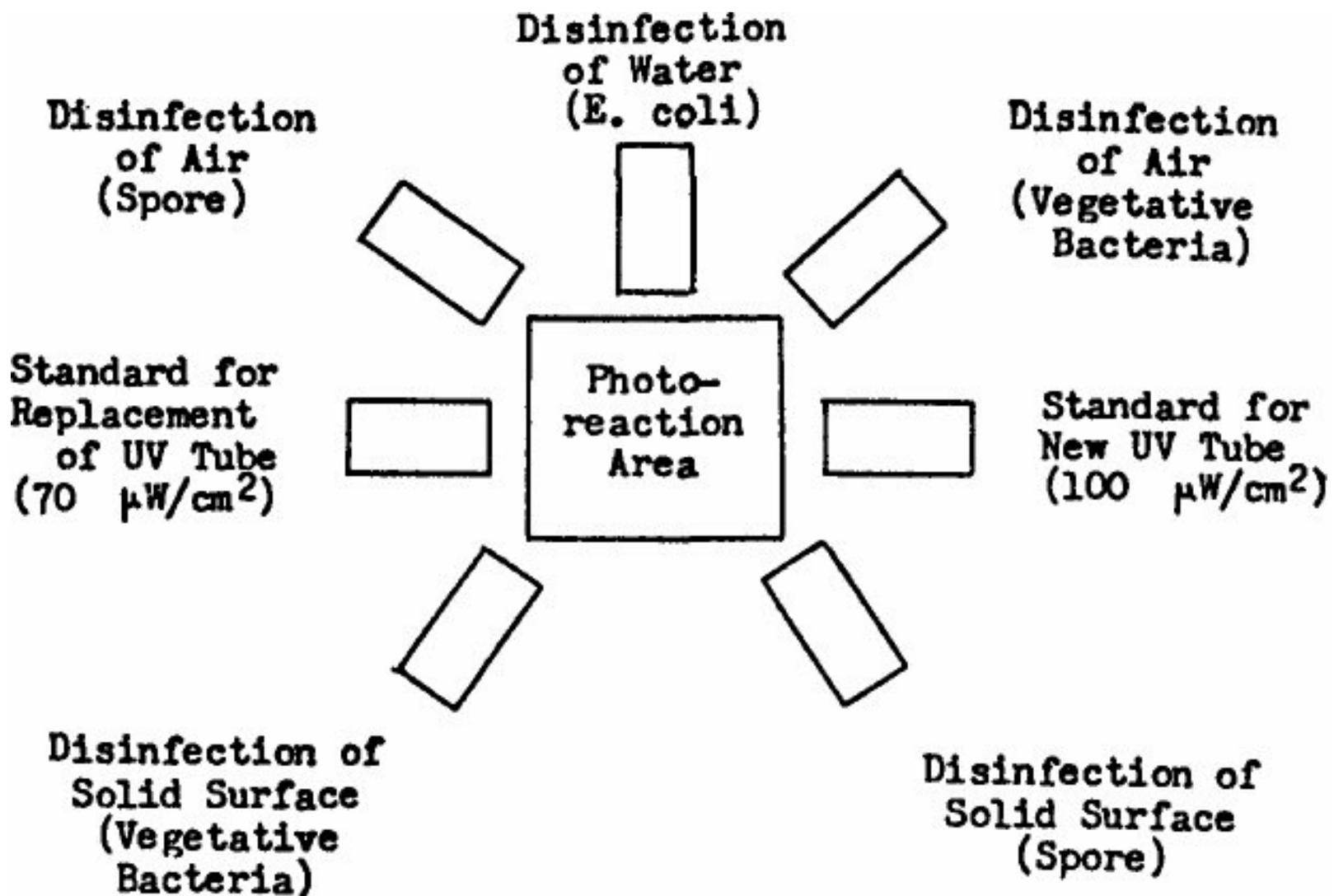


Figure 3. Distribution of Standard Color Columns on Chemical Indicator Card.

All these results of the experiments were the average value of ten repetitions:

(1) Color change under various light sources.

When the painted card was put under artificial lights or sunlight (9:00 a.m. on sunny day, outdoors), the color change only occurred obviously under UV irradiation (Table VI).

(2) Effect of temperature and humidity.

The painted card was tested under various temperatures and relative humidity. There was no significant difference between the average PV under different temperature and relatively humidity (Table VII, Table VIII).

**Table VI. Photosensitivity of Testing Card to Various Sources**

Source of Light	Average Intensity of UV irradiation (W/cm <sup>2</sup> )	Average PV of Testing Card After Treatment
Incandescent lamp	0	0.00
Fluorescent	0	0.00

lamp		
Sunshine	16	0.03
UV lamp	60	0.84

**Notes:**

- (1) The card was put under artificial lights 1 m apart.
- (2) The exposure time was 30 min.

**Table VII. The Effect of Temperature on Color Change of the Testing Cards**

Temperature (°C)	Average PV of Testing Card After Treatment
10	0.10
20	0.09
25	0.08
30	0.08

*Note:* The intensity of UV irradiation from the tube was 130 W/cm<sup>2</sup>. The exposure time was 1 min.

**Table VIII. The Effect of Relative Humidity on Color Change of the Testing Cards**

Relative Humidity (%)	Average PV of Testing Card after Treatment
20	0.10
40	0.10
60	0.11
80	0.10

*Note:* intensity of UV irradiation from the tube was 130 W/cm<sup>2</sup>. The exposure time was 1 min.

**(3) Effect of dose of UV irradiation.**

The color change of painted card was assessed with various tubes, under different exposure times and at different distances. The results showed that the color change expressed by PV is closely correlated to the dose of UV irradiation. The correlation coefficient (r) = 0.99, p 0.01. The regression equation (Figure 4) is:  $y = 0.001 - 0.00002x$ .

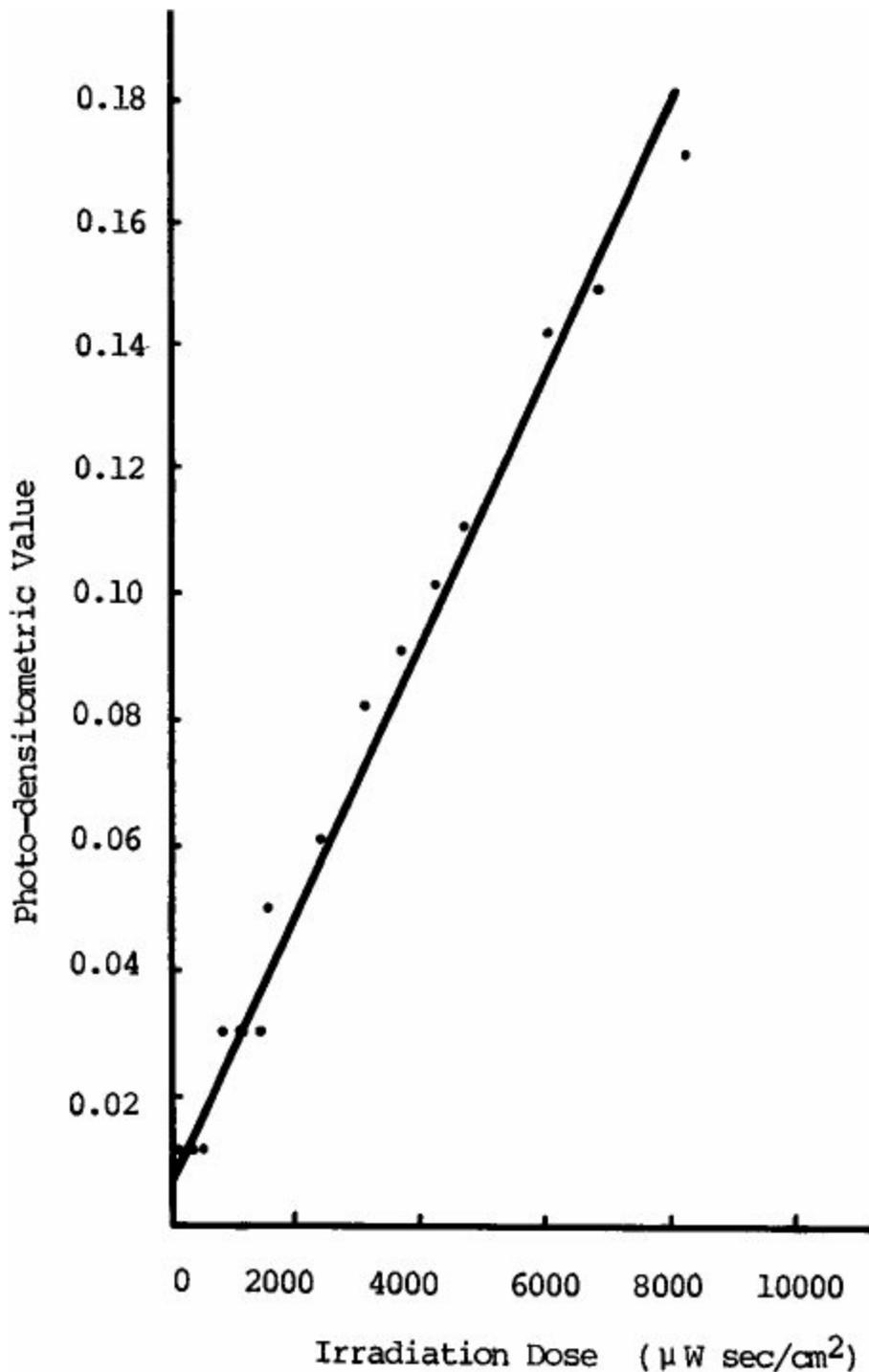


Figure 4. The Relationship Between the Color Change of Testing Card and the Dose of Irradiation.

The experiment also showed that similar PV could be obtained so long as the doses of UV irradiation were the same, no matter what UV tube was used (Figure V).

## Comment

Disinfection with UV irradiation is convenient in practice. It would play a more important role in the prevention of nosocomial infection with the progress of the research work. In hospitals, however, UV lamps are usually used by members lacking knowledge about the UV irradiation. So it is important to simplify and popularize the monitoring method as well as

to improve the methods and equipment of UV disinfection. The radiometer and chemical indicator presented above have been used in several hospitals and some serious problems of UV disinfection were thus found. Some UV tubes bought from market were not up to the standard and the UV intensity produced by some of them was even lower than  $30\text{W}/\text{cm}^2$ . Seventy percent (245/350) of the UV tubes in use showed a UV intensity lower than  $70\text{W}/\text{cm}^2$  which could not meet the lowest requirement for disinfection. Some tubes were fitted incorrectly and the object to be disinfected could not receive sufficient dose of irradiation etc. Although some of the preliminary methods to monitor the process of UV disinfection are available at present, how to estimate the intensity of germicidal UV irradiation more accurately and conveniently needs further investigation.

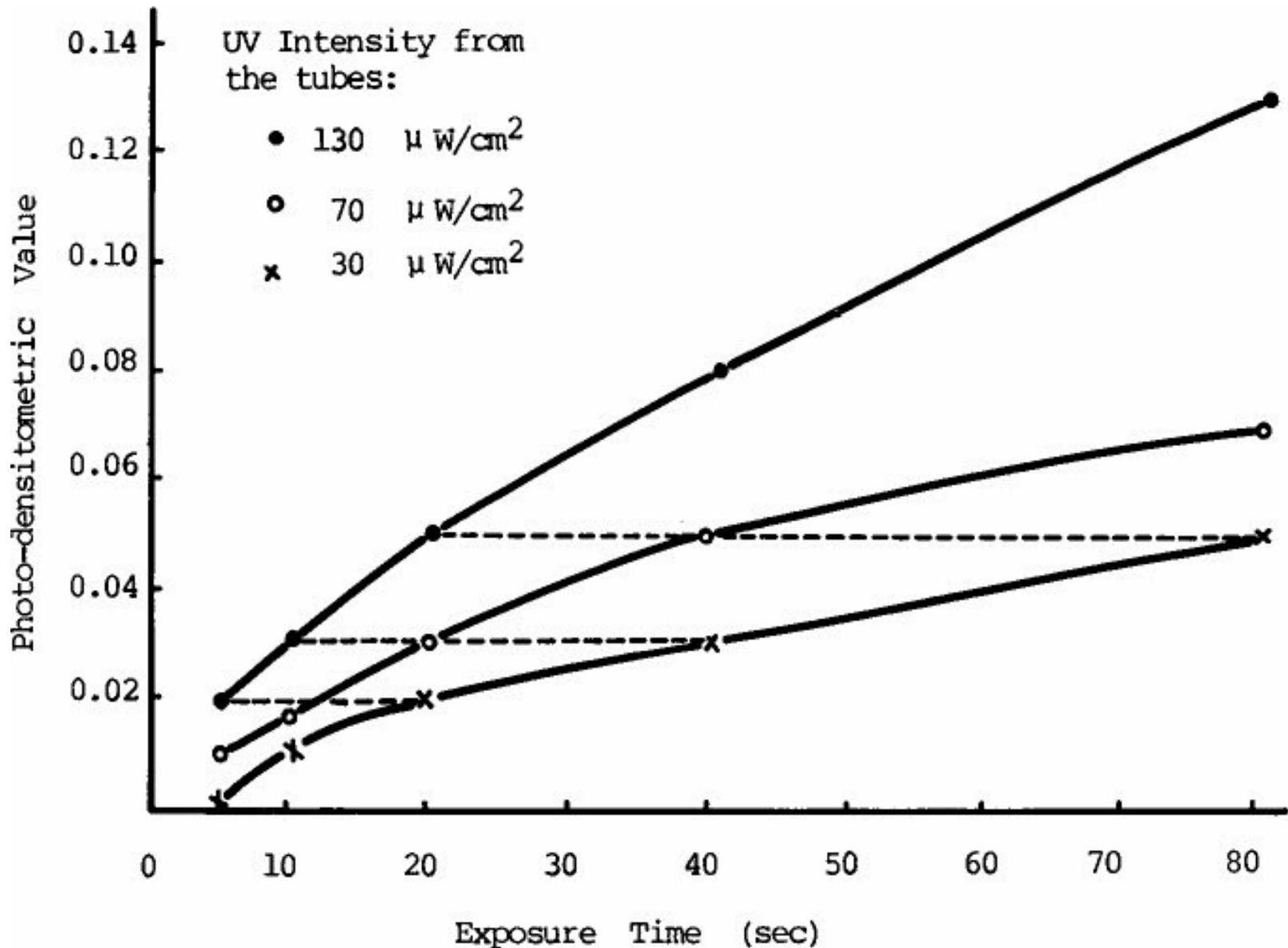


Figure 5. The Color Change of Testing Card (expressed in PV) Irradiated for Various Times by Different Tubes.

# Experimental Observation on the Sterilizing Effect of Ozone on *Pseudomonas aeruginosa* in the Air.\*

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The problem of burn infection has not been solved until now. We have observed that a low concentration of ozone is able to sterilize *Pseudomonas aeruginosa* after screening some chemicals and Chinese herbs.

Ozone is an unstable gas with a peculiar "fresh" smell. It is able to decompose to oxygen at normal temperatures. It is a disinfectant with the advantage of broad-spectrum high, quick-potency, and without any smell and drug residue. Ozone is easy to prepare for it only needs air and electricity. There are some advances in preparing an ozone generator and measuring ozone concentration in recent years, so ozone is likely to be used extensively.

In this experiment we studied several concentrations of ozone's sterilizing effect.

## Material and Method

### Strain:

The strain of *Pseudomonas aeruginosa* (0025) is kept dry and frozen in our Institute Laboratory. Taken out of the container bottle and inoculated into ordinary culture medium of meat broth at 37C, cultured for 18 to 24 hours, and after a continuous growth for two generations and subsequent identification, it was kept in the refrigerator for further use.

### Sampling:

A Porton model sampler was adopted for sampling. The sampling solution was 10 ml of physiological saline.

### Ozone Generator:

The ozone generator (model CYF-1) was purchased from the Institute No. 718 with an

ozone generation of 700 to 1000 mg/hour.

## *Sprayer:*

A JM-2 aerosol model sprayer was employed.

## *Aerosol Chamber:*

The aerosol chamber was homemade with 8mm thick organic glass, and a volume of one and one half (1.5)M<sup>3</sup>. The upper part was arched while the lower part was rectangular in shape. The chamber consisted of a sampling tube connection, window for material introduction, an opening for spraying, filter tip for air suction as well as four hand windows. The hand windows were taped with rubber gloves. The chamber also contained the air compressor, the air suction device and an operation table.

## *Method of Determining the Concentration of Ozone:*

The concentration of ozone was determined colorimetrically with potassium iodide-phosphoric acid buffer solution.

## *Procedure of Operation:*

The aerosol chamber was filled with ozone until the required concentration was obtained after measurement, followed by spraying 5 ml of culture solution containing *Pseudomonas aeruginosa* with the sprayer under a pressure of 3 kg/cm<sup>2</sup>. The strain was sampled at once. This was followed by collecting the samples every one and one half minutes. The blow rate was maintained at 5 litres per minute. The collected samples were diluted 10 times and subsequently cultured as usual. Counting the living bacteria and calculating the germicidal ratio was accomplished by comparison of the samples with the same procedure as used in the samples except without any ozone supply.

The rate of bacterial natural mortality and sterilizing was computerd by the formulas below:

Baterial natural mortality rate

$$N_t = \frac{V_0 - V_1}{V_0} \times 100\%$$

N<sub>t</sub> = natural mortality rate

V<sub>0</sub> = Control: the quantity of bacteria in the air before treatment.

V<sub>1</sub> = Control: the quantity of bacteria in the air after treatment.

Bacterial sterilizing rate

$$K_t = \frac{V_0' (1 - N_t) - V_t'}{V_0' (1 - N_t)} \times 100\%$$

$V_0'$  = ozone: the quantity of bacteria in the air before treatment

$V_t'$  = ozone: the quantity of bacteria in the air after treatment

## Electron-Microscopy:

The 1.5 m<sup>3</sup> aerosol chamber was filled with ozone until the required concentration was reached after measurement, the ozone filling was stopped and then followed by spraying the fluid containing the strains. The samples were collected after a specified period of time, followed by cooling and centrifuging it at 12000 rpm for 30 minutes. After adding 2 drops of normal rabbit serum, an extra thin film was prepared and examined under the electron-microscope.

## Results

1. The results of sterilizing *Pseudomonas aeruginosa* by a low concentration of ozone in the air:

Experiments were done as described above. We have experimented 13 times, (See results in tables)

2. The natural mortality of *Pseudomonas aeruginosa* in the air:

In the same process described above, we have observed the natural mortality of *Pseudomonas aeruginosa* in the air without ozone. We have observed 5 times. (results in Table II). The natural mortality rate of samples at 15 min. is 26.77%.

**Table I. The results of sterilizing *Pseudomas aeruginosa* by low concentration of ozone in air.**

Concentration of ozone (ppm)	Temperature (C)	Humidity	Killing rate (%) of times (minute)			
			15	30	45	60
0.15	21	84	98.41	100	100	100
0.15	21	84	95.00	99.52	100	100
0.15	19	84	96.23	99.57	99.86	100
0.15	19	94	97.17	99.36	100	100
0.15	19	94	98.94	99.59	100	100
0.15	19	94	98.35	98.94	100	100
0.14	19	94	97.17	98.21	100	100
0.13	19	94	96.70	98.55	99.20	100
0.10	20	89	96.48	97.14	100	100
0.10	19	89	92.94	97.63	99.62	100
0.085	20	94	96.47	98.23	99.56	100
0.051	20	89	98.94	99.15	99.62	100
0.051	21	84	97.76	99.15	99.62	100

Average of killing rate: The samples at 45 minutes is 99.80% and at 60 minutes is 100%.

**Table II.**

Temperature (C)	Humidity	The natural mortality (%) of times (minutes)			
		15	30	45	60
21	93	34.78	71.73	71.73	83.15
22	93	37.59	50.00	62.50	62.50
23	93	26.45	59.78	83.06	97.30
22	93	20.00	60.00	68.00	76.00
20	94	15.15	51.51	45.45	74.62
21	93.20	26.77	58.60	66.14	76.71

Average of natural mortality at 15 minutes is 26.77% and at 60 minutes is 76.71 %. Obviously, the natural mortality of the bacteria is only little. The discrepancy between ozone treated and the control is significant, especially at 15 min. The longer the time, the less the discrepancy. The sterilizing rate of *Pseudomonas aeruginosa* at 60 min. is 100%, while natural mortality is 76.71%.

# Discussion

Ozone was discovered early, but it has not been used widely for problems of the technological level of ozone generators, lack of sensitive measurement method of ozone concentration and the toxicological knowledge of ozone. It was only used in drinking water disinfection and waste water treatment. It is better in sterilizing drinking water, for example, than chlorine (0.3 mg/l) which needs 10 minutes to kill *Escherichia coli* while ozone (0.4-0.5 mg/c) needs only 1 minute. Kinnman<sup>1</sup> has shown that *Escherichia coli* was sensitive to ozone at concentrations as low as 0.01 mg/l. In fact chlorine could not kill bacterial spores. For example, chlorine (100 mg/l) *Clostridium bifermanta* required treatment for 6 hours,<sup>2</sup> while ozone (2.2mg/l) only needs several minutes to kill *B. cereaus*.

Viruses are known to be much more resistant to chlorine than bacteria. Early work by Kessel et al<sup>3</sup> showed that exposure to a chlorine residual of 0.1-1.0 mg/l for 1.5-3 hours was needed for inactivation of poliovirus, while ozone at a concentration of 0.045-0.45 mg/l gave the same results after only 2 minutes. The short time required for poliovirus inactivation by ozone has been confirmed by Coin et al<sup>4</sup>.

In addition, the cysts of *Entamoeba histolytica* (the pathogen responsible for amoebic dysentery) are fairly resistant to chlorine, requiring treatment with 0.5-1.0 Cl<sub>2</sub>/l for 30-120 minutes for inactivation<sup>5</sup>. Here again, ozone proves to be the more efficient disinfectant, requiring a contact time of 2-4 minutes. With 0.3 mg O<sub>3</sub> (residual) for inactivation<sup>5</sup>. Using a higher concentration of ozone (0.7 mg/l) Newton et al<sup>6</sup> found that over 96% of cysts were inactivated within 1 minute and than 99% in 5 minutes.

We tested several concentrations of ozone for sterilizing in air and found that ozone (about 0.15 ppm) has a good sterilizing effect. We have tested it 13 times. The killing rate was 96.96% at 15 minutes, 100% at 60 minutes. The average concentration of ozone was 0.11 ppm. It is suggested that this concentration has a good sterilizing effect and it could be used in room air sterilizing during which people need not go out. So it is easy to use.

## On the natural mortality rate of bacteria in air.

After spraying bacteria, the large droplets dropped quickly and so did the visible bacteria. We considered that the killing rate of bacteria must be calculated after spraying bacteria 15 minutes, so do ozone killing rate. Ozone is able to kill 96.96% bacteria in the air at 15 minutes and the longer time the more, till 60 minutes the 100%. At standard condition, ozone T 1/2 is 16 minutes in liquid, and 3 ppm ozone is 30 minutes in the air ozone, from zerosol chamber and simulat ward. The more concentration the longer T 1/2, vice versa, 0.15 ppm ozone could be measured no more after 0.5 hour. So it can be used easily.

Though ozone is an oxidant, no change could be seen in several kinds of clothes and medical rubber in 0.40-0.57 ppm. We have experimented 28 times per times for 0.5 hour.

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# Research on the Sterilizing Effect of Low Concentrations of Ozone in the Air. An Observation on the Applied Effect in Burn Wards.\*

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As to burn infection, so far some problems still remain unsolved. Through our laboratory experiments we found that low concentrations of ozone can be used to kill *Pseudomonas aeruginosa* with a high and quick effect. It leaves no odd smell and residues after use. Since only electricity and air are basic needs for ozone production, we have an easy access to the resources and the production is economical also. The concentration we use is standard and ozone can decompose by itself into oxygen under normal temperatures. So we have acquired the desired result that air sterilization can be achieved.

## Material and Method

### I. Ozone Generator:

Type CYF-1, bought from 718 Research Institute. Ozone can be generated at 700-1000mg per hour. The machine should be assembled before used in the ward. The remodeled machine should be fixed 1.8M above the ground.

### II. Measurement of ozone concentration:

We used neutral potassium iodide and phosphate buffer to do a colorimetric analysis and took air sample, similar to the way it was done in the ward. The concentration of ozone for the burn wards was determined by the size of the room, thus we figured out the time needed to fill the room with ozone.

### III. Bacteria Sampling:

Sample was taken in the ward by means of natural sedimentation. The culture medium was ordinary agar. Samples had been taken, one from the centre of the room, the other four from the four corners of the room. The sampler was as high as the patient's bed. The room was exposed for 15 minutes before being filled with ozone to

take sample for later comparison. When ozone was concentrated as needed after 15 minutes the agar plate was opened and exposed for another 15 minutes for sample taking.

#### IV. Case Study:

Extensive burns and the burns which are in the recovery stage.

##### I. Effect of Low concentrated Ozone's Eradication of Bacteria in Simulant Ward:

The simulant burns room was 20.48M in volume with concrete floor and 1.2 square metre painted walls. During experiments two doors were covered with plastic film Inside there was an ozone generator and also a sampling tube that was of the same height as the patient's bed. The room was empty during experiments. Through the means of bacterial sedimentation and from five sampling-taking points, we took the sample and then compared them with the sample taken before the fill of ozone. Table I. illustrates the results.

Condition for such a result was that the room was concentrated with ozone. After 15 minutes, the agar plates were exposed for another 15 minutes to take samples. The experiments have been repeated for eleven times and the average ozone concentration was 0.14 ppm (0.3mg/M).

##### II. Effect of Sterilization by a low concentration of ozone in the Burn Ward

The volume of the ward is 48.54M. In the centre was a bed with a patient who has a burn area of 40%. Doctors and nurses were working as usual. No patient's relatives came to visit. Doors and windows were closed. Ozone was filled according to the accumulated time. After 15 minutes the agar plate was exposed for 15 minutes. Samples were taken from five different points each time. Agar plate from before and after ozone was filled were incubated at 37C temperature for 18-24 hours. We calculated the number of bacteria, then we figured out the average number and percentage of bacteria killed by the formula. See Table II.

**Table I. Percentage of Eradication of Bacteria in Simulant Ward for Burns**

Ozone concentration (ppm)	Temperature (C)	Humidity (%)	Eradication Rate (%)
0.10	18	79	99.44
0.13	19	74	99.20
0.14	18	74	99.67
0.14	19	79	99.40
0.14	19	79	99.62
0.14	19	79	99.54
0.14	18	79	96.18
0.15	19	79	99.75

0.15	16	79	99.45
0.15	18	79	99.12
0.15	18	79	99.81
0.14	18.45	78.09	99.21

**Table II. Eradication of Bacteria by a low concentration of Ozone in the ward for burns**

Frequency of observation	Temperature (C)	Relative Humidity (%)	Eradication Rate
1	29	73	99.60
2	22	89	99.90
3	22	89	99.84
4	24	90	99.46
5	24	90	99.85
6	30	74	99.86
7	29	69	99.87
8	22	89	99.63
9	24	90	99.74
10	24	90	77.92
11	26	80	98.05
Average	25.09	83.90	99.70

### Case:

A single room is for the patients with extensive burn. The total burned area is above 40%, with superficial and deep wounds. The burn was caused by a gas explosion and accompanied by light burns in patient's respiratory passage. In the recovery room were those who were burned by toluene, electric shock, alkali and diesel oil. Having been treated, most of them recovered while some still had small injured area unhealed. They were being treated. The above mentioned patients were observed eleven times in the course of the experiment and reacted positively in terms of respiration. They also appeared normal after a regular check-up in their chest, blood, urine. The amount of sodium, potassium and chlorine, combination of CO<sub>2</sub> and liver function tested normal. All the patients are now fully recovered.

### III. Dissipation of Ozone in the Simulant Ward:

In a simulant room of 20.48M in volume, we put a sampling tube to take up ozone, when ozone was concentrated. We from time to time took ozone to see its

concentration by means of neutral potassium iodide and phosphate buffer colorimetric analysis. Results you can see from Figure 1. From it we can see that ozone in 0.87 ppm will dissipate until it can no longer be detected in 60 minutes. We also found that ozone concentration can disappear in 30 minutes within standard concentration (0.71 ppm). The dotted line represents in the illustration the standard concentration.

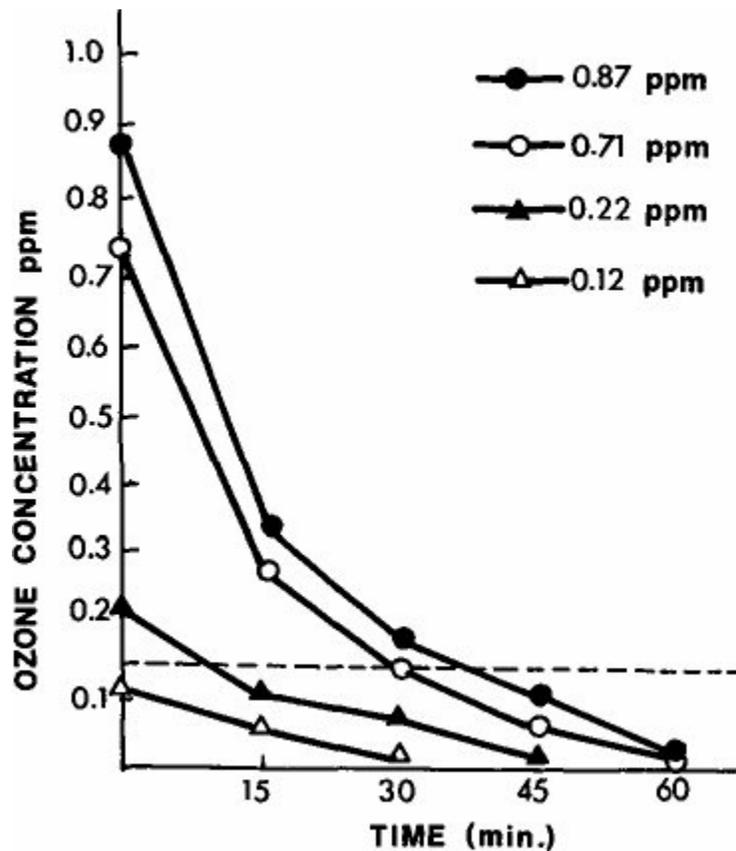


Figure 1. Illustration of Dissipation of Ozone in the Simulant Ward.

#### IV. Curve for Existing Bacteria in the Burn Ward:

Patients selected for the experiment are burned above 40% in degree. Doctors and nurses work in their normal routine way. We use ordinary agar plate to take samples by means of bacteria sedimentation. Observations began from 6 a.m. until 9 p.m. Samples were taken every other hour from five different locations and there followed the usual practice of culture and calculations of the number of bacteria for a continuous three-day observation. Figure 2 is the result that was derived from that three day observation. From the illustration can be seen that the number of bacteria taken at different times fluctuates but not much. There were about four peaks in the curve, namely 6 a.m., 10 a.m., 5 p.m. and 8 p.m.

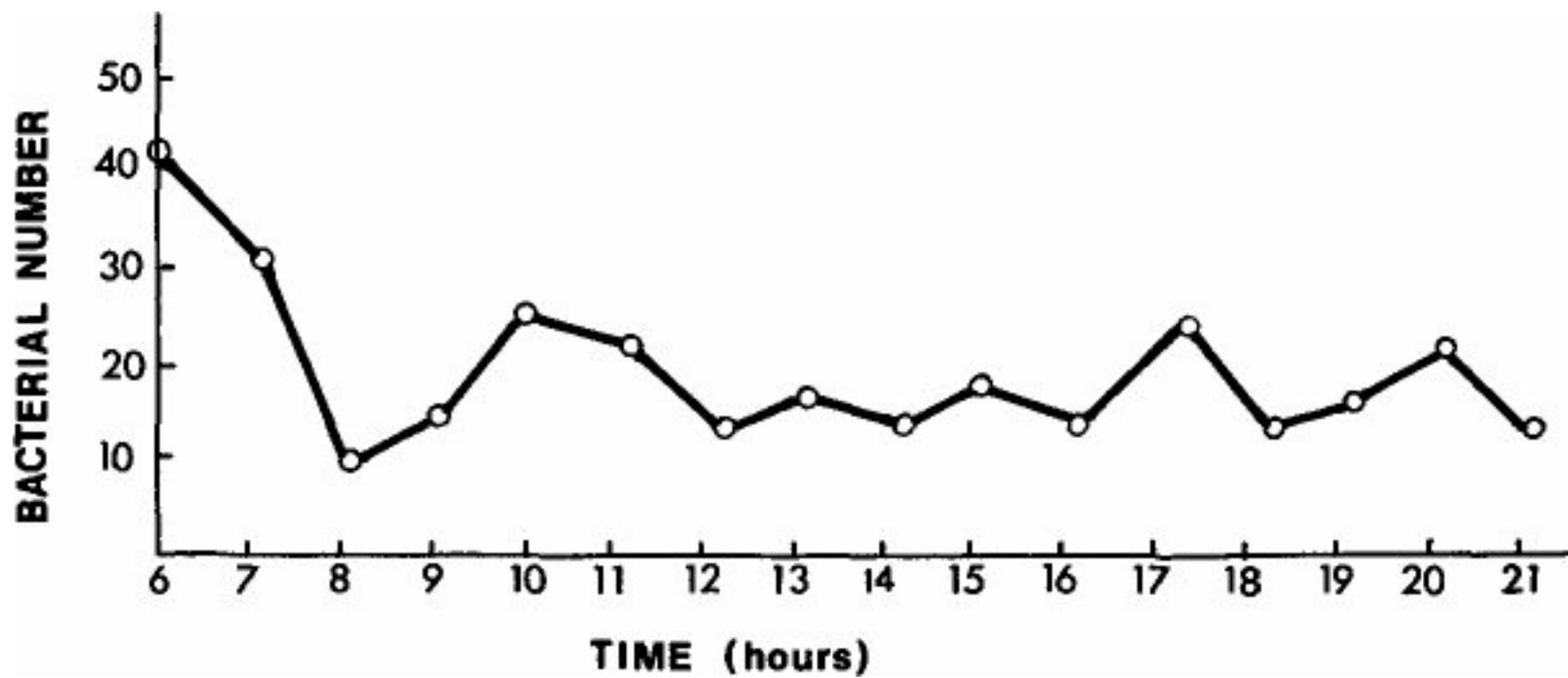


Figure 2. Curve for Existing Bacteria in the Burn Ward.

## Discussion

There is a concern that ozone is toxic; therefore, its use is limited. The toxicity of ozone is connected with its concentration and contact time. The high concentration and longer contact time, the more toxicity and vice versa. After experiments, we believe that low concentration of ozone (0.3 mg/M) is able to kill bacteria in the air.

There are some advantages when ozone sterilizes the air, such as high efficacy, wide spectrum and quick antibacterial action; no residue or smell remains; easy preparation for ozone is made of air and electricity; ozone's price is cheap and when using low concentration people need not get out, while now other disinfectants do not satisfy this.

There are measurements to detect ozone. After our experiments, we found that it need not measure ozone's concentration every time. According to the volume of the ward, we fill ozone to the room. When measurements show the concentration adequate, we take down the time and then we use that fill time to get the adequate concentration thereafter.

The killing rate of bacteria by ozone is 99.21% in simulated wards and 85.89% (average) in burn wards. The difference may be related to the patients. We found that patients' respiration, cough and turn over in the room are able to increase the concentration of bacteria in the air. Therefore the killing rate of bacteria in the burn wards is lower than the simulated wards.

The air humidity also affects the effectiveness of sterilization. From the results, it is clear that the higher relative humidity, the higher the killing rate.

In experiments in hospitals, the burn patients showed no adverse reaction, no stimulative effect and no *Pseudomonas aeruginosa* infection. The doctors suggested that the wounds heal quicker than before.

The ozone negative ion generator we used is able to produce ozone and negative ions, which freshen the wards air as in the forest and at the seashore.

# Rapid Methods and Automation in Microbiology

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The concept of automation in clinical microbiology embraces both fact and fantasy. The fact is that clinical microbiology is at least semi-automated. There are procedures and instruments which provide answers to microbiologic questions rapidly with less technologist intervention than was possible ten years ago. The fantasy is that there have been dramatic improvements in delivery of health care of industrial quality control as a result of microbiologic high technology and that we can afford it in the future. A more accurate focus of this paper would be clinical microbiology in a changing technological world.

The product of the efforts of most microbiology laboratories is available for a price. Whether the product is useful and whether it is worth the price will eventually effect the future marketability of microbiology. How will we respond as clinical microbiologists when fee schedules will be set on a regional or a statewide basis for clinical laboratory tests? In order to confront microbiology in this changing health care world, we must appreciate how the science has developed. There are three issues to address: (1) the retrospective nature of microbiology, (2) the nature of pathogenic bacteria and clinical specimens, (3) emerging pathogens in both the clinical and industrial world.

## The Retrospective Nature of Microbiology

Sir Robert Williams, speaking at the 2nd International Symposium on Rapid Methods and Automation in Microbiology in Cambridge, England, in 1976, said that “the clinical microbiologist has developed as a diurnal species.” Until recently, the microbiologist has been a slave to the 18 hour growth cycle. Virtually every procedure that was carried out in the laboratory was subject to a built-in overnight delay with the possible exception of the Gram stain. Almost no information on the etiology of infectious disease was transmitted while it was of immediate benefit to the product or the patient. Consequently, microbiology developed as confirmatory clinical science. Microbiology has not developed as a unique laboratory discipline which directs action but rather one which confirms a decision or denies it in the face of a resolving or exacerbated condition. Only when information is available in a timeframe which is of consequence to the process will microbiology truly make a

contribution.

## The Dynamic of Specimens and the Nature of Pathogenic Bacteria

Unlike specimens such as blood which are analyzed for chemical and cellular constituents, specimens for micro-biological analysis are labile populations of microorganisms still interacting with the host, with antimicrobial agents, with products and with each other. The removal of the specimen from the immediate environment does not stop this interaction. Excessive delay in transportation and improper storage are adverse environmental conditions which will inexorably harm the specimen. Additive to the problem of the dynamic of the specimen is the lack of a clear answer to the question, "What is a pathogenic bacterium?" In the past, the definition was relatively easy. "A pathogenic bacterium is one which fulfills Koch's Postulates." For the microbiologic historian, Koch's Postulates may be alive and well, but for the contemporary microbiologist, rigid adherence to this definition may be dangerous. Isenberg and Balows (1983), writing in "The Prokaryotes," have said that "the early medical microbiologists had no choice but to seek and establish a close, almost one-to-one relationship, between a bacterium and a specific disease." They further stated that "polymicrobial infections are not uncommon and may well be the most likely types of disease for certain patients. Several microorganisms may act symbiotically to cause disease and that the combination of these microbial particles is dynamic.

Microbiologists must recognize the danger of antimicrobial therapy (as it affects the balance of microbial populations), as well as the immunologic paralysis that is rendered by cytotoxic and immunosuppressant therapy." It is obvious, then, that our definition of a pathogen, once clear, is not clouded. What, then, is the role of the microbiologist? It is to sort out small numbers of microorganisms which may or may not be harmful in an environment which is changing as a result of environmental pressure and in which microorganisms are interacting, not only with host and with the product, but with each other. Microbiologists expose these offending microorganisms to unnatural nutrition and test them after subculture to provide a generic name and subsequently determine their susceptibility to a series of antimicrobial or disinfecting agents. That the results of this exercise correlate as well as they do with the ultimate response to both the "bug" and the "drug" should come as a surprise to everyone.

## Emergent Pathogens in Clinical Microbiology

Microbiologists are experiencing the second golden age of microbiology, the first being at the turn of the 20th century. During the past five years, microbiologists have seen the many species and few genera of *Legionella*, and the acid-fast parasite, *Cryptosporidium* as a causative agent of diarrhea in not only AIDS patients, but also in the general population. How many positive blood cultures have been signed out as "diphtheroids" when,

in fact, they were *Corynebacterium* JK? It is now-possible, with specialized techniques, to diagnose from 2/3 to 3/4 of human diarrhea. Parvoviruses such as the Norwalk agent contribute to many cases of adult diarrhea and rotavirus is a significant cause of pediatric diarrhea. Recent recognition of a T-lymphotropic virus (HTLV-3), or lymphadenopathy associated virus (LAV) as the causative agent of AIDS is exciting as is the report that HTLV-1 and HTLV-2 may be viruses associated with cancer (Gallo et al, 1983). *Vibrio* species, especially *Vibrio vulnificus*, are newly recognized pathogenic bacteria in the marine environment. Because of their proclivity for oysters and clams, they have the potential for changing the seafood eating habits of many knowledgeable people.

The development of microbiology and the micro- biologist has left both the discipline and the disciple relatively unprepared for the increasing demands of the consumer and the industrial technologic revolution. For example, a large number of physicians were surveyed a few years ago by a well-known commercial laboratory publication. Although most respondents were satisfied with laboratory services in general, the most common complaint was late reporting of results. The physician and also the quality control supervisor expect "real time" reporting. Failure of the microbiology laboratory to respond to these needs may be reflected in some major changes that are taking place in where clinical microbiology testing is being performed. Physicians' offices routinely performed urinalysis and CBC's but relatively few of them did their own microbiology, with the exception of pediatricians. The microbiology that was done in the physician's office was often of poor quality. Industry has identified a major market for microbiology in the physician's office. One only has to witness the proliferation of screening tests for urinary tract infection, streptococcal, pharyngitis, infectious mononucleosis and candidiasis to recognize that a major portion of the uncomplicated clinical microbiology may not be done in the hospital laboratory. It is also foolish to ignore the home care consumer product group. Available for self-diagnosis are pregnancy tests and glucose test strips for diabetics. In the future, the diagnosis of urinary tract infections, streptococcal sore throat and even sexually transmitted diseases, may be done at home. Even more insidious is the transfer of procedures formerly done in the microbiology laboratory to other sections of laboratory medicine. Examples are serology performed in chemistry, hepatitis virus components in blood bank and any microbiologic test using enzyme immunoassay or radioimmunoassay being performed in another lab. While such a change in venue may not be directly applicable to the pharmaceutical industry, certainly the change in markets should be noted by all.

The most visible consumer problem confronting the clinical microbiologist today is that of prospective reimbursement (PR). The obvious effect of PR is the changing of the financial character of the laboratory from a revenue center to a cost center, or in less formal terms, from a bonanza to a millstone. The publicity given PR may adversely affect the laboratory's development during the decade of the 80's. While it has been reported that some laboratories have suffered decreases in personnel, workload, salaries, etc. because of PR, it may be, particularly in microbiology, "much ado about nothing." Dr. Mary Jane Ferraro, of the Massachusetts General Hospital, has recently analyzed the extent to which microbiologic analysis contributes to patient bills. With a few exceptions, such as bacterial endocarditis, microbiology contributes less than 1% to the total, hardly a drag on the

medical economy. The fear is that microbiologists may overreact and stunt the growth of their specialty. This modification of the laboratory from a revenue to a cost center has essential parallels in industry.

Recognizing that PR and Diagnosis Related Groups (DRG) might have a profound effect on how a company views the microbiology market, I asked research and development people in 12 major companies serving clinical microbiology how they viewed the problem. To the person, they concluded that microbiology is a prime market in laboratory medicine and that the PR/DRG problem would have little or no effect on their decisions to market products or instruments for the detection of infectious disease. The picture then, is not all black nor obviously white. Microbiology has come of age and it is on the verge of a technologic revolution. This technologic revolution may be tempered only by its ultimate cost and by the ability of we who are the last of the “scientific green thumbs” to accommodate a radical change in how clinical microbiology is performed. To predict the status of the world of microbiology for the rest of this century, it is necessary to address three areas: (1) instrumentation, (2) immunology, (3) molecular biology.

## **Instrumentation**

The thrust of present instrumentation in microbiology has been to automate the traditional process of microbial identification. There are few instruments which are truly innovative and do not rely on the growth of microorganisms. A multipurpose instrument is not available which will process the specimen, isolate and identify the organisms and perform antibiotic susceptibility testing on them. Neither is the microbiologic climate “right” for the development of such an instrument. The decade of the 70’s witnessed the introduction of the Autobac, the Autobac MTS, the Abbott MS-2, the Abbott Advantage and the Vitek AMS. The Vitek AMS, developed initially for the space program, comes as close to any instrument in the laboratory to being a hands-off automated microbiology analyzer. Also in the 1970’s, the Bactec became very popular. There has been a gradual shift away from disc diffusion susceptibility testing to MICs. Some say there will be a shift back not to agar diffusion but to a “breakpoint” microdilution system in which only 1 or 2 concentrations of an antibiotic are tested, not multiple ones. More recently, the microdilution susceptibility test has been streamlined by the use of computers and automatic readers. Labor saving devices such as the automatic gram stainer, media maker, agar plate pourer, and agar plate streaker, are popular. While the utility of all these instruments cannot be denied, with few exceptions, they have not provided that “diagnostic edge” so critically needed in microbiology. I see instrumentation developing in three areas: (1) multifunctional machines, (2) screening and (3) rapid susceptibility testing.

The next decade will require laboratories to maximize their instrumental resources by the use of multi-functional machines. A good example of such an instrument is the Abbott Quantum. The Quantum is not a microbiologic robot nor does it provide a bacteriologic assembly line. Rather, it is a small instrument that is capable of doing a wide variety of enzyme immunoassays for antigens and antibodies ranging from hepatitis to rotavirus to cytomegalovirus to Group A streptococci. Additionally, it is capable of identifying bacteria in

four hours or less and soon will provide antibiotic susceptibility results.

Instruments that are small, simple and economical, even though “single purpose,” are needed. A good example is the Bac-T-Screen (Marion Scientific) which screens urine samples for the presence (or absence) of microorganisms, and enables the laboratory to discard up to 80% of their urine cultures without culturing.

Instruments which provide rapid antimicrobial susceptibility test results have a place in today and tomorrow's laboratory. An example is the Gibco Sensititre. The Sensititre System consists of a computer, video monitor, a printer and an automatic microdilution plate reader. Not only can the instrument read antibiotic susceptibility tests automatically in five hours or less, but it has the capability of automatically identifying bacteria in the same amount of time. The unique aspect of this system is that microbial growth is detected by measuring a fluorogenic compound cleaved by enzymatic activity. The system's computer is multifunctional and can be used for various epidemiologic and data handling tasks in the laboratory.

## Immunology

One of the few available techniques that circumvent the growth cycle of microorganisms are reagents and kits for the direct detection of microbial antigens in body fluids. These methods are based on counterimmunoelectrophoresis, coagglutination, latex agglutination, enzyme immunoassay, or fluoroimmunoassay. Much has been written about their application to the identification of bacterial antigens in cerebrospinal fluid for the rapid diagnosis of meningitis. Antigen detection is widely used, sensitive and provides a distinct diagnostic advantage, enabling the identification of one of the three or four main etiologic agents of meningitis in a few minutes or less. Accompanying the availability of these immunologic techniques has been the advent of monoclonal antibodies which impart very high specificity to these immunologic reactions without reducing sensitivity. Recently, a number of antigen detection tests distinct from those used for the detection of meningitis, have become available. They include 10 minute tests for the detection of Group A streptococci in the throat, rapid detection of *Chlamydia*. Herpes simplex virus (HSV), and respiratory syncytial virus. One laboratory in Finland headed by Dr. Halonen routinely uses time-delayed fluoroimmunoassay to analyze respiratory specimens for influenza virus, parainfluenza virus, adenovirus and respiratory syncytial virus.

## Molecular Biology

As exciting as the applications of immunology have been and will be to clinical microbiology, a technique of the future with equal importance is that of recombinant DNA. It is the most revolutionary development in biology in recent years. Although recombinant DNA technology may have the most immediate effect in medical genetics, the long term contributions to infectious disease diagnosis will be inestimable. The technology essentially consists of removing fragments of DNA from microorganisms which contains specific genes

of interest. These fragments are then put into a suitable vector such as *E. coli*. *E. coli* functions as a clone machine to make multiple copies of these genes. The copies containing the relevant DNA fragment are selected, harvested and used as DNA probes. That is, they are used as molecular traps to search for complimentary DNA in clinical specimens. There are commercially available probes for HSV and cytomegalovirus. The routine availability of DNA probes should enable the microbiologist to rapidly detect specific DNA in patient specimens, particularly for those infectious diseases whose diagnosis is hindered by slow growth of the agent.

Microbiology will never be the same again. While it is still premature to preside over the funeral of the agar plate, there are enough signs to suggest that the laboratory of the future will be different. It will be computer assisted and contain two basic types of instruments, ones that are dedicated for screening tests and others that have broad utilization within the laboratory. The laboratory's main diagnostic reagents will be antibodies and DNA probes. Specimens received in the morning will be reported out by noon time. Rarely will specimens be carried overnight for identification or susceptibility testing. It may be possible to detect antibiotic resistance genes directly in living specimens. The clinical laboratory can already detect antibiotic inactivating enzymes (beta lactamase) in clinical specimens. While some of the simpler microbiology may be done in the clinic, the physician's office, or even at home, the clinical microbiologist will be responsible for the detection and identification of the diverse array of both old and new pathogens that invade both the immuno competent as well as the compromised host. The process of change has already begun and we are a vital part of it.

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# Preservative Systems for Parenterals and for Nonsterile Products

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The elaboration of an adequate preservative system is generally not the most spectacular stage of the development of a new product. The synthesis of the effective agents, the investigation of their action, the elaboration of the basic receptor or the optimization of the technology are far more impressive. Nevertheless, the significance of the right choice of development of an effective preservative or preservative systems should not be underestimated. It should be reminded that according to recent economic data, about 10-20 percent of the produced goods decays in each year and the microbial change is one of the most important factors.

The effective antimicrobial preservative is especially important in the industrial branches we are dealing with, that is in the pharmaceutical, cosmetic and household-chemical industries, where the last two decades have brought considerable progress. These changes were elicited by the rapid increase in the amount and number of products and basic materials, as well as by the more and more rigorous regulations concerning the microbial purity of the products.

The extent of the change was less significant in the field of parenteral products, because they had been subject to strict regulations previously. Here the changes were two-fold: first, the ophthalmologic preparations have contributed to the number of sterile products, second, the regulations have become more rigorous with the advent of special culture media and the increased sample volumes.

A markedly rapid expansion was seen in the field of the so-called nonsterile preparations. Twenty years ago, these products were not even controlled microbiologically, while now their rigorous examination is compulsory.

The attention was called to this subject by several accidents revealed in the mid-sixties, in which serious infections were caused by several contaminated pharmaceutical and cosmetic preparations. These problems were dealt with first by Kallings<sup>1</sup> and co-workers. Their pioneering work resulted in a system of overall regulation in the pharmaceutical industry, though the criteria are still not uniform. In the cosmetic industry, the system of regulations is not complete up to now, though many manufacturers are following the

suggestions of the *Cosmetic, Toiletry and Fragrance Association (CTFA)*. In some areas, there are so-called non-official regulations, these requirements are not only met but even surpassed by the products of the leading firms, especially the large exporters.

## Preservation Procedures

The partial or total destruction or elimination of the viable germs is the foundation of effective antimicrobial preservation. A reduction of the germ count can also have a preservative effect provided that recontamination of the system is excluded by protective measures. Nevertheless, in the majority of the cases, the application of preservative substances are necessary for the prevention of the growth of contaminating microorganisms.

Table I summarizes the various antimicrobial preservation procedures, their relative merits and the limits of their application.

**Table I. Antimicrobial Preservation Techniques**

Basic Method	Techniques	Merits and Shortcomings
Drying	Air flow drying fluidization lyophilization,	Instability, increase of germ count in case of humidity
Cooling	Cold storage deep freezing	Full virulence at normal or warm ambient temperature
Heat treatment	Dry heat damp pasteurization	Moderate effect adequate for sterilization
Gas	Ethylene oxide gas	Widespread, application decreasing because of toxicity
Irradiation	UV gamma	Only in combination with other methods application increasing, adequate for sterilization
Antiseptic methods	Heat treatment after irradiation or bacterium filtration	When other methods are inapplicable, danger of recontamination
Combined procedures		Frequently synergetic effects, when other procedures are inapplicable.
Chemical preservation	Different chemicals and their combination	Most important, basic method of antimicrobial preservation.

Heat treatment is still the most frequently applied procedure. Out of the more recent methods, gas sterilization by ethylene oxide has gained wide application especially in the

USA. However, it has been abandoned in many places because of the toxicity of the remanent gas<sup>3</sup>. An opposite tendency can be observed in the case of ionizing radiations, particularly gammairradiation. After initial hesitations, it is increasingly applied for the reduction of germ count in numerous basic materials of cosmetics and pharmaceuticals, as well as in biochemical products, enzymes, ointments, ointment bases, talcs and other half and finished products.

The most important factors are still chemical preservatives, which are applied in increasing number and in combinations in the industry. The number of the preservatives used in the food, pharmaceutical, cosmetical and household chemical products is compiled in Table II on the basis of well-known *Pharmacopeas*<sup>6-8</sup> and the publications of the FDA<sup>9</sup> and CTFA<sup>10</sup>.

**TABLE II. Number of Applied and Licensed Chemical Preservations in Different Product Categories**

Category	Number of Preservatives and Combinations			
	Basic Compounds	Derivatives	Combinations	Total
Foods	6	4	5	15
Pharmaceuticals	20	10	ab.15	45
Cosmetics	40	20-30	ab.50	110-120
Technical product, detergents, dyes, drill oils, etc.	ab.60	ab.40	10-20	110-120

The different columns of the Table contain the basic compounds and their derivatives and the most frequent preservative combinations. Their number is impressive although it is hardly increasing in the last few years, since the introduction of new compounds has been made difficult by the rigorous regulations.

## **Combined Technological Procedures with Special Regard to Combinations of Heat Treatment and Irradiation**

The increasingly rigorous requirements have made the application of earlier technologies (heating or different irradiations) impossible, since a more intensive treatment (by increasing the temperature or the radiation dose) has not been feasible. This circumstance has directed the attention toward the combined procedures.

The combined procedures were applied first in the food industry,<sup>11</sup> because these complex products are the most sensitive to external effects. Then the other branches, first of all, the pharmaceutical and cosmetical industry also recognized this possibility.

All possible variations have been tested: different combinations of dry and wet heat, ionizing and UV irradiation, ultrasonic treatment, ethylene oxide gas sterilization and diverse chemicals. Such an example is the antimicrobial treatment for pancreatin by the combination

of isopropanol with gamma irradiation by *Lussi-Schlatter* and *Spoise*<sup>12</sup>. Others found successful applications for the combinations of PHB<sup>13</sup> chlorocresol<sup>14</sup> formaldehyde<sup>15</sup> and chloramin<sup>16</sup> with irradiation or occasionally with heat treatment. The synergistic effect of the treatments could not, however, conceal the fact that a decomposition of the applied chemicals took place depending on the dose of radiation.

The most favorable experiences have been gained with the combination of heat treatment and irradiation. An additive effect is always guaranteed, furthermore, a synergism can be expected in the majority of the cases. The procedure can be performed basically in three variations: irradiation followed by heat, the reverse succession, and finally the simultaneous application.

An interesting problem is that of the order of succession of the treatment, which is still unsettled. The first data on a synergism were published by Morgan<sup>17</sup> as early as 1954. He found that a synergism was found only when the irradiation was followed by heat treatment. This is still the opinion of the majority of authors. An example of the contrary was reported by Levison and Hyat<sup>18</sup> who investigated the response of *B. megaterium* spores.

The first step for the resolution of this apparent contradiction was made by Padwal-Desai<sup>19</sup> who demonstrated that the variations can be species specific to the same extent as the individual procedures are. Those who preferred the order of succession of gamma irradiation then heat treatment tested the sensitivity of soil spores and *Clostridium* strains, whereas the other party investigated yeasts and molds. These choices, of course, predestined their opinions.

On the basis of the different published data<sup>17-20</sup> and our own results, the apparent contradiction can be completely resolved. In fact, the right choice of the parameters of the treatment are even more important than their order of succession.

The majority of the investigators applied very low doses (0,4-1,6 kGy) and dose rates (0,11-0,54 kGy/h) of gamma radiation for the sensitization of the microorganisms before a relatively more intensive heat treatment (80-125°C). Inversely in the case of the successful heat-then-gamma irradiation combinations a moderate heat treatment (35-70°C) was followed by a relatively intensive irradiation (2,5-15 kGy). Therefore, it is not justified to compare the efficiency of these procedures. The order of succession should be set to conform with the production of each preparation, then the parameter of the operations should be optimized.

This principle has been observed in our practice, as shown by the following examples for the combined treatments of pharmaceutical and cosmetic products. Since a moderate, mostly dry heat treatment is involved in, or can easily be introduced into all technologies, our experiments were based on the heat-then-irradiation variation.

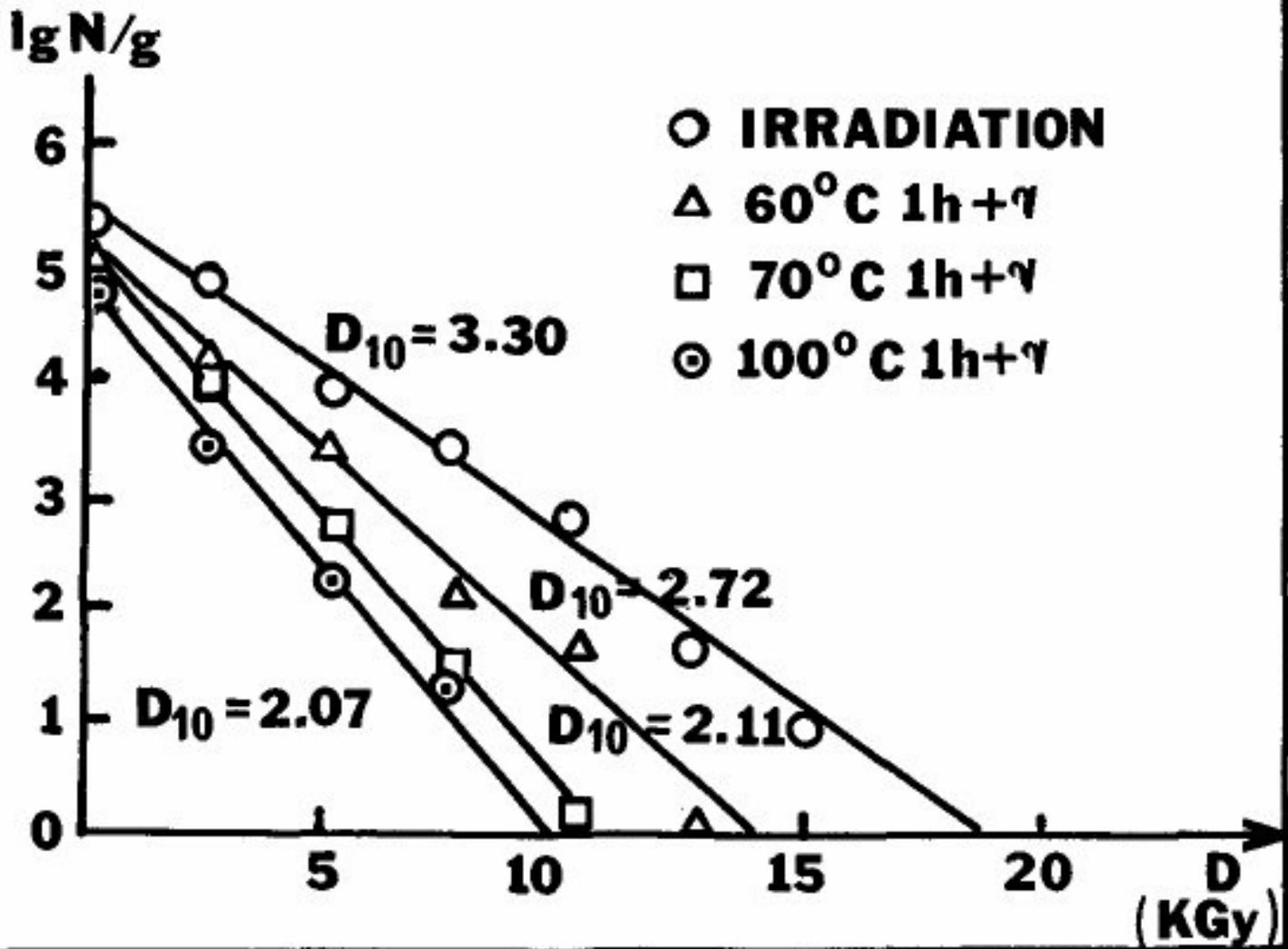


Figure 1. Synergetic Effect of Combined Heat and Radiation Treatment on Microbes in Neopankreatin.

○ irradiation    ▲ 60°C 1h + γ    □ 70°C 1h + γ    ● 100°C 1h + γ

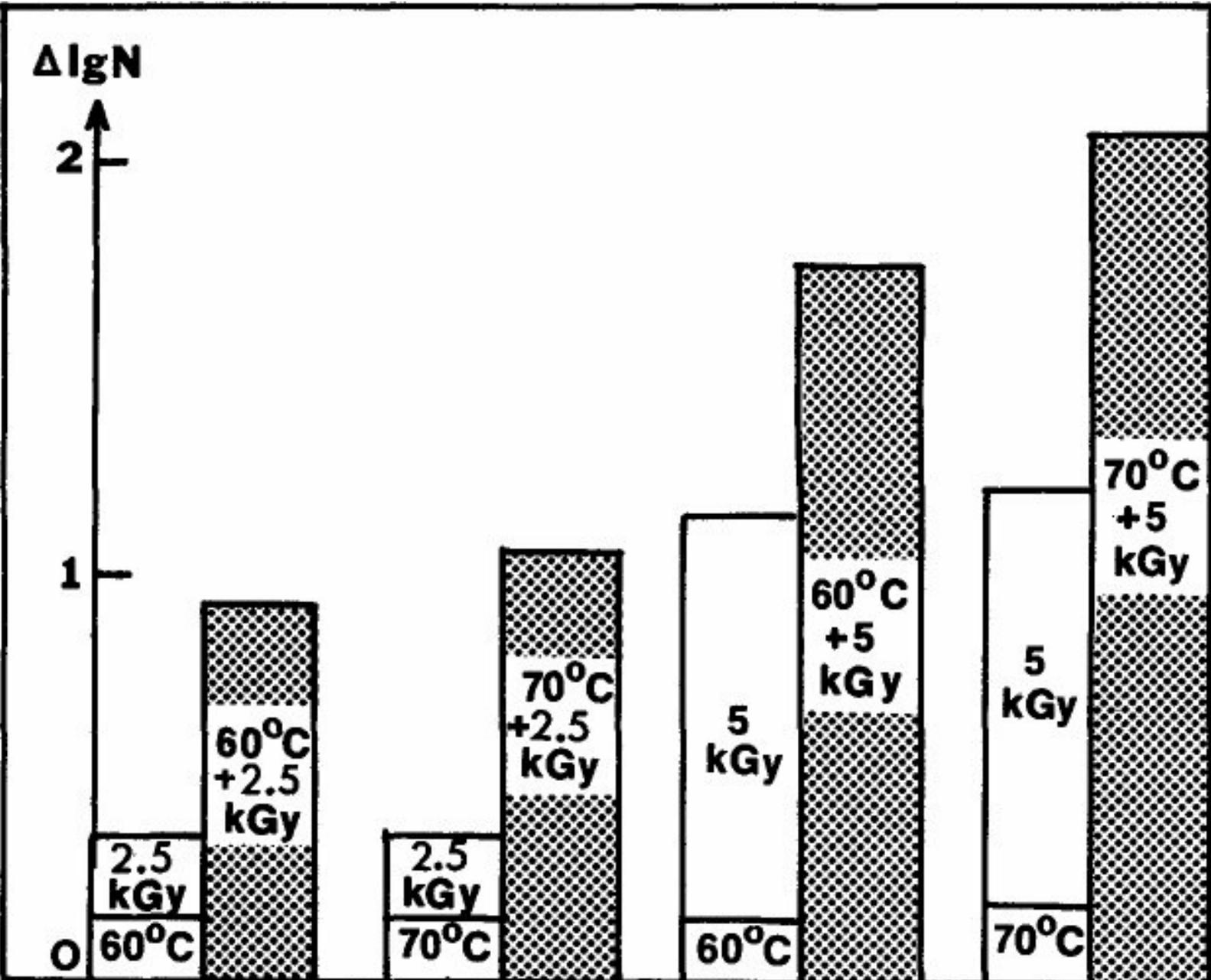


Figure 2. Combined Heat and Irradiation Treatment of Neopankreatin/Graphical Representation/

The optimization of the parameters was performed as follows. Three or four temperatures generally between 60 and 100°C, were chosen in the knowledge of the heat sensitivity of the materials. After the heat treatments, the irradiation was performed with different doses according to the radiation resistance of the product. The dose range was generally 2,5 to 12,5 kGy, sometimes from 2,5 up to 25 kGy.

Figure 1. Shows the results of the combined treatment of a biochemical product. Neopancreatin. It can be seen on the figure that a heat treatment at 70°C for one hour followed by gamma irradiation proved to be the most successful technology. Figure 2. Shows graphically the synergistic effect of the combined procedure.

Table. III. Microbiological Examination of Ointments Produced by Different Technologies

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Ointments model Total germ count g<sup>-1</sup>

No. Procedure and Its Main Parameters	Ointments model Total germ count g <sup>-1</sup>			
	A	B	C	D
1. Conventional warm technology	75	50	20	20
2. Heat treatment/80°C, 1h	20	10	5	10
3. Heat treatment/80°, 1h and antiseptic filling	10	1	5	5
4. Heat treatment/140°C, 1h and antiseptic filling	steril	1	steril	steril
5. Irradiation with different doses				
2.5 kGy	5	2	5	1
5 kGy	1	0.1	0.2	0.2
10 kGy	0.2	0.1	0.1	0.2
15 kGy	0.1	steril	steril	0.1
25 kGy	steril	steril	steril	steril
6. Irradiation then heating adequate pretreatment and heating at 80°C for 1h	steril	sterile	sterile	sterile
7. Heat then irradiation pretreatment at 80°C for 1 h				
2.5 kGy	2	1	0.2	0.2
5 kGy	0.1	0.1	0.1	0.2
10 kGy	sterile	sterile	sterile	sterile

Table III shows the germ count reduction achieved by seven-different technologies in the case of four model ointments. Although a germ count reduction also resulted by many other technologies, the majority of the sterile samples suffered intolerable physical and chemical damage. The samples treated by heat plus gamma irradiation were the exceptions which tolerated well the applied dose (10-15 kGy). The so-called radiation resistant receptor tolerated even a dose of 25 kGy.

It is concluded that the previous heat sensitization reduced the dose requirement of the irradiation, which can result in an expansion of the field of application of this technology. Table IV was compiled with regard to the dose-requirement reducing effect of the combinations showing the sterilizing and pasteurizing dose requirement for three families of products.

**Table IV. Sterilizing and Pasteurizing Doses for Three Groups of Products**

Groups	Sterilizing Dose kGy	Pasteurizing Dose kGy
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Ointments	10.0-27.5	2.5-12.5
Tales	10.0-25.0	5.0-12.5
Biochemical products	—	5.0-17.5

It should be remembered that the need for pasteurization and preservation increased considerably the application of irradiation technology. The way of the introduction of this technology has been long and full of troubles since 1956 when Johnson & Johnson(22) first marketed the radiation sterilized products, two kinds of ointments in capsules.

## Chemical Preservation

The main method of conservation of pharmaceuticals, cosmetics and the so-called technical products is chemical preservation. It is necessary even after a suitable pretreatment or sterilization in order to protect the product against recontamination.

The selection of suitable preservatives which meet all requirements is a very difficult task. The aspects of selection are the mechanism of action, the action spectrum, pH optimum, solubility, the distribution coefficient in case of complex systems, further the stability and compatibility, finally the toxicity and tolerance.

The problems have become even tougher in the recent years. The use of several traditional preparations was limited officially. A classic example is hexachlorophene, but the quaternary ammonium salts are also criticized because of the increasing incidence of hypersensitivity reactions, though high, a growing number of the cases may be connected with their increasing application rather than with an increase in the sensitivity rate. Although we do not have the data for the pharmaceutical industry, nevertheless, the application of the parabens increased considerably in the cosmetical industry. According to the data published by the FDA,<sup>10</sup> its share increased from 63 to 68% in the USA between 1977 and 1984.

Further problems have to be accounted with, such as the hidden incompatibility,<sup>24</sup> most frequently the inactivation effect of the so-called nonionic emulsifiers, and the problems with packaging materials.

New preparations are rarely introduced for cosmetics and technical products. This is connected with the considerable restrictions in the licensing. This tendency characterizes the last 10-12 years.

After the above discussion, it can be seen that the number of the antimicrobial agents suitable for pharmaceuticals is rather limited. The repertoire consists of not more than 20 basic compound and about 10 analogues. Their most important characteristics—their action spectrum and pH optimum—are listed in Table V, mostly on the basis of the data published by Wallhauser<sup>24</sup>.

The hegemony of the parabens is evident, even among the analogues. The methyl and propyl esters of PHB are dominating, though the ethyl and butyl esters are also favored mostly in combinations. They exert an excellent preservative effect, especially in combinations. Their application is limited mostly by their incompatibility with a number of

auxiliary materials, such as sorboxethen, saccharose and poly-oxyethylene-esters, PVP, cellulose and carboxycellulose derivates, plant resins and gums, etc. On the other hand, the effectivity of PHB-esters is very much increased by 2.5% propylene glycol.

Out of the other compounds, the most versatile agents are the chlorhexidine derivatives (hydrochlorid, diacetate and digluconate) sorbinic acid (frequently in combination with potassium sorbate), benzoic acid and the quaternary ammonium compounds (benzalconium chloride, benzethenium chloride and cetyltrimethy-lammonium chloride). In the ophthalmological preparations phenyl-mercury-nitrate and Merthiolate are specifically applied, but benzalconium chloride is also favored. The rest of the agents are used less frequently for individual purposes.

There are, of course, preparations which do not require preservation either because they are supplied in a closed system for a single use (like the majority of injections) of the system is autosterile or it is at least strongly bactericidal. Such preparation are the antimicrobial agents or the systems containing bactericidal basic materials (e.g., high concentrations of ethanol or propylene glycol.

In the field of cosmetics, the selection is better (24,25). As shown in Table II, the number of preservatives is two to three times more. Nevertheless, the freedom of choice is not much greater, since unexpected problems of incompatibility may arise because of the higher number of the applied auxiliary materials and the effective agents. Moreover, the latters include many composite preparations, which are difficult to characterize (plant extracts, protein hydrolysates, etc.)

Table VI contains statistics on the preservatives which are most frequently used in cosmetics. The table was compiled on the basis of an analysis of the preparations registered by the FDA between 1977 and 1984<sup>10</sup>.

## **Table V. Effectiveness of Chemical Preservatives Applied in Pharmaceuticals\***

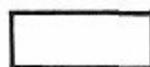
Preservatives	Active spectrum				pH optimum					
	G/+/ G/-/	Yeasts	Molds	4	5	6	7	8	9	
Benzoic acid	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Benzly Alcohol	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Benzalkonium Chloride	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Benzethonium Chloride	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
CETAC	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Chloracetamide	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Chlorbutanol	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Chlorhexidine	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Cresol	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Dehydroacetic Acid	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Ethanol	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Hexachlorophene	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Parabens	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Phenol	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Phenylethyl Alcohol	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Phenylmercuric Esters	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Propionic Acid	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Sorbic Acid	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Thiomersal	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	
Thymol	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	efficient	



efficient



weak



no effect

\* = Based on K.H. Wallhäusser, Sterilization-Desinfection-Konservierung, Stuttgart, G. Thieme Verl., 1978.

The hegemony of the parabens is even more pronounced here than in the field of pharmaceuticals, where their share is 68%. Their chief merit is their versatility in application. Many preservatives can be used in a few classes of products only, e.g., formaldehyde only in shampoos and FAA concentrates and certain mercury compounds only in ophthalmological preparations, etc. Other agents do not provide a complete protection in themselves, therefore, they are used in combinations, e.g., Germall 115 with potassium sorbate. Germall is most frequently combined with PHB-esters, while potassium sorbate is used together with sorbinic acid.

**Table VI. Chemical Preservatives Which Were Most Frequently Applied in the Cosmetics Industry of the USA Between 1977 and 1984 FDA Registered**

Preservatives	Applications Registered			
	1977	1980	1982	1984
Methylparaben	5693	6785	7140	7694
Propylparaben	5349	6174	6274	6796
Imidazolidinyl urea	1254	1684	1820	2315
Quaternium 15	599	1001	1079	1126
Butylparaben	483	668	739	803
Formaldehyde sol.	888	874	734	711
2-Bromo-2-nitro-propane-1,3-diol	366	566	546	429
Sorbic acid	455	393	361	396
Ethylparaben	31	159	174	365
Sod.dehydroacetate	145	191	242	231
2-Methyl-4-iso-thiazolin-3-one	0	38	135	222
5-Chloro-2-methyl-4-isothiazolin-3-one	0	38	124	222
DMDM hydantoin	15	79	169	195
Benzoic acid	65	84	116	132
Phenylmercuric acetate	99	147	144	131
Dehydroacetic acid	73	107	111	124
Triclosan	52	88	95	102
Sodium benzoate	118	89	99	98
Potassium sorbate	71	76	87	94
Phenoxyethanol	17	25	48	72
Chloroxylendol	36	71	70	65
Trisodium EDTA	26	40	47	58
Diazolidinyl urea	0	0	0	52
Disodium EDTA	42	60	65	91
Benzyl Alcohol	10	35	47	49
Thimerosa	17	22	40	42

New compound can rather be found among the cosmetic preservatives. The description “new” is relative, of course. Several “new” compounds, such as Bronopol, Dowicil 200, Germall 115 and Irgasan DP300 have been present on the market for more than 10 years. This fact indicates that a certain conservatism is prevailing in the selection of preservatives and very long time is necessary for the introduction and general application even of a very potent new preservative on the market.

One of the most recent compounds is KATHON<sup>25</sup> which was introduced in 1978-80 in the United States, where it achieved the 11th place by 1984 as shown in Table VII. Though we do not know the European data, but we estimate that its introduction is even more significant here in the last 2-3 years. This preparation can be regarded as a combined preservative, since it contains two components: 2-methyl-4-isothiazoline-3-on and 5-chloro-2-methyl-4-isothiazoline-3-on. Its effectiveness is supported by the data of Table VII which were kindly provided by the firm Rohm and Haas. This table shows the results of the antimicrobial preserving efficiency of KATHON in case of two commercial preparations, Johnson Wax’s Foam Bath and L. C. Douche.

Among the cosmetics, there are also such systems which are antimicrobial or bactericidal in themselves, e.g., those preparations which contain 20-95% ethanol or isopropanol, about 20% propylene glycol or 30-50% glycerol. Evidently, additional preservatives are needed in such cases. Natural volatile oils and fixed oils should also be mentioned because several of them (alone or in combinations) exert antibacterial, moreover some of them antifungal, effects<sup>27</sup>. For example, one could mention camomile, thyme, liquiritae, cinnamon and coriander. These can be regarded as traditional agents which are being rediscovered now.

**Table VII. Biological Activity of Kathon CG in Johnson Wax’s Foam Bath and L.C. Douche**

Johnson Wax’s	0	1,000	400,000
Foam Bath	400	10	10
	800	10	10
	1,000	10	10
	1,000	10	10
L.C. Douche	0	4,000,000	3,120,000
	400	10	10
	800	10	10
	1,000	10	10
	1,500	10	10

\* Based on Rohm and Hass, Ltd. publication.

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# Combinations of Preservatives

The limitations of the introduction of new, effective preservatives is counterbalanced by the new trend of the combination of two, three or even five chemical preservative agents. The aim of the combination can be the widening of the action spectrum, the increasing of the effect or the broadening of the effective interval of pH. There are multi-component systems which contain a single genuine conservant and another substance which is not a preservative itself but enhances the antimicrobial effect of the former, these systems are also combinations in our nomenclature. Finally, a combination of genuine preservatives can be combined with another kind of additive.

**Table VIII. Combinations of Preservatives and Potentiating Additives**

Trade name combination <sup>R</sup> or of the Basic CompoundComposition	Remark
Nipostat <sup>R</sup> Methyl, propyl and butyl esters of PHB	Germ count reduction 2-3 times faster
Phenonip <sup>R</sup> Methyl, ethyl, propyl & butyl esters of PHB and Phenoxetol	Wide antimicrobial & pH spectra
Liqua Par <sup>R</sup> Butyl, isobutyl & isopropyl combination pos, bacteria	Effective against molds, yeasts & Gram
Lauriciden Plus 41 <sup>R</sup> Lauridin, methyl & propyl ester of PHB & EDTA	Suitable for O/W & W/O type emulsions
Emericidine <sup>R</sup> Phenoxetol & p-chloro-meta-xyleneol	wide pH spectrum
Germaben II <sup>R</sup> Germall 115, methyl & propyl ester of PHB propyleneglycol, 30:11:3:56	Wide antimicrobial spectrum, good compatibility
Phenonip + Germall 115 combination, 0.5:0.3%	Not inactivated by non-ionic emulgeators and collagen
PHB-esters + Bronopol 0.01-0.02%	Potentiation
+ Kathon CG	Full action spectrum
+ 2-5% propyleneglycol	Potentiation
+ Pionin	Effective also at pH 8
Dowicil 200 + PHB esters or phenol	synergetic effect
Sorbinic acid + PEG or PE-propyleneglycol	Potentiation
Zinc-omadin + EDTA, 1:1	pH optimum at 4,5 potentiation
KATHON CG + Irgasan dP 300	Potentiation outstandingly active

The number of the possible combinations is very large. We listed in Table II, the commercially applied or experimentally proven, effective combinations only (about 15 for pharmaceuticals and 50 for cosmetics). Examples for the different types of combinations are shown in Table VIII.

In the pharmaceuticals, the combinations of PHB-esters are favored mostly. Out of them, Nipacombin and Nip = Nip have been in use for some time, while Nipastat is more recent. The effectiveness of the earlier combination of PHB-methyl and propyl esters, 4:1, was further increased by the addition of the butyl ester. Table IX shows the biological effectiveness of Nipastat combination based on the report of Maddox<sup>28</sup>, the killing of the microorganism was achieved two to three times faster.

The examples for the combinations of a genuine preservatives with a potentiating additive are the joint application of PHB-ester with propylene glycol and of sorbinic acid with PEG and/or propylene glycol.

In the cosmetic industry, the number of the possible combinations is also greater. For the PHB-esters, we mention the following additions: Dowicil 200, Bronopol, KATHON CG, Pionin, Phenoxetol and Germall 115. Among these, Dowicil is synergetic, KATHON and Phenoxetol enlarge the action spectrum, while Germall prevents the inactivation of the PHB-esters by the nonionic emulsifiers and collagen. Best known of the Germall-combination is Germaben II<sup>30</sup> containing Germall 115 (30%) and methyl and propyl esters of PHB (11 and 3%, res.) and propylenglycol (56%). Liqua Par is a similar combination of butyl, isobutyl and isopropyl esters of PHB.

A relatively new combination is that of the PHB-methyl-esters, Lauricidin and EDTA, which is equally recommended for W/O and O/W types of emulsions. The chelator EDTA which was regarded as a chemical stabilizer earlier, is increasing the effect of the preservatives. Figure 3 shows the investigation of the 1:1:1 combination after Kabars.<sup>31</sup>

The preparation Lauricidin Plus 41 is a further development of the combination of Nipagin and EDTA to which PHB-methylester was added. The potentiating effect of EDTA manifests itself not only in the PHB-ester-Lauricidin combination, similar phenomena were observed in the case of other preservatives, too, e.g., with zinc-omadine (in the preparation of 1:1).

**Table IX. Increased Effectiveness of the Nipastat Combination as Compared With the Combination of the PHB Methyl and Propyl Esters, 4:1 in a Liquid Emulsion**

Test Organism	Initial Germ Count per Gram	0.25% PHB Methyl & Propyl Esters	0.25% Nipastat
Ps.aeruginosa	$3.8 \times 10^{10}$	14 days	7 days
E.coli	$7.5 \times 10^6$	4 days	1 day
S.aureus	$4.3 \times 10^6$	7 days	4 days
Citrobacter sp.	$4.0 \times 10^6$	21 days	7 days
C.albicans	$2.5 \times 10^6$	14 days	4 days

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\* D.N. Maddox: Cosm.Toiletr. 97,85/1982.

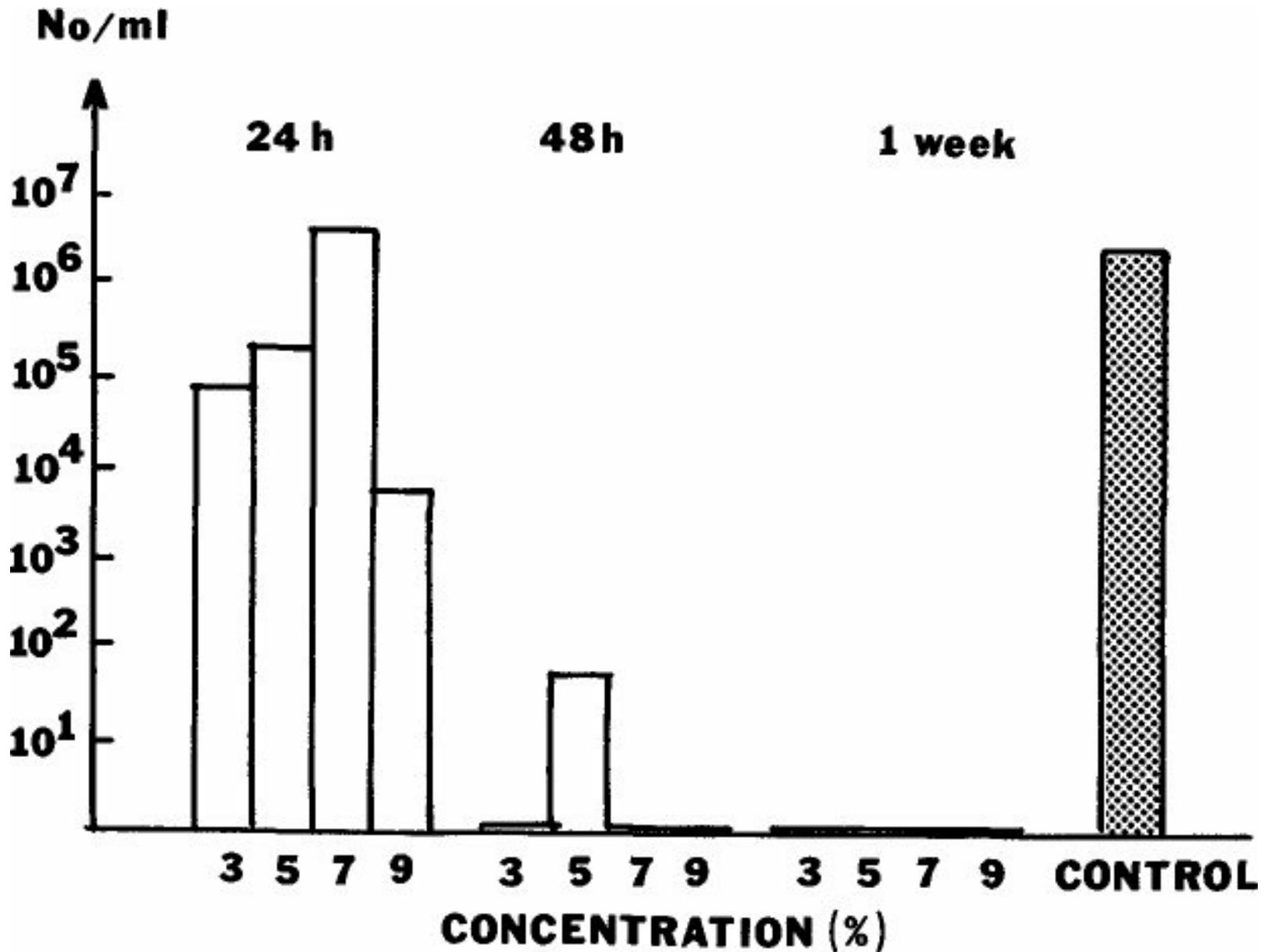


Figure 3. The Effect of Lauricidin-methylparaber-EDTA/Na<sub>2</sub>/ Combination /1:1:1/ Against *E. coli* in O/W Emulsion\*

\* J.J. Kabara-C.M. Wonette: Cosm. Toiletr. 97,77/1982/

The modification of the distribution coefficient is one of the potentiating measures in two-phase or multi-phase systems. In emulsions, for example, the poorly water-soluble PHB-esters are concentrated in the lipid phase. This should be prevented by the addition of some apolar solvent<sup>24</sup> in amounts which should be determined in the knowledge of the composition of the given system (e.g., 10% glycerol or ethanol, or 2-5% propylene glycol). An overdose is also disadvantageous since it decreases the concentration of the preservative in the lipid phase.

Finally, I would like to present the results of one of our experiments,<sup>32</sup> which might be of interest in regard to the applied method and the preservative effect. For the conservation of O/W type cosmetic, ointments, we applied the combination of KATHON CG and Irgasan DP 300. We performed the usual microbiological stability test according to the USP XX, which consists of the addition of microbes to a germ count of  $10^5$ - $10^6$ /g, followed by storage at roomtemperature for four weeks: samples are taken weekly and the test cultures are examined.

Since the results were very good, the experiment was performed applying an even higher germ count (about  $10^{11}$  and further amounts of microbes were added after the weekly sampling procedures as superinfection. The results of this test are shown in Table X.

**Table X. Effect of Kathcon CG-Triclosan Combination on Germ Counts in O/W Type Costmetical Ointments After Repeated Addition of Microbes.**

Type	Day 1	Days 7	Days 14	Days 21	Days 28		Days 35
	Initial Germ Count	Before-After Addition of Germs	Before-After	Before-After	Before-After		Final Germ Count
Regenerating cream placebo*	$2.0 \cdot 10^9$	$10 \cdot 1.1 \cdot 10^9$	$10 \cdot 5.5 \cdot 10^8$	$10 \cdot 5.0 \cdot 10^8$	$1.2 \cdot 10^3$	$4.2 \cdot 10^8$	$3.0 \cdot 10^2$
Regenerating cream	$3.3 \cdot 10^9$	$10 \cdot 1.3 \cdot 10^9$	$10 \cdot 5.5 \cdot 10^8$	$10 \cdot 3.5 \cdot 10^8$	$1.2 \cdot 10^3$	$5.0 \cdot 10^8$	10
Moisturing cream placebo*	$2.0 \cdot 10^9$	$10 \cdot 2.3 \cdot 10^9$	$10 \cdot 4.7 \cdot 10^8$	$10 \cdot 5.3 \cdot 10^8$	$1.6 \cdot 10^7$	$6.6 \cdot 10^8$	$6.0 \cdot 10^6$
Moisturing cream	$1.2 \cdot 10^9$	$10 \cdot 1.1 \cdot 10^9$	$10 \cdot 1.2 \cdot 10^8$	$10 \cdot 4.7 \cdot 10^8$	$4.0 \cdot 10^2$	$2.3 \cdot 10^8$	10

\* Placebo ointments contained Triclosan only.

It can be seen that Triclosan was outstandingly effective in itself, since its antimicrobial effect was not decreased until the fourth superinfection of microbes and only in the case of the so-called moisturizing ointment. On the other hand, KATHON CG combination resulted in the reduction of the germ count by 4-5 orders of magnitude in one week even after the fourth and fifth superinfection of microbes. Further investigations are still in progress, nevertheless, our combination proved to be outstandingly effective in the preservation of emulsions.

## Summary

Surveying the present state and scope of preservation procedures, some prospective trends can be observed. New methods, mostly various pretreatment procedures, are enlarging the technological repertoire, from which the combinations of chemicals and heat treatment, and especially the heat and gamma irradiation procedures are remarkable.

In the field of chemical preservatives, the effective, new compounds are scarce which is

mainly due to rigorous regulations concerning the introduction of new compounds. This can be observed mainly in the pharmaceutical industry, but the number of the new products is decreasing in the cosmetic industry too.

To compensate this negative trend, a rapid development occurred in the research of preservative combinations. The new and effective combinations can be classified in three groups: first, combinations of several genuine preservatives, second a genuine preservative plus potentiating additives, and third, multicomponent systems containing several genuine preservatives and potentiating agents.

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# Where does the Pharmaceutical Industry get Pure Water?

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Water is an excellent solvent, so naturally it is one of the important raw materials in such industries as cosmetics and electronics. But in the manufacture of medicines in particular, water is important as a transport medium when medicines are administered orally or parenterally to people and animals. Most of us regard the drinking water out of the tap to be quite pure, and it is. (Figure 1). However, the quality requirements by the pharmaceutical industries are considerably higher.

Let us take, for example, the extremely strict purity requirements for water used in manufacture of medicines, especially parenteral injection solutions. The “Bible” of medical manufacturers, the national pharmacopeia<sup>1</sup>, defines purified water as an extremely pure chemical. For the permissible maximum amount of salts in the water, it states that the evaporation residue can be a maximum of 1 mg in 100 ml of water, (10 p.p.m.) which already means 99.999% purity.

There must not be any viable bacteria in the water, but also not dead bacteria-based pyrogenic material, i.e. endotoxins<sup>1</sup>. The maximum is 0.25 EU/ml, which would be only 0.000025 mg (0,000025 p.p.m.) in a single liter of water. Only then is such water considered safe to be injected. It will not create the need for product recall or danger for patients.

How is water as pure as this obtained? In practice, there are two permissible methods nowadays, i.e.

- reverse osmosis (R.O.), which means hyperfiltration of water<sup>2</sup> or the other way, which has been in use in ancient times already
- Distillation, i.e. evaporation of water and condensation of the produced steam back into water.

When a practical-minded, industry representative sees an advertisement such as Figure 2, distillation of water appears to him to be a totally inefficient, unbelievably expensive process. Are things really all that bad?

## Purification by Reverse Osmosis

The United States Pharmacopeia permits use of reverse osmosis for production of water in this highest quality category, i.e. water for injection. The membrane material used in reverse osmosis stops bacteria, viruses, and their residues. Part of the salts however, get through anyhow. RO-technology is rather complicated, and requires a number of other devices to complete the system but as well a strict control of the product. If a leak should occur in the membrane, dangerous impurities would not be filtered<sup>2</sup>. Thus, careful quality control of the product is essential. Nevertheless, when the system is finally in operation, operating costs are relatively low. Costs begin to increase when it becomes necessary to replace fouled or blocked membranes.

### DEGREE OF PURITY FOR VARIOUS TYPES OF WATER

Water	Content mg/liter (p.p.m.)	Purity %
<u>Naturally occurring</u>		
Atlantic Ocean	35000	96.5
Brackish waters	~10000	~99.0
<u>Treated waters</u>		
Mineral waters	~ 2700	~ 99.73
Good drinking water	200	99.98
<b>BUT</b>		
Purified water, at least	<10	~ 99.999
Water for injection made by distillation	<1	>99.9999
Absolutely pure water (theoretical)	0	100

Figure 1. Degree of Parity for Various Types of Water.

# Purification by Distillation

The device used in the primitive distillation process is shown in Figure 3. Water is evaporated with heat generated electrically, by steam, or by combustion. The vapor produced this way is conducted into a condensing device where a pipe coil with cooling water circulating through it carries off heat from the vapor, permitting it to recondense back into water. And thus, pure, distilled water is obtained. Some of the used cooling water, now heated, can be reused as feed water to the still. Thus, a small part of the energy investment stays in the circulation. So just why is distillation such an expensive process? To start with, water has exceptional thermo-dynamic characteristics (Figure 4). Distillation means that the water temperature will first have to be raised to its boiling point (100°C at sea level). Just 85 kilogramcalories (= 355 kj) is needed to heat 1 kg of water to the boiling point, if we assume the feed water from the tap is 15°C. But, the conversion of water into 100°C steam requires more than six times as much energy, i.e. 539 kcal per 1 kg (= 2250 kj). And conversely, to condense that steam vapor back into water, all that energy used for the evaporation must be removed from the steam by condensing it with cold water. As 1 kg of cooling water can only carry off about 50....60 kcals, getting rid of 539 kcals will require 10 times as much cooling water as distillate is produced!

R.O. System

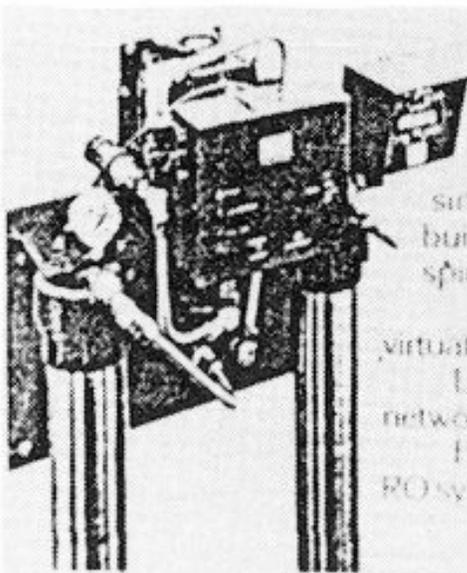
Can beat

your

distillation

costs

by 80%



Reverse osmosis systems do essentially the same job as distillation. For one-fifth the cost.

And water processed by our RO systems meet USP XIX standards for "water for injection" when fed by deionized water.

Our RO systems also give you features that make water purification simpler and more efficient: automatic operation, solid-state controls, built-in conductivity monitoring, and a water saver. Plus the most reliable spiral membranes in the industry--guaranteed for a full year.

The system shown can process 1600 gallons per day. And it's virtually maintenance free.

Like all our water purification systems, it's backed by our complete network of support centers and field service engineers.

For more information on our full line of cost-saving RO systems, write.

Thus, the reverse osmosis advertisement is quite right about the efficiency of process described above. This type of single-effect i.e. conventional, primitive still can be used in practice only for very small-scale production.

The days of the distillation method in mass production would indeed have been numbered unless better methods had been developed. Clearly, to attain better distillation methods, one has to create a situation wherein the heat of evaporation is transferred back to the process to be used again and again.

Several technical solutions have been developed over the last 100 years. The most popular method nowadays is multiple-effect distillation<sup>4</sup>. Its popularity is partly also based on the fact that such a distillation device has no moving parts.

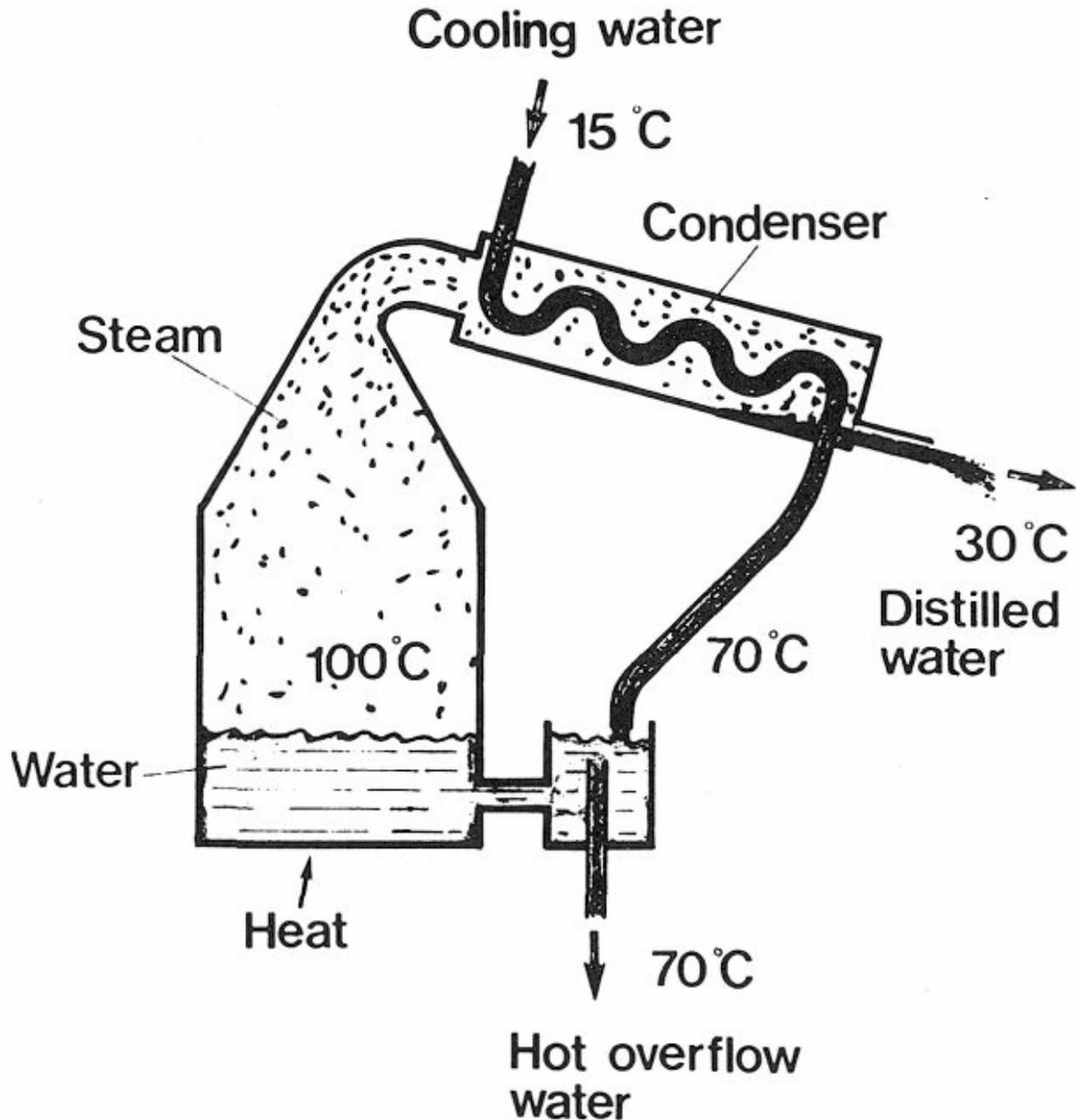


Figure 3. Conventional Distillation Set-up.

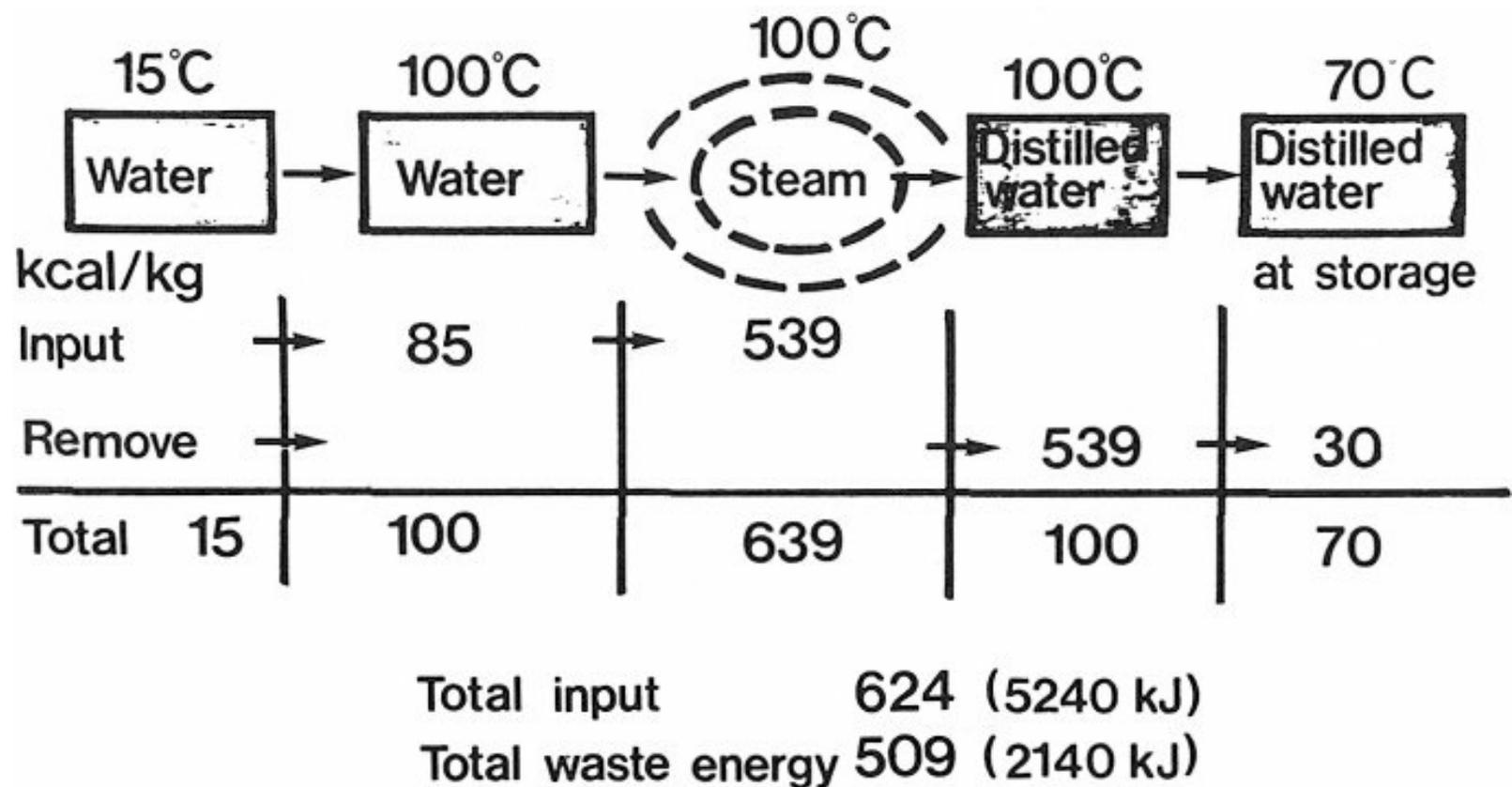


Figure 4. Thermodynamic Characteristics of Water.

## Multiple-Effect Distillation

Multiple-effect distillation means that distillation is performed in several phases, stages<sup>5</sup>. This concept is illustrated in Figure 5. When the steam temperature difference can be created, the energy input can be reused several times. This is achieved by using step-by-step decreasing steam pressure. The operation becomes more economical the more there are stages.

The heating energy is fed in a multiple-effect only into the first stage. This stage generates over-pressure steam, which is used for heating Stage 2; the over-pressure steam from 2 is used for heating Stage 3, and so on. When the steam is used for heating, it condenses and comes out as distilled water.

A condenser is only needed after the last stage to cool the steam generated by this stage. Now the energy consumption values are already totally different! 20 kW is enough to distill 75 kg an hour and a mere 250 kg of cooling water is needed. And we only have three stages!

As the feed water to be distilled is first used as cooling water, it and its heat are not wasted, and thus, the still can be made even more economical.

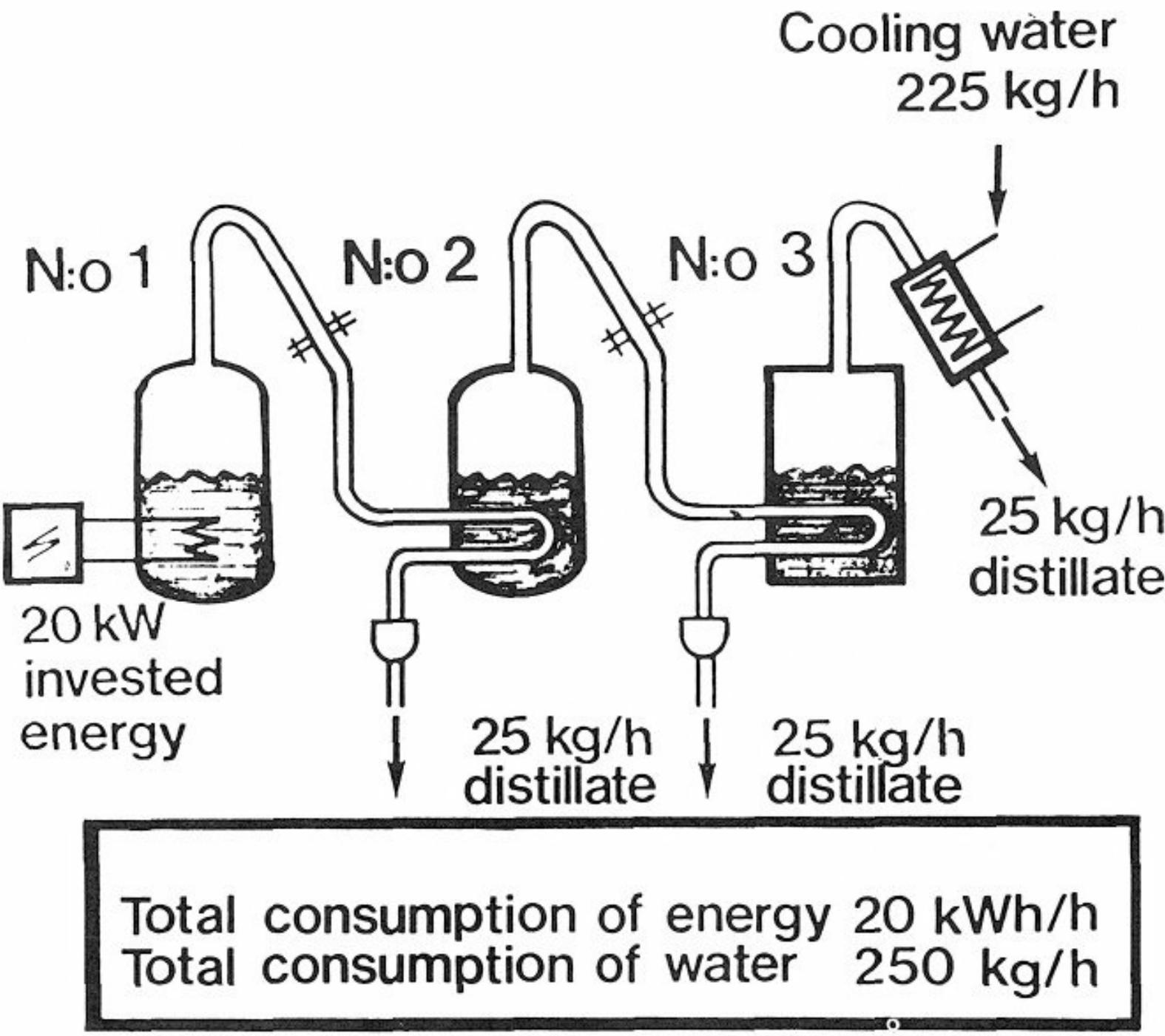


Figure 5. Multiple-effect Process 20 kW – 75 kg/h.

Example:  
 With six stages, 80% of the energy fed into the still circulates in the process, and no additional cooling water is needed at all. Figure 6. Thus, our first problem is solved, i.e. how we can distill water at low cost.

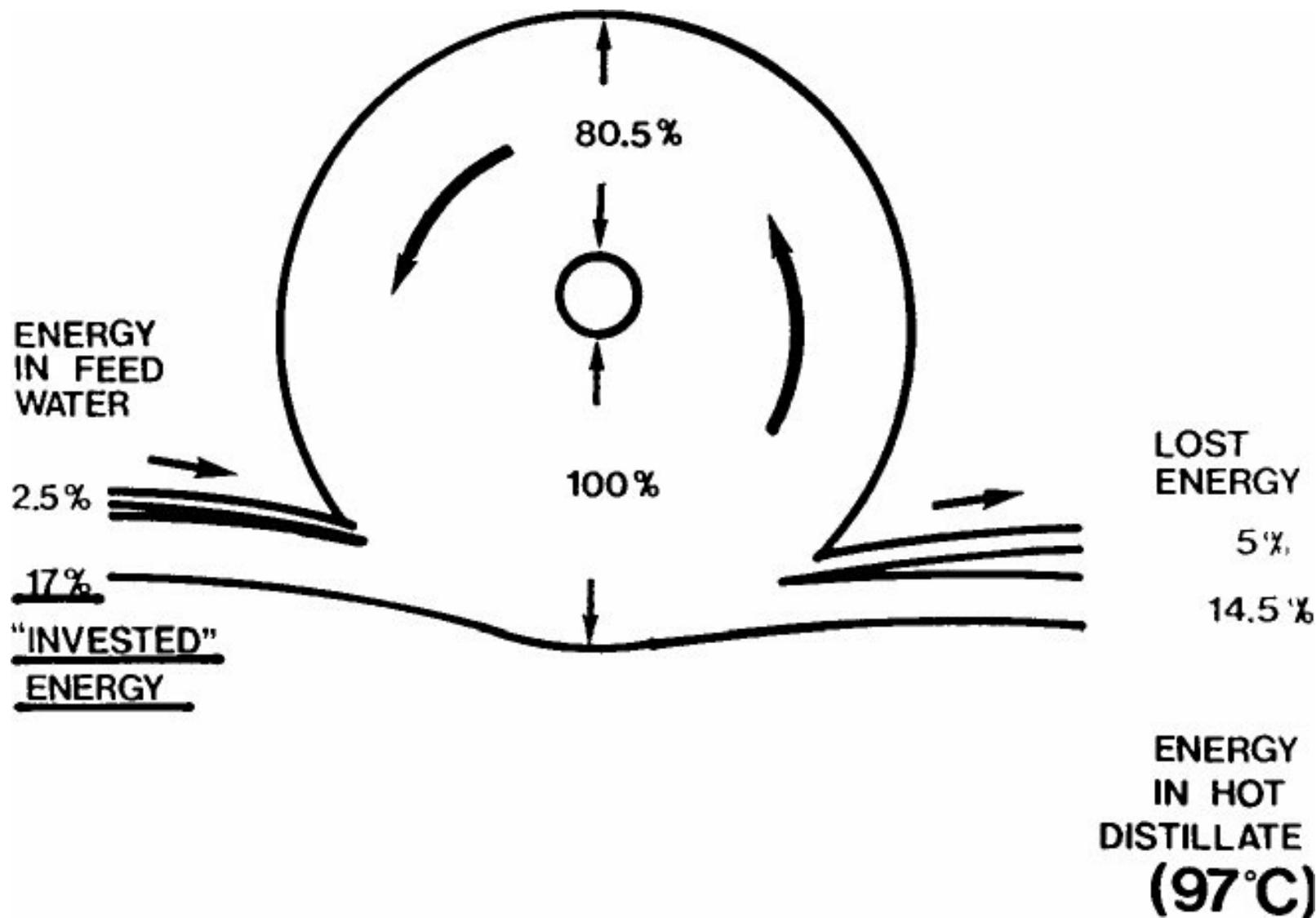


Figure 6. Energy Circulation in a 6-effect Still.

## Production of High-Quality Distilled Water

Energy requirements and water consumption are reduced enormously by the multiple-effect method, but that is not the same as to meet Water for Injection (W.F.I.) requirements: extremely high water quality creates another set of challenges to the process designer.

I mentioned in the beginning, the greatest permissible salt content residue and the maximum amount of bacterial waste, endotoxins, known as pyrogens in the water for injection. Further, three other important features are required of pure medical water:

- gases in the water must be removed
- the size and amount of the particulate matter i.e. particles must not exceed some very low set values
- volatile substances in the water which are also distilled have to be removed

Thus, the requirement has several parts (Figure 7). A "perfect" still has to be economical, but also fulfill all the five conditions of purification efficiency. A complete solution

for all this is the FINN-AQUA distillation column<sup>4</sup>, developed in 1971. So there is an answer to our theme question today. The pharmaceutical and cosmetic industries can now obtain highest quality pure water reliably and economically using FINN-AQUA multiple-effect stills.

## **WHAT A MODERN STILL MUST DO:**

---

### **To have**

- low energy consumption**
- low water consumption**

### **The product must have**

- low total solids < 10 mg / liter**
- very low endotoxin content < 0,0000125 mg/liter**
- very low number of particles**
- very low amount of volatile pollutants and gases**

**The  
answer  
is  
the  
FINN -  
AQUA  
water  
still**

Figure 7.

## **The Operating Principle of the Column**

The FINN-AQUA column ingeniously combines the feature of small size, great efficiency and an exceptionally high degree of purification<sup>4</sup>. Figure 8 shows a cross-section of the column. The central section is composed of a group of parallel tubes surrounded by steam which heats them. The water to be evaporated flows downwards through these tubes. It flows down the surface of the tube as a film, and due to heat exchange, part of the water evaporates. A mixture of steam and water arrives at the bottom of the column with enormous speed. The vapor then makes a sudden turn upwards, while most of the droplets are shaken off at this point to remain at the bottom of the column. Spiral vanes conduct vapor through a large ring shaped vapor path. The vanes force the rising vapor to rotate faster and faster as it moves upwards around the central section of the column. Viewed from above, the process appears as follows: (Figure 9)

The heavier parts in the high-velocity vapor, which include the remaining smaller droplets and particles as well as endotoxins, move towards the outer rim of the moving vapor flow due to centrifugal force. Convenient openings in the jacket allow impurities and droplets to pass through to the space between jacket and the outer wall. Extricated from the vapor flow, the particles and droplets fall back to the bottom of the column. Only gas, the purified

steam passes out of the top of the column.

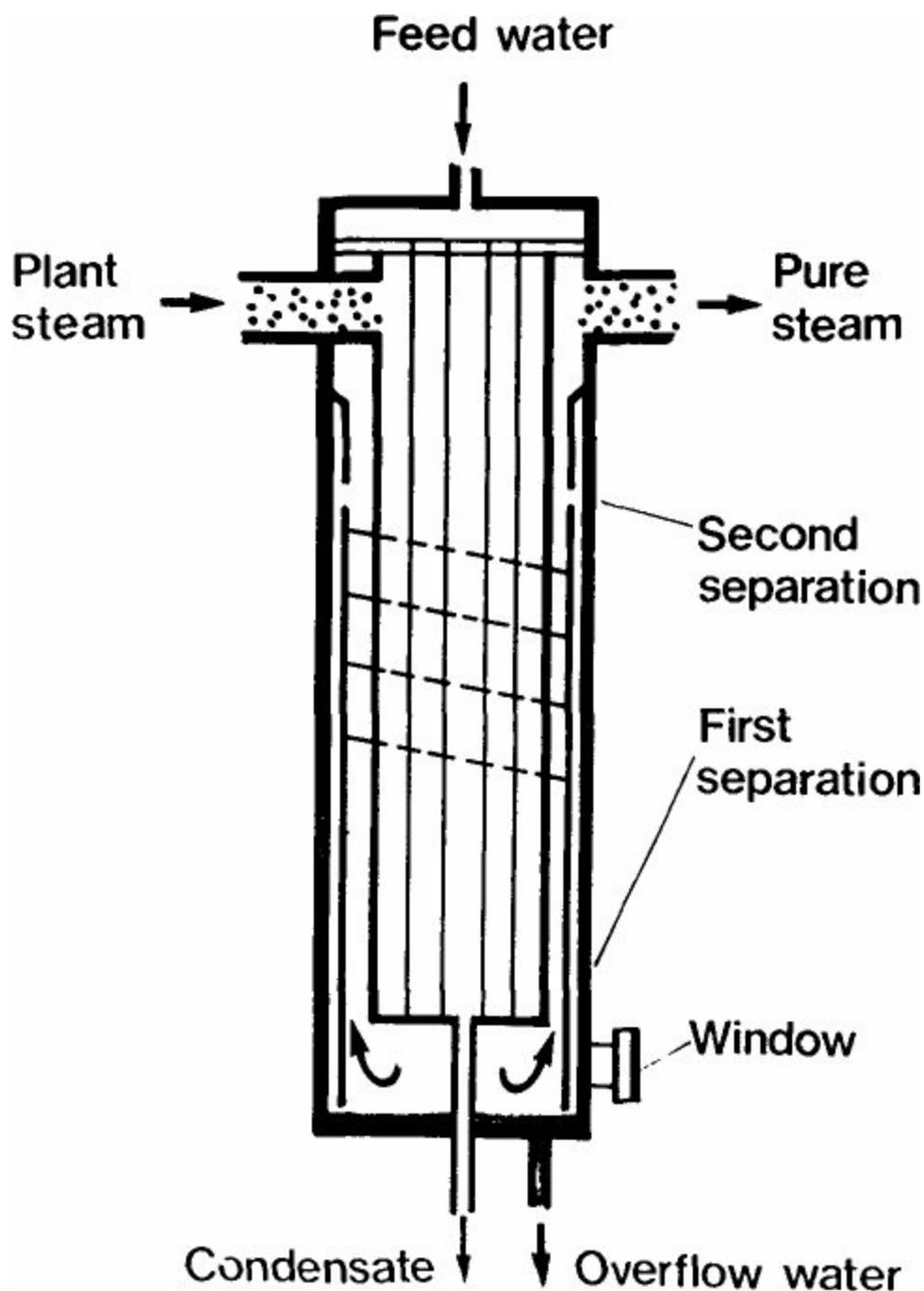


Figure 8. FINN-AQUA Columns

The separation of impurities is efficient precisely because of the immense centrifugal force as high as 100 Gs. The rate of fall of particles from the steam is thus very great in relation to free fall in earth's gravitational field. Particles down to 3-6  $\mu\text{m}$  leave the steam in this way<sup>6</sup>, without the use of any filtering devices. This is far better performance than required for production of WFI according to the U.S. Pharmacopeia.

Thanks to these features, steam of extremely high quality is produced easily by the FINN-AQUA column with only a single evaporation cycle. There is an appreciable safety margin with regard to the required maximum limits.

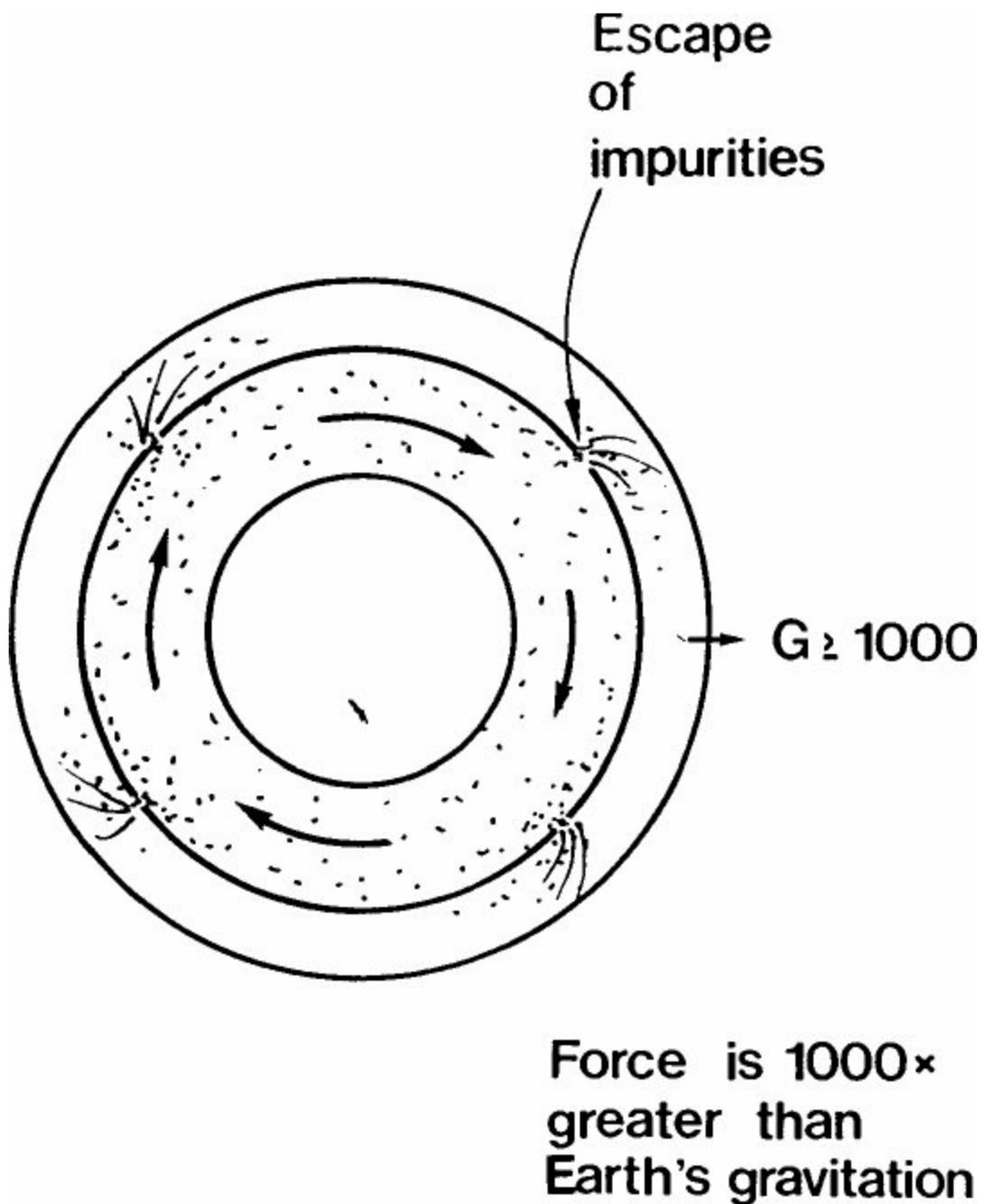


Figure 9. How Steam is Cleaned by Centrifugal Field.

Applied as a multiple-effect still several FINN-AQUA columns have been arranged in series<sup>5</sup>, usually a minimum of four and a maximum of six stages. After the final stage in the series, there is a condenser and gas remover (Figure 10). The first stage, column 1, is heated by an external energy source such as plant steam or electricity. The feed water is first used as cooling water in the condenser. Thus, the feed water is already preheated and further heated as it passes through each column. When it finally arrives in Column 1 for evaporation, it is very close to boiling. Thus, the process features a remarkably rapid start-up. All distillate and the steam produced by the last column is collected in the condenser, which is kept a little over 100°C so that nearly all of the gases in the distillate leave with a small amount of steam which flows through a gas exit opening. Very little additional cooling water is actually needed because it is only required to complete the operation of the condenser, in fact, cooling water is hardly needed at all in stills with 5 to 6 stages,

depending on the temperature of tap water. I still would like to present a diagram (Figure 11) showing how increasing the number of columns reduces operating costs; however, the price of the still naturally rises with more columns. The most economical number of columns is determined by comparing the costs to the yearly operating time of the still.

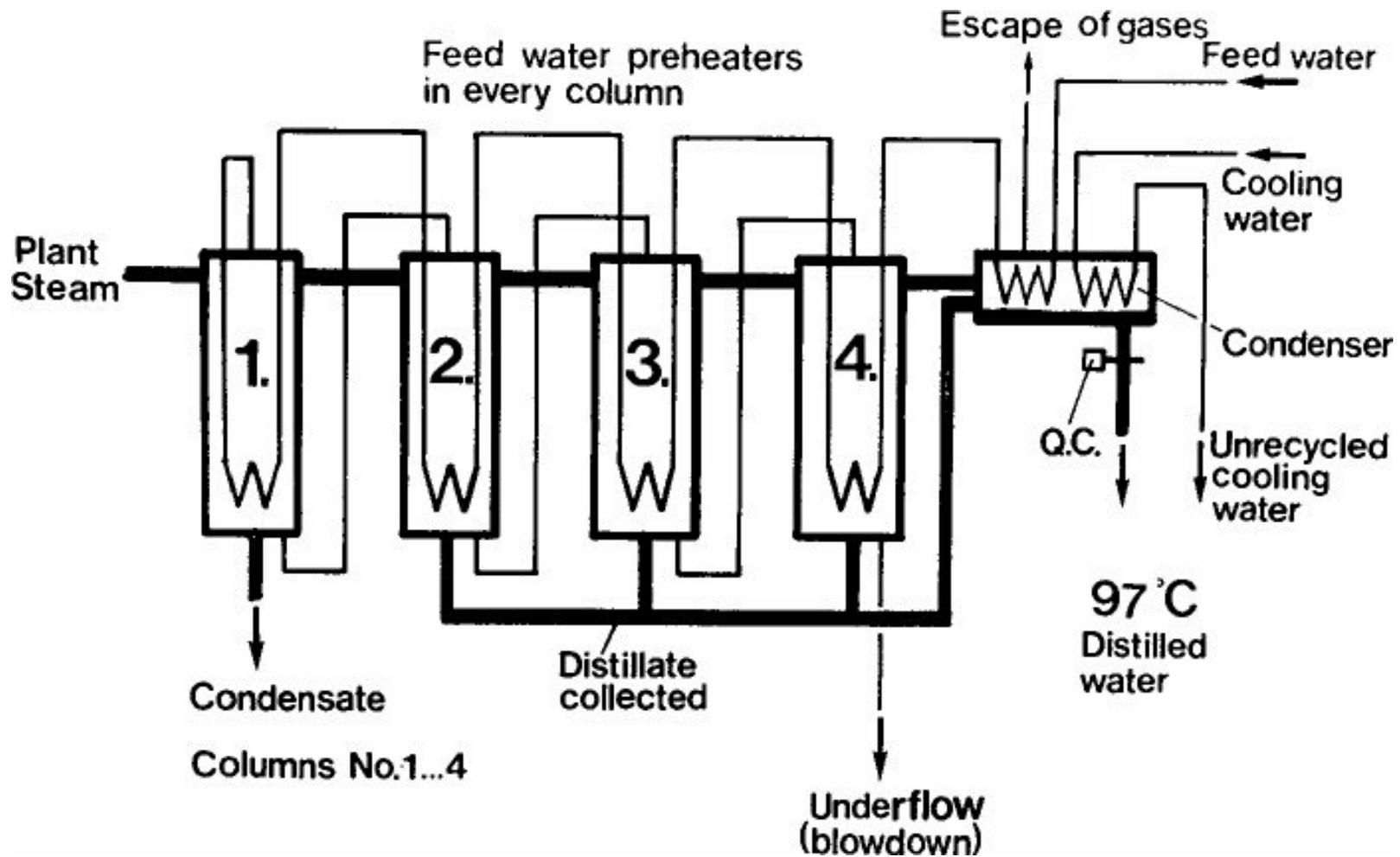


Figure 10. Parts of a Complete FINN-AQUA Still.

## The Construction of the Still

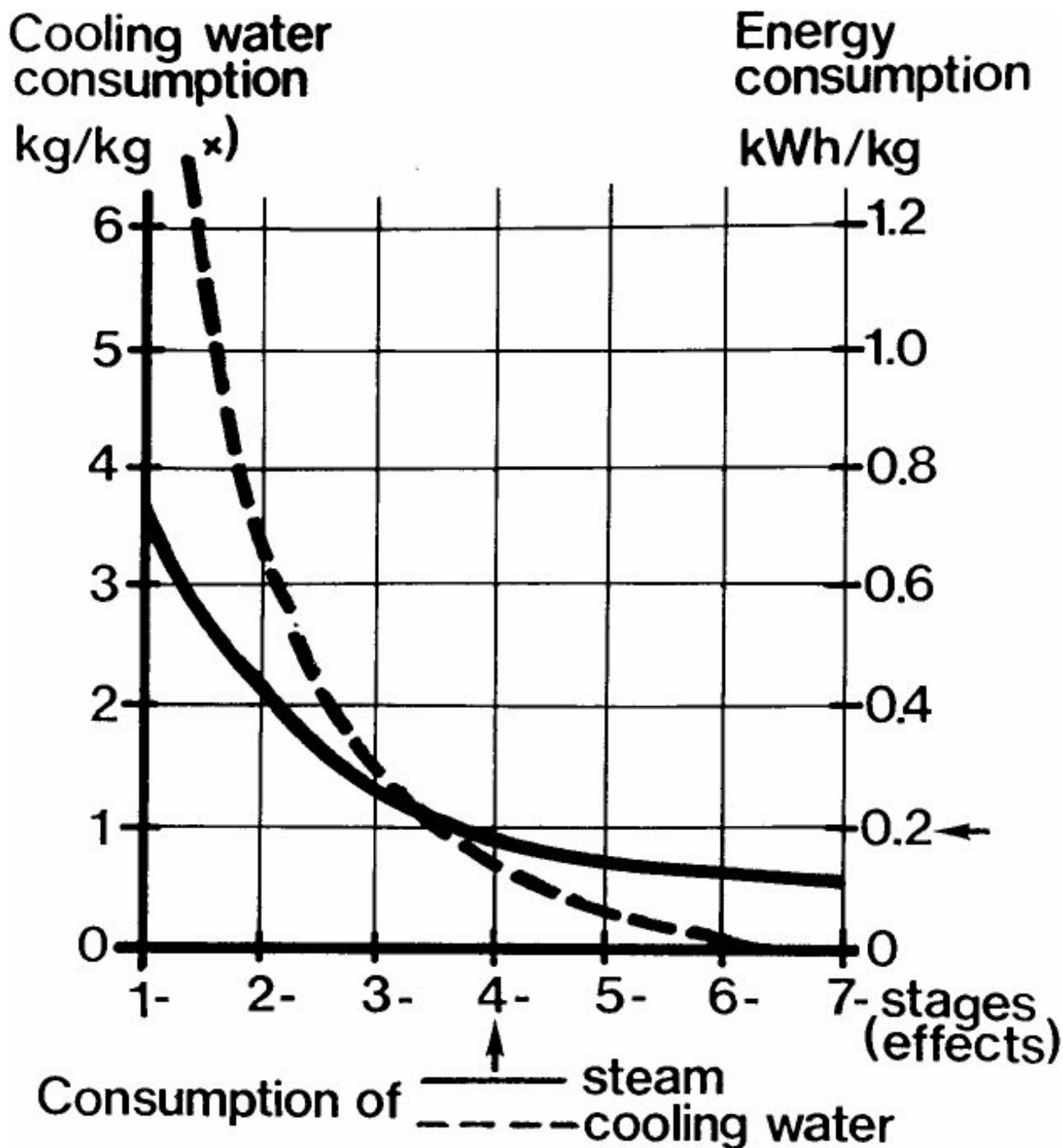
One of the most important reasons for the popularity of the multiple-effect still must be that it has no moving parts. The wear of the still is negligible and the need for service practically nonexistent as long as operators observe the natural precaution that no hard water is fed into the still.

Since the stills are made of a highly durable material, low-carbon acid-resistant steel, their operating time will be appreciably longer. The surfaces in contact with steam are recommended to be electro-polished so that corrosion caused by wear is insignificant<sup>7,8</sup>.

As there are no internal pumps, the still is noiseless. It is so reliable that it can be fully-automated and used 24 hours a day, but there are also other advantages for the buyer of FINN-AQUA multiple-effect still.

# Production of Pure Steam

Cleaning and disinfection of containers, tools, pipes and so on is increasingly done with pure steam, i.e. steam which is as pure as W.F.I. If such steam is only needed for short intervals, it can be taken from the pipe between the columns 1 and 2 of the multiple-effect still (Figure 12). This is an additional advantage achieved by only adding one valve<sup>4</sup>. The still continues to produce distillate despite this, although the efficiency is reduced.



x) single stage still takes 10 kg cooling water / kg!

Figure 11. The More Columns, the Greater the Economy of the Still.

But when there is a continuous need for such steam, an ordinary pure steam generator is recommended for production of such steam only (Figure 13)<sup>4,6</sup>. The machine can be provided with a small additional condenser so that during times of no pure steam production, it can function as a laboratory size still for W.F.I. In this case, the machine operates naturally as a single effect still and does not save energy (Figure 14).

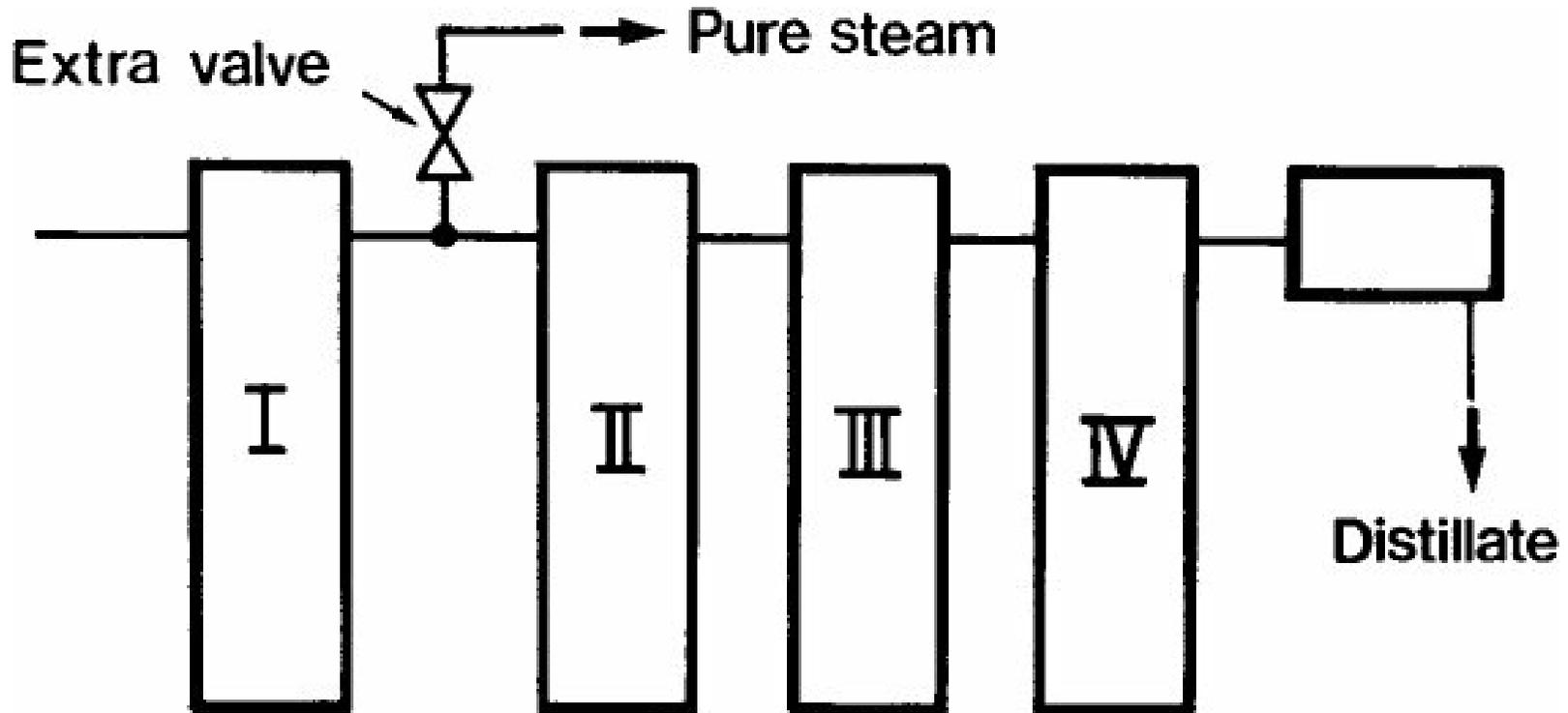


Figure 12. How Pure Steam Can be Received from a Still During Operation.

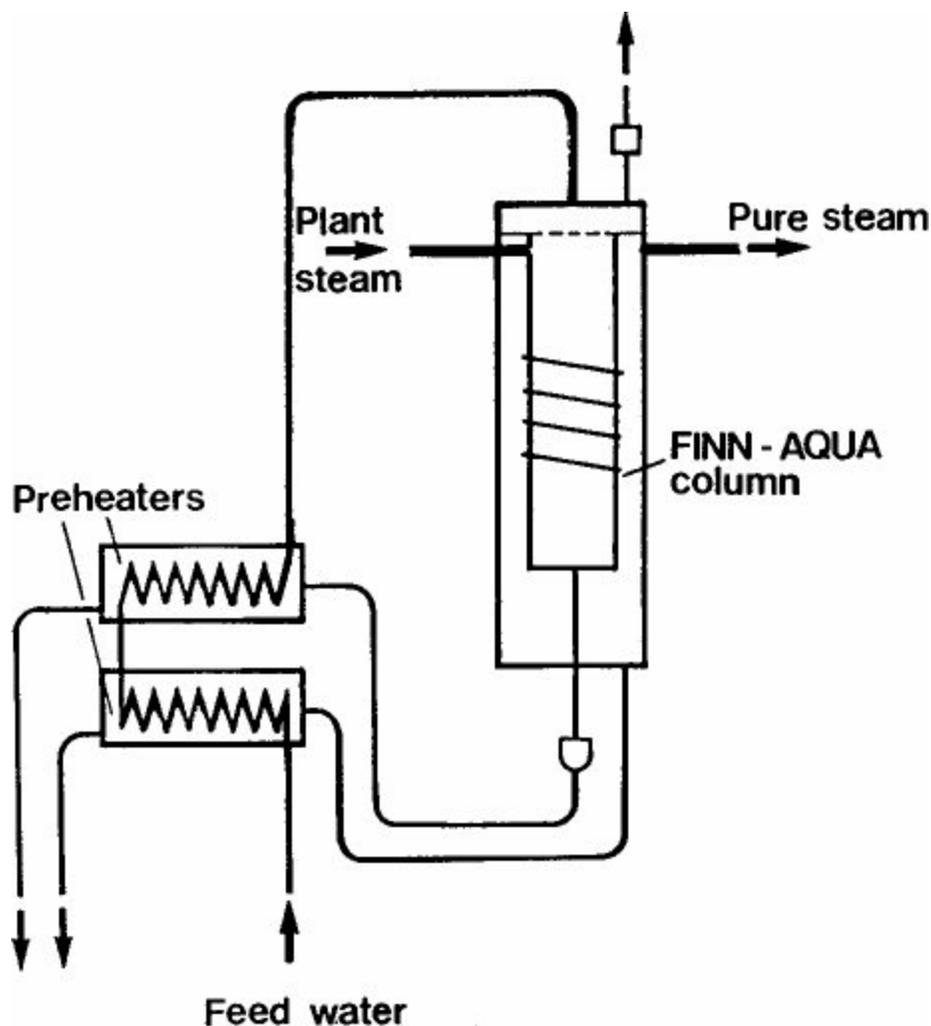


Figure 13. Pure Steam Generator.

## Dimensions of the Stills

The features of multiple-effect stills make them best suited for mass production of pure water. The smallest still of the series distills 75 kg and the largest 11000 kg an hour. When such a still is put to generate pure steam as a by-product, it can produce it as 3 to 10% of its distillation capacity.

## Dimensions of the Pure Steam Generators

Actual pure steam generators are built between the dimensional limits 110-3750 kg production of pure steam an hour. A distillate condenser attached to such a still can produce 50 — 200 kg of distilled water an hour.

## Storage and Distribution of Distilled Water

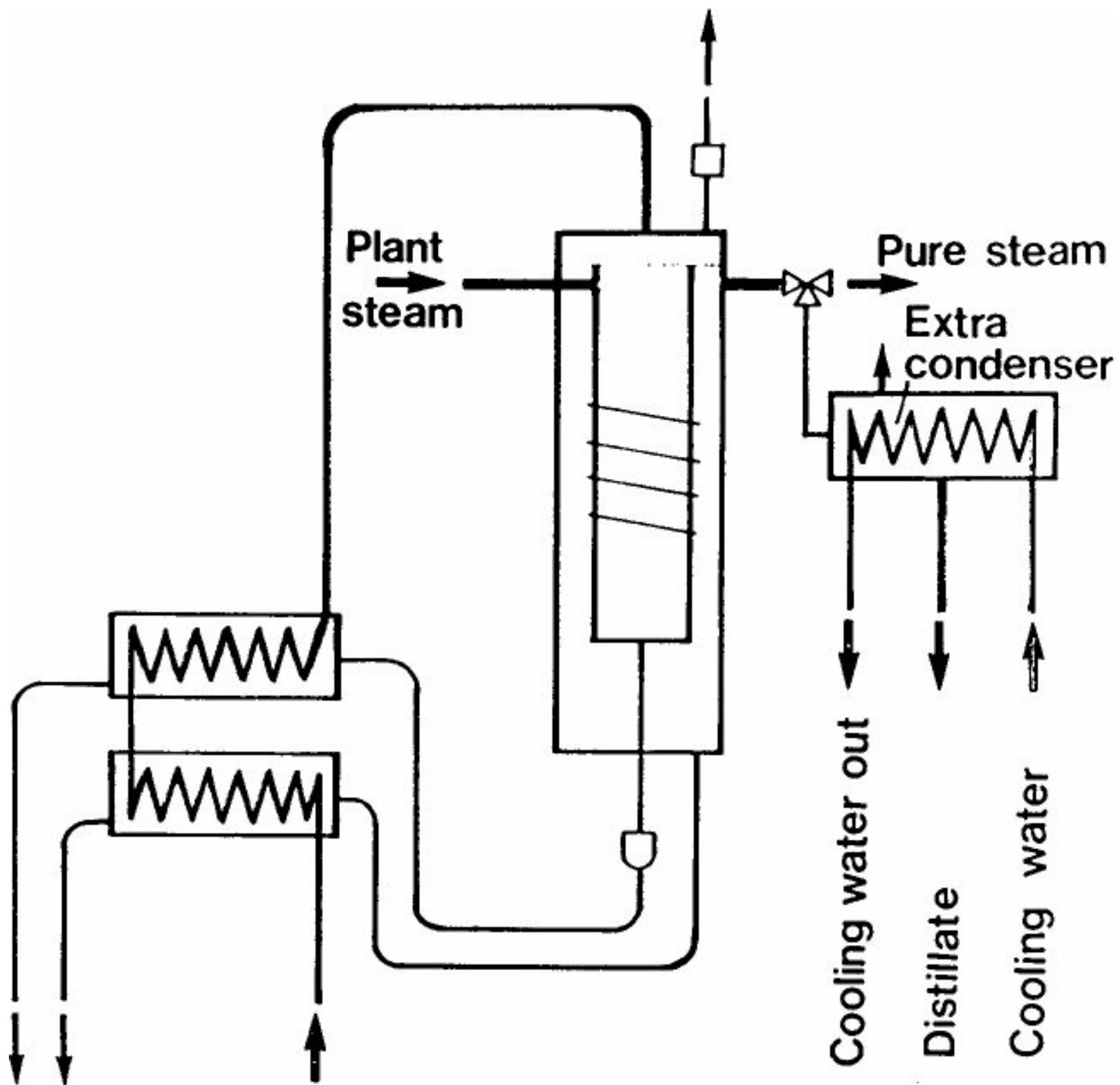


Figure 14. Pure Steam Generator as a Still

Storage and distribution of distilled water intact and of almost as high quality as when it first came from the still requires particular attention and special equipment. To this purpose, we have developed a complete technology which is available to our clients (Figure 15). However, due to lack of time, it is not possible to present this technology here, but we are happy to get acquainted with our client's needs and make suggestions.

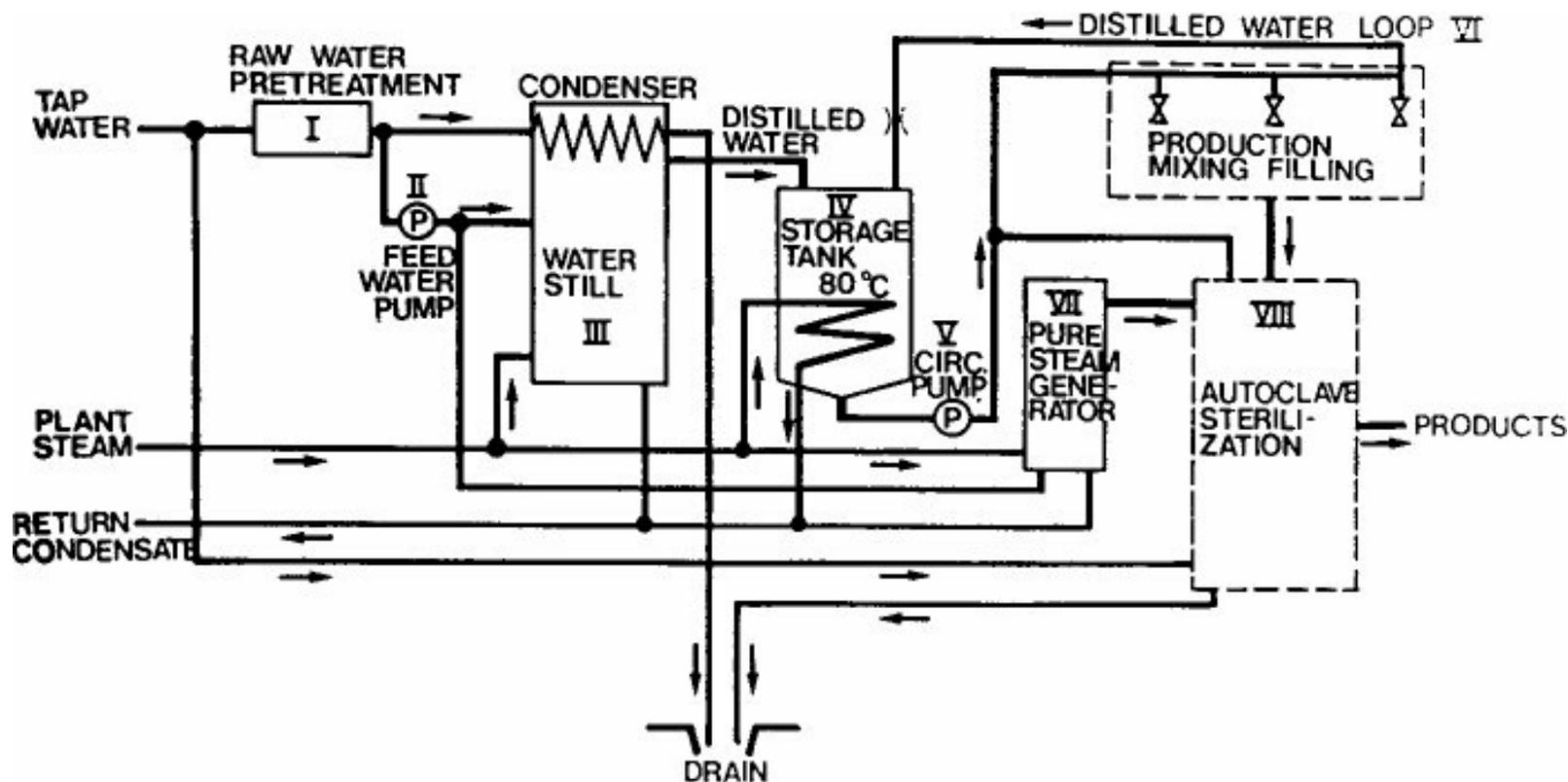


Figure 15. "Injection Water" Production, Storage and Distribution System "FINN-AQUA".

Before we finish up this morning, I would like to show you some slides which clearly show the small dimensions and configurations of our stills.

I thank you for your patient attention, and naturally I would be happy to answer any questions you may have during the break or later.

## Summary

From the point of view of economy and the quality of distilled water, the most efficient method to produce pure water is multiple-effect distillation. When this principle is applied so that the still unit is made up of FINN-AQUA columns, an extremely high quality of the product is also achieved<sup>9,10,11</sup>. Thus, the FINN-AQUA multiple-effect still competes successfully with other similar devices and is usually the winner. If necessary, the multiple-effect still functions as a generator of pure steam.

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# Session VI

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Canadian Health and Welfare  
Ottawa, Canada.*

# Overview Worldwide Regulations Governing Sterile Medical Products

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Mr. Chairman, Ladies and Gentlemen, the topic of my presentation is Overview—Worldwide Regulations Governing Sterile Medical Products outside the Peoples' Republic of China. It is indeed a vast subject to cover in 30 minutes, especially considering the abundance and diversity of such regulations. As this is the only regulatory presentation during the conference, and the first one on this subject for many of the participants, I feel it is my duty to cover all the pertinent fundamental issues. I must therefore limit the scope of my paper in such a manner that these issues can be presented in a comprehensible manner. I shall therefore:

## *(1) Focus on medical devices*

Sterile medical products tend to fall into four distinct categories: drugs, parenteral solutions, medical devices and diagnostic products. Each class has its own set of problems in terms of the regulation of sterility and should be dealt with separately. To keep the presentation within a manageable scope, I shall focus on medical devices as this is my particular area of expertise.

## *(2) Focus on Europe*

The major areas of medical device regulation are currently North America, Europe and the more developed countries of the Pacific Basin. I shall focus on Europe as there is here a diversity of regulatory models and an intensity of emphasis on regulating sterility assurance that is unparalleled in other regions of the world.

## *(3) Focus on the Objectives, Methods and Context of Regulatory Control of Sterile Medical Devices*

The regulatory authorities define certain objectives that manufacturers must meet in order to sell sterile products. The correct carrying out of these objectives must be verified by regulatory authorities using suitable methods of regulatory control. Finally, we must take

a critical look at the role of sterility assurance in the general context of regulating medical device safety and efficacy.

## 1. Regulatory Objectives in the Control of Sterile Medical Device

These objectives fall into five categories as shown in Table I.

**Table I. Objectives in Regulating Sterility Assurance.**

1. Define sterility in a manner that its achievement can be verified.
2. Promote a sterilization process that ensures sterility of the product.
3. Eliminate adverse effects resulting from sterilization.
4. Establish packaging and storage requirements that maintain sterility.
5. Impose labeling requirements that ensure that the end-user is supplied with adequate information related to the sterility of the product.

### *1.1 Definition of Sterility*

The traditional approach to sterility assurance was based on defining sterility as absence of viable microorganisms. Sterility was proven by sterility testing. Samples would be taken, incubated in a suitable growth medium and, if microbial growth was observed, the product was not sterile. Control of sterility from the regulator's point of view is very simple, all he needs to see are batch certificates of sterility. This approach is recognized as inadequate by the modern regulator.

The necessity of an absolute assurance of sterility has become imperative with new medical device technology. For instance, infection at a prosthetic implant site can lead to a risky reoperation with consequent removal of implant and additional tissue trauma.

The answer of the regulators is to define sterility in terms of an overkill. A product can be considered as sterile if there is a probability of only one product in a million still carrying a viable microorganism. It is usually represented in the context of sterilization as a log reduction of viable microorganisms to the  $10^6$  level.

There is no doubt that the overkill approach results in better sterility assurance. But is its indiscriminate application by the regulator rational? The purpose of regulation is to define what is an acceptable benefit/risk and benefit/cost ratio from the society's point of view. An overkill approach of this magnitude raises the economic cost of sterilization and subjects the device and its package to the harsh conditions of sterilization longer than might be necessary.

While that might be necessary for highly critical devices such as cardiovascular or orthopedic implants, it could be argued that this is not the case for less critical products, e.g., wound drainage devices.

**We should not lose sight of the fact that the overkill concept is a statistical one, not one**

of absolute certainty. This issue is debated in North America, but European regulators do not seem to be willing to depart from the definition of sterility as a probability of  $10^6$  yet. There is no product release test than can certify a SAL of  $10^6$ . The regulator must therefore look at the process of sterilization itself to exercise regulatory control.

## 1.2 Sterility Process

Regulations deal increasingly with the whole sterilization process rather than the end result. Sterility testing has survived in the form of testing of biological indicators or mass produced medical devices. But the purpose is to detect any gross failure of the sterilization cycle. In this sense, sterility testing has become a test of the process rather than of the product.

Questions asked by regulatory authorities from manufacturers are even more explicit in this regard. Typical items of information that must be provided to the authorities are given in Table II. This is actually a composite of requirements as no single European country asks all of the information listed.

**Table II. Sterilization Process Information Required by Authorities of Various European Countries.**

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1. Average bioburden and their alarm limit.
  2. Sterilization cycle parameters, e.g., for ethylene oxide (preconditioning, gas concentration, humidity, pressure, temperature gas dwell time, aeration cycle).
  3. Product load configuration.
  4. Sterilization equipment (type, technical drawings, maintenance schedule).
  5. Type, number, location and D-values of biological indicators.
  6. Incubation media for biological indicators.
  7. Reference of sterility test method (e.g., USP, Euro. Ph.).
  8. Inactivation level claimed (e.g.,  $10^{-6}$ ).
  9. Packaging materials used.
  10. Description of any validation procedure.
- 

There is recognition by the regulator in this line of questioning that sterility assurance should be based on process validation, i.e., all the relevant factors related to initial contamination and the efficacy of microbiological inactivation must be considered and subsequently a process to reach the appropriate level of sterility must be developed and tested. This implies that each combination of factors (bioburden, device type and sterilization method) is unique and cannot be regulated in detail.

Yet the regulator is tempted to establish universal guidelines for the sterilization process. So far, these guidelines have remained voluntary in practice and too general to be really constraining. The manufacturer is still relatively free provided that he can convince the authorities that an adequate sterility assurance level is reached.

Unfortunately, there are also disagreements between authorities of different countries on some aspects of the sterilization process, e.g., the use of biological indicators. Until now, these disagreements have not resulted in trade barriers but such development is not inconceivable.

### *1.3 Adverse Effects Resulting from Sterilization*

Sterilization of medical devices by irradiation is authorized and even encouraged in virtually all European countries. Regulatory authorities in these countries do not seem to be concerned about radiation-induced changes on materials. The one notable exception is West Germany, which has largely restricted the use of this method.

Ethylene oxide sterilization is rapidly becoming a nemesis of the regulators. There is general agreement that it is dangerous. The real issue is how far its use should be regulated. While outright banning of this highly toxic chemical would be impractical, exposure to it is being regulated both as a work place contaminant and as a residue on the products.

Typical proposals and texts on the control of ethylene oxide in work place environment tend to limit its time weighted average concentration in the air of 10 to 50 parts per million. Some countries have gone very far in limiting residues on product, e.g., 2 ppm in France and Italy. Unfortunately, the economic cost of these regulations is heavy. Practical considerations are sometimes also overlooked, e.g., is the product likely to release EtO residue into the patient, is there a method available to detect a low level of residue, etc.

Steam and dry heat sterilization are often mentioned in regulatory texts but do not present any major problems to the regulator. Some countries (e.g., Holland and Spain) are now considering the four above mentioned methods as the only allowed ones. Any other method, e.g., formaldehyde solution, would require specific authorization.

### *1.4 Packaging and Storage*

The package of the sterile product should play the twin role of being permeable to the sterilizing agent, and impermeable to contaminating microorganisms. Apart from a few exceptions (e.g., French Pharmacopeia), regulatory texts on packaging of medical devices tend to be very general. They might state the obvious need of an effective sterile barrier and mention that any opening of the package should leave the breach of the sterile barrier visible.

The reason for this regulatory omission is probably partly due to the lack of serious scientific studies on the performance of packaging materials and seals as sterile barriers. Regulations on storage are also very general. More specific is the tendency of some countries to limit shelf-life to five years. There is no scientific basis to this other than it is

longer than the average stock dwell time of a sterile medical device and seems to be a psychological dividing line between certain and uncertain future. It must be stressed that sterility and package integrity are much more event related than time-related.

## *1.5 Labeling*

All European regulatory texts on sterile products also list labeling requirements. This is to provide essential information to the end user, such as listed in Table III. Labeling has become a problem, not so much because of the nature of the information required, but because various countries require this information in their local languages. It is not unusual to see one label with the same information in English, German, French, Spanish, Italian and Japanese, for instance. As more countries insist on local language labeling, the likelihood of efforts becomes greater as does the cost of separate inventories and computerized labeling systems.

One can only hope that an international system of symbols will be agreed upon some day. We have seen what the objectives of the regulators are. But it is not enough to define these, the regulators must also make sure that the manufactures comply with these requirements.

**Table III. Typical Labeling Requirements in Europe for Sterile Medical Devices.**

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1. Description of the product
  2. Name of manufacturer
  3. Statement that the product is sterile
  4. Statement that the product is for one use only
  5. Method of sterilization
  6. Instructions for opening
  7. Storage conditions
  8. Shelf-life
  9. Batch code
  10. Date of sterilization
  11. Registration number of the product
- 

## **2.0 Methods of Control**

Generally speaking authorities control regulatory compliance in three ways: product batch, process or product type control.

## *2.1 Batch Control*

Product batch control involves controlling every lot that is placed on the market. At its simplest level, it is a documentary control, i.e., the manufacturer must supply appropriate batch certificates that certify that the product has been sterilized according to a specific method and that an appropriate sterility assurance level has been reached. This is a relatively simple control mechanism and can be integrated with normal customs procedures.

A physical control on the batch itself is rarer. It is still practiced in Belgium where all sterile products have to be resterilized or tested for sterility. This type of batch control system is of course expensive and of doubtful scientific value. Moreover, resterilization can be dangerous, even if the original method is used. The cumulative irradiation dose or wrong cycle parameters in EtO sterilization can have an adverse effect on the product.

## *2.2 Process Controls*

The most comprehensive form of process control involves inspection of manufacturers by authorities, usually to determine if they comply with a good manufacturing practice guide developed by the regulatory authorities. While GMP guides provide general ground rules for manufacturing (e.g., process documentation, product traceability, etc.), which in themselves are supportive of sterility assurance, they can also address the issue of sterilization itself. In practice, the efficacy of this system of control depends on the quality of the inspectors and the frequency of the inspections. At its best, this system can be of great assistance to the manufacturer in detecting systematic problems. But obviously too much of a good thing can be a nuisance.

Fortunately for the medical device manufacturers worldwide, the only two authorities that currently carry out systematic international inspections are the UK and the US authorities. The manufacturers' nightmare is to have 10, 20, perhaps even 30 or 40 different countries carrying out these inspections. Imagine the cost, the disruption and the demands on management time if there was a week long inspection every second or third week, most of which would result in requests for changes in the manufacturing process.

Inspection schemes, however, are amenable to a solution of mutual recognition of factories and exchange of information. The success of the Pharmaceutical Inspection Convention, which groups both European market economy and socialist countries is proof of this.

Process controls need not involve factory inspection, however. In Holland, foreign manufacturers must submit sterilization process information to the distributor who will hold the documentation for an eventual inspection by authorities. Process control is carried out also to some extent in the context of product type control, which we shall examine next.

## *2.3 Product Type Control*

This involves registering each product type with the authorities of the country of sale. Application dossiers can vary from three pages to several thick binders and cover, besides

the sterilization process, such matters as component materials, R&D tests, clinical experience, quality controls, etc. Registration might be of a pre-market approval type, i.e., authorities' approval of the product is required prior to sales or of a simple notification type.

The notification system is useful in order to know what products are used in the country. The pre-market approval system is important in screening new materials, technological innovations and products that are new for the manufacturer. However, it is a system fraught with pitfalls. In general, countries that have adopted this approach have underestimated the number of applications that will be generated and the complexity of dealing with these applications. The review and approval process can last a long time (e.g., 3-48 months in Spain) as the authorities repeatedly request additional information. It can be characterized by communication difficulties, especially in the international setting, and thus jeopardize the transmission of accurate information to the authorities. Imagine the language problems resulting from a typical situation where a multinational corporation whose official language is English, has a manufacturing plant in Germany where the manufacturing documents are in German, and must apply for product authorization in Spain. Within Europe, the problem is still manageable as multi-lingual personnel can be found. On a worldwide scale, with different writing symbols being used, the problem is compounded.

Finally, the theoretical rationale of the pre-market approval system is to scientifically evaluate the safety and efficacy of the device, including the claimed sterility assurance level. Evaluation of the same product by several countries constitutes a substantial waste of resources. It can only be hoped that some day there will be international agreement for the mutual recognition of product approvals.

### **3.0 The Context of Regulation of Sterile Medical Devices**

The regulation of medical devices does not start in a vacuum, but usually in a context where a well-established regulatory system is already in place to control pharmaceutical products. The responsibility to develop regulatory controls and to implement them with respect to medical devices is often initially given to the same institutions and the same people who are in charge of regulating pharmaceuticals. The initial approach to medical device control is thus colored by pharmaceutical thinking. This pattern can be clearly seen in Table IV—Evolution of Medical Device Regulation.

Sterility is the most obvious aspect of product safety that is common both to medical devices and pharmaceuticals. Thus in most countries medical devices were defined by using the qualification *sterile* (Norway, Sweden, France, UK, Holland, Spain). In practice, this excluded the scope of the corresponding control of products that were not sold in sterile condition, e.g, products sterilized by the hospitals.

Additional aspects that reinforce this pattern are worth mentioning. In France, sterile medical devices are controlled using the Pharmacopeia mechanism. Pharmacopeia monographs are implemented by pharmacists employed by the industry and the hospitals. Generally speaking, the involvement of pharmacists as enforcers and persons responsible for the control of medical devices is characteristic of the Latin countries (Spain, Italy, France, Belgium). No other profession plays an equally important role in Europe in the

control of medical devices.

While the issue of sterility is important, there are other problems with the safety and efficacy of medical devices. Catheters break, heart valves can promote thromboemboli, pacemaker leads can disintegrate, etc. But I would like to briefly mention two examples that illustrate how the current regulatory approach to sterility might miss other potential problems caused by actual lack of sterility.

**Table IV Evolution of Medical Device Regulations**

Country	Devices Covered	Initial Emphasis	New Trends
Norway	Single-use sterile devices	Sterility	Biological safety of materials
Sweden	Disposable sterile devices	Sterility	Structural engineering safety; regulation of implants; reporting of defects
Germany	Implants, sutures and dressings	Chemically active devices of biological origin	GMPs sterility
France	Sterile surgical materials, sutures	Sterility	Packaging
UK	Sterile surgical products	Sterility assurance through GMP	Orthopedic product GMPs
	Pacemakers	GMPs	
Holland	Sterile medical devices	Sterility	Structural engineering safety; reporting of defects; warehousing
Spain	Sterile medical devices, implants	Sterility	Improvements in the administration of medical device control
Italy	Specifically named devices	Plastics, devices in contact with blood	New medical device classification system establishing graduated requirements depending on invasiveness, use of power source, etc.

The regulation of sterile medical devices is frequently structured so that only industrially sterilized medical devices are controlled. In western countries, this is virtually synonymous with single-use medical devices, i.e., devices that are used in one medical procedure only. However, hospitals will sometimes repeatedly resterilize and reuse these products that were intended for one use only. Such practice might be a much more important source of infection and product failure than any process carried out by the manufacturer. Of course, in the pharmaceutical field, this problem does not arise since drugs cannot be reused once they are administered.

My second example is based on the fact that the concept of sterility is, in practice, defined as an absence of bacteria or more complicated microorganisms. Viruses and the new suspected class of microorganisms, prions, are absent from these considerations. Yet diseases such as multiple sclerosis, AIDS and Creutzfeldt-Jakob disease and, possibly even various cancers, are thought to have causative mechanisms involving the smaller microorganisms. Medical devices may play a role in the transmission of these diseases. This is not only true of medical devices of biological origin but also of more “device” like products, e.g., brain electrodes in Creutzfeldt-Jakob disease and syringes and trial contact lenses in AIDS.

There is now growing realization in western Europe that the initial approach (as indicated in Table IV) has been too limited. This is particularly true for the two Scandinavian countries, Norway and Sweden, and UK and Holland, where authorities have started to ask questions from manufacturers about other safety aspects than sterility. Interestingly enough, these have concerned actual problems as they have occurred rather than dealt with systematic inquiries mandated by regulations. Thus, they are not representative of the regulatory focus of the country but reflect actual, real problems that in some cases have been politically very visible, e.g., problems related to the engineering and the component materials of pacemakers and heart valves.

The initial momentum of the original orientation is still continuing as can be seen from Table IV: It is now being diverted from the original pharmaceutical bias towards a more medical device specific bias. In some cases, the departure is not great, e.g., Norway, where the regulation allows this evolution. In other countries, there is a possibility of radical departure, e.g., Holland and Sweden, where the omissions of the regulatory control scheme has been widely publicized in the press, or because the need of reform is obvious to all, e.g., Germany and Italy. In the end analysis, the degree to which the old system can dominate the new emerging one depends on the political will to change and adapt to modern times.

I understand that, in the present audience, there are probably many participants from countries that have not yet gotten themselves into some of the very difficult situations in which the European countries find themselves now. I would like to conclude by making some constructive comments on what a rational control system might look like based on the European experience. I feel that it is very important to proceed in stages by first assessing the problems and then deciding what to do about them. These stages could be:

During the first stage: the basic framework of regulation of medical devices would be established. This would be composed of:

- (1) Registration of all institutions manufacturing, distributing and selling medical devices.
- (2) A notification of medical devices to a central authority giving the name of the device, the name of the manufacturer, the clinical purpose of the device, a list of the component materials used, a brief explanation of the sterilization process and copies of any labels, instructions and promotional material related to the device. It would be important to formulate this as a notification rather than as a pre-market approval system in order not to paralyze the control system.
- (3) A legal obligation for the hospitals and for the doctors to report any adverse reaction or event related to a particular device and to send this report to the same central authority that holds the file on notified products. These reports should then be duly attached to the central record for that product. Only this way will it be possible for the authorities to carry any sort of realistic statistical analysis of the problems encountered.
- (4) Powers given to regulatory authorities to deal with any problems as they arise, i.e., authority to request information, obtain samples, to inspect facilities, to seize and ban products.

The second stage would be to evaluate the experience received over a period of a couple of years to assess the nature and magnitude of problems.

The third stage would be to implement controls to solve what have been identified as the most important problems. For instance, it might be found out that a significant issue is sterilization of medical devices in hospitals. The regulatory answer might be to institute guidelines for hospital sterilization and verify compliance through inspections as well as require that certain imported products must be sterilized by the manufacturer.

The important lesson of the regulatory experience of the developed countries is that there is a real risk of initiating regulatory control schemes that cannot be implemented because of underestimated resource requirements. This will result in waste which surely is not the best way to serve the people.

# Contract Sterilization of Medical Products

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Since World War II, the medical disposable industry worldwide has shown phenomenal growth. This growth was stimulated in large part by the convenience of properly packaged, ready-to-use, off the shelf items, whose cost was low enough to allow a single use. In point of fact, the cost was often lower than the reprocessed equivalent, due in part to the increasing labor cost to reprocess. Further, the user had a real comfort factor with respect to the sterility of these disposable items since they were manufactured by companies like Johnson & Johnson, Becton Dickinson, etc., with extensive experience in sterilization methods and technology.

The net effect has been a dramatic proliferation of sterile disposable items ranging from simple gauze pads, cotton balls and bandages, through labware such as, petri dishes, pipettes, tissue culture flasks, to more complex devices like balloon catheters, dialyzer cartridges and total procedural kits, and the list grows daily. The plastic syringe shown in Figure 1 is the perfect model of a single use product. As a result, its predecessor, the glass syringe, requiring intensive labor for cleaning, reassembly and sterilization, is seldom used.



Figure 1.

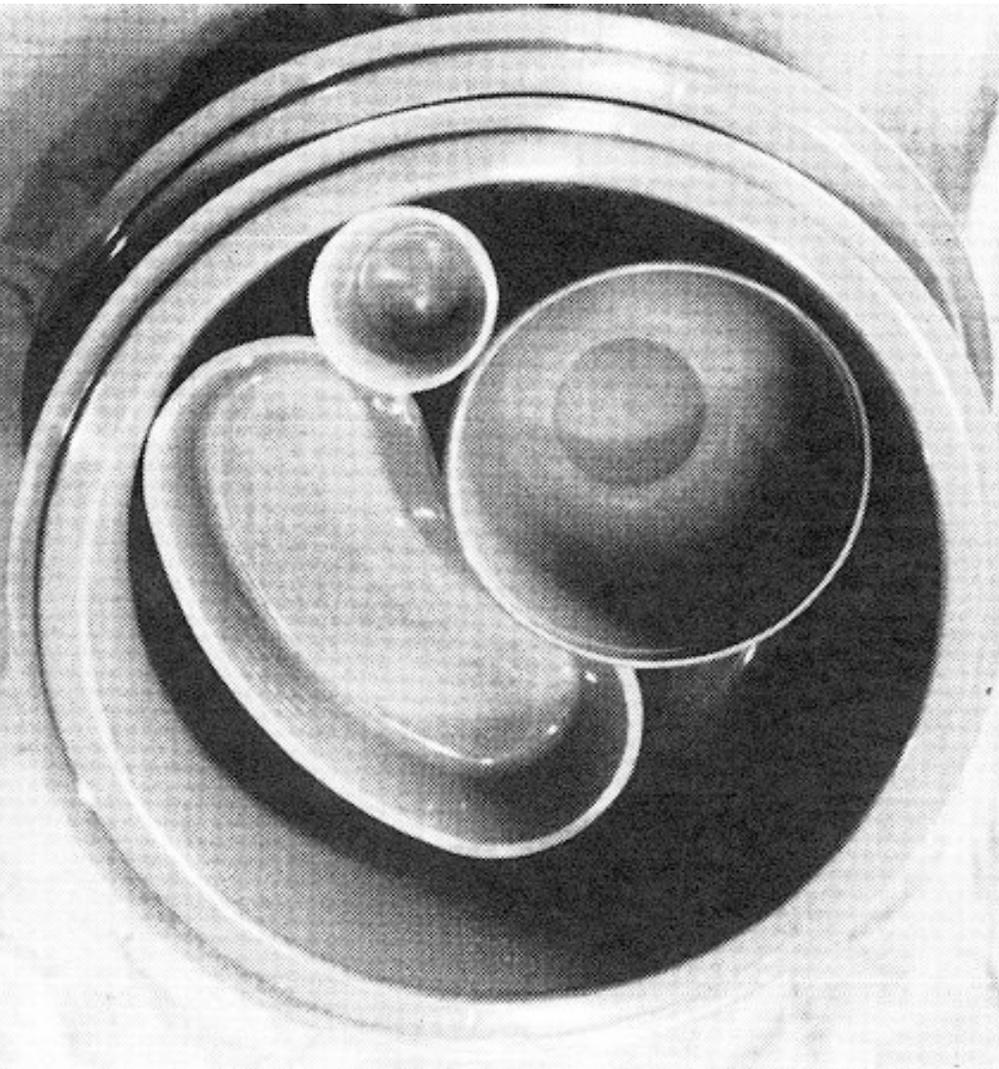


Figure 2.

**RECENT PERFORMANCE AND FORECAST:  
SURGICAL APPLIANCES AND SUPPLIES (SIC 3842)**

	<u>1982</u>	<u>1983</u>	<u>1984</u>	<u>1985</u>
Value of shipments* (1972\$)	2,790	2,978	3,176	3,390
Percent charge	6.7%	6.6%	6.8%	

\* In millions of dollars

Figure 3.

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However, one might question the wisdom of plastic throwaway basin sets, Figure 2, which not only use resources but impact our environment. What is wrong with reusable stainless steel sets, which can be reprocessed hundreds of times, packaged in sealed pouches, radiation sterilized with sterility assurance levels comparable to that of the disposable, and at a lower cost? Obviously, nothing. Coming back to the medical disposable industry, its growth was documented in a report issued by Predicast, Inc. in 1975, which showed the market for sterile disposables in the USA to be approximately \$1 billion, with a projected growth to \$2.3 billion by 1985. This indicates an annual growth rate of 9.5%. Other sources have indicated comparable rates of growth over the last two decades. For example, the latest four year summary for the U.S. Department of Commerce's category for surgical appliance and supplies is shown in Figure 3. Included in this category are sterile medical disposables, estimated as approximately 50% of the total. The shipment values as tabulated here, are in terms of 1972 dollars and show a compound annual growth rate of approximately 6.7%.

Although statistical information is difficult to obtain for the rest of the world, Predicast estimated the worldwide disposable market, without the USA, to be approximately twice that of the USA. Consequently, today we are looking at a total worldwide market of about \$7.5 billion in 1985 dollars. The consequence of this rapid growth in the medical disposable industry has been to give rise to a smaller but growing service activity providing sterilization of medical devices on a contract basis. More specifically, these contract sterilizers provide, on the main, either ETO or radiation sterilization services. Steam sterilization as a contract service is very limited in the United States, since by their very nature the vast majority of medical disposable devices are heat sensitive and therefore unsuitable for steam, or dry heat, sterilization. Figure 4 shows the overall growth worldwide for sterile medical disposable products and the corresponding growth of the irradiated portion. The total growth rate is about 400 million cubic feet per decade, with the radiation portion showing the fastest growth. ETO is the sterilization modality used for the remainder. Of this total of sterile disposable products, approximately 20-25% is sterilized by outside contractors.

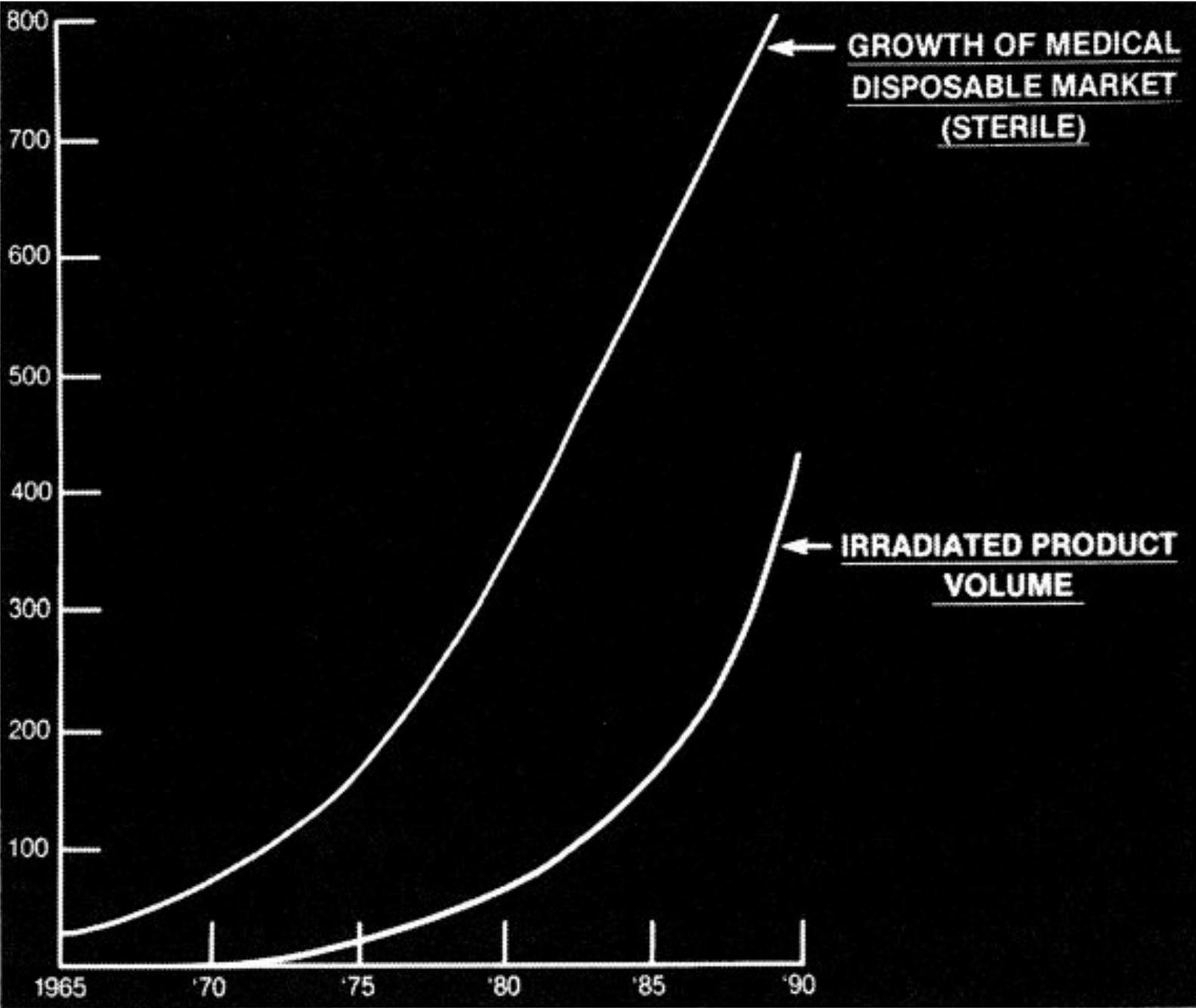


Figure 4.

Before describing the opportunities for contract sterilizers, let me first mention some responsibilities primarily to the FDA.

A review of the FDA's basic philosophy states the following:

“Contract sterilization is considered an extension of the finished device manufacturer’s process. The manufacturer is ultimately responsible for assuring that all sterilization operations and quality assurance checks used for products are appropriate, adequate and correctly performed;”

and further:

“While the finished device manufacturer bears overall responsibility for the safety and effectiveness of the device, both the contract sterilizer and the finished device manufacturer are legally responsible for an effective sterilization process;”

Finally, with respect to Regulatory Actions, the FDA position asserts:

The contract sterilizer should be held accountable for:

- assuring process specifications are met
- equipment qualification
- personnel training
- equipment calibration and maintenance
- cycle records
- environmental control
- proper handling to prevent mix-ups of sterile and non-sterile devices
- conformance to agreement or contract

Because of the complex interaction between manufacturers and contract sterilizers, FDA insists upon an agreement which shall cover the device to be sterilized and cover the required actions of the contract sterilizer, actions of the manufacturer and mutual activities. The activities include:

- |                           |                                 |
|---------------------------|---------------------------------|
| validation                | shipping                        |
| bioburden control         | approvals                       |
| labeling                  | documentation control           |
| secondary packaging       | process control                 |
| receipt and handling      | information transfer & contacts |
| preconditioning           | non-conformance                 |
| biological indicators     | reprocessing                    |
| loading                   | maintenance                     |
| cycle parameters          | calibration                     |
| cycle control and records | training                        |
| post handling             | audits                          |

Consequently, the contract sterilizer's business is heavily impacted by the FDA's policies, procedures and people.

Returning to the contract sterilizer, he generally fulfills three important functions:

- To provide sterilization for a small manufacturer who does not have his own in-house (internal) sterilization capability;
- To offer sterilization to manufacturers whose peak production demands exceed the existing in-house sterilization capability;
- To cover emergency situations, when manufacturers lose their internal sterilization capability.
- In addition, and particularly of late, is a fourth situation—where the contract sterilizer provides an alternative, radiation, to the manufacturer who wants to replace his ETO for

any one of many reasons.

With respect to the first situation, no internal sterilization capacity, it is estimated that of the over 1000 medical disposable manufacturing plants in the United States, fewer than half have an ETO sterilization capacity, and a much smaller percentage, less than 3%, have a radiation capability. Consequently, one can easily see the reason for the growth of contract service.

Back in 1977, in a talk presented at the second Johnson & Johnson meeting in Vienna on "Sterilization of Medical Products by Ionizing Radiation", I presented the slide on the relative use of the three major sterilization techniques (see Figure 5). As one can see, at that point in time, ETO had reached its peak and it was evident even then that radiation was going to replace ETO for substantial portions of the future sterilization requirements of the medical disposable industry, as well as replacing some existing ETO capacity. As it turned out, the projections were rather prophetic. The long dotted lines in the figure were estimates on the expected shift, due in large part to the basic advantages of radiation over ETO. The short dotted line, indicating a faster cross-over, was the projected situation if external forces, i.e., regulatory agencies, came into the picture.

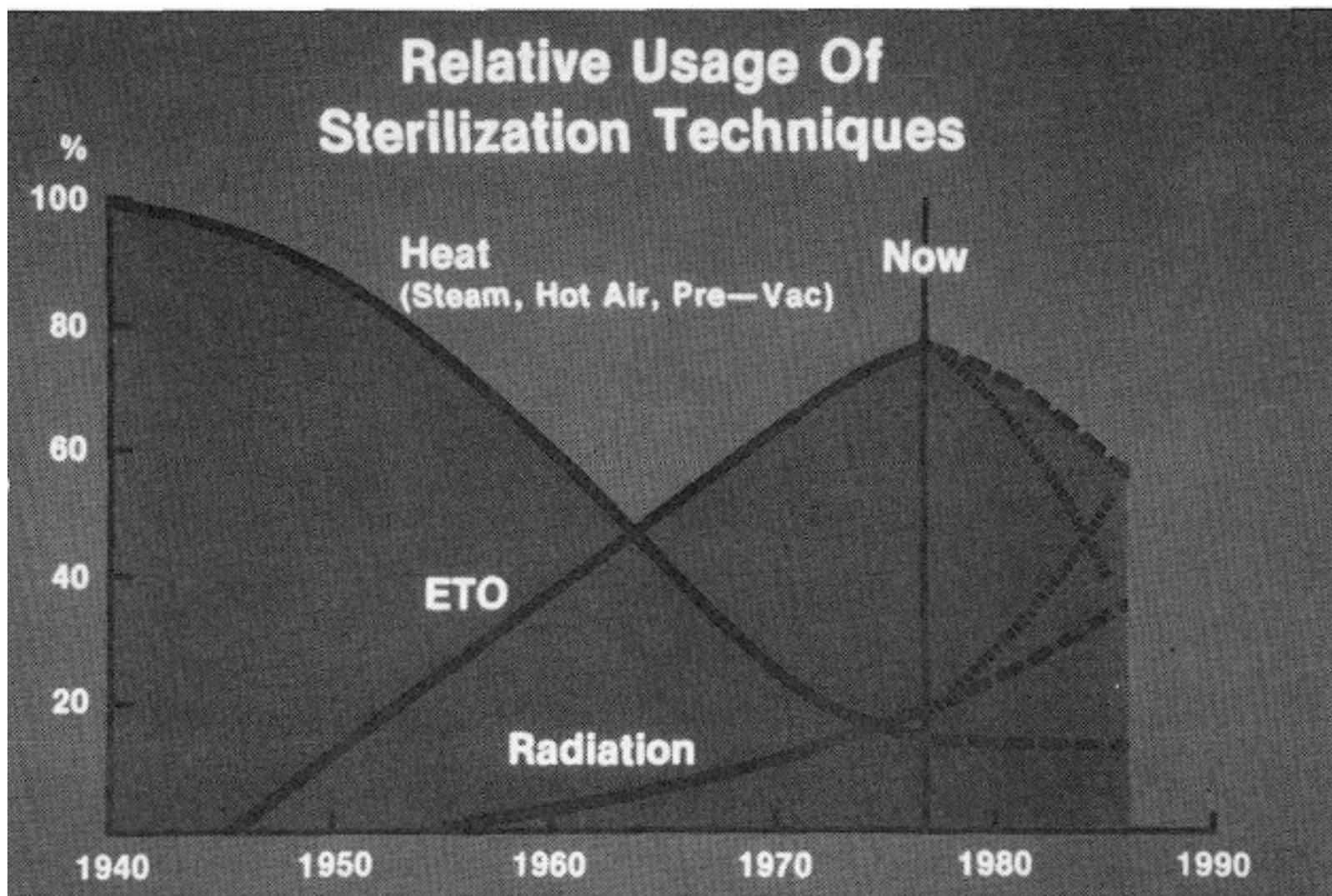


Figure 5.

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As it turned out, the regulatory impact has taken place. The Occupational Safety and

Health Administration (OSHA) has reduced the worker ETO levels in the USA by 98%, from 50 PPM down to 1 PPM. The effect of this regulation on the relative use of ETO vs. radiation has been to hasten the cross-over.

Going back to the early mention of basic advantages of radiation over ETO, let's examine that statement. Earlier speakers have already made reference to the difference in variables that one has to contend with in using steam, ETO and radiation. What is evident is the overwhelming simplicity of using radiation from a process control standpoint, since time is the only variable. Testifying to both the simplicity and reliability is the fact that product can be released—dosimetric release—based solely on the process parameters, obviously after proper validation.

In addition, other factors favoring radiation include:

- Lower cost (partly from reduction in inventory and testing costs)
- Lack of toxic residuals
- Improved packaging
- Speed
- Reduction in environmental impact

A further advantage of radiation over ETO relates to energy needs. As we are all aware, energy is still a critical element in the growth of all countries, and the energy requirements for radiation sterilization are considerably less than that for ETO, since ETO is a derivative of oil.

This shift from ETO, due to reasons cited above, has served to accelerate the growth of the contract sterilization activity over and above the ordinary growth of the disposable industry. Whereas a manufacturer of medical devices could have initiated or increased his ETO capacity in the past with a relatively modest capital investment, the capital requirements for radiation has excluded most small and medium manufacturers from even the contemplation of going the radiation route. Because of the large investment, only major producers of medical devices have been able to justify their own radiation facilities. This fact opened up opportunities for entrepreneurial radiation contract sterilizers who could provide radiation contract facilities capable of processing a range of products efficiently and economically for numbers of manufacturers. The largest of such contract sterilizers is Isomedix with 11 Cobalt plants.

The listing, Table I, of radiation contract sterilizers furnished here by AECL, is as of 1983. As one can see, the majority of the developed countries have one or more facilities.

Historically, the majority of the radiation sterilization facilities have used Cobalt 60 as their radiation source, although lately a few Cesium 137 plants have started up. The growth of this activity is indicated in Figure 6. Added to the Cobalt growth is the growth of electron beam (EB) accelerators, providing a 70,000 curie (Ci) equivalency for each kilowatt (kw) of electron power. At the present time, there are approximately 135 gamma irradiators in the world with a Cobalt activity in service amounting to about 100 Megacuries. Table II, compiled by AECL as of 1984, shows their geographic distribution. By contrast, there are no more than a handful of EB accelerators in use for sterilization with estimated power output of about 400 kw—mostly located in Denmark, U.K., Germany and USA.

**Table 1 Contract Gamma Sterilizers**

	Location	Licensed Capacity (kCi)
<i>Brazil</i>		
Embrarad	Sao Paulo	1,000
<i>Canada</i>		
Isomedix Corporation	Whitby	3,000
Sterirad	Toronto	500
<i>Denmark</i>		
NUNCATOM <sup>R</sup>	Roskilde	850
<i>Germany, Federal Republic of</i>		
Gammamaster	Allershausen	3,000
Willy Rusch	Rommelshausen	1,500
<i>Israel</i>		
Sor-Van Radiation, Ltd.	Yavne	1,000
<i>Japan</i>		
Koka Laboratory	Koka	1,500
<i>Korea</i>		
Korea Advanced Energy Research Institute	Seoul	1,000
<i>Mexico</i>		
Institute National de Investigaciones Nucleares	Salazar	1,000
<i>Netherlands</i>		
Gammamaster	Ede	1,000
Gammamaster	Ede	3,000
<i>United Kingdom</i>		
Gamma Radiation Services	Reading (Tilehurst)	1,000
Irradiated Products, Ltd.	Swindon	3,000
Irradiated Products, Ltd.	Swindon	2,000
Irradiated Products, Ltd.	Bradford	3,000

## *United States*

Applied Radiant Energy	Lynchburg, VA	500
International Nutronics, Inc.	Irvine, CA	3,000
International Nutronics, Inc.	Dover, NJ	60
International Nutronics, Inc.	Palo Alto, CA	750
Isomedix, Inc.	Columbus, MS	2,000
Isomedix, Inc.	Morton Grove, IL	500
Isomedix, Inc.	Northborough, MA	3,000
Isomedix, Inc.	Parsippany, NJ	2,000
Isomedix, Inc.	Puerto Rico	
Isomedix, Inc.	Spartanburg, SC	4,000
Isomedix, Inc.	Whippany, NJ	3,000
Neutron Products, Inc.	Dickerson, MD	1,000
Neutron Products, Inc.	Dickerson, MD	200
Radiation Sterilizers, Inc.	Tustin, CA	3,000
Radiation Sterilizers, Inc.	Schaumburg, IL	5,000
Radiation Technology, Inc.	Burlington, NC	3,000
Radiation Technology, Inc.	Providence, RI	3,000
Radiation Technology, Inc.	Rockaway, NJ	2,000
Radiation Technology, Inc.	West Memphis, TN	2,000
3M Company	Brookings, SD	3,000

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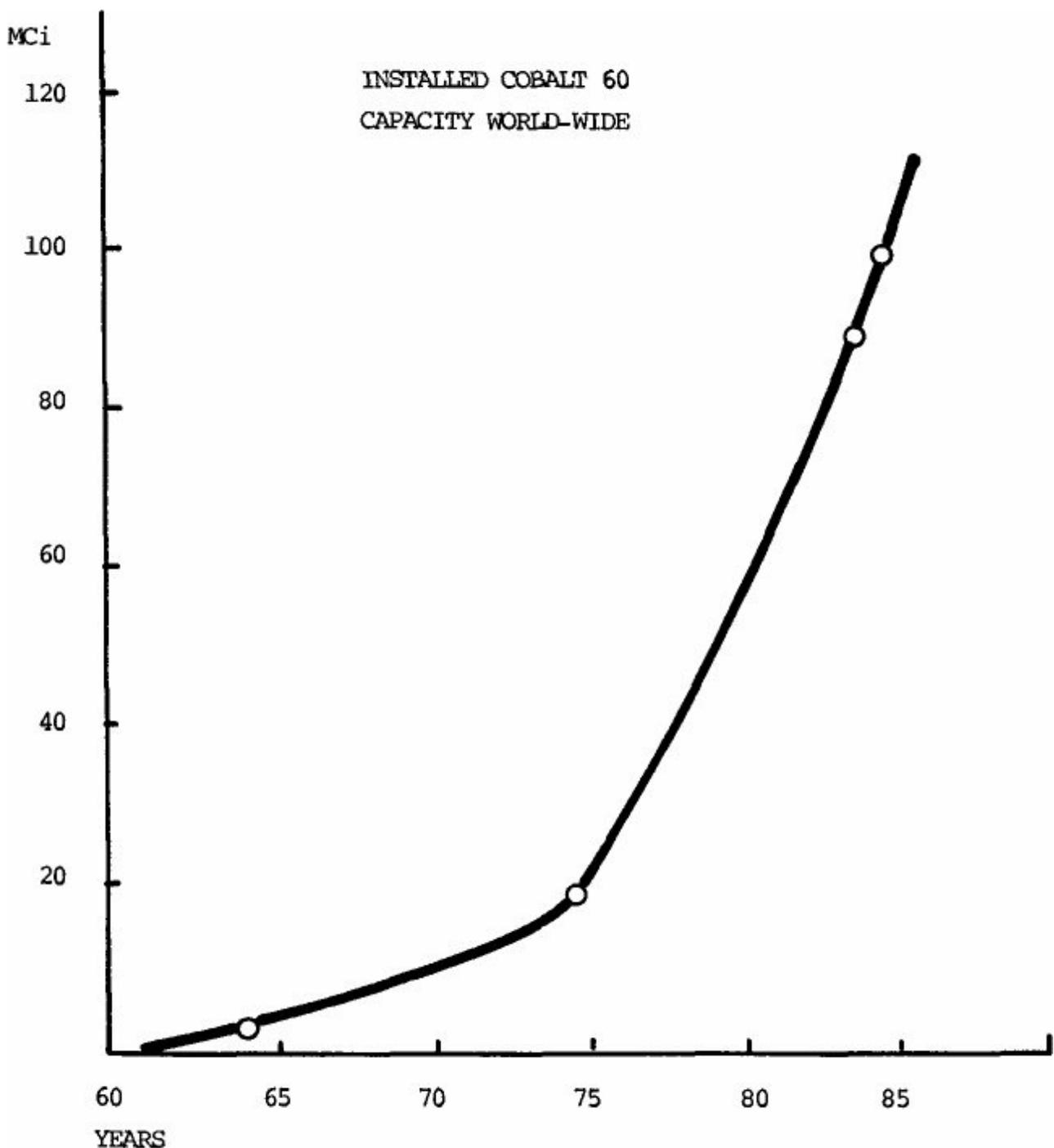


Figure 6.

Of the industrial gamma irradiators, approximately 40 facilities are in the USA with installed capacity of about 50 million curies. Of these 40 facilities, 21 are contract service centers, using approximately 50% of the 50 Megacuries or 25 Megacuries. At one cubic foot per curie per year at 2.5 Megarads, the contract radiation sterilization industry has a capacity of 25 million cubic feet per year. By contrast, the ETO contract facilities in the USA have a capacity of approximately 350 pallets per cycle or 1000 pallets per day for three cycles. This equates to approximately 72,500 cubic feet per day or 22 million cubic feet per year, or an approximate equivalency to the Cobalt 60 capacity. However, even though the cross-over for radiation vs. ETO has taken place in the contract area, it is not yet true for "in-house" sterilization since a greater proportion of ETO sterilization is performed in-house (approximately four to one) when compared to radiation.

**Table II. World List of Industrial Gamma Irradiators Compiled by Atomic Energy of Canada Ltd., Kanata, Canada March, 1984**

Country		Operator	Location	Plant Design
Argentina	(3)	CNENA	Ezeiza	CNENA
		CNENA	Buenos Aires	CNENA
		CNENA	Mar del Plata	CNENA
Australia	(3)	Ansell	Dandenong	AECL
		J & J	Sydney	AECL
		Ansell	Sydney	AECL (U.C.)
Belgium	(1)	IRE	Fleurus	Sulzer
Brazil	(3)	J & J	San Jose Dos Campos	AECL
		IBRAS-CBO	Campinas-Est	AECL
		Embrarad	Sao Paulo	AECL
Canada	(4)	Ethicon	Peterborough, Ont.	AECL
		Isomedix	Whitby, Ontario	AECL
		Sterirad	Toronto, Ontario	AECL
Chile	(1)	CAED	Santiago	AEC-Spain
Czechoslovakia	(1)	Kovo	Brno	AECL
Denmark	(3)	Nunc	Roskilde	AECL
		Novo Steritex/Induplast	Copenhagen	AECL
		Molnlycke	Espergaerde	AECL
German Democratic Rep.	(1)	GAECD	resden	AEC E. Germany
Egypt	(1)	NCRT	Cairo	AECL
Eire	(2)	B-D	Dublin	AECL
		Imed	Erie	AECL
El Salvador	(1)	Delka	San Salvador	AECL
England	(8)	Gillette	Reading	Marsh
		Swann Morton	Sheffield	March
		IPL	Swindon	IPL

		IPL	Swindon	IPL
		IPL	Bradford	AECL
		J & J	Slough	Marsh
		Gamma Radiation Serv.	Tilehurst	March
		B-D	Plymouth	AECL
Finland	(1)	Kolmi-Set Oy	Ilomantsi	AECL
France	(3)	Conservatome 1	Dagneux	Conservatome
		Conservatome 2	Dagneux	Conservatome
		Conservatome 3	Dagneux	Conservatome
Greece	(1)	Dimes	Athens	AECL
Hungary	(1)	Medicor	Debrecen	AECL
India	(1)	Isomed	Bombay	Marsch
		IDEA	Trombay	AECL
Indonesia	(1)	Girikencana	Jakarta	AECL
Iran	(1)	AEOI	Teheran	AECL
Israel	(1)	Sor-Van	Yavne	AECL
Italy	(5)	ICO	Ascoli Piceno	AECL
		Thicon	Rome	AECL
		Gammatom	Como	Gammatom
		Gammarad	Bologna	Marsh
		Italgamma	Nettuno	Italgamma
Japan	(7)	Tochigi-Siki	Tochigi	JAERI
		Radia Industry	Takasaki	JAERI
		JAERI	Takasaki	JAERI
		JAERI	Takasaki	JAERI
		JRA	Koka	AECL
		Terumo 1	Kofu	AECL
		Terumo 2	Kofu	AECL
Korea	(1)	KAEC	Seoul	AECL
Malaysia	(1)	Ansell	Melaka	AECL

Mexico	(2)	ININ	Salazar	AECL
		USDA	Tapachula	AECL
Netherlands	(2)	Gammamaster 1	Ede	AECL
		Gammamaster 2	EDE	AECL
New Zeland	(1)	ICI Tasman	Upper Hutt	AECL
Saudi Arabia	(2)	Alshifa	Dammam	AECL
		King Faisal Res. Center	Riyadh	Int. Nutronics
Scotland	(2)	Ethicon	Edinburgh	Nuclear Chem. Plant Co.
		Arbrook	Livingston	AECL
Singapore	(1)	Baxter Travenol	Singapore	AECL
South Africa	(3)	Nucor	Pelindaba	AECL
		Hepro	Tzaneen	AECL
		Iso-Ster	Johannesburg	AECL
Spain	(1)	Arago	Barcelona	Arago S.A.
Sweden	(2)	Radona/LIC	Skaerhamn Island	Marsh
		J & J	Rotebro	AECL
Switzerland	(1)	Steril Catgut	Neuhausen	Sulzer
Taiwan	(2)	ITRI	Taipei	BNL
		INER	Kneeling	INER
Thailand	(1)	Krung Dhon Hosp. Corp.	Marsh	
USA	(40)	AHSC/Convert.1 (2 plants) 2	El Paso (TX)	AECL (2x)
		Ethicon	Somerville (NJ)	AECL
		Ethicon	San Angelo (TX)	AECL
		B-D	North Canaan (CT)	AECL
		B-D/Falcon	Oxnard (CA)	AECL
		B-D	Sumter (SC)	AECL
		B-D	Broken Bow (NB)	AECL
Isomedix	Spartanburg (SC)	AECL		

Isomedix	Morton Grove (IL)	AECL
Isomedix	Columbus (MS)	AECL
Isomedix	Vega Alta, Puerto Rico	AECL
Isomedix	Northborough (MA)	AECL
Isomedix	Whippany (NJ)	AECL (U.C.)
Isomedix	Parsippany (NJ)	Isomedix
Isomedix	Sandy City (UT)	AECL (U.C.)
Isomedix	Groveport (OH)	AECL (U.C.)
Surgikos	Sherman (TX)	AECL
Surgikos	Arlington (TX)	AECL
Sherwood	Norfolk (NB)	AECL
Sherwood	Deland (FL)	AECL
Sherwood	Commerce (TX)	AECL
3M	Brookings (SD)	AECL
(Proctor & Gamble/Buckeye	Huntsville (AL)	AECL
Int. Nutronics	Irvine (CA)	Int. Nutro.
Int. Nutronics	Palo Alto (CA)	Int. Nutro.
Int. Nutronics	Dover (NJ)	Int. Nutro.
Baxter Travenol	Puerto Rico	AECL
Radiation Sterilizers	Tustin (CA)	Radiation Sterilizers
Applied Rad. Energy	Lynchburg (VA)	Applied Radi.
Atomics Int.	Canoga Park (CA)	Atomics Int
Neutron Prod.	Dickerson (MD)	Neutron Prod.
Inc. Radiation Tec.	Burlington (NC)	Radi. Tech.
Radiation Tec.	West Memphis (AR)	Radi. Tech.
Radiation Tec.	Rockaway (NJ)	AECL
Radiation Ste.	Schaumburg (IL)	Radi. Ster.
Radiation Ste.	Westerville (OH)	Radi. Ster.

		Radiation Ste.	Atlanta (GA)	Radi. Ster. U.C.
USSR (1981)	(11)	Grand Total:	11 industrial gamma irradiators (4 medical, 3 food, 4 chemical)	
Venezuela	(1)	IVIC	Caracas	AECL
Federal Rep. of Germany	(5)	Ethicon	Hamburg	Marsh
		Braun	Melsungen	Sulzer
		Beiersdorf	Hamburg	CEA/Conservatome
		Willy Ruesch	Rommelshausen	AECL
		Gammamaster Muenchen	Allershausen	AECL
Yugoslavia	(1)	Nuclear Inst.	Vinca	CAE/Conservatome

One somewhat different organization in the contract sterilization business is my own company, Medical Sterilization, Inc (MSI). We are relatively new, having only gone operational this year, 1985. A number of things set MSI apart from the typical contract sterilizer. First, the major thrust of our company is the off-site processing and sterilization of surgical instruments for hospitals, clinics, and surgi-centers. By using industrial techniques and larger equipment, we can provide economy of scale, together with a higher sterility assurance level. Basically, we are bringing the industrial philosophy of GMPs to the hospital. The net effect of our operation is to provide our clients, primarily hospitals and clinics, with cost effective, higher quality sterile instrument sets and trays. Second, as far as I know, we are the only contract sterilizer with all three major sterilization modalities under one roof. This allows an objective evaluation of what is the best sterilization technique for a given product and also makes radiation available for the first time for hospital use. Third, our radiation is machine-produced. The essential difference in using an accelerator rather than an isotopic source, i.e., Co<sup>60</sup>, is that the penetration of the electron beam is limited by the voltage. This is illustrated in Figure 7 showing the penetration for various voltages compared to Co<sup>60</sup>. Since both EB and gamma rays interact with matter through ionization, the only difference is their penetration. As a practical matter, the choice affects what product size, density and configuration can be accommodated. The reason we chose an accelerator was our feeling that it provides us with greater flexibility for both the hospital activity, as well as the contract services. However, we are now seriously considering a Cobalt source as well. Since in our hospital activity, daily delivery of product is vital, the absolute reliability of an isotopic source is attractive.

# DOSE DISTRIBUTION IN WATER

RELATIVE DOSE (%)

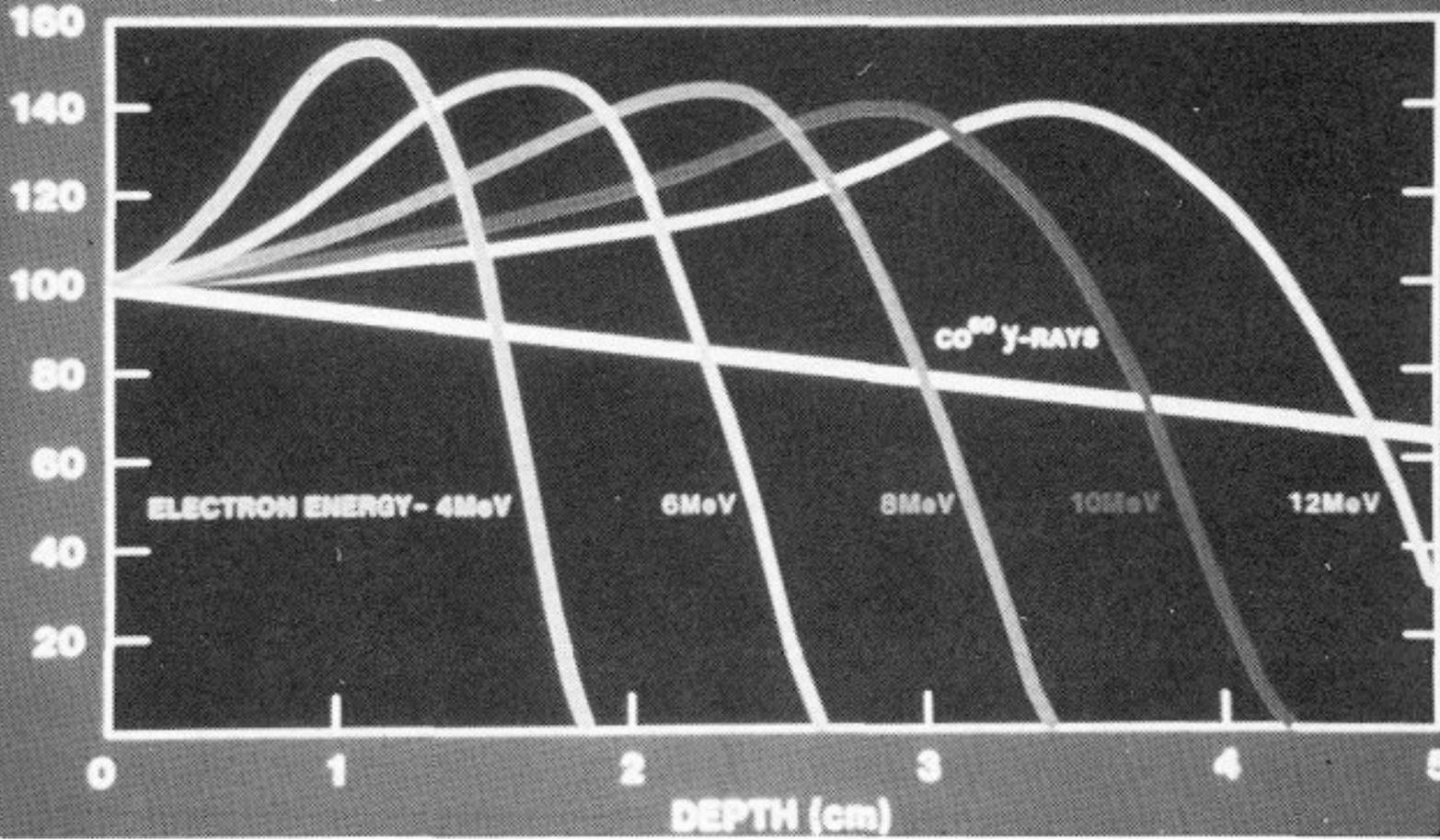


Figure 7.

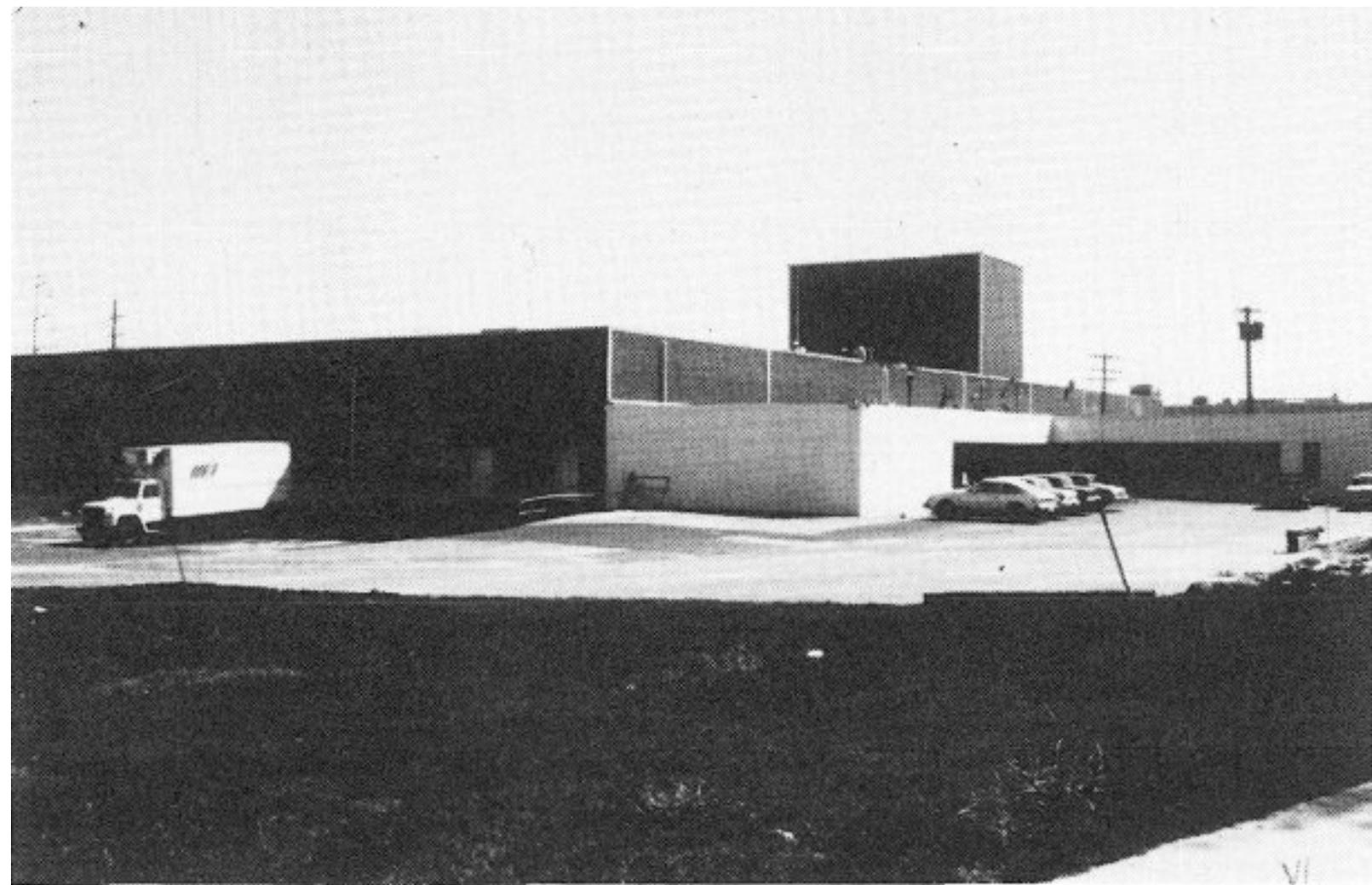


Figure 8.

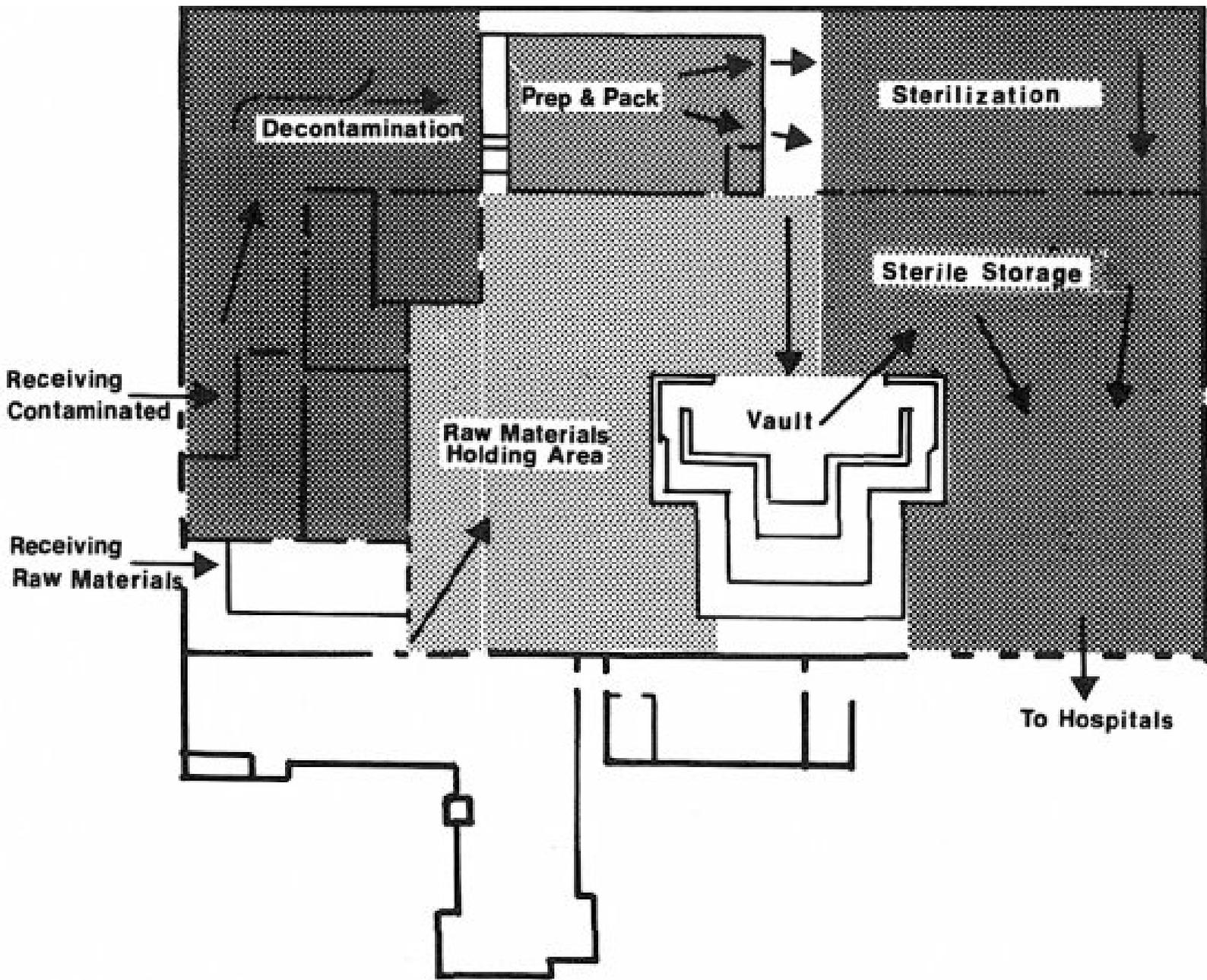


Figure 9.



Figure 10.



Figure 11.

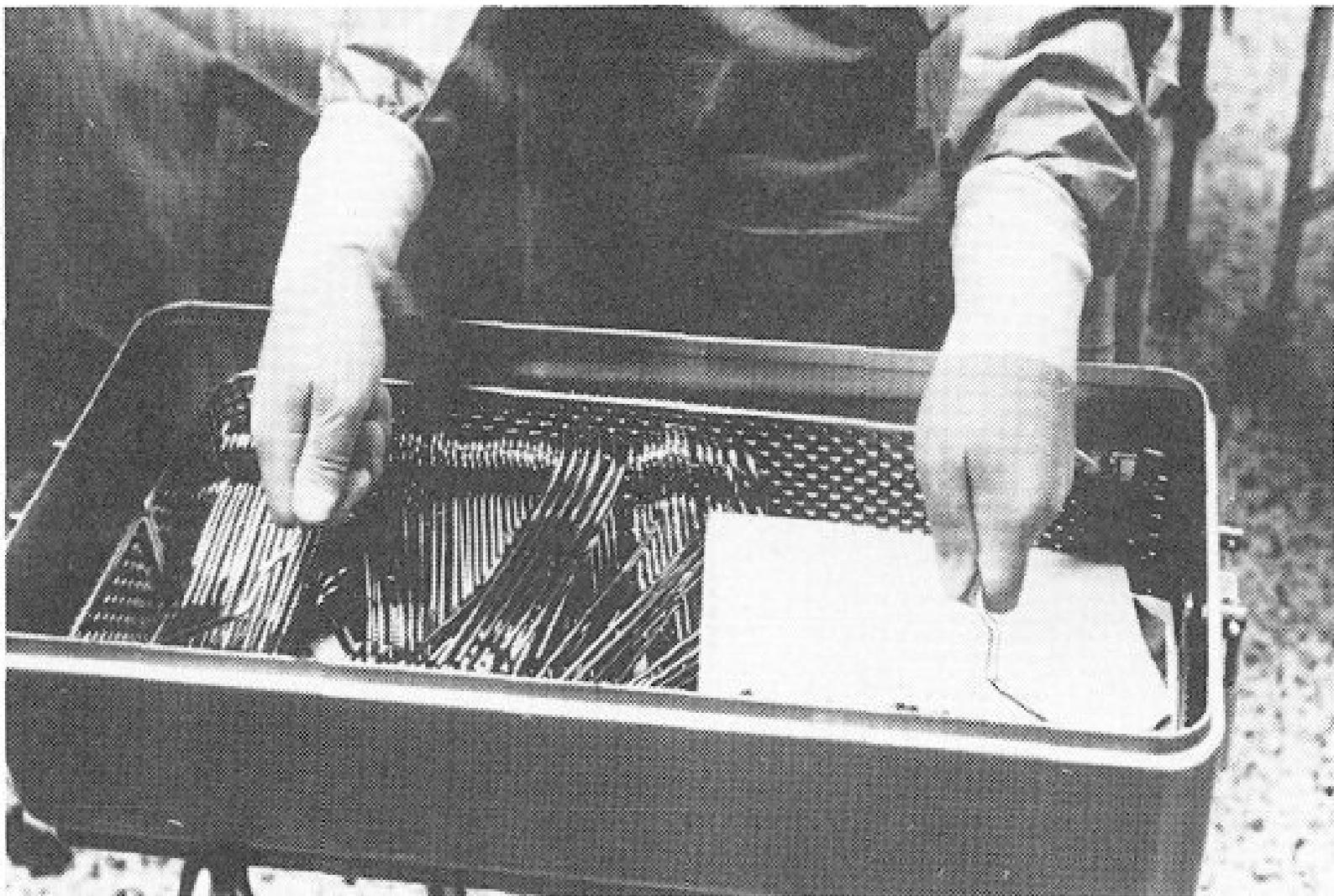


Figure 12.

MSI's home is shown in the photo, Figure 8. Our building contains 100,000 square feet with the accelerator located in the super-structure. The facility layout is diagrammed in Figure 9. Hospital product flow is as follows: First, "dirty product" (contaminated surgical instruments and peripheral equipment) arrive from client hospitals in the "Decontam" area where it is staged to go through either the tunnel washers or ultrasonic cleaners for decontamination and cleaning. The "Decontam" area (Figure 10) is under negative pressure with 15 air changes per hour; daily air and surface samples are taken for monitoring the microbial population. After cleanup, all apparatus goes through a "pass-through" sterilizer and then into the "Preparation and Packing" area, which is under positive pressure. In the "Prep & Pack" room inspection, refurbishment and assembly takes place (Figure 11). The instrument set is then locked in a metal container ready for terminal sterilization. The use of the container provides a better, more secure and more easily transportable unit than the conventional tray double-wrapped with muslin cloth or non-woven paper. After terminal sterilization, the units then go into "Sterile Storage" prior to delivery to client hospitals. The choice of the terminal sterilization technique is based upon a number of factors, including cost, convenience and the sterility assurance level desired. Typical of the hospital instrument sets which we process is the Major Basic set (Figure 12) being removed from its container by a scrub nurse.

MSI's sterilization capacity, as mentioned earlier, consists of all three modalities. The photo, Figure 13, shows the 700 cubic foot ETO chamber with its two aeration rooms in the rear, and the 310 cubic foot steam sterilizer in the foreground. The steam unit and the ETO chamber are both microprocessor controlled.

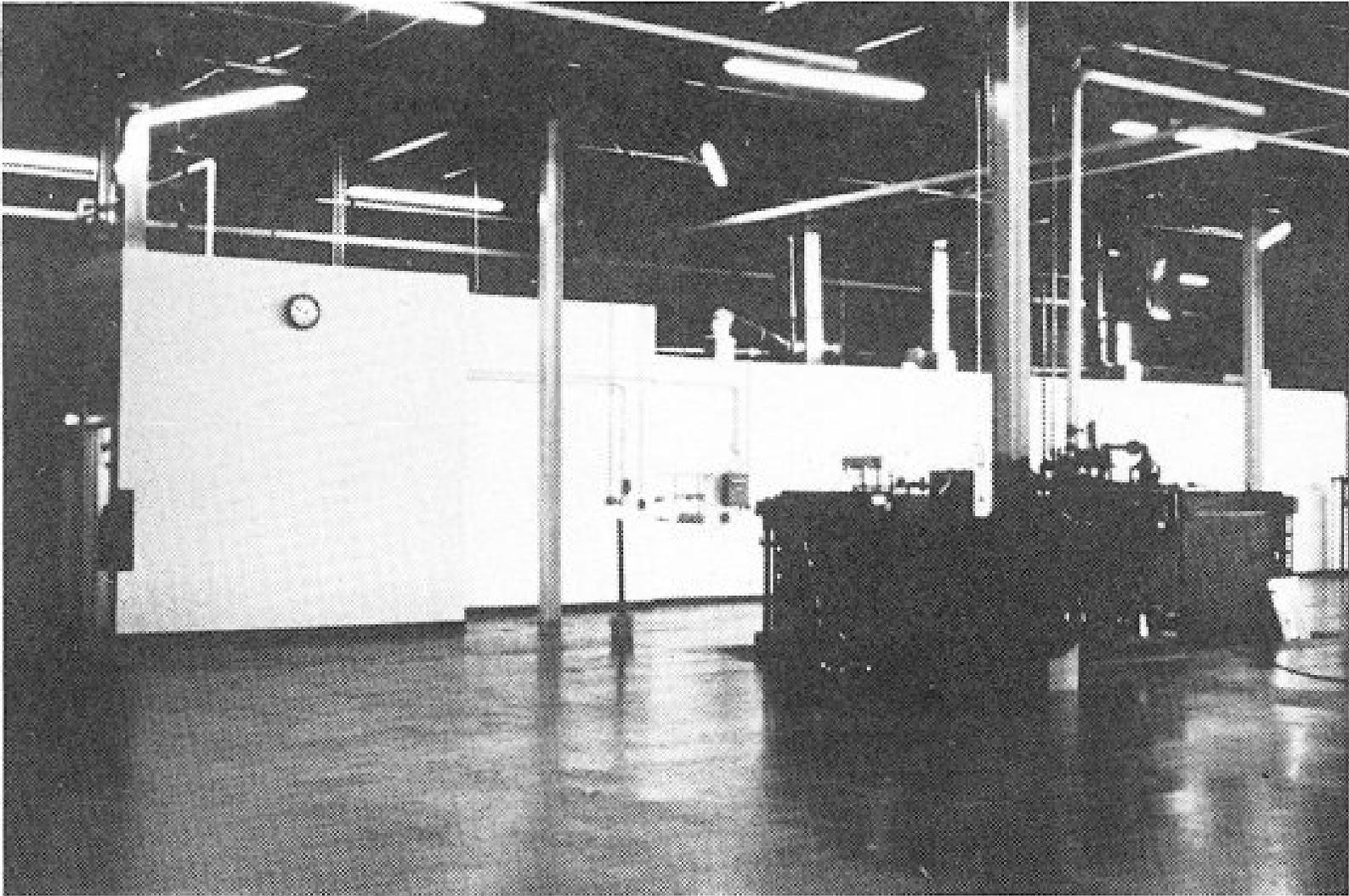


Figure 13.

Perhaps the most interesting aspect of MSI's facility is the 4.5 MeV, 150 kW radiation unit housed in a large concrete shield (Figure 14). Product, palletized, comes into the plant by truck load. Individual carts, computer-controlled, transport the individual cartons through the labyrinth. A plan view of the vault area is shown in Figure 15. Carts entering the vault go around three corners before going under the downward directed electron beam from the vertical accelerator and then the product exits.



Figure 14.

Laser readers identify each cart individually going into and exiting the vault. Therefore, traceability and control is cart-by-cart.

When we require more penetration than we can get with 4.5 MeV electrons, we can convert to X-rays. Figure 16 shows the relative dose vs. depth for X-rays and  $\text{Co}^{60}$  gamma rays. As one can see, there is little difference in penetration. Consequently, the consideration in choice between Cobalt 60 and machine radiation becomes one of convenience and cost. Although in the past, for penetrating radiation, cost considerations dictated Cobalt 60, today the situation is not crystal clear. As shown in Figure 17, Cobalt 60 cost per kw has been increasing since 1975. By contrast, X-ray cost has been decreasing due in large measure to the increasing power level of EB accelerators, so a cross-over is now at hand. However, in spite of this, as mentioned earlier, we are now looking for a  $\text{Co}^{60}$  unit, specifically for hospital-type products.

In closing, let me state that MSI was established to bridge the gap between manufacturers of sterile disposables and the reprocessing of sterile items in the hospitals and I believe we have succeeded. Although MSI is atypical of most contract sterilizers today, it may be an indication of what I believe may be an ever increasing role for contract sterilization into the 1990s – servicing both the medical disposable manufacturer and the health care institutions.



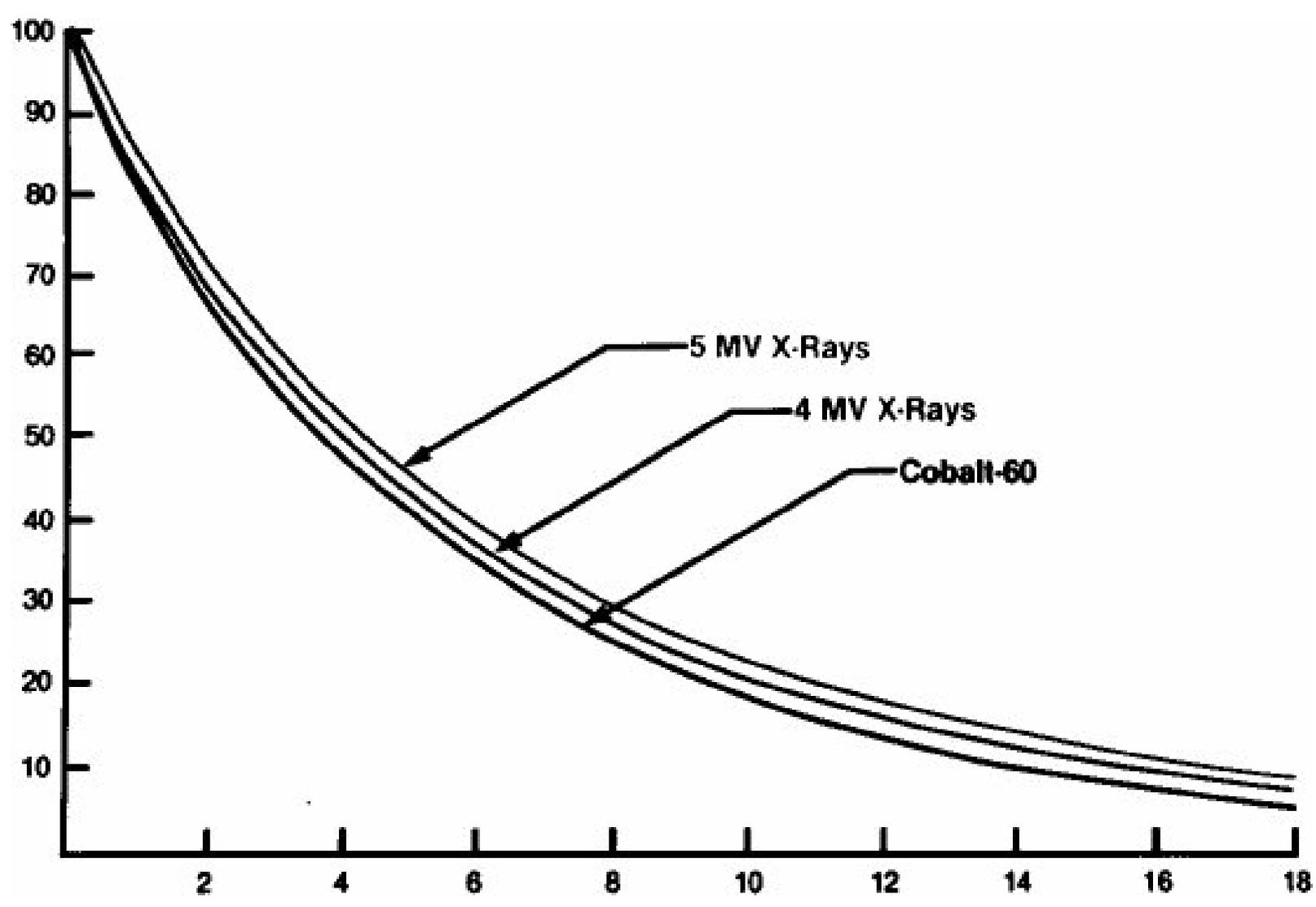


Figure 16.

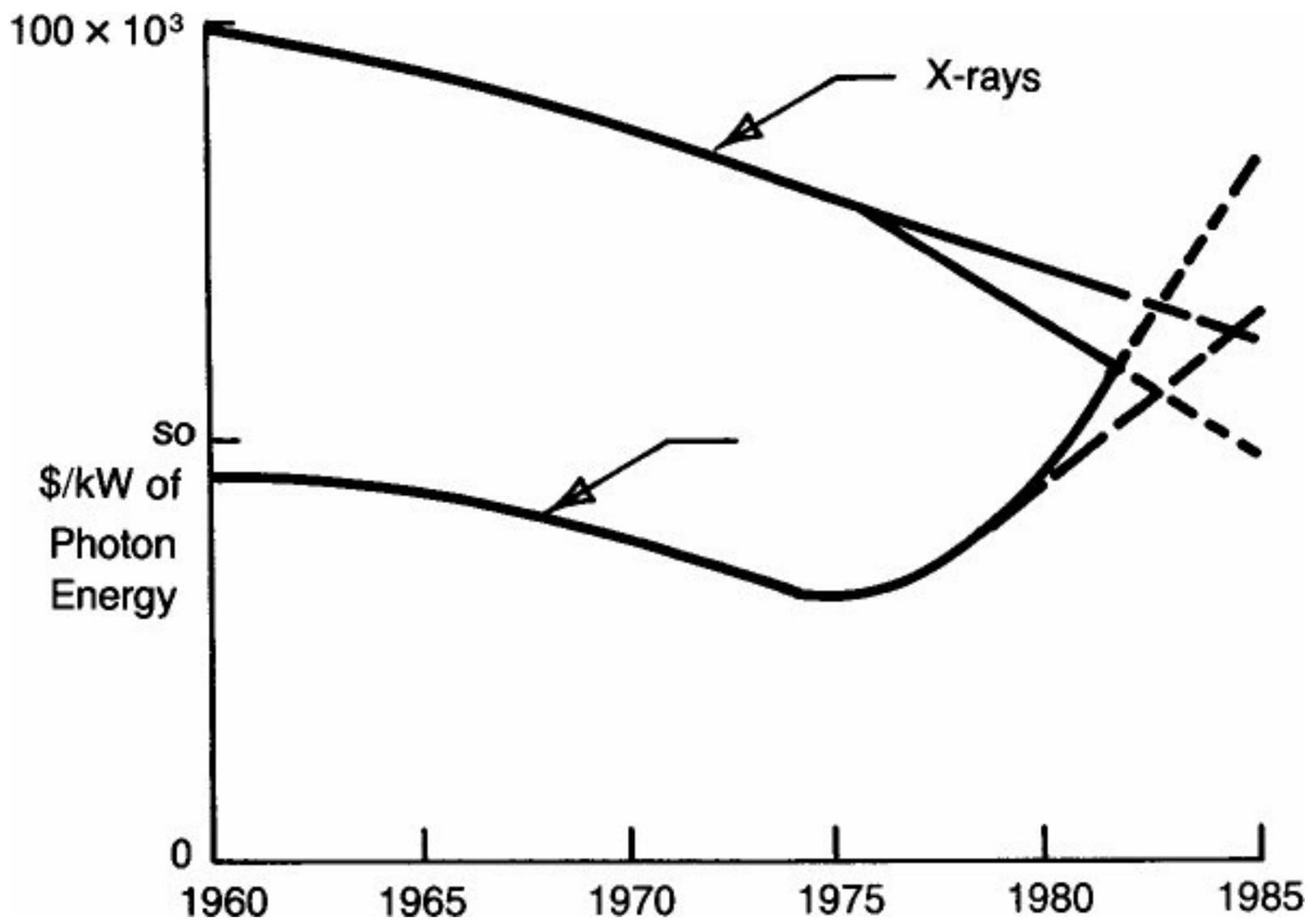


Figure 17.

# Studies on the Preservation of Fruits

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## Introduction

Preservation of food is an important problem for human health and closely related to economic development. Because lack of refrigeration facilities and other effective means of preservation, fruits in China suffered from heavy loss due to deterioration. It was estimated that the annual loss due to fruit deterioration are 200 million yuans. Therefore, it is an urgent task to develop effective and feasible methods for preservation.

From 1982, scientists in the fields of pharmaceutical chemistry, microbiology, plant physiology, biochemistry and toxicology, jointly carried out a series of research projects in the preservation of citrus and litchi, two important varieties of fruits in the southern part of China.

Based on the preliminary research results in 1982, we expanded our experiments in 1983-1984 and increased the amount of fruits studied to 150 thousand kg. In 1984, we conducted several long distance shipping of litchi and citrus to Beijing at ambient temperature by trains.

## Materials and Methods

- (1) Investigations on microbiological contamination of litchi and citrus: The sampling, isolation and identification of fungi from litchi and citrus are referred to as methods in Manual of Microbiology of Food Hygiene.<sup>1</sup>
- (2) Screening for preservatives: Plant method was used for preliminary sensitivity test and tube method for MIC determination.
- (3) The use of preservatives in field trial:
  - (1) Preservatives: RQA paper and thiabendazole (TBZ). RQA paper and paper bag were bought from Lingling paper factory. TBZ was dissolved in hydrochloride, then diluted

with water and adjusted to pH 5.0.

(2) The methods tested for the preservation of citrus are listed in Table I.

(3) The method for the preservation of litchi were as follows:

A' Dipped in TBZ solution for one minute.

B' Packed in RQZ paper bag after dipped in 0.2% TBZ solution for one minute.

C' Packed in RQA paper bag.

D' Untreated

\* These tests were carried on July 19-24, 1984, the temperature was 27°-24°C.

(4) Residue analysis

i RQA analysis: The HPLC method<sup>4</sup> was used with a sensitivity of 0.15 µg/5µl and recovery rate is 80-90%.

ii TBZ analysis: The fluorometric method<sup>5</sup> was used, the sensitivity was 0.02 µg/5ml.

**Table I. Methods Tested for the Preservation of Citrus**

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A. RQA:	Wrapped in RQA paper after dipped in 200 ppm of 2,4-D, solution for one minute.
B. TBZ:	Dipped in 0.2% TBZ solution + 2 00 ppm of 2,4-D solution for one minute.
C. Control-1:	Untreated.
D. Control-2:	Dipped in 2,4-D solution (200 ppm) for one minute.
E. Control-3:	Dipped in 2,4-D solution (200 ppm) and wrapped in untreated paper.

Storage Condition: Ambient temperature 8-16°C

Underground store house 9-18°C

Moisture 85-90%

---

## Experiment Results

(1) Fungal flora of citrus and litchi

Fungi were isolated from 75 samples of moldy citrus and during the determination of aerobic bacteria counts of litchi. The results are listed in Table II.

In the 59 strains of yeast isolated, the predominant strains were *Saccharomyces fructum* and followed by *Sacc. kluyveri* and *Pichia membranaefaciens*. We also isolated some strains of fungi from citrus with different diseases, the results as follow (Table III):

(2) Screening of Preservatives

Twenty-eight preservatives were used screening in which nine chemicals were found effective (Table IV).

RQA and TBZ were further tested for MIC determination. The results are listed in Table V.

As the results in Table IV and V showed that RQA is a most effective chemical to test fungi strains. It is more powerful than TBZ, especially to yeast.

**Table II. Fungi Isolated from moldy Citrus and Litchi**

	Citrus 11/82	05/83	Litchi 06/83
Number of Strains	52	74	96
<i>Fusarium moniliforme</i>	21	17	1
<i>Verticillium</i>	21	15	1
Yeast	5	2	59
<i>Penicillium sp.</i>	2	12	12
<i>Alternaria</i>	5	3	
<i>Lachnea</i>	4	4	1
<i>Aspergillus sp.</i>	2	13	9
<i>Geotrichum candidum</i>	2	1	
<i>Cladosporium</i>		4	8
<i>Mucor</i>	2		
<i>F. solani</i>		2	
<i>F. lateritium</i>			2
<i>Sclerotium</i>			1
<i>Trichoderma</i>	1		
Unidentified		1	3

**Table III. The Distribution of Fungi from Diseased Citrus**

Disease	Brown Rot	Siberian Plague	Steam Rot	Green Molds
Strains	29	21	24	25
<i>Fs. monliforme</i>	17	9	9	3
<i>Verticillium</i>	6	9	4	3
<i>F. solani</i>	2			
<i>Penicillium sp.</i>	3			2
<i>Lachnea</i>		3	2	

<i>Alternaria</i>		5	
<i>Asp. flavus</i>		1	5
<i>Asp. sydowii</i>			5
<i>Asp. versicolor</i>			1
<i>Cladosporium</i>			4
Yeast			2
<i>Geotrichum candidum</i>	1		
Unidentified		3	

**Table IV. Fungi Inhibitory Effects of Nine Preservatives**

	Control	TBZ	RQA	FC-3	FC-4	FC-5	FC-6	FC-7	FC-8
<i>A. flavus</i>	+++	-	-	-	-	-	-	-	-
<i>A. niger</i>	+++	-	-	-	-	-	-	-	-
<i>F. moniliforme</i>	+++	-	-	-	-	-	-	-	-
<i>Alternaria</i>	+++	++	-	-	-	-	-	-	-
Yeast	+++	++	-	-	-	-	-	-	-
<i>P. citrinum</i>	+++	-	-	-	-	-	-	-	-
<i>Paecilomyces</i>	+++	-	-	-	-	-	-	-	-
<i>Geotrichum candidum</i>	+++	++	-	-	-	-	-	-	-

Remark:-: no growth

+: growth

### (3) Field trial

Based on the small scale experimental results in 1983, we increased the amount of citrus tested in 1984. In the same year, we tried to transport litchi from Zhanzhou to Beijing by train (five days) at very warm temperature (27-33°C). Both results are shown in Table VI.

As above results indicated that RQA paper or paper bag are very effective in the preservation of both citrus and litchi, RQA + TBZ might be the most effective method for litchi preservation.

In 1985, middle preservative experiments for litchi were carried out in July. We increased the amounts of fruit kept refrigerated (3-5°C) until 30 days for observation, the results are summarized in Table VII.

While we take 270 kg of litchi for observing the the fruits' life on goods shelves, the

results as Table VIII showed.

(4) The residue of RQA and TBZ in the fruits were analyzed and the results were as follows: Table IX

As above described that there were very little residue of TBZ and RQA in the pulp of fruits.

(5) Toxicological results and referred data of RQA.

(1) Acute toxicity: Rat: Intraperitoneal administration LD<sub>50</sub> 200 mg/kg  
(Oral administration LD<sub>50</sub> 3000 mg/kg)

(2) Mutation tests

(1) Ames test negative

(2) Testicular chromosome aberration negative

(3) Myelocytic micronuclear test negative

(3) Accumulation test

Accumulation index: 6 slight accumulation

**Table V. Inhibitory Effects of Different Concentrations of RQA and TBS to Different Strains of Fungi**

Control	1:100		1:500		1:1000		1:2000		1:3000		1:4000		control
	R	T	R	T	R	T	R	T	R	T	R	T	
<i>A. flavus</i>	-	-	-	-	-	-	-	-	-	-	-	-	+++
<i>A. niger</i>	-	-	-	-	-	-	-	-	-	-	-	-	+++
<i>F. moniliforme</i>	-	-	-	-	-	-	-	-	-	-	-	-	+++
<i>Alternaria</i>	-	++	-	++	-	++	-	++	-	++	-	++	+++
<i>P. citrium</i>	-	-	-	-	-	-	-	-	-	-	-	-	+++
<i>Paecilomyces</i>	-	-	-	-	-	-	-	-	-	-	-	-	+++
<i>Geotrichum candidum</i>	-	+++	-	+++	-	+++	-	+++	-	+++	-	+++	+++
Yeast	-	+++	-	+++	-	+++	-	+++	-	+++	-	+++	+++

Remark: R: RQA

T: TBZ

-: no growth

+: growth

**Table VI. Preservative Effects of RQA and TBZ in Citrus and Litchi**

Treatment	Species of Fruits	Period of Storage (days)	Amount of Fruits (kg)	Good fruits 9 (%)	X2 test*
A. RQA	Miju	80-130	20,000	95.1	p 0.001
	Tiancheng	102-142	30,000	94.3	p 0.001

B. TBZ	Miju	80-130	30,000	92	p 0.001
	Tiancheng	102-142	50,000		p 0.001
C. Control	Miju	80-130	900	31	
	Tiancheng	102-142	900	49	
D. Control	Miju	900	55.9		
	Tiancheng	900	59.4		
		80-130			
		102-142			
E. Control	Miju	80-130	900	61.2	
	Tiancheng	102-142	900	72	
A' RQA	Suye (litchi)	9	36	90.3	p 0.001
B' TBZ	Wuye	9	360	81.8	p 0.001
C' RQA + TBZ	Wuye	9	360	92.1	p 0.001
D' Control	Wuye	9	348	33.7	

**Table VII. The Preservation Effects of RQA and TBZ for Litchi (Lanzu) Kept by B6 Refrigerator Trains (1985)**

Groups	Species	Amounts of Fruits (kg) Experiments	Sampling	Period of Storage (days)	Good Fruits (%)	Best Fruits (%)
RQA	Lanzu	5000	120	17	95.9	94.8
		2500		30	93.8	87.5
TBZ	Ditto	4000	150	17	87.8	84.6
		2000		30	85.8	68.4
RQA + TBZ	Ditto	2500	120	17	94.6	97.6
		2000		30	94.3	89.2
Control	Ditto	5000	150	17	9.9	0
		50		30	0	0

**Table VIII. The Good Shelves Life of Litchi after 17 Days Kept in Refrigerator**

Groups	Shops	Amounts of Fruits (kg)	Tem. of Shelves	Shelves Time (hr)	Good Fruits (%)
RQA	A	20	10-12°C	72	84.2
	B	20	22-24°C	48	92.96
	C	200	30-32°C	48	81.2
TBZ	A	25	10-12°C	48	70
	B	25	22-24°C	48	84.4
	C	25	30-32°C	24	84
RQA + TBZ	A	20	10-12°C	72	90
	B	20	22-24°C	72	90.94
	C	20	30-32°C	48	88.5
Control	A	25	10-12°C	12	60
	B	25	22-24°C	48	0
	C	25	30-32°C	12	52

**Table IX. The Residue of TBZ and TQA in Fruits Preserved**

Species	Residue	
	TBZ	RQA
Huapiju	peel 1.38 mg/kg	0.6 mg/kg
	pulp 0.0145 mg/kg	0.3 mg/kg
Houcheng	peel 1.39 mg/gkg	0.6 mg/kg
	pulp 0.039 mg/kg	0.3 mg/kg
Litchi	peel 58.1 mg/kg	ND
	pulp 0.77 mg/kg	0.3 mg/kg

## Discussion

### (1) Microflora and sensitivity test

Citrus and litchi are very difficult to preserve in our country. It is necessary to learn the distribution of microflora in citrus and litchi and their sensitivity to different chemicals, before carrying out field trial. We found that most fungus strains are sensitive to RQA at

1:4000 concentration.

## (2) Toxicity of RQA<sup>6</sup> and TBZ

The oral administration LD<sub>50</sub> in rat was 3200 mg/kg.<sup>7</sup> After feeding 2500 ppm of RQA in fed for 16 weeks, no abnormal symptoms and pathological changes were found in rats. Ames test and chromosome aberration test in rat test is cells were negative. The non-effective dose for rats in subacute toxicity test was 2500 ppm (125 mg/kg b.w.).<sup>6</sup> Therefore, RQA is a safe antifungi drug if we compare with the residue levels in fruit pulp.

The acute toxicity (LD<sub>50</sub> 3400 mg/kg p.o.) of TBZ is lower than RQA and it was approved in a number of countries to be used as fruit preservative<sup>8</sup>. However, it was found to be teratogenic by Ogata, et al, in 1984.<sup>8</sup>

## (3) RQA paper or RQA paper bag are very effective and feasible to use for fruit preservation. In early stages after harvest, RQA paper or RQA paper bag could adsorb the fruit sweat and also inhibit or kill the fungi in the peel. In the later stage, it can protect the fruit from recontamination. This will keep the fruit in good quality and greatly increase their commercial life.

## Summary

RQA is a highly effective and safe preservative for citrus and litchi by using RQA paper or RQA paper bag. The percentage of good fruit for citrus and orange were up to 94.3-95.1% (in four to five months) and by using RQA paper bag, the percentage of good fruit for litchi were up to 90.3% in nine days and 95.9% in 17 days, 93.8% in 30 days keep by B6 type refrigerator trains.

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# Efficacy Testing and Market Research for the Pork Industry\*

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## Abstract

Low dose (less than 30 krad) gamma irradiation of *Trichinella spiralis* infected pork renders the parasites sexually sterile and blocks maturation of the ingested larvae in the host gut. Irradiation of freshly slaughtered, market weight hog carcasses indicate that larvae throughout the carcass have essentially identical sensitivities to radiation. The research data indicate that 30 krad of 0.66 MeV gammas can be delivered to market weight, split carcasses with acceptable uniformity, and that such a dose can provide a substantial margin of safety for human consumption of even heavily infected meat.

Feasibility studies of pork irradiation in commercial operations have shown the process to be technically, economically and financially feasible. Treatment during the first four years of operation in a 2,000 hog per day plant will cost about 0.0034 dollars per pound and 0.0011 dollars per pound thereafter. Social and political feasibility are addressed in a 1,000-family consumer survey completed in the first quarter of 1984.

## Introduction

The National Pork Producers Council (NPPC) Committee on Trichina-Safe Pork has recommended that the NPPC pursue a policy to provide a nationwide supply of trichina-safe pork by January 1, 1987. Irradiating pork to eradicate the trichina is one of the approaches identified by the Committee to accomplish this goal.

This paper presents the preliminary findings of studies to evaluate the feasibility of producing the marketing pork certified as "trichina-safe". The studies are being conducted as part of the Department of Energy, Byproducts Utilization Program (BUP). These studies include (1) low-dose irradiation research to verify the control of trichina under conditions that simulate the modern pork industry, (2) an economic feasibility study of a large-scale pork irradiation program, and (3) a consumer attitude survey to determine the consumer reaction to the irradiation concept.

# The Trichinosis Problem

Trichinosis is a parasitic disease caused by the microscopic nematode, *Trichinella spiralis*. The disease is found in numerous species of wild and domestic carnivorous animals, such as hogs, as well as in humans.

When meat containing encysted trichina larvae is eaten, the muscle and cyst walls are digested, releasing the larvae. The free larvae quickly pass to the small intestine and burrow into the wall of the intestine where they mature and copulate in two to four days, producing a second generation of 1,000 or more larvae. Newborn larvae pass through the lymphatic and circulatory systems, eventually filtering throughout the body. Although the larvae attempt to penetrate all tissues and organs, they can only survive in the striated or voluntary muscles such as legs or arms. Larvae within the muscle cells coil up, increase in size and become fully encysted in 17 to 21 days after the initial infection occurred. The encysted parasites may remain alive in a dormant state for the life of the host (the normal course in human infection), or until the second generation trichinous meat is again ingested by a carnivore.

The incidence of trichinosis in both humans and swine has declined dramatically in recent years. In 1947, when trichinosis became a reportable human disease, 451 cases and 14 deaths were reported. Over the five year period of 1976-1980, there was an average of 119 cases and 0.6 deaths per year.<sup>1</sup> The types, sources, and methods of preparation of meat products incriminated as the source of trichinosis in 1981 have been summarized.<sup>1</sup> Of the 188 reported courses, pork products were incriminated in 146(77 percent), 35 were from nonpork products and 7 were from unknown sources. Sausage was involved in 93 (49 percent) of the cases. Supermarkets, butcher shops, or other commercial outlets were the source of meat in 101 cases (54 percent). Meat obtained directly from the farm was the source in 47 cases (25 percent). In 121 cases, the meat was not cooked. Many of these cases were from eating raw, smoked sausage.

Swine are usually infected from eating infected meat scraps in garbage that has not been properly cooked or from eating infected wildlife such as game animal residuals, rats, etc. Consequently, the incidence of the disease is substantially less in areas where hogs are grain-fed in confined areas compared with those garbage-fed or that roam in woods or pastures.

The symptoms of trichinosis in humans depend in large part on the number of trichinae eaten. Eating a moderate amount of lightly infected raw or undercooked pork may result in no noticeable illness. However, eating even a small amount of heavily infected raw or undercooked pork may result in a serious case of trichinosis. If the infection is heavy, symptoms such as vomiting, diarrhea, and abdominal pain may occur with 24 to 48 hours. However, these initial symptoms do not always occur. The characteristic symptoms of trichinosis occur during the period when larvae are traveling through the body and becoming encysted in the muscle tissue. During this period, the symptoms include fever, edema, extreme muscular pain, petechial hemorrhage, and eosinophilia. Permanent disability or

death may occur with heavy infections. Treatments for trichinosis are directed toward relieving the distressing symptoms. There is no cure presently available for the disease.

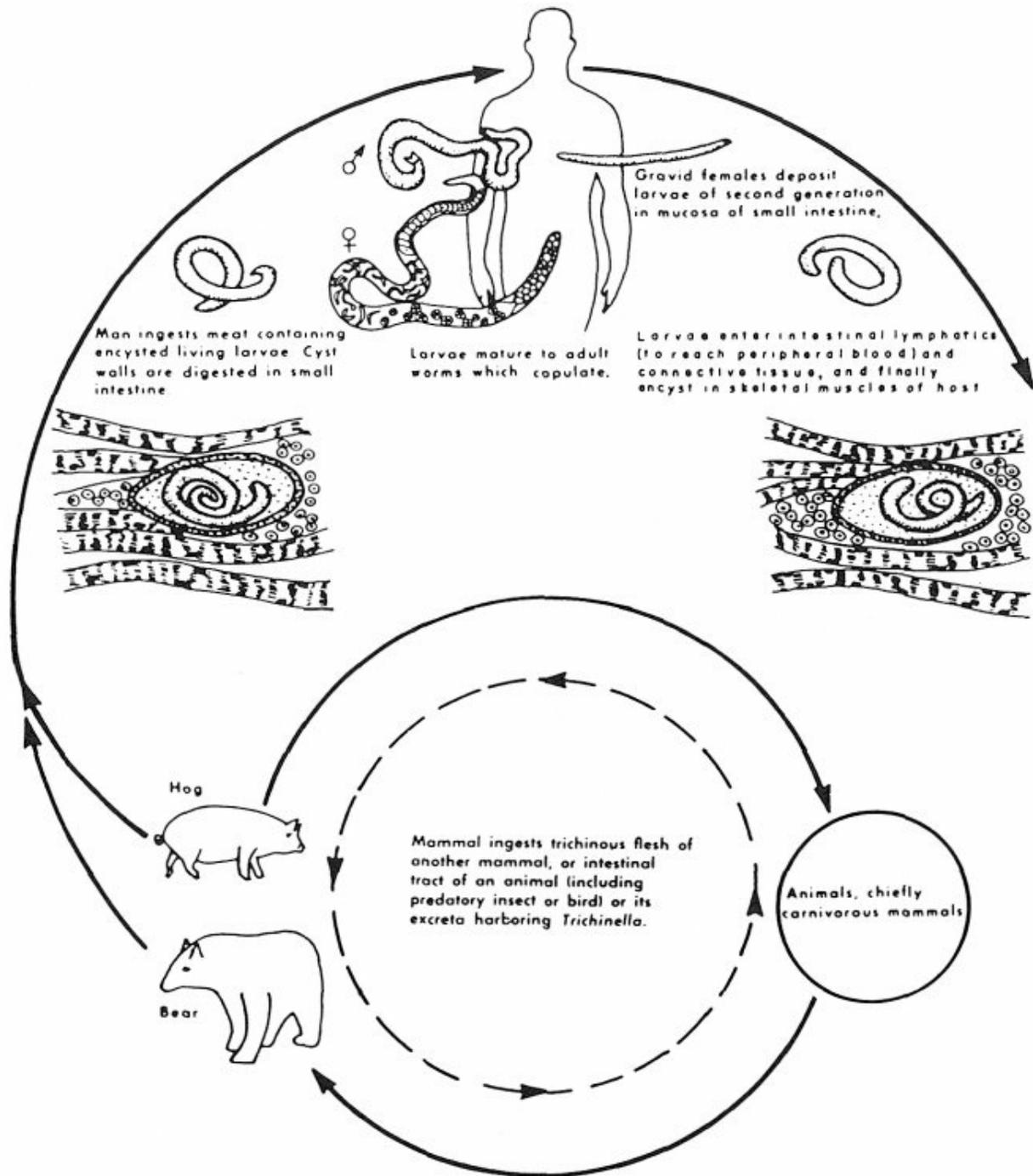


Figure 1. The Trichinosis Cycle.

## Control of Trichinosis by Irradiation

A food sterilization program administered by the U.S. Atomic Energy Commission and the U.S. Army found that foods irradiated with high doses (10 to 60 kGy) were generally wholesome, although in some cases, they were objectionable in flavor and aroma. Notably, pork and pork products were especially resistant to these effects and were deemed satisfactory, both aesthetically and nutritionally, even after doses of 30 kGy.<sup>2</sup>

Wholesomeness and toxicity studies were also performed at lower doses up to 1 kGy (sufficient to inactivate trichinae) and, as expected, showed no detectable deleterious effects from radiation.<sup>3</sup> In fact, taste panel results in this work showed that such irradiated pork was preferable to the unirradiated samples, due to the extended shelf life of the former.

If the literature indicates that when animals are fed irradiated meat containing encysted larvae, only very small radiation doses (5 to 10 Gy) are required to reduce the number of second generation larvae able to encyst in the muscle tissue. A higher dose of 100 to 200 Gy sexually sterilizes the trichina larvae encysted in the irradiated meat, as evidenced by complete loss of infectivity in test animals.<sup>4,5</sup> Microscopic examination of adult female trichinae developed from irradiated larvae reveal a complete degeneration of the reproductive system after these doses. At doses of 300 Gy, encysted larvae died in the enteric phase without reaching maturity. These results have also been confirmed by others.<sup>6,7</sup> Work recently completed by the U.S. DOE and their contractors on pork irradiation is also very supportive of previous findings.<sup>8</sup> This work indicates that (1) gamma irradiation of infected pork to a dose of 0.15 to 0.30 kGy renders the trichina sexually sterile and blocks maturation of ingested larvae in the host gut; (2) irradiation of freshly-slaughtered hog carcasses indicates that the radiosensitivity of the larvae is relatively unaffected by the age of the cyst, location of the cyst in the skeletal muscles, or the oxygen tension in the meat; and (3) post-irradiation holding of irradiated meat shows no significant recovery of trichina viability. The research shows that 0.30 kGy of cesium-137 radiation “can be delivered to split market-weight hog carcasses with acceptable uniformity, and that such a dose can provide a substantial margin of safety for human consumption of even heavily infected meat.”(8)

## Costs of Irradiation

Costs for pork irradiation facilities were developed for processing plants having a wide range of slaughter capacities.<sup>9</sup> These costs are summarized in Table 1. The average cost in dollars per pound decreases from 0.0056 to 0.0016 as hog plant capacities expand from 1,000 to 10,000 hogs per day. In terms of dollars per hog, the average cost decreases from 0.954 to 0.271 over the same range of plant capacities.

### Table I. Pork Irradiator Cost Summary.

1,000 Hog/Day   2,000 Hog/Day   4,000 Hog/Day   6,000 Hog/Day   8,000 Hog/Day   10,000 Hog/Day

**Capital Costs**

Facility Costs	\$589,700	\$634,800	\$ 716,100	\$ 806,700	\$ 886,200	\$ 964,500
Source Costs <sup>a</sup>	<u>177,120</u>	<u>354,120</u>	<u>708,240</u>	<u>1,062,360</u>	<u>1,416,480</u>	<u>1,770,600</u>
Subtotal Capital Cost	\$766,820	\$988,920	\$1,424,340	\$1,869,060	\$2,302,680	\$2,735,100
Annualized Cap. Cost (25% ROI)	\$191,705	\$247,230	\$ 356,085	\$ 467,265	\$ 575,670	\$ 683,775
(\$/lb) <sup>b</sup>	0.0036	0.0023	0.0017	0.0015	0.0014	0.0013
(\$/hog) <sup>b</sup>	0.614	0.396	0.285	0.250	0.231	0.219

**Annual Operating Costs**

Labor	\$ 90,000	\$ 90,000	\$ 90,000	\$ 90,000	\$ 90,000	\$ 90,000
Outside Services	3,000	3,000	3,000	3,000	3,000	3,000
Power	2,250	2,500	3,000	3,500	4,000	4,500
Source Recharge	5,810	11,060	21,550	32,450	42,970	53,470
Materials & Supplies	<u>5,000</u>	<u>5,600</u>	<u>6,700</u>	<u>7,800</u>	<u>8,900</u>	<u>10,000</u>
Subtotal Annual O&M	\$106,060	\$112,160	\$ 124,250	\$ 136,750	\$ 148,870	\$ 160,970
(\$/lb) <sup>b</sup>	0.0020	0.0011	0.0006	0.0004	0.0004	0.0003
(\$/hog) <sup>b</sup>	0.340	0.180	0.100	0.073	0.060	0.052
<b>TOTAL ANNUAL COSTS</b>	<b>\$297,765</b>	<b>\$359,390</b>	<b>\$ 480,335</b>	<b>\$ 604,015</b>	<b>\$ 724,540</b>	<b>\$ 844,745</b>
<b>TOTAL AVERAGE COST<sup>b</sup></b>						
(\$/lb)	0.0056	0.0034	0.0023	0.0019	0.0017	0.0016
(\$/hog)	0.954	0.576	0.385	0.323	0.291	0.271

<sup>a</sup> Source used for estimate was the Cs-137 (WESF) capsule.

<sup>b</sup> Assumes a plant operating 6 days a week and 52 weeks per year, and assumes an average eviscerated weight of 170 pounds per hog.

Table I also shows how total average costs are partitioned into capital and O&M costs. Because capital costs are recovered in four years (25% ROI), the total average costs apply only to the first four years of plant operation. After that time, costs for irradiation are due to O&M only. Using a 4,000 hog-per-day plant as an example, irradiation costs would be about 39 cents per hog during the first four years of operation. Beginning the fifth year, irradiation costs would drop to about 10 cents per hog.

There are presently about 150 large-scale gamma (cobalt-60 and cesium-137) irradiators installed in the U.S., but none has yet been adapted to commercial hog processing facilities. Using standard radiation safety and control principles, however, the adaptation of current irradiator technology to commercial hog facilities is expected to be a matter of standard design practice.

In a current processing sequence in a hog slaughter facility, the live hogs are first stunned, exsanguinated, and hung from an overhead conveyor for easy transferral throughout the plant. After dehairing, gutting, heading, splitting and cleaning, the prepared carcasses are placed in refrigerated storage for approximately 24 hours. The chilled carcasses are then ready for cutting, handling and packaging.

An irradiation facility could be designed to treat the pork at any of several stages in the

processing sequence, such as after packaging or before dehairing. After passing through the facility, the pork could be processed as usual. The irradiation facilities would not usually require significant amounts of floor space compared to the cutting and packaging floor.

## Economic Impacts of Pork Irradiation

An economic analysis supported by the U.S. DOE identified and assessed the likely impacts of pork irradiation on demand, prices and profits at producer, packer and retailer level of the market chain.<sup>9</sup> The anticipated effects on demand were examined by considering alternative scenarios using plausible assumptions with respect to consumer and industry acceptance of irradiated pork. A summary of the economic analysis is given as follows:

- The trichinosis stigma causes pork demand to suffer in both the domestic and foreign markets.
- Irradiation is economically feasible if consumers react positively to trichina-safe pork.
- The irradiation of pork appears financially feasible, using a 25 percent rate of return on investment in the irradiation facilities. This rate of return is considered adequate to attract the funds necessary to finance the irradiation facilities.
- The contribution of irradiation processing to finished product unit cost is relatively small. Therefore, additional cost due to irradiation is not an overriding consideration in consumer acceptance. However, initial investment costs in irradiation facilities appear significant for packer/processors.
- There is a lack of conclusive evidence on consumer acceptance of irradiated pork at this stage of the research program. However, it appears plausible that trichinosis elimination would result in a 2 percent increase in the domestic demand for pork in the short run. In the long run, an additional 1 percent increase in domestic demand and an expansion of exports by one-third (or 1 percent of domestic production) appears plausible. On this basis, industry profits would increase by a total of \$493 million per year in the short run, primarily as a result of increased prices. Economic theory suggests \$402 million would accrue at the farm level, \$74 million at the packer and processor level and \$17 million at the retail level. In reality, market power and other considerations could influence the share of the profits realized at the different levels. In the long run, the quantities of pork produced and handled would increase. Producer and packer/processor costs would increase accordingly and prices would change. The increased profits would decline to about \$30 million annually at the farm level, but would increase to about \$100 million at the packer/processor level.
- An analysis also was conducted of the economic impacts if consumers reacted negatively to the irradiation issue and domestic demand was reduced by 2 percent. Farm and retail sectors of the industry would suffer from reduced income but packer benefits exceed losses. In the short run, profits would decline by a total of \$393 million per year. In the long run, the decline would be about \$7 million per year at the producer level. However, profits of \$38 million at the packer/processor level might be

expected. This illustrates the importance of carefully evaluating and developing consumer acceptance.

## Consumer Acceptance

In order to gather information on the consumer reaction to irradiation, the U.S. DOE conducted a consumer attitude research study among a representative cross-section of U.S. adults.<sup>10</sup> Although the survey results are statistical in nature and can be used only as estimates in making projections, the survey data base can help the U.S. DOE to better develop project goals and outline consumer education plans to facilitate technology transfer. Some of the survey results are shown below:

- (1) The majority of people are concerned about current food preservation treatment methods, disease risk from food and food spoilage methods. The salt and nitrite content in pork, as well as the disease of trichinosis, were a major concern. It was projected that if these concerns could be alleviated, then the demand and consumption of pork would increase.
- (2) Consumer concerns with irradiation are significant but still less dominant than other alternative food treatment processes such as chemical sprays or preservatives. Some volunteered initial concerns for the irradiation process were the potential harm to people, possible side effects, long-term effects, if radiation were left in the food, the amount of testing done and the effect on the food.
- (3) Communication and education efforts should focus on the ability of food irradiation to ease world hunger and to reduce or eliminate chemicals and preservatives in food, as these were the advantages of food irradiation perceived to have the greatest impact on people. However, the concerns with irradiation do not go away easily and any education program will need to be clearly presented, carefully structured and extensive. A shallow or superfluous education program would tend to raise more questions in the consumers mind than reduce the original concerns.

## Future Activities

The BUP has determined that pork irradiation appears technically, economically and financially feasible. There do not appear to be any insurmountable obstacles from the view point of political feasibility. It appears likely that FDA will regard food irradiated at doses of 1 kGy or less as wholesome and safe for human consumption. The decision on whether the products must be labeled as "irradiated" may have an important influence on consumer acceptance.

Although the prospects for pork irradiation are encouraging, several issues must be addressed in more detail before the pork industry makes the decision to implement such a technology. These issues include: (1) developing more conclusive evidence on consumer acceptance and preparing a consumer education plan; (2) developing a reliable estimates

of the demand for irradiated pork in both domestic and foreign markets; (3) operating a pilot plant to validate costs and engineering issues; (4) analyzing the economies of small-scale irradiators to serve small plants or to irradiate pork products presently treated for trichinosis at relatively high costs by refrigeration; (5) determining the adequacy of transportation facilities for increased exports; (6) confirming any potential benefits from reduced spoilage; (7) assessing other potential benefits and costs (such as from reduced use of additives); and (8) assessing packer/processor interest and developing an implementation program.

Several activities are being initiated through DOE's Byproducts Utilization Program to address these issues. They include organoleptic studies and construction of a demonstration pork irradiator.

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# **Byproducts Utilization Program\*** **(including the U.S. Department of Energy Food Irradiation Program) Technology Update and Future Initiatives**

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## **The Peaceful Atom**

Nuclear energy activities are carried out according to the provisions of the Atomic Energy Act of 1954, as amended. The Act states that “atomic energy is capable of application for peaceful as well as military purposes...”. It sets the policy that the “development, use, and control of atomic energy shall be directed so as to make the maximum contribution to the general welfare...”. It also provides for a “program of conducting, assisting, and fostering research and development in order to encourage maximum scientific and industrial progress...”. It is in this sense that use of the atom in isotope form as a “weapon for peace” achieves its highest potential for improving the quality of the human condition.

The peaceful uses of atomic energy, expressed in general terms above, were given focus under President Eisenhower’s Atoms for Peace Program. Under this program, research efforts in food irradiation and nuclear medicine were initiated. The mandate for development of peaceful uses of the atom has been passed on through predecessor agencies to the U.S. Department of Energy (DOE). This history has established the significant potential that the ‘peaceful atom’ can have in alleviating hunger and disease around the world.

## **BYPRODUCTS UTILIZATION PROGRAM (BUP)**

Nuclear byproducts contain many useful and valuable materials. These materials have a wide range of applications in food technology, agriculture, energy, public health, medicine and industrial technology. The DOE has aided in the supply of useful byproducts and assisted in the research, development and demonstration of their uses. Transfer of this federally-developed technology to industry will ensure full realization of the benefits of the peaceful atom. Specific byproducts include isotopes such as cesium, krypton, strontium and tritium.

# Byproducts and Applications

## CESIUM

- Food Treatment
- Medical Product Sterilization
- Sewage Disinfection

## KRYPTON

- Lighting
- Non-Destructive Testing

## PLUTONIUM (238) (POWER SOURCES)

- Space Exploration
- Artificial Organs
- Cardiac Pacemakers

## STRONTIUM

- Remote Power Sources

## TRITIUM

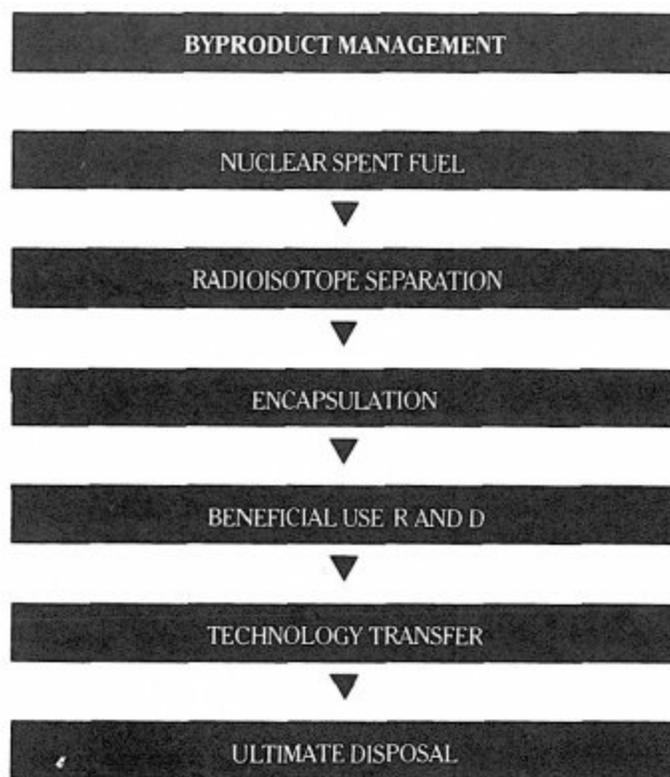
- Lighting

## XENON

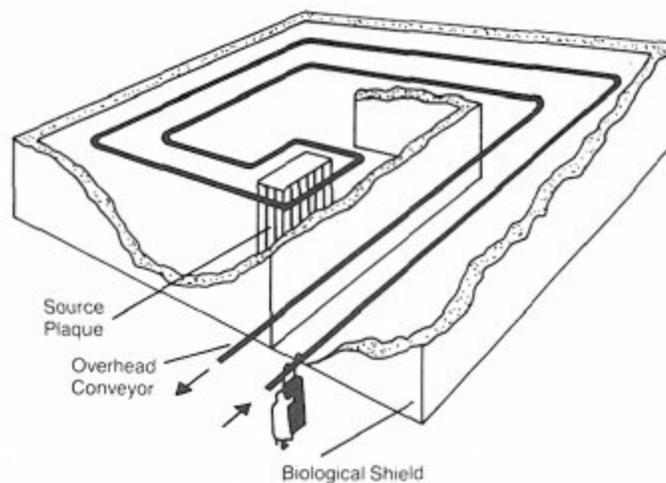
- Lasers
- Lighting
- Anesthetics

## CATALYSTS

- Palladium
- Rhodium
- Ruthenium
- Technetium



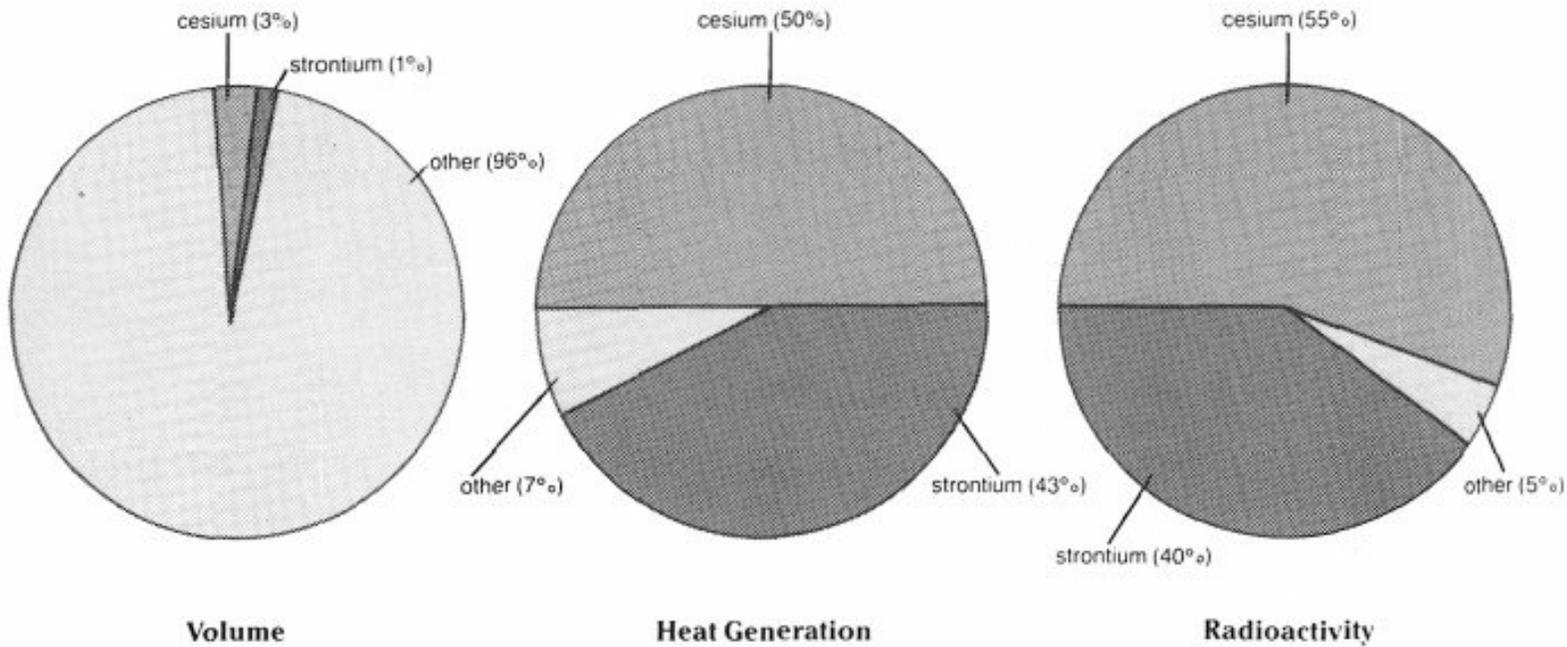
Cesium-137 and strontium-90 are two of the highest activity byproducts. The separation of these isotopes from nuclear waste removes more than 90 percent of the gamma radiation and heat load. In 1974, the DOE began the separation of cesium from defense waste at Richland, Washington, and in addition, purified and encapsulated this material for interim storage at the Richland Waste Encapsulation and Storage Facility (WESF). Such separation has simplified interim waste management and will significantly reduce the cost of ultimate disposal of the remaining fractions of nuclear waste. This approach has also provided a usable inventory of safe, approved cesium and strontium capsules for the BUP.



## Cutaway of a Cesium Irradiator

Gamma irradiation is a physical means of treating materials for purposes such as disinfection or sterilization. Irradiation with cesium 137 does not induce any radioactivity in the products being irradiated.

# Characteristics of Nuclear Waste\*

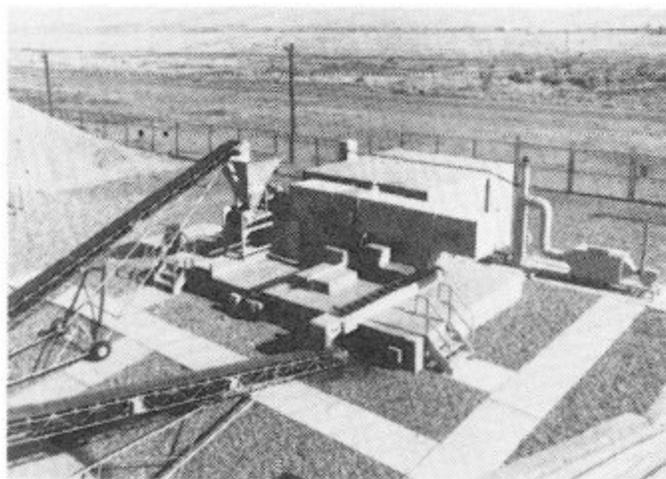


**Volume**

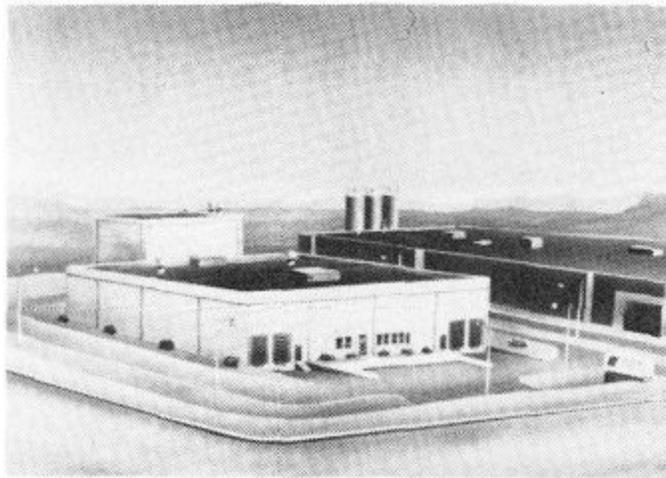
**Heat Generation**

**Radioactivity**

\*33,000 MWd tonne: 10 years after discharge



**Sandia Sludge Irradiator**



**Medical Products Irradiation Facility**

# Radiation Dose Required For Various Objectives

## LOW DOSE (100KRAD)

- Sprout Inhibition
- Insect Disinfestation
- Delay of Ripening
- Parasite Inactivation

## MEDIUM DOSE (100-1000 KRAD)

- Reduction of Microbial Load
- Improve Food Shelf-Life

## HIGH DOSE (1000-5000 KRAD)

- Commercial Sterilization

## DOE IRRADIATION ACTIVITIES

Current areas of emphasis in the BUP include sewage sludge disinfection, medical products sterilization and food processing.

### *Sludge:*

Research by Sandia National Laboratories and New Mexico State University has demonstrated the efficacy of cesium-137 irradiation in destroying harmful pathogens in municipal sewage sludge. The value of irradiated sludge as a soil amendment and as a ruminant animal feed supplement has also been demonstrated. Sludge irradiation technology has been successfully pilot tested since 1978 at Sandia National Laboratories. These successful research and pilot-scale programs coupled with favorable economic feasibility have established the foundation for commercial sludge irradiation processing.

### *Medical Products:*

One of the most rapidly growing uses for irradiation technology is in the medical products industry. Currently, 30 to 40 percent of sterile medical products are treated with cobalt-60 gamma radiation as an alternative to fumigation with the gaseous sterilant ethylene oxide (ETO). Irradiation is expected to replace ETO treatment as concerns over worker exposure to chemical residues increase. The first large-scale medical products irradiator using cesium-137 is now under construction and will be operational in 1985. More widespread use of cesium irradiation technology to sterilize medical products is dependent on a sufficient supply of isotope.

### *Food:*

The focus of DOE food irradiation activities is on low-dose applications. Increasing the food supply by reducing post-harvest losses can be achieved with these doses—a potentially significant application for developing countries. Extending shelf life and reducing the use of chemicals in food processing will expand domestic and export markets of numerous commodities. The FDA proposes to allow low-dose food irradiation in this country. In so doing, the U.S. will join 25 other countries currently using food irradiation processing. International expert committees have determined that foods irradiated to 1

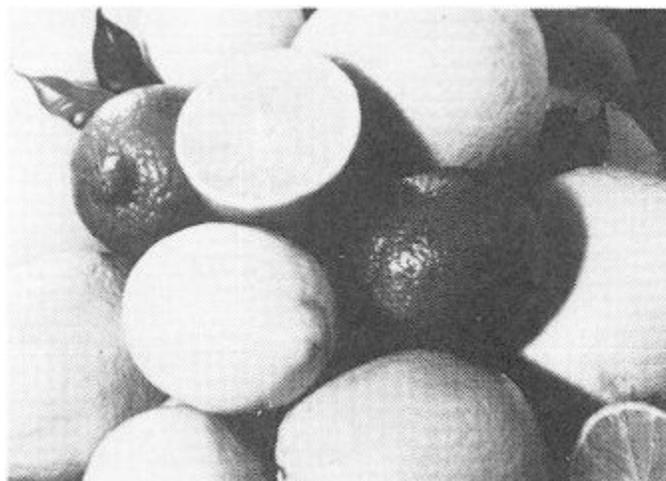
Mrad are wholesome and safe for human consumption.

## **DOE Food Irradiation Program**

Specific food irradiation activities being supported by the DOE include research, feasibility studies and development of full-scale irradiation facilities.

Research and feasibility studies are being conducted in cooperation with the U.S. Department of Agriculture (USDA), several universities and private contractors. The objectives of these efforts are to define the parameters critical to the irradiation treatment of agricultural commodities and to evaluate the feasibility of implementing an irradiation technology program in specific agricultural industries.

Full-scale irradiation facilities are being designed and constructed to accomplish the technology transfer goals of the BUP. These facilities will serve as a validation of cesium irradiation technology and will address technical and institutional issues such as licensing, economics and operational reliability.



## **Research/Feasibility Studies**

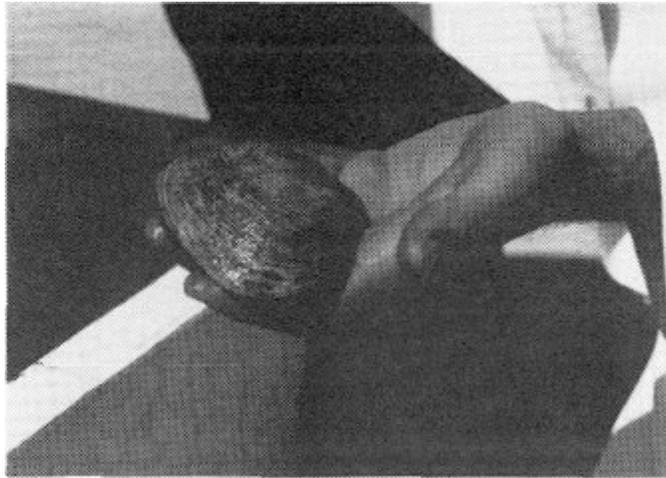
### *Citrus:*

The DOE is cooperating with the USDA-ARS to determine the feasibility of irradiating grapefruit as an alternative to fumigation with ethylene dibromide (EDB) for quarantine treatment. DOE has conducted several small-scale irradiation treatments of grapefruit at Sandia National Laboratories' cesium irradiation facilities. These and larger-scale studies conducted by USDA in commercial cobalt-60 irradiation facilities have shown irradiation to be an effective treatment for citrus disinfestation. The Environmental Protection Agency's recent ban on the use of EDB as a fumigant provides additional incentive to develop irradiation technology for the citrus industry.

### *Dried Fruits and Nuts:*

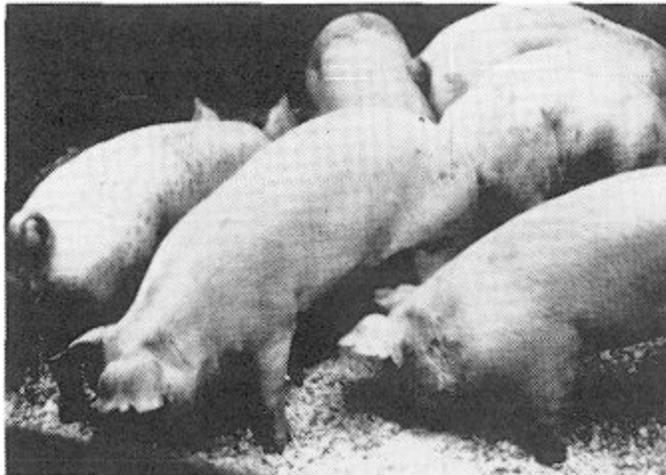
The future of methyl bromide as a fumigant for crops such as almonds, raisins, walnuts and prunes is uncertain. Cooperative efforts are underway with the USDA (ARS and ERS), Oregon State University, the California Almond Board, the California Prune, Raisin, and Walnut Board, and industry leaders to assess the feasibility of replacing fumigation of these

commodities with irradiation. Elements of this study include efficacy testing, engineering/economic considerations and taste panel evaluations. Preliminary results indicate that irradiation technology may have specific applications in treating these commodities for insect disinfestation.



### *Bivalves:*

The DOE is supporting the University of Lowell, Massachusetts, in efforts to assess the potential for irradiation treatment of bivalves (clams, oysters) harvested from polluted beds along the eastern coast of the United States. Clams from lightly polluted beds are now cleansed (depurated) in clean seawater exposed to ultraviolet light where pathogens are destroyed. This technique is not applicable to clams harvested from moderately or highly contaminated beds. Initial research has shown that shellfish can withstand substantial doses of gamma radiation during depuration; thus, radiation may offer a potential way to harvest clams from contaminated beds and safely cleanse them prior to introduction into the human food market.



### *Pork:*

Research on the effectiveness of irradiation to inactivate trichina parasites in ground and fresh pork has shown that very low doses of 30 krads are required. Taste panels have determined that no adverse qualities result from these low doses. These results have prompted the National Pork Producers Council (NPPC) petition to the FDA to include pork in their final food irradiation regulations. An economic feasibility assessment indicates that

large processors (greater than 1,000 hogs per day) can irradiate carcasses for less than 1 cent per pound. An assessment of consumer attitudes conducted by NPPC regarding food irradiation in general and pork irradiation in particular, indicates that important issues such as efficacy and wholesomeness must be addressed if irradiation treatment of foods is to be accepted by consumers. This pork research program is a cooperative effort among DOE, NPPC, USDA, New Mexico State University, Iowa State University, the Inhalation Toxicology Research Institute, and the Sandia and Los Alamos National Laboratories.

## **Agency for International Development (AID)**

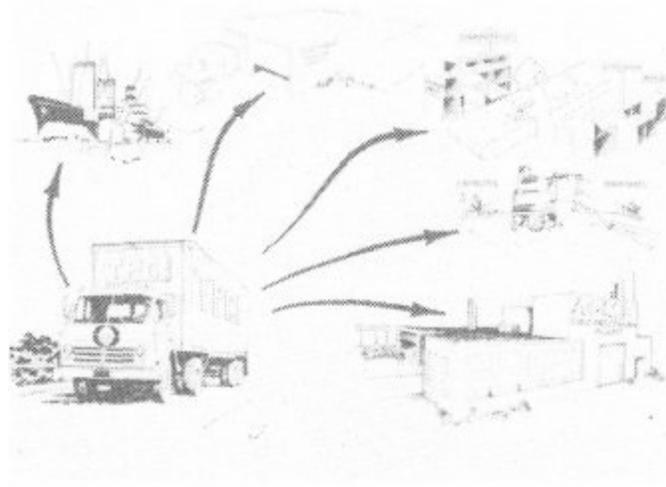
The DOE has recognized the international interest in irradiation technology and its potentially dramatic applicability to many developing countries of the world. The magnitude of postharvest food losses for developing countries is estimated to average about 20 percent but run as high as 50 percent in specific areas. The DOE is cooperating extensively with AID to assist in evaluating the potential for this technology in reducing these losses in the Caribbean Basin, Central and South America and Southeast Asia.

### *Transportable Cesium Irradiator (TPCI):*

In order to conduct meaningful research on irradiation treatment of fresh commodities, the effects of complicating variables (such as time since harvest, temperature of storage and shipment, distance and time of shipment) must be minimized. Further, for research results to be valid, irradiation of the particular pests of concern must be conducted at the appropriate stage in the life cycle of the pests. These factors have dictated the need for a small-scale research irradiation facility that can be transported to sites where infested commodities are harvested or collected for investigation of irradiation applicability. The TPCI is such a flatbed-mounted cesium irradiation unit. Operation should commence in 1985. TPCI will be capable of irradiating unit-size cartons of commodities as part of research initiatives on various products. Use is projected at multiple USDA locations throughout the country, as well as by commercial firms interested in gaining limited basic research data on the feasibility of irradiation treatment of their specific products.



**Trans Portable Cesium Irradiator  
USDA Research Support**

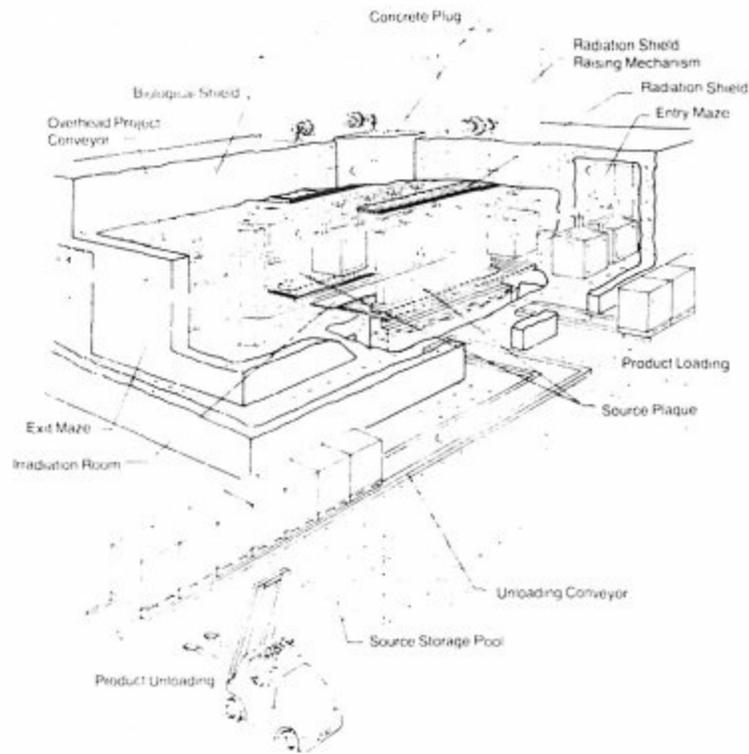


## **Technology Transfer—Full-Scale Demonstration Facilities**

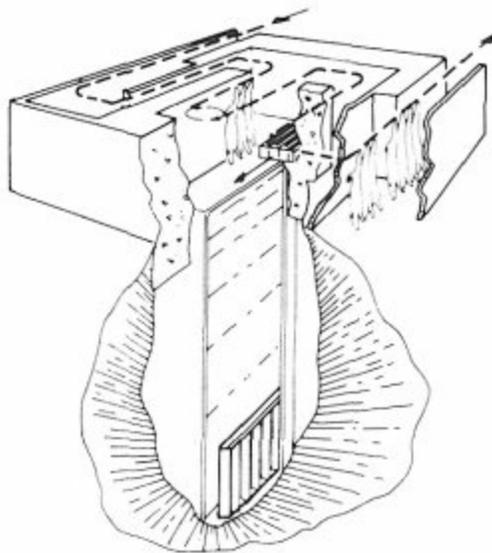
### *Cesium Agricultural Commodities Irradiator (CACI):*

The CACI will demonstrate the irradiation of agricultural commodities under conditions simulating commercial operations and will provide sufficient quantities of irradiated commodities to conduct economic, storage and marketing studies. The irradiator will utilize cesium-137 to treat tote loads (six standard cartons each) or pallets of agricultural commodities. The cesium will be stored in a water pool and raised into the irradiation chamber during operation. The irradiator will be used by USDA, various food industries, and by visiting scientists, faculty and students from developing countries.

## Agricultural Irradiator Isometric



## Conceptual Pork Irradiator



"The Cs-137 source is shown in its secure position at the bottom of the storage well. In use, the source is raised to a position between the two conveyor rails (shown by the dashed lines) which carry the hog carcasses."

## Meat Irradiation Technology Center (MITC):

MITC will provide a large-scale validation of irradiation technology to the fresh meat industry. The MITC will be a panoramic, wet source storage gamma irradiator which will consist of a concrete shielded structure containing cesium-137 source capsules and an overhead conveyor system capable of moving several hundred carcasses or boxed products per day into and out of the irradiation chamber. The MITC will address technical, economic, social and institutional issues as a precursor to commercial implementation of irradiation technology. The MITC will be operated by an organization, such as a university,

having complete meat processing facilities and research support in the areas of veterinary science, food and meat science, biochemistry, consumer science and nutrition.

## THE FUTURE

In addition to food irradiation research and facility validation initiatives being supported by the DOE, several efforts supporting the technology in a broader fashion are being pursued.

### Isotope Supply

Commercial irradiation technology currently utilizes the isotope cobalt-60 and machine-generated radiation as its radiation sources. However, the U.S. produces very little cobalt-60—most of the world's demand is met through production at a nuclear reactor complex near Pickering, Ontario, Canada.

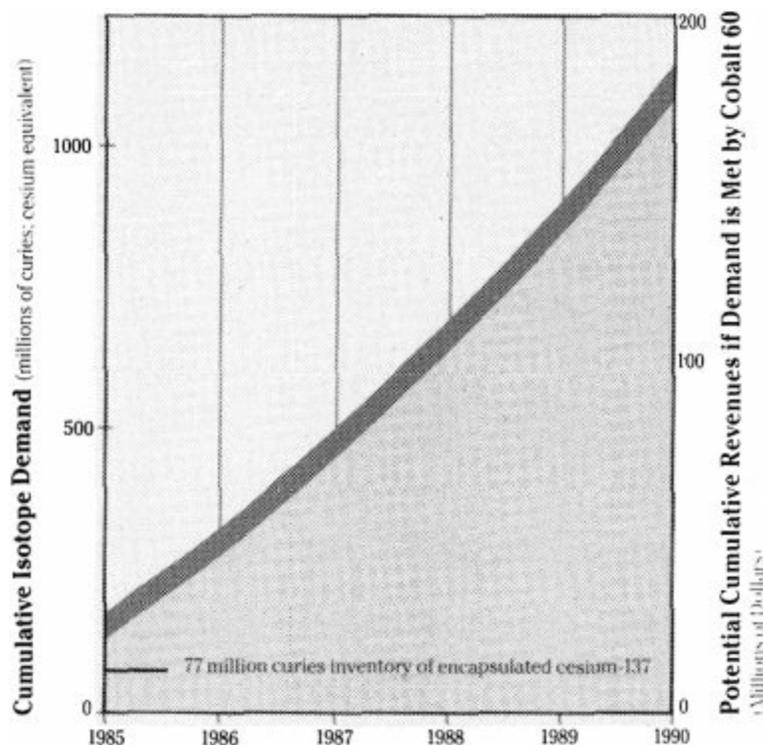
An alternative to cobalt-60 is cesium-137. Since 1974, the AEC/ERDA/DOE has encapsulated 77 million curies of cesium-137. This supply is a small fraction of the total amount of cesium potentially available from all sources over the next 20 years. Most of the isotope is contained in spent fuel rods from commercial power reactors that are currently being stored onsite at these facilities.

### Isotope Demand

Currently there exists a shortage of isotope (both cobalt-60 and cesium-137) and machine-generated radiation sources to meet existing demand in the medical products sterilization field. Thus, any major utilization of irradiation in the food industry is precluded in the near term. In order to assure that the promise of food irradiation technology is realized, the DOE is investigating options for increasing the supplies of radiation sources. Barring any increase in U.S. supplies of isotopes, the domestic radiation industry will be left totally dependent upon foreign supplies of radiation sources.

### Estimated Inventories of Cesium-137 From Various Sources

	Source	Effective Inventory Date	Million Curies
Hanford	• Cesium Chloride Capsules through 6/84 (1575 Capsules)	1/83	77
	• Future Purex Wastes	1/91	38.7
Savannah River Plant	• Current Wastes	1/83	102
	• Future Wastes	1/2001	109
Commercial Spent Fuel	• Accumulated through 1981	1/83	520
	• Accumulation through 2020	1/2021	11,000



## Initiatives

The U.S. DOE Byproducts Utilization Program has had singular success over the years in structuring technology transfer objectives which meet the needs of the country. For example, the availability of research results, based on the last 5 years of DOE food irradiation work, will lead to early solution of the critical national and allied nation food production and food chain problems resulting from the ethylene dibromide (EDB) ban. The actualization of these years of research and development can only be met by maintaining the momentum of the DOE initiatives with the following activities:

1. *Technology Transfer Irradiator Support:*

The TPCI, CACI, and MITC are “investments in excellence” for solution of postharvest loss, disease reduction and world hunger problems. Utilization of the facilities must be encouraged and supported to maximize the technology transfer potential to areas such as the Caribbean Basin and Central America.

2. *Establishment of a Food Irradiation Information Network:*

Technology potential can only be achieved if information is evaluated and then shared, especially with lesser developed countries where the needs are greatest.

3. *Development of Radiation Sources:*

The current isotope shortages can only become more severe as the technology grows unless isotope resources such as cesium-137 are retrieved by spent fuel conditioning, and unless isotope generation such as cobalt-60 production is reevaluated. Options to “foreign source only” must be critically studied and resolved in the near future by pulling aside the curtains on the myths which currently surround the issues.

#### 4. *Evaluation of Machine-Generated Irradiation:*

Feasibility of machine-generated irradiation must be evaluated as one leg of the triad of cesium-137, cobalt-60 and electron beam irradiation sources. Social and political issues surrounding public health, world hunger and economic stability in regions such as Central America and the Caribbean Basin are inexorably linked to export of agricultural commodities. Irradiation is being recognized as a major potential solution to current export quarantine constraints. Because of isotope shortages, machine-generated sources will be critically needed in the short term. Irradiation technology development and demonstration—as a weapon for peace—is crucial to a timely solution of these regional problems.

# Status and Future of Radiation Processing of Medical Devices, Pharmaceuticals, Food and Toiletries

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*Vienna, Austria*

## Introduction

Ionizing radiations are increasingly being used in the commercial practices in the medical, pharmaceutical, public health, cosmetic and food fields. This involvement in the technology basically relies upon the induction by the radiation energy, of microbicidal effects and other chemical and physical modifications of the materials concerned in the desired directions. Consequently radiation processing has often formed an integral part of the processes of sterilization of patient-ready medical devices and decontamination of raw materials to be used in the pharmaceutical formulations and in the preservation of food stuffs to extend storage life and supplies to the consumers.

In these and other relevant industrial applications, radiation processing techniques often hold an important edge over the alternatives using heat and toxic chemicals. These particularly relate to the ability to penetrate and a negligible rise in temperature during treatment. Other significant attributes of radiation compared to its conventional alternates are the energy economy and conservation, as well as preservation of environmental quality through a pollution-free operation of the technology and presumably, a superior sterility assurance of the finished product.

The International Atomic Energy Agency (IAEA) has continuously supported programs to promote beneficial applications of all the above-mentioned radiation processing fields in the developing and developed Member States. These programs follow the line of its statutory responsibilities “to accelerate and enlarge the contributions of atomic energy to peace, health and prosperity throughout the world,” and in particular response to the identified welfare needs of its Member States<sup>18</sup>.

For ease of handling the discussions on the “status” and “future outlook” will be separately treated in the following sections:

- (i) the geographical distribution of the medical devices, sterilization and food irradiation technology with particular regard to the developing regions in the world;
- (ii) the current developments in the regulatory process and product control aspects;

- (iii) any potential bearing of these development on the industrialization and international trade;
- (iv) the international services for the dosimetry calibration of the irradiator facilities as a promoter of the beneficial scopes from these technologies;
- (v) food industrial processes with considerations for the developing countries;
- (vi) future outlook and concluding remarks.

## *I. Radiation Sterilization of Medical Devices*

Among the prospective areas of industrial applications, radiation processing technology has made its most significant mark and impact in the field of sterilization of medical devices in a ready-to-use prepacked hermetically-sealed state. Since over twenty-five years, the medical supplies manufacturing industry has made use of the microbicidal effects of radiation. Many millions of medical devices are currently being sterilized by radiation each year and a continuing rapid growth is experienced, which is attributed to (a) a wider spectrum of medical devices being brought into industrial operation; (b) a greater cumulative bulk throughput of each species of medical device being processed to meet further demands of health-care; (c) a larger number of developing countries and regions is introducing this nuclear technology and/or the sterile products thereof in the national health care.

One obvious outcome of this development has been a marked reduction in the incidence of cross-infection (nosocomial) diseases. These sterile single-use items are particularly valuable when healthcare services are to be delivered in an unequipped rural camp hospital, a situation often met within the developing regions. The health authorities in many developing countries have opted to introduce this apparently expensive alternate, i.e. radiation-sterilized single-use devices in their national healthcare system in consideration of the long-term economic welfare advantages gained due to “prevention” of manpower loss and other societal burdens through reduction of cross-infection hazards. Nevertheless, in the context of the entire world regions with rapid population growth, the current production capacity of the technology falls far short of the actual demands. There is still a long way to go before the full potential welfare scope is realized.

This statement, however, needs to be brought in the due context of the available statistics. I believe as reasonably up-to-date, that there are about one hundred and thirty cobalt-60 gamma sterilization plants in operation in the world, which represents a total activity of approximately 75 million curies of installed cobalt-60 radioisotope. Considering that the age of this technology is even less than thirty years, it is indeed a commendable record. This installed radiation energy inventory is equivalent to a potential annual production output of sterile medical devices of over two million cubic meters, when calculated on the basis of a sterilizing dose of 25 kGy. In the light of the current conceptual advances in North America in the regulatory process control and dose-setting criteria potentially permitting an even lower dose for specified products, this throughput equivalence could be still higher.

## II. *The role of IAEA programs*

In keeping with the objectives of the statutory tasks of the IAEA and in particular with regard to the health and welfare interests of the technologically lesser-advanced developing Member States, the directives of the Agency programs have continued to attach a great deal of importance towards an appropriate development and dissemination of current technical know-how for this nuclear technology. Particular emphasis has been laid in these programs on helping the development of suitable practices pertinent to the indigenous medical and pharmaceutical items, with due regard to the local conditions of the tropical environment. Development and implementation of the action plans were carried out through periodic panels, expert advisory groups, topical symposia, research support and coordination programs, publications and primarily through the instrument of an elaborate technical cooperation and support service to the Member States.

Encouraging outcome is noticeable, particularly in some developing countries of Asia and the Far East in the implementation of the various integral steps of the practices for radiation sterilization of medical supplies which are summarized in Table I. Following the successful commissioning of the two cobalt-60 irradiator facilities in India (1974) and in South Korea (1975) respectively, both through the combined resources of the United Nations Development Program (UNDP) special fund, IAEA and the national government concerned, a spurt of interest in this nuclear technology was evident in most other countries in the region.

Today production-scale cobalt-60 irradiator facilities are operating in the region in Indonesia, Malaysia, Singapore, Bangladesh and Thailand, while smaller pilot capacity gamma-cell facilities are engaged in the test-process research and development and preliminary marketing orientation surveys in the Philippines, Pakistan, Burma, and recently in Sri Lanka. Since last year the Chinese cobalt-60 facilities, respectively in Beijing and Shanghai, have sought and received IAEA assistance in research support as well as the provision of microbiological standard preparations for their dosimetry and process calibration. Through these recent developments the "radiation sterilization map" in the Asia and Far East region has succeeded to better bridge the "gap" between Japan and Australia. Further progress is impending in the region.

The corresponding recent advances in this nuclear technology for healthcare services in the developing regions of Europe, the Middle East, Africa and Latin America are also summarized in Table I. The status in the Africa, Middle East and Latin America regions is marked by a high degree of heterogeneity and inadequacy, leaving much scope for future development. In the African region, Egypt and Saudi Arabia have already commissioned and are operating cobalt-60 facilities producing sterile medical supplies for indigenous healthcare. In striking contrast, many other countries in the region do not have even at the elementary level a technical and manpower infrastructure, while some others, e.g. Algeria, Ghana, Morocco, Zambia and Zaire, are in an advanced planning stage of this technology implementation. A promotion of regional cooperation is desired.

## Processing Applications.

Region and country	Large-scale irradiation facilities: (setting-up/commissioning)	Relevant research support and coordination (practices development)	Advisory assistance on technical economic and marketing services for planning/implementation	Training of technical manpower on radiation processing training courses/fellowships
<b>I. Asia and the Far East</b>				
Bangladesh	“ + ”	+	+	+
Burma	( <sup>60</sup> Co-gamma cell)	+	+	+
China	“ + ”	+	+	+
India	+ <sup>a</sup>	+	+	+
Indonesia	+	+	+	+
Korea (South)	+ <sup>a</sup>	+	+	+
Malaysia	“ + ”	+	+	+
Pakistan	( <sup>60</sup> Co-gamma cell)	+	+	+
Philippines	( <sup>60</sup> Co-gamma cell)	+	+ <sup>c</sup>	+
Sri Lanka	( <sup>60</sup> Co-gamma cell)	+	+	+
Thailand	+ <sup>b</sup>	+	+	+
Vietnam	( <sup>60</sup> Co-gamma cell)	+	+	+
<b>II. Europe and the Middle East Region</b>				
Bulgaria				+
Czechoslovakia	“ + ”	+	+	+
Greece	“ + ”	+	+	+
Hungary	+ <sup>a</sup>	+	+	+
Iran	+		+	+
Israel	“ + ”		+	
Jordan		+		
Poland			+	+
Portugal			+	+
Romania			+	+
Spain	“ + ”	+	+	+
Turkey	+ <sup>b</sup>	+	+	+
Yugoslavia	+	+	+	+
<b>III. African Region</b>				
Egypt	+	+	+	+
Ghana	+ <sup>ab</sup>	+	+	+
Kenya		+	+	+

Nigeria		+	+	+
Saudi Arabia	“ + ”	+		
Zambia				+
Zaira	“ + ”			+

#### IV. Latin America Region

Argentina	“ + ”	+	+	+
Brazil	+ b	+	+	+
Chile	+ b	+	+	+
Mexico	+ b	+	+	+
Peru	+ b	+	+	+
Venezuela	“ + ”	+	+	+

Notes: a = <sup>60</sup>Co-gamma irradiation for medical devices sponsored by UNDP/IAEA/Government project.

Recently some countries in the Latin America region have been considering a possible regional cooperation in the industrial uses of radiation processing, for which medical supplies sterilization is a likely candidate. This regional cooperation is expected to follow the pattern of the existing IAEA Regional Cooperative (RCA) for the countries of the Asia and Pacific region. The IAEA RCA program has been established through the concluded Agreements between the Director General of the IAEA and the Governments of the Member States in Asia and the Pacific region. Active coordinating programs are operating since over five years in the specified radiation processing fields to advance and sustain economic and welfare returns through the national technological grid of the RCA Member States. The Agency's RCA program as well as the UNDP/Industrial Project component in the RCA framework provide large scale support for the radiation sterilization practices technology through job-oriented training courses, research support and equipment supply among others.

Almost all of the cobalt-60 facilities installed in the developing Member States through IAEA support are administered by the respective governmental establishments, such as the Atomic Energy Establishment or the Ministry of Science and Technology. Consequently, their operation involves the service sterilization of medical products derived from the local manufacturers. This implies that a close cooperation be maintained with the facility user customers at all stages, including an education and technical guidance of the product manufacturers for specifications, compatibility and standardization as per good manufacturing practice (GMP) and the national process regulatory criteria.

### III. *International Dose Assurance Service (IDAS) of the IAEA*

It is well recognized that a reliable dosimetry is among the key requirements for successful technological developments in all the radiation processing industries, including those of particular interest for this conference. Process and quality control in these

endeavors have been based on the data and assurance criteria that the final product has received the correct quantity of radiation energy through the delivered process dose and this requires that the in-product dose be accurately measured<sup>4</sup>. With the increasing international trade in irradiated products, the authorities who assess their safety will more and more require to base their clearance judgment on these process validation criteria<sup>6</sup>.

With this objective in view the IAEA Dosimetry Section initiated international coordinated programs to help develop and evaluate the dosimetry systems for reliable use in high dose range radiation processing industries. Through the participation of leading dosimetry research institutes the international experts have elucidated in detail the technical characteristics of a series of physical and chemical dosimeters together with the advantages and limitations associated with them. These data provide valuable guidance in the choice of appropriate dosimeter systems for a specified situation of radiation technology. The program further involved an intercomparison of a series of suitable high-dose level dosimetry systems "in the product" during normal operation of an irradiation facility, in parallel with the operator's own routine dosimetry system. The aim is to confirm that no unexpected errors are arising in the facility's routine dosimetry system.

These preparatory developmental research and intercomparison programs have successfully culminated in the recent establishment of an International Dose Assurance Service (IDAS) by the IAEA<sup>13</sup>. The IDAS is expected to have multifarious promotional impacts through the concerted international efforts to achieve measurement standardization of dosimetry and dose assurance for large radiation sources and their advancement. In addition the service can be used by national authorities for quality control of radiation processing as well as for licensing and safety inspection. The standardization of dosimetry provides a sound justification for the regulatory approval of irradiated products and the basis for the international clearance for free trade of the irradiated products.

#### *IV. Food Preservation by Irradiation*

About thirty years of research and development work on the preservation of food by irradiation has shown that this radiation processing technology holds the potential to reduce post-harvest losses and produce safe foods. Compared to the conventional methods, radiation preservation is an energy-conserving "cold" process and can replace or drastically reduce the need for food additives and fumigants which can pose health hazards for workers and general consumers. Food irradiation processing policies Food and Agriculture Organization (FAO) and programs have been developed<sup>7,8</sup> through support by the Food and Agriculture Organization (FAO). (?)IAEA and World Health Organization (WHO) and by a number of individual Member States with an aim to achieve their general acceptance and practical implementation, facilitating an unimpeded movement of irradiated foods in international trade.

The Food Preservation Section of the Joint FAO/IAEA Division is responsible for implementation and supervision of all program activities in this field. My brief comments in this report have been based upon the information gathered from Dr. J. van Kooij of Food Preservation Section, to whom I am grateful for this help.

FAO/IAEA sponsored coordinated research projects on wholesomeness studies generated data on toxicology and other specific interactions with irradiation and on topics of chemistry of treated foods and food components. On the basis of these and other relevant data, a joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food (JECFI) was able to recommend the acceptability from a toxicological standpoint of any food commodity irradiated up to an overall average dose of 10 kGy. The Committee further concluded that up to an irradiation dose of 10 kGy no special nutritional or microbiological problems are envisaged from irradiated food<sup>7,8</sup>.

## **Table II. Unconditional and Provisional Clearances of Food Products in Different Countries\***

Foods	Argentina	Bangladesh	Belgium	Canada	Chile	Denmark	France	Hungary	Israel	Italy	Japan	Netherlands	Norway	Philippines	Poland	South Africa	Spain	Thailand	Uruguay	USA	USSR
Potatoes	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Onions		x	x	x	x		x	x	x	x	x			x	x	x	x				x
Garlic			x				x		x							x					
Shallots			x				x														
Wheat, flour, whole wheat flour	x		x	x																	x
Spices	x	x		x			x	x				x	x								x
Chicken	x			x					x			x				x					
Fish and fishproducts (chilled, frozen)	x			x								x									
Frozen Shrimps	x											x									
Froglegs	x											x									
Rice and ground rice products	x			x								x									
Ryebread												x									
Egg powder												x									
Blood Proteins												x									
Cocoa beans					x							x									
Dates					x																
Pulses	x			x																	
Papaya	x			x													x				
Mango	x			x													x				
Strawberries		x		x								x					x				
Paprika		x																			
Mango achar																					
Bananas (fresh, dried)																					
Litchis																					x
Dry food concentrates																					x
Grain																					x
Dried fruits																					
Mushrooms												x									
Endive												x									
Asparagus												x									
Battermix												x									

\* Information source from Dr. J. Van Kooij, 1984.

Another significant breakthrough has been encountered in the recent adoption by the FAO/WHO Codex Alimentarius Commission (CAC) of the Codex General Standard for Irradiated Foods<sup>8</sup>. The scope of this standard refers only to the processing of food with ionizing radiation up to a dose of 10 kGy. Such products, however, will be subject to general food regulations like any other food. This adoption by the CAC Standard is a recognition

that food irradiation has been established as safe for general application at least up to an absorbed dose of 10 kGy<sup>8</sup>.

Food irradiation for preservation has been to date approved in some 26 countries and about 140 specific commodities including fish, poultry, fruits and vegetables have gained governmental clearances in recent years (Table II). Although the irradiation process is not yet commercially applied in all countries that have granted clearances, an estimated world production of irradiated foods amount to over 65,000 tons per year, including the data for grain disinfestation.

Today this promising radiation process stands poised for a rapid growth following the important regulatory breakthroughs. Residual problems seem to be connected to questions of public information and industrial economics. To overcome these obstacles the future steps should concentrate upon a demonstration of the economic feasibility of the technology of food irradiation processing along with the formulation of a uniform legislative framework. Success and achievement in these regards should be facilitated by closer international cooperation<sup>10,19</sup>.

## **Regulatory control aspects of the sterilization process and the products**

Like all other sterilization processes, the radiation sterilization process and the sterilized medical products for clinical use need to fulfill the validation criteria as stipulated and implemented by the national Food and Drug Administration and other relevant health regulatory authorities. The purpose of this regulation is the imposition of a strictest quality control on the radiation processed items to ensure the fulfillment of the desired objective pertaining to the consumer safety. Often such radiation processed items may have to be consumed beyond the national boundary of production. Under such circumstances, the items must as well comply with the regulatory requirements of the consumer country. This is facilitated by the availability of the criteria of international standardization and their dissemination for coordination to help implement suitable regulatory guidelines.

Countries pioneering in these efforts, such as the United Kingdom, USA, and Australia have formulated guidelines to good manufacturing practices (GMPs) for sterile medical devices and surgical products and also for pharmaceutical products<sup>24,26</sup>. Since the inception of radiation sterilization process for medical supplies the sterilizing dose of 25 kGy was generally followed in most countries. In the light of further research and development and an enlightenment on the nature of the sterilization process through progressive experiences, however, to date there are in practice some distinctive specifications of the concept of the required sterilizing doses.

This problem, for example, is illustrated by the situation in North America and Europe. There happens to be no specified fixed minimum sterilizing radiation dose in North America, which progressively implements the guidelines as formulated by the Association for the Advancement of Medical Instrumentation (AAMI) in this regard. Those guidelines refer to the dose setting approaches based upon the estimated radiation resistance characteristics

of naturally occurring microbial bioburden on the medical products concerned. Different sterility safety levels are thus achievable for the different categories of medical items according to their clinical end uses. Consequently, many devices could in practice be radiation sterilized at doses lower than 25 kGy, while still some others may justify an even higher one. In contrast, the European health regulatory authorities continue to follow and recommend a minimum sterilizing radiation dose of 25 kGy. This situation is expected to lead to some problems of international clearance of the sterilized medical items and towards the attainment of the implied health welfare objectives. Further joint analysis and review of the problems and the necessary technical steps should be facilitated through international standardization approaches.

In 1967, an IAEA expert group recommended the basis for an international Code of Practice for radiation sterilization of medical products<sup>3,5</sup>. The IAEA in cooperation with WHO and the national health regulatory authorities in the Member States since remains responsive to the periodic updating and revisions of those recommendations in the light of added new operational experiences in the fields concerned. One such review discussion of the document is planned during the IAEA Advisory Group Meeting (AGM) scheduled to be held in Sri Lanka late in 1986. It is anticipated that this meeting's discussions should involve relevant expertise from the health regulatory authorities, medical professionals, biomedical researchers and manufacturers of sterile medical supplies.

## Irradiation sources

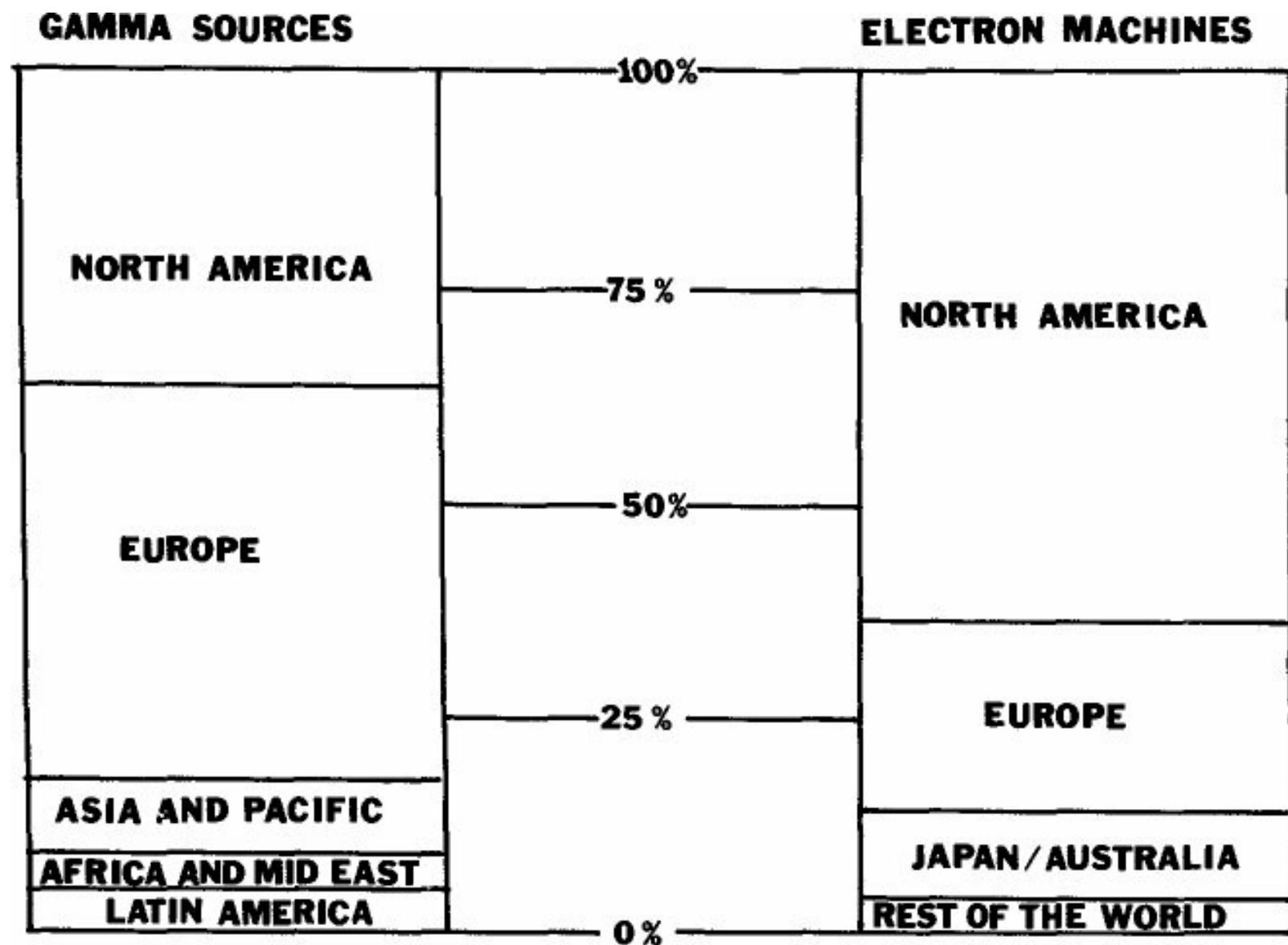
The most important irradiation sources used in the industrial radiation processing in general are: (i) gamma facilities, utilizing primarily <sup>60</sup>Co radioisotope and to a very limited extent <sup>137</sup>Cs isotope; and (ii) electron beam accelerators of varying capacity<sup>17,18,19</sup>. During the past fifteen years radiation processing, as a whole, is estimated to be grown steadily at about the rate of 10 to 15% per year. The main indicator of this growth is the number and total installed power of radiation sources, a topic extensively reviewed at this conference as well as in several recent meetings<sup>18,19</sup>. Currently, more than 130 industrial gamma irradiators are in operation and/or under implementation process (Table III) and the state-of-the-art practices are showing an upward trend<sup>17</sup>. These seem to indicate that radiation processing, based upon both gamma and electron beam sources, has become an established and accepted method in the manufacturing industries.

The status, as it stands today is that gamma sources from <sup>60</sup>Co are most often the preferred choice for radiation sterilization, food irradiation and in general for treating bulky and voluminous products. In some overlapping areas, both radiation sources can be advantageously used. The final choice, however, may depend upon detailed techno-economic analysis and other factors of local significance including support manpower, as the case may be in many developing countries. Often the developing countries seem to prefer a "multi-purpose" irradiator plant to be able to deal with medical devices, food, and other relevant items. Such irradiator plants are already in existence or are being planned in more than a dozen countries, such as Egypt, Bangladesh, Belgium, Brazil, Hungary,

Indonesia and Israel.

Distribution of radiation processing facilities in the major geographical regions (Table III) reveal that despite a recent rapid growth in North America in the  $^{60}\text{Co}$  capacity, Europe still leads somewhat. The situation is the reverse in respect of electron-beam machines. There has been a remarkable advance in the area of  $^{60}\text{Co}$  irradiator installation in the Asian region. In Africa and the Middle East and Latin America regions respectively there are several gamma irradiators operating and/or under construction. The electron machines outside North America and Europe are mainly in Japan and Australia (Table III).

**Table III. Radiation Capacity (Distribution particular regard to sterilization of medical devices)**



World-wide geographic distribution. In absolute quantities, the gamma capacity is approximately four times that of the electron capacity. (Derived after Morgenstern [1978] and updated)

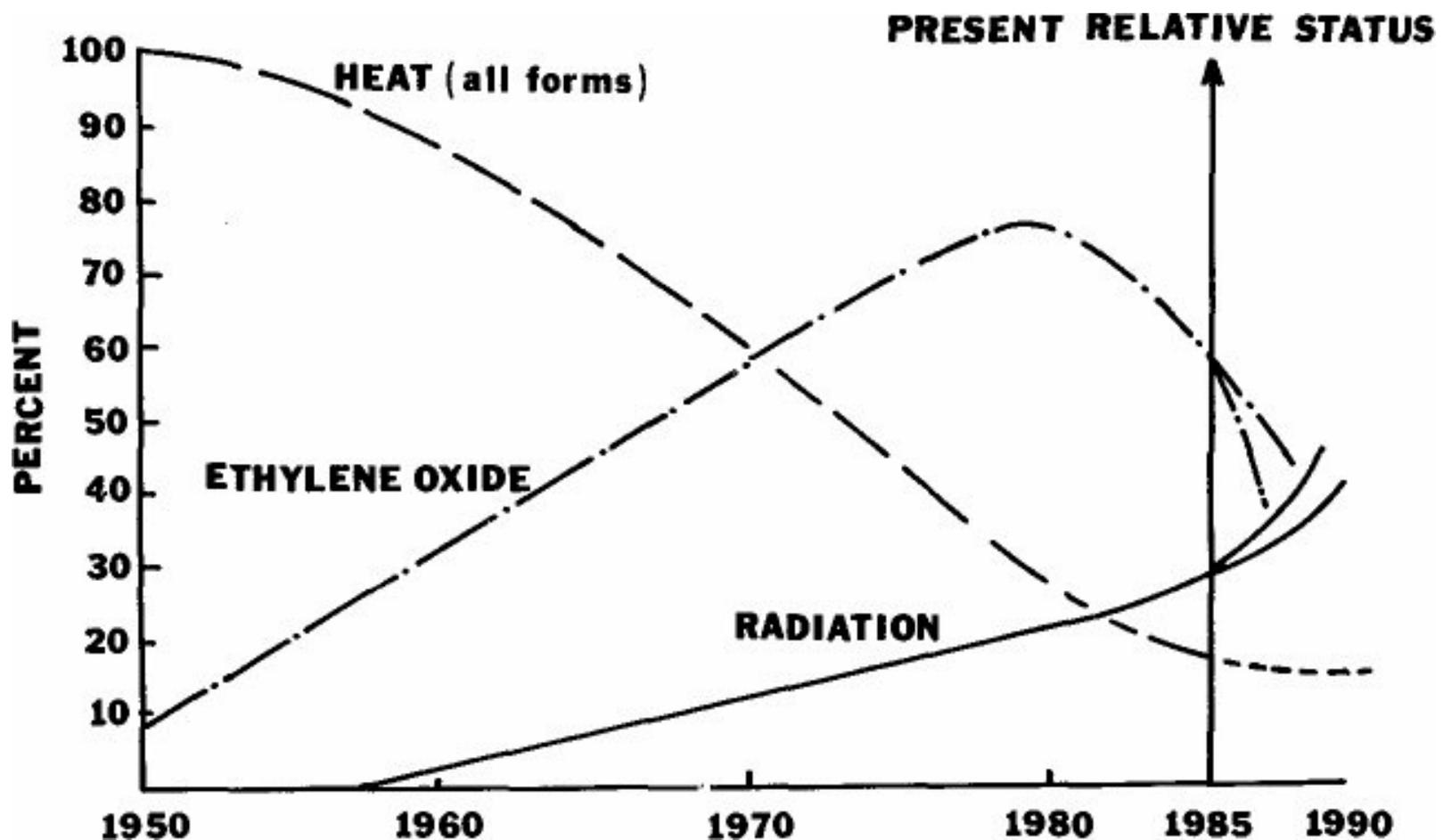


Figure 1. The Relative Proportion (not in exact scale) of the Different Sterilization Techniques Through the Years. (Derived after Morganstern [1978] and updated)

In the overall field of processing industries there is a noticeable trend (Fig. 1) in favor of radiation sources as compared to ethylene oxide gas, although the latter still comprises a considerable proportion world-wide. This is particularly prominent in the countries where the regulatory control guidelines for environmental quality conservation and occupational health protection considerations progressively disfavor the use of toxic ethylene oxide gas as a sterilant.

## Status of pharmaceuticals, toiletry and cosmetic products for radiation processing

Radiation sterilization has recently been attempted on certain specific pharmaceuticals. Being a cold process, this sterilization mode is expected to be particularly suitable for pharmaceutical formulations containing heat sensitive agents. Radiation, by virtue of its penetrating power, offers the advantage of application as a terminal sterilization step. Radiation also associates a very high microbial inactivation factor and most of the pathogenic organisms such as *Pseudomonas* and *Staphylococci* are claimed to be eliminated with relatively low doses of 5 to 10 kGy, even when the contamination levels are relatively high. Ethylene oxide, although it provides a cold sterilization method, is claimed to have a comparatively lower sterility assurance and may leave behind residues which are

reported to be carcinogenic and mutagenic.

Despite the encouraging and challenging nature of the pharmaceuticals field, it would be anticipated that there would be only rather slow growth so far as the radiation processing industrial practice is concerned. One major reason being the requirement that each irradiated drug be considered as a “new drug” with all the attendant time, efforts and expenses in providing necessary efficacy of the process and safety clearance of the product. The volume of anticipated throughput as well is expected to remain relatively rather small.

A number of research establishments are active in generating and accumulating data on the radiation chemistry and pharmacology aspects of a select group of pharmaceutical substances. Some of the relevant findings are reported and reviewed in the open literature<sup>15,2,11,16,25</sup>. Pharmaceutical formulations in aqueous media are generally unstable to radiation processing, owing to interactions of the solutes with hydrogen and hydroxyl radicals. Substances in dry solid state mostly retain stability under radiation processing conditions and doses (20 to 45 kGy). This has been reported in favor of many antibiotics, sulphonamides, anaesthetics, vitamins, enzymes, and other biological materials<sup>25,15</sup>. Separate sterilization of the drugs in dry solid state and aseptic mixing with water in the final formulations thus could be a feasible approach. A number of bulk antibiotics and ophthalmic ointments in their dosage forms are known to have been sterilized by radiation in a number of countries including the UK and India<sup>16</sup> although the details of the process are unpublished.

Pharmaceutical and toiletry basic raw materials, such as talc, bentonite, kaolin among others, belonging to the siliceous earths are prone to be contaminated with bacteria and pathogens. Radiation processing for decontamination of these bulk substances holds great promises. The processes and techniques need to be further improved through processing research in which the joint efforts of the national and international resources should enhance cooperation.

## **Future outlook and conclusion**

The wealth of data and information presented at this conference by the various experts, in the particular context of the successful radiation processing for sterilization, disinfection and preservation allows to conclude that the future of this technology looks still brighter. Through broadbased implementation strategies the radiation processing should be promoted to the significant areas of food, environment, and energy developments aiming at the harmonious international standard setting to help determine the radiation sterilization dose guidelines should be furthered. Such developments through joint efforts of the national and international organizations should further the early realization of the health and welfare objectives of the developing and developed countries.

In this context it needs to be acknowledged that this international goal of health standard upgrading has indeed been fostered by the present International Conference in Beijing, jointly organized by the Chinese Government Authorities and Johnson & Johnson. I am most

grateful to the organizers of this excellent educational meeting for enabling me to attend.

## Disclaimer

Materials, data and interpretations as presented in this paper are the sole responsibility of the author and those views expressed are not necessarily shared by the International Atomic Energy Agency.

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# **General Discussion and Concluding Session**

**Moderator: Irwin W. Sizer, Ph.D.**

*Dean, Emeritus, Graduate School  
Massachusetts Institute of Technology  
and President,  
Whitaker Health Sciences Fund, U.S.A.*

# Introduction to General Discussion and Concluding Session

Irwin W. Sizer, Ph.D.

Now that this superb conference is coming to a close, it needs to be pulled together in a General Discussion. My qualifications for leading this discussion are not very impressive. In the 1940's a student of mine, Dr. Samuel A. Goldblith, worked at M.I.T. on sterilization of foods and pharmaceuticals using electron beam irradiation. Another student, Dr. Eugene R. L. Gaughran, our Conference Co-Chairman, studied the enzymes of thermophilic bacteria at high temperature.

Fortunately for me, I served later on as a consultant for Johnson & Johnson and its subsidiaries for over thirty years. I was deeply involved at Ethicon in the sterilization of surgical sutures with the electron beam. This was its first commercial application, announced in 1956, to the sterilization of medical products. I have also consulted for Johnson & Johnson on sterilization using steam, cobalt 60, ethelene oxide, glutaraldehyde, etc. It was also my pleasant role to participate in a number of international symposia on sterilization sponsored in several countries by Johnson & Johnson.

During the past three days, this Conference on Sterilization of Medical Products, Disinfection and Preservation has focused on ways of inactivating microorganisms, or creating conditions under which they will not cause contamination. Methods proposed for achieving sterility have emphasized wet and dry heat, gases such as ethylene oxide and formaldehyde, irradiation with electrons and gamma rays, filtration and also chemical germicides. As pointed out by several speakers, a combination of these approaches can often be used with unusual effectiveness. Very special attention has been given to problems of sterilization and disinfection of the hospital with emphasis on equipment, special facilities, medical products for the purpose of insuring safety against contamination and infection of both patients and hospital personnel. In the hospital, and especially in its surgical rooms, chemical germicides and disinfectants have proven useful in preventing infection. In a similar way, barrier materials, including rubber gloves for blood diagnosis, play an important role. It was pointed out that the design of hospital rooms and sterile areas in industry can be important and that the use of standardized ultraviolet light can contribute to the overall cleanliness of hospital rooms. Materials brought into the hospital for medical use, including parenterals and even water, must be free of contaminants as determined by sterility tests. For such tests, as well as those performed by the clinical laboratory, automated analytical methods combined with new approaches utilizing monoclonal antibodies and the new

molecular biology can contribute in a major way to the problem. Many of these new methodologies can utilize computer technology for rapid and reliable results.

In the area of industrial production of sterile medical products, the emphasis has shifted from the final testing of sterility of the product to a concern for the process itself, in which emphasis is given at every step to a reduction of the bioburden and an attempt to develop a process whereby maximum cleanliness, minimum bioburden and, to the extent possible, sterility, which is a prime goal at every step in the manufacturing procedure. Such "process control" has become a major concern of manufacturers of sterile medical products around the world as contrasted with former emphasis solely on the sterility of the final product.

Certain products, including fruits and other foods, pharmaceuticals and toiletries, may on an individual basis, require special techniques, such as radiation processing or treatment with germicides to insure sterilization or at least the inactivation of most of the contaminating organisms. With these materials under practical conditions, germicides and disinfectants, such as glutaraldehyde, alcohols, iodophors and sodium dichloroisocyanurate are especially useful and economically appropriate.

To conclude my introduction to the General Discussion, I can say that this international conference has made it apparent that tremendous advances are being made in the sterilization of medical products and the disinfection and preservation of a variety of materials vital to human health. It is especially rewarding for everyone at this symposium to learn how much progress is being made in the field of sterilization by investigators in the People's Republic of China. The future looks promising indeed in view of the opportunities for cooperation between scientists, clinicians and industrialists in China and the rest of the world.

# General Discussion

**Question: by Dr. Yuan Qia-kuang, Institute of Epidemiology and Microbiology, Chinese Academy of Preventive Medicine, Beijing.**

Dr. Tilton just told us a lot of methods to detect microbe by microbiological, immunological and molecular-biological means. I believe that most of these methods will be used in the detection of disinfection. However, I have some questions.

The first, at present, is there any simplified method to detect microorganism? If we want to determine the enzymatic activity of microbe by the gas chromatography, is it possible to differentiate between active and inactive bacterium?

The second question, in order to differentiate bactericidal from bacteriostatic, is there a more rapid method in addition to prolonged incubation?

**Answer: by Dr. Richard C. Tilton, University of Connecticut, U.S.A.**

Yes, I understand those questions. Thank you. They are very good questions. Let me cover first the enzyme activity profiles. There are a number of products available which identify bacteria by their enzymatic activity profiles with the exception of the identification of an enzyme such as Beta-Lactimase directly in a product or process. That seems to be the only enzyme detection product available. But, yes, the detection of bacterial growth by the detection of enzyme activity profiles is certainly a possibility and probability.

With regard to the problem of bactericidal vs bacteriostatic activity, while I do not necessarily comment on it with regard to sterility testing, my comments vis-a-vis its role in antibiotic susceptibility testing are probably relevant. There are only, at least in the host, certain situations where the bactericidal activity of an antibiotic or an antimicrobial agent may be important. Usually, bacteriostatic activity seems to be sufficient and then the hosts normal immune defenses clear the organism from the system. However, in a process or in process control where there are no immune defenses so to speak, then I would suggest that a product should be bactericidal and not bacteriostatic. There are no quick ways at the present time, to determine bactericidal vs. bacteriostatic activity with the possible exception of using some of the supervital dyes.

## **Question: by Dr. Han Chi, Institute of Health, China National Center for Preventive Medicine, Beijing.**

1. The joint FAO/IAEA/WHO Expert Committee on the Wholesomeness of Irradiated Food announced that “no toxicological hazard is caused by irradiating, for conservation of any food up to a dose of 1.0 kGy.” But at present, irradiated food has been banned in W. Germany and only irradiated potatoes and spices (condiments) have been given clearance in USA and Japan, why?
2. What are the prospects of irradiated food?

## **Answer: by Dr. Mukherjee, International Agency of Atomic Energy, Vienna, Austria**

With regard to your question I wish to add the following comments. The pioneering International Project in the Field of Food Irradiation (IFIP) was primarily membered by the European nations including West Germany which even acted as the host and largely contributed to the development of the scientific basis of the “historical conclusion” of the Joint FAO/IAEA/WHO Expert Committee on Wholesomeness of Irradiated Food (JECFI) in 1980 to provide a blanket clearance up to an overall average dose of 10 kGy which this Conference has discussed. However, with regard to the different national standings on the food and drug specifications, there might remain still other technical questions and/or legislative considerations which need to be adequately settled. The countries might therefore be concentrating on those matters on an item by item basis, so far as the clearance of irradiated foodstuffs is concerned. This situation should not be construed as a banning and/or opposition to the application of the radiation process concerned.

Referring to your second question, I would refrain from repeating what I have already discussed in my main paper and other experts have also commented upon, for example Dr. Morgenstern made remarks on the future outlook on radiation preservation of food. The technology for food irradiation has currently reached a status whereby it is poised for a rapid widespread development. In the course of the recent years we have noticed that an increasing number of national approvals have been granted to individual irradiated foods or groups of products.

However, at present, a large-scale use of radiation processing is still somewhat hampered by the lack of worldwide legal acceptance of irradiated food commodities. It is not possible to utilize irradiation facilities economically, if only a few commodities may be irradiated and if even those may not be freely exported. Therefore, many of the countries which have already granted clearances cannot in actual practice use the process. An important task ahead is therefore to transfer the technology to the food producers, processors, and distributors and to convince consumer organizations and consumers of the safety of the process. Persistent policy of information and education is required to overcome misapprehensions. In line with the current climate it is expected that within the next few years many more countries will allow the commercial implementation of the

process.

## **Comment of paper by Li Zhi-gui by Dr. Li Shi-xin, Director, Shanghai Huangui Fine Chemical Institute, Shanghai.**

1. The use concentration as 0.2-0.5% of peracetic acid referred to in the report is quite proper. It is calculated according to the products whose concentration is 20 percent produced in China. Therefore, the real content of peracetic acid in the disinfectant is 0.04-0.1%. Sterilization with this concentration can be reached.

2. Various kinds of disinfectants used all over the world can be produced at present in China and the technology of producing these kinds of disinfectants has relatively reached a high level. Some of them have been exported and contributions have been made to mankind.

3. A variety of preparations of disinfectants are more applicable for various purposes so the research of preparations obviously becomes important and we are now striving to carry on such work. A series of disinfectants for different uses have been developed and manufactured, such as "Food-Utensil Clean-333" and "Quick Disinfectant", etc..

## **Question: by Dr. E. Gaughran, Johnson & Johnson, USA.**

Would Dr. Kallings be kind enough to list for us in order of decreasing importance, the sources of the organisms that are responsible for postoperative infections?

## **Answer: by Dr. Lars Kallings, National Bacteriological Laboratory, Stockholm, Sweden.**

That is a very good question, because the discussion could help explain why there is a difference in the priorities which have to be set in the clinical situation as compared to the pharmaceutical situation when manufacturing large amounts of drugs or devices with defined degree of quality. If there is a need for clean rooms in the factories, why is there no such need in the clinical setting except for very special procedures?

Most microorganisms causing hospital infections originate from the patient himself—at least when a baseline hygienic standard is maintained. Since the most common microorganisms on the skin and on the mucous membranes are anaerobic bacteria, these anaerobic bacteria also belong to the most common causes of hospital infection. The content of the colon contains about  $10^{11}$  bacteria per gram and there are several hundred different species present in the normal anaerobic flora of the human body. Probably, *Bacteroides fragilis* is the species that top the list in postoperative infections in bowel surgery. *B. fragilis* and *E. coli* often symbiotically causes surgical wound infections, deep abscesses, peritonitis and sometimes septicaemia with Gram-negative shock.

Among the hospital infections the urinary tract infections (UTI) are the most common.

The most common cause of UTI is *E. coli*. Therefore, *E. coli* will also belong to the bacterial species that top the list.

In burns still *S. aureus* and *Pseudomonas* are the most common, often due to cross-infection. *S. aureus* is also the most common cause of skin infections and often found in surgical wound infections. At least in some of the neighbouring nations to China, *S. aureus* is a common cause of septicaemia in hospitals with subsequent abscesses in different organs, as in the brain. Therefore, *S. aureus* should be high up in the list before *Pseudomonas*.

In wound infections and in maternity wards *Streptococcus pyogenes* is still a cause of cross-infection in many countries as it was in the days of Semmelweiss. In pediatric clinics Respiratory Syncytial Virus (RSV), rotavirus, *Shigella* and enteropathogenic *E. coli* are commonly spread with some geographical differences.

Hepatitis B was discussed yesterday. We shall not forget the common causes of pneumonia as pneumococci and *H. influenza* and Gram-negative rods from the intestinal flora, particularly in the bed-ridden elderly patients that have received antibiotics.

Generally, in patients that have been treated with antibiotics, as is often the case with patients referred to central hospitals, the list will be somewhat different and include e.g. multiresistant *Klebsiella*. *Klebsiella* and *Pseudomonas* are examples of bacteria often spread to the patients from a common source in a ward unit, e.g. from water used for rinsing and cleaning. In immunosuppressed patients *Candida* and other fungi should be added as well as parasitic infections as for instance *Pneumocystis carinii* pneumonia and Toxoplasmosis, mycobacteria infections of various species, legionellosis, herpesvirus incl. cytomegalovirus infections.

This is by no means a complete list but I hope that I have answered in a way that gives an idea of the prevailing agents causing nosocomial infections. I may add that in intensive care units device-related infections due to multiresistant *S. epidermidis* may be a problem, e.g. when using central venous catheters.

This was sort of an international hit list of nosocomial infection and of course we would be very interested to learn the Chinese experiences.”

## **Comment and Question: by Mr.. Tu Ying, Department of Epidemiology, Third Military Medical University, China**

Concerning the source of nosocomial infection, I would like to say a few words. As I know someone considered that *Staphylococcus aureus* and *Pseudomonas aeruginosa* in burn wound mainly originated from normal flora of patients themselves. But recently we have performed an epidemiological investigation in the wards of a burn unit. From patients, medical personnel and environment, the bacteria were sampled, cultured, isolated, identified and phage-typed or serotyped. The results showed that the cross-infection rate of wound surfaces (34.5%) was higher than the auto-infection rate (13.8%). The main sources of nosocomial infection was patients, and the infective agents were mostly transmitted through indirect contact of hand, furniture, equipment and air from patients to patients.

Therefore, we may say the sterilization and disinfection are very important for the prevention of nosocomial infection.

I was very pleased to participate in this international scientific conference. It affords me an opportunity to share the world's current knowledge of sterilization and disinfection. I would like to ask a question of Mrs. Fran Koch.

**Question: Is it necessary to study residual disinfection to enforce the role of barrier materials in preventing nosocomial infection?**

**Answer: by Mrs.. Fran Koch.**

Residual disinfection is important as barriers may be disrupted during a surgical procedure, i.e., a hole in a glove.

**Comment and Question: by session moderator.**

I have one for the audience. So far, I do not understand the true target within the microbial cell of the various means of destroying that cell. I have somehow the impression that heat sterilization denatures proteins within the bacterial cell. Maybe irradiation by electron beam or gamma irradiation with a single hit target theory might act on the DNA. Other things I have read suggest that bacterial membranes are important, but I submit that we still do not understand in detail what actually happens within the bacterial cell when it is inactivated. This is not just an academic question because, with reference to the future in combinations of ways of the destroying microorganism, it would be very helpful if we knew very intimately how different methods of sterilization actually work at the molecular level. I wonder if any of you would care to comment about this particular problem?

**Comment: from the floor.**

I will be happy to tackle at least a part of that question in the hope that some of the rest of you might join in. In fact, we have known or we do know the microbial targets for many of the disinfectants, sterilizing agents, antibiotics and so forth. However, the problem is that, as we learn more about the bacterial cell, the modes of action that were once considered to be inviolate, somehow are no longer inviolate.

Let me give you one example and I will use the example of penicillin. In the early fifties, Dr. Jack Strominger published on the mode of action of penicillin and for about 15 or 20 years, we were very secure in that we knew that penicillin essentially inhibited the final cross-linking of the cell wall of microorganisms by interfering with D-alanyl-D-alanine moiety. However, penicillin binding proteins were discovered in the middle seventies and we now

have quite another story and we recognize that there is more to the story than the intercalation of D-alanyl-D-alanine. That the relationship of penicillin or the binding of penicillin to the penicillin binding protein plays a major role in how penicillin inactivates or kills a microorganism. That is a very crude and homely example of my initial remark. That is, as we learn more about the bacterial cell, as we learn more about cell surface interaction, about the role of the glycocalyx, about the synergistic relationship of the microorganisms and their antagonists, some of our early ideas about modes of action of antiseptic, germicidal, antibiotic agents become outmoded.

**Question: by Madame Chen Yao-jun, Inspection Department Director, Institute of Food Safety Control and Inspection of the Ministry of Public Health, Beijing.**

What kind of chemicals are suitable for disinfection in food establishments, such as big containers, machines, etc., which have direct contact with food?

**Answer: by Dr. Martin Favero, Center for Disease Control, USA**

If chemical germicides are used, the types might be the quaternary ammonium compounds, dilute solutions of hypochlorite, iodophors and, in some cases, phenolics. So it would be more towards the low-level and sanitizer types. With respect to food processing, I would say the same thing. I think the food processor many times has an analogous problem as the manufacturer in the pharmaceutical house. It has its own GMPs, if you will. I think the difference is one of magnitude and it is there that sanitization is very important. A good example of this would be in the processing of milk, in the cleansing of canisters and cans. When this is done with chemical germicides, quite often it is the lower level to intermediate germicides that are used like hypochlorite solutions or chloramines or iodophors or things of that activity range.

# Concluding Remarks

Chen Chun-Ming, M.D.

During the past three days, we have heard presentations covering a wide range of health-related subjects. From the concepts and various methods of sterilization, to process monitoring, tests for sterility, pyrogens and sterilant residues. We reviewed infections in the hospital environment and ways of detection and prevention of infectious agents. Information was shared on problems both inside and outside of China. We even touched on international regulations and improvements in food preservation. But these three days were not solely concerned with technical dialogues, they were concerned with developing mutual understanding and friendship. An understanding that comes from the realization that many of us have the same problems, and working together, we can accomplish great advances.

I would like to talk about how the Chinese participants are feeling about the Conference. They enjoy the meeting very much, to their impression, the speakers have made not only an overall description of the current development and the trend of sterilization work, but also a systematic statement on the statistics, principle and methodology of sterility monitoring and regulations. Since China is on the way to modernization, we have foreseen the future industrialization and centralization of sterilization. Your analysis of the problems and the action taken in this respect are indeed very useful. So I would say the presentations have enhanced much inspiration of my Chinese colleagues and provided plenty of information. Moreover, it is really a wonderful beginning for the future communication between Chinese and foreign scientists and I believe you have already made good friends during the past three days. I believe that this Conference is only a beginning. We must continue to share our research findings in order to improve the health of the people of the world. I would like to take this opportunity to invite my new friends to return to Beijing in the near future to continue the efforts that we have made here this week. Personally, I would like to thank the Vice-chairman Dr. Gaughran and Dr. Schwenker and all the session chairmen for your cooperation and to the members of organizing committee Dr. William Yu, Dr. Robert Morrissey and Mr. Herbert Kramer, Dr. Zhao Tong-bin, Mrs. Situ, Dr. Wang yu-sen, Mr. Xiao enpei who made great contributions to the success of the Conference.

I should again express our sincere gratefulness to the CPPC and the Ministry of Public Health of China for their sponsoring, and on behalf of the organizing committee, I thank all the speakers and participants for their kind cooperation and contributions they have made. I should also, on behalf of all the participants, express our high appreciation to the enthusiasm and contributions of the Johnson and Johnson in promoting the scientific

exchanges and future development of sterilization work.

I wish you good health and a pleasant trip back home and look forward to your future success.

This is my concluding remarks. Thank you.

Now please let me announce the closing of International Scientific Conference on the Sterilization of Medical Products, Disinfection and Preservation.

# Concluding Remarks

William H. Yu, Ph D

*China Office, Johnson & Johnson, Beijing*

I am most happy, on behalf of Johnson & Johnson and its world wide family of companies, to welcome you tonight to the Great Hall of the People for the closing banquet in celebration of the completion of this international conference.

Many institutions and Chinese government agencies have contributed to the success of the International Scientific Conference on the Sterilization of Medical Products, Disinfection and Preservation. We are grateful for the kindness and hospitality of the Chinese government. It is impracticable, at this time, to individually extend our gratitude to all the contributors. However, I would like to express our sincerest thanks to China's Ministry of Public Health and the Medical and Health National Committee of the Chinese People's Political Consultative Conference for their endorsement and full support. We, Johnson & Johnson Family of Companies, are also delighted with the wonderful experiences that we have in cooperating with the China Academy of Preventative Medicine and the China Medical and Health conference ought to be congratulated. But you, the participants, are the most important contributors. By the sharing of your discoveries, ideas and experiences among other distinguished scientists from all over the world has made this conference extremely rewarding. We have witnessed the success of this outstanding conference during the past few days. Tonight, we also have the privilege to witness the presentation of the prestigious Kilmer Awards given to the recipients who have made exceptional contributions in the fields of sterilization, disinfection and preservation.

It is now my pleasure to present Dr. Robert A. Fuller, Corporate Vice President of Science and Technology of Johnson & Johnson, for the presentation of the Kilmer Awards.

# Kilmer Award Presentation

Robert A. Fuller, Ph.D.

*Johnson & Johnson, U.S.A.*

I think that the hopes expressed at the opening banquet for the success of this Conference have been fully realized and indeed its accomplishments have exceeded even the optimistic expectations of the organizing committee. I would particularly like to acknowledge the enormous contribution of our Conference Chairman, Professor Chen Chun-Ming to the success of our meeting.

The sharing of knowledge and information on the sterilization of medical products, disinfection, and preservation between such an outstanding group of highly regarded international scientists and their Chinese colleagues has been an exciting and inspiring event to witness. I am confident that this is only the beginning of this sharing process because many new contacts and new friendships between the participants have been established.

I have been privileged to attend all six international conferences on sterilization sponsored by Johnson & Johnson and I can say unequivocally that the hospitality we have received here has never been exceeded. I know that I can speak for all of the foreigners present in expressing to our Chinese hosts our sincere appreciation for the warmth of their reception and their kindness in accommodating our needs and requests.

In his remarks at the beginning of the Conference, Mr. Clare referred to the outstanding contribution of Dr. Fred B. Kilmer to the field of sterilization and disinfection.

To honor international scientists who have made significant contributions to the field of sterilization and disinfection in the tradition established by Dr. Kilmer, an award in his name was established several years ago.

It is my great privilege this evening to present two scientists whose contributions have been recognized as deserving of the Kilmer Award.

Dr. Liu Yujing from the Institute of Microbiology and Epidemiology of the Academy of Military Medical Sciences China.

Dr. Irving J. Pflug of the University of Minnesota, USA.

## Biographical Notes on Receptients

**Lin Yujing** graduated from the Medical College of Nanjing University and took a special course in Public Health at the China Medical College. He has been engaged in research on disinfection in the Institute of Microbiology and Epidemiology of the Academy of Military

Medical Sciences. He sponsored a national conference on disinfection and sterilization in 1984 and won the third award of PLA in the same year for his work on disinfection and sterilization.

**Irving J. Pflug** obtained his B.S.A. degree in 1946 and his B.S.A.E. degree in 1948 from Purdue University, USA and his M.S. (1950) and Ph.D. (1952) from the University of Massachusetts. At present, he is Professor of Environmental Microbiology in the School of Public Health and Department of Food Sciences and Nutrition of the University of Minnesota. Dr. Pflug's many contributions to sterilization microbiology as it relates to pharmaceuticals, foods and medical devices has made him a frequent advisor to industrial and governmental organizations. He is internationally renown for his course and his two teaching volumes, on the microbial and engineering of sterilization processes.

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  - \* Not presented at the conference.
  - \* Not presentation at Conference.
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