Validation of Pharmaceutical Processes

Third Edition

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Validation of Ethylene Oxide Sterilization Processes

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INTRODUCTION

The recent history of EO sterilization has been dominated by advances in engineering technology. These advances include not only the computerized controls for the operation of sterilizers, but also the physical environmental controls that permit safe use of 100% EO gas. Most of these improvements have been driven by economics. Faster, cheaper processes are indeed worthy objectives. However, sometimes it seems we have lost the focus on what we are really attempting to accomplish in the sterilization processes. Any sterilization process must deliver a lethality that kills the naturally occurring bioburden microbes that contaminate the products and materials. If the process does not render the products or materials free from living microorganisms, then sterilization has not been achieved.

The microbiological dimension of the EO process has been overwhelmed by the recent strides in engineering and the physical process controls. The increasing complexity of medical products would be much more difficult to sterilize without these corresponding engineering process improvements. However, failure to properly address microbial lethality renders all of these engineering advancements meaningless if the resulting product is not sterile.

Process validation means establishing by objective evidence that a process consistently produces a result or product meeting its predetermined specifications (1).

The EO sterilization process is expected to deliver sterile products that possess all other specified quality attributes. Validation must document all critical process controls. The products to be sterilized must be challenged with an appropriate microbial system located in the "worst case" or "least lethal" product location. In addition, this microbial challenge product must be positioned in the worst case, least lethal location(s) in the production load. If the microbial challenges are not located in these least lethal locations, then the resulting documented evidence may be biased and result in false conclusions about the adequacy of the sterilization validation program.

The validation of the EO gas sterilization process is one of the more complex programs facing process engineers and microbiologists because some critical process parameters are interactive. EO gaseous sterilization has been shown to be an extremely effective process that can be performed with an infinite number of combinations of parameters. Key parameters that affect sterilization efficacy are (*i*) concentration of EO gas, (*ii*) RH, (*iii*) temperature of the process, (*iv*) accessibility of the product and packaging for these parameters, and (*v*) time.

A validation program must demonstrate that the selected combination of these interactive process parameters result in an effective physical and biological process. The effectiveness of this process is measured by calibrated physical instruments and a calibrated microbial challenge. These process parameters must then be correlated to a calculated SAL for the product. SAL is the probability of a single viable microorganism occurring on a product. The required assurance level may vary depending on the product itself or the end use of the product, but is typically less than one chance in a million of a non-sterile unit or SAL of 10^{-6}.

Another challenge is the task of assuring that the EO gas used does not create a health hazard for the employees in the working area or leave unacceptable residuals in the product delivered to the consumer. Adsorbed EO gas is removed fairly rapidly from processed materials, while absorbed EO gas is released much more slowly. This absorption rate is highly dependent on the specific process conditions, material being processed, as well as the geometry of the product, which affects material surface-to-volume ratios. Appropriate measures must also be taken to assure that EO gas used in the sterilizing environment is controlled and contained so that environmental insult in affected work areas is within acceptable regulated limits.

During the EO gas sterilization process, the gas interacts with the materials processed by reaction, absorption or adsorption. The EO gas is also trapped in the air spaces within the product or material being

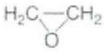
Abbreviations used in this chapter: AAMI, Association for the Advancement of Medical Instrumentation; BI, biological indicator; BIER, biological indicator evaluator resistometer; CO_2, carbon dioxide; DEC, dynamic environmental conditioning; DUT, device under test; EO, EtO, ethylene oxide; FDA, Food and Drug Administration; GC, gas chromatography; IP, inoculated product; IR, infrared; MW, molecular weight; NIOSH, National Institute of Occupational Safety and Health; NIST, National Institute of Science and Technology; PEL, permissible exposure limits; RH, relative humidity; RTD, resistance temperature detector; SAC, static atmospheric conditioning; SAL, sterility assurance level; SLR, spore log reduction; TAR, test accuracy ratio; TC, thermocouple; TUR, test uncertainty ratio; TWA, time-weighted average.

sterilized. Unreacted residual gas is rapidly removed through evacuation, heated nitrogen or air exchanges. Product that is removed from a sterilizer must be controlled to prevent environmental insult to the workers. The best procedure is to place the sterilized materials in an environment that aids the desorption of the gas and is environmentally controlled to minimize workplace contamination.

CHARACTERISTICS OF EO

Chemical Properties

EO is also referred to as EtO, 1, 2-epoxyethane, and dimethylene oxide (2). It has a formula of C_2H_4O. The following structure is illustrated:



It is a colorless gas, with a molecular weight of 44.05. It has a characteristic ether-like odor at toxic levels. EO has a boiling point of 10.7°C (51.3°F) at 760 mmHg pressure, a melting point of −112.6°C (−170.7°F), a specific gravity of 0.8711 apparent at 20°C (60°F), or a specific gravity of 0.897 at 4°C. EO has a vapor density of 1.5, with dry air being equal to 1.0, and a vapor pressure at 20°C of 1095 mmHg. It is completely miscible in water, alcohol, acetone, benzene, ether, carbon tetrachloride, HCFCs, and most organic solvents, and is a powerful solvent for fats, oils, greases, waxes, some rubber formulations, and paints. It is highly exothermic and potentially explosive when heated or mixed with (*i*) alkali metal hydroxides, (*ii*) highly active catalytic surfaces such as anhydrochlorides of iron, tin, or aluminum, and (*iii*) the oxides of iron and aluminum. The explosive limits are 3% to 97% by volume in air. It has a flash point of −6°C (20°F). It is relatively noncorrosive for materials. EO is relatively stable in neutral aqueous solutions and when diluted with liquid or gaseous carbon dioxide or halocarbons such as HCFCs. EO is relatively unstable in either acidic or alkaline aqueous solutions and may rapidly form ethylene glycol.

Biological Activity

EO reacts irreversibly with numerous chemical moieties on cellular molecules by an alkylation reaction where the [CH_2OH–CH_2–] alkyl group is covalently bonded with the available moiety via an addition reaction. Reactions with –NH_2, –SH, –COOH, and CH_2OH groups are common and illustrated in Figure 1 (3).

Reaction rates vary and depend on the specific pK_a for each moiety and the existent pH. For a more comprehensive review of possible reactions we refer the reader to Russell (4). First-order lethality kinetics require that only one molecule per cell is the critical target (5–7). Reactions other than the critical reaction leading to microbial inactivation must be considered collateral damage reactions. Not all microbial inactivation obeys first-order kinetics. However, even where multiple sites or molecules may be required for inactivation, the concept regarding critical reactions and collateral reactions is the same. Where inactivation is the result of cumulative damage, which is not first-order kinetics, then some damaging reactions must be considered more important to the events leading to microbial inactivation (critical)

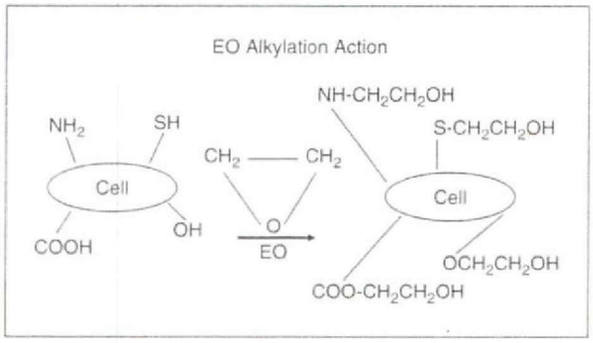


Figure 1 Illustration of the alkylation reaction of ethylene oxide with chemically active moieties in the bacterial cell.

than other reactions (collateral). Winaro and Stumbo (8) identified EO reactions with DNA as the critical reactions resulting in microbial inactivation. Lawley and Brookes (9) defined specific reactions of EO with the nucleic acid tertiary heterocyclic nitrogen sites (=N-) in numerous experiments resulting in more than 10 publications between 1957 (10) and 1963 where they state (9):

1. Sites in the nucleic acids reactive towards alkylating agents are shown to be, in order of decreasing reactivity: for RNA, N-7 of guanine, N-1 of adenine, N-1 of cytosine and N-3 of adenine for DNA, N-7 of guanine, N-3 of adenine and N-1 of cytosine. Denatured DNA behaves in this respect like RNA.
2. The observed differences between DNA and RNA are ascribed to the involvement of N-1 of adenine and of cytosine in hydrogen bond formation in DNA.
3. In all cases alkylation results in destabilization of the nucleosides or the corresponding moieties in the nucleic acids. At neutral pH, with DNA, 7-alkylguanines and 3-alkyladenines are slowly liberated by hydrolysis, the latter at the greater rate, whereas with RNA slow rearrangements occur, 1-alkyladenine moieties yielding 6-methlaminopurine moieties and 1-alkylcytosines giving the corresponding 1-alkyluracils.

More recent studies suggest that disruption of the DNA molecule may occur differently depending on various repair mechanisms (11). In the case of certain repair mechanisms, the reactions with cytosine may be the injury which ultimately leads to the inactivation of the microbe.

VALIDATION OF EO STERILIZATION PROCESSES

Validation of the EO process is divided into two phases: Engineering Qualification and Process Qualification. When these activities are completed successfully and all aspects of the process are documented, the process can be certified for routine use for manufacturing goods.

Engineering Qualification

Engineering Qualification deals with the sterilizer and associated equipment used in the process. This phase is divided into three segments: Installation Qualification, Calibration, and Operational Qualification.

Installation Qualification

Installation Qualification requires an audit of the equipment as it has been installed in the facility. This audit includes checking all utilities and supplies to the equipment to make sure that they meet the manufacturer's recommended specifications. Engineering drawings must be evaluated to assure that (*i*) the equipment is assembled according to the manufacturer's prints, (*ii*) the equipment is installed according to the installation schematics, and (*iii*) all aspects of the equipment are documented with appropriate engineering drawings or sketches. These drawings are essential for future reference to compare the hardware validated to any future configurations. This segment of the validation program is probably the most abused with sterilizers using nonexplosive containers of EO. Systems using 100% are extremely well documented, which is driven by the safety issue. Once the equipment is hooked up and it "runs," little more is ever documented. With the pressure to get things working, little attention is paid to the documentation for future reference. Inadequately treated items typically include documentation of utilities, spare parts lists and preventive maintenance procedures. Many validations have been performed with all the necessary tests on the hardware relating to product loads, but with no record as to the exact configuration of the equipment when the validation was executed. Since any mechanical device will routinely malfunction, or wear out and require replacement, it is absolutely essential that a well-prepared Installation Qualification document be assembled for each piece of equipment to be validated. If this is not done, subsequent validation data may prove meaningless.

Calibration

The second segment of the Engineering Qualification is the calibration of all process sensing, controlling, indicating, and recording devices on the sterilizer or independent systems associated with it. Recording instruments that appear on the control panel are typically calibrated, but many of the control instruments are often located out of sight and should not be ignored since they may have a tremendous impact on the cycle function. For example with the DEC phase of an EO sterilizing process, it is extremely important to calibrate the stall point of the vacuum pump before the actual pressure or temperature set points are calibrated. This measurement is critical to balance the steam input into the chamber in relation to the capacity of the vacuum pump to remove the steam from the chamber. All critical process control instruments that are recorded and displayed by the control system must be calibrated. This is even more complicated when micro processor control units are employed, because not only are there specific operating set points for those systems, there are also high- and low-limit alarm and other default systems that must be documented and calibrated. The calibration program will also vary depending on the type of computerized system.

The calibration program should be performed with instruments referenced as secondary standards. The secondary or transfer standard is a standard that can be transported to and from the actual sterilization equipment because most instruments associated with the sterilizer must be calibrated at the sterilizer's location. Secondary standards must be traceable to a recognized standard such as those maintained by the NIST.

A measurement or calibration compares a DUT to a standard or reference. This standard should outperform the DUT by a specific ratio, called the "TUR" also known as the TAR. As a rule of thumb, the TUR should be greater or equal to 4:1 (12).

Primary standards should have an even greater sensitivity. It is recommended that these primary standards be submitted to the NIST for calibration and recertification on a periodic basis. Primary standards are usually recertified annually. It is extremely important that detailed procedures be established including limits and acceptable correction variances allowed and calibration frequency for all the instruments on the sterilizer. Adequate records must be maintained. A tracking system is essential to assist metrology, assuring that required calibrations occur at their designated frequencies. A history file should be maintained for each instrument, and the records reviewed to assure established calibration frequencies are appropriate.

Operational Qualification

The third segment of Engineering Qualification is Operational Qualification that deals with the operating parameters of the sterilizer: their function, adjustment, and control. These tests are performed with an empty chamber. The various parameters for the cycle are evaluated to determine if they perform as specified by the manufacturer. Temperature controllers are set and evaluated to determine performance. The temperature distribution within the sterilizer is documented. The unit is sequenced through its operating steps to assure that the sequencing is appropriate. Every operating parameter must be documented to determine its compliance with the manufacturer's operating specification. The Operational Qualification protocol will serve as the basis for developing the Standard Operating Procedure for routine operation of the sterilizer. The Operational Qualification testing specifies in detail how the equipment operates.

Process (Performance) Qualification

The final phase of validation deals with Process Qualification. Even though the unit functions appropriately with an empty chamber, it must now be demonstrated that it sterilizes product. This phase may require repetition with different products and loads.

Load Configuration

There are several key aspects of Process Qualification. First, the specific product and all its packaging must be defined. The next step is to define the way master cartons are arranged into pallets. Pallet arrangement within the sterilizer is also part of the load configuration definition. Many manufacturers have numerous products that must be mixed together in order to achieve effective sterilizer throughput.

Categorizing product for the sterilizing load is an extremely important element. It is important that the particular product mix is configured with a rationale that packaging is similar and products should be of consistent mass and materials and actual product

configuration. It is possible that a manufacturer may have in its catalog hundreds of different products. If these all have the same characteristics and packaging, it is possible that they could be sterilized within one or two different sterilization cycles. It is also possible that a manufacturer may produce only a few products, each being so different from the other products manufactured that each product sterilization process will have to be validated in different cycles.

Once product categories have been identified, it is also possible to vary the load configurations. Loads must be extremely specific in the way they are defined. Small tolerances are permissible without changing the overall impact on the biological effectiveness of the sterilization. However, changes in the qualified load must be evaluated and properly documented to determine the potential impact on the biological effectiveness of the process.

Conditions which influence the lethality delivered by the process are mass, density, packaging, product design and materials. External preconditioning of product loads is common and allows easy process measurements. Preconditioning time may vary with different loads. Determining moisture, EO gas and temperature penetration into the palletized load is much more difficult in the sterilizer. If time to achieve acceptable levels of these parameters is similar to the originally defined load, then loads can be considered equivalent. Measured lethality should be similar with similar loads. It should be noted that configuration changes may influence the location of "worst case–least lethal" position. Confirmation will have to be performed and appropriate adjustments may have to be made to assure that a proper monitoring location is documented.

Once the product and load have been defined, then the worst case–least lethal locations in the product, within each pallet and within the vessel must be determined. These locations will have to be monitored physically and biologically to provide data on all critical process parameters.

Pallet Configurations

Pallet construction may depend in part on how much shipping will take place between the time of construction until sterilization. When processing was performed in-house, it was easy to construct pallets with "chimneys" configured between columns of master cartons. These chimneys assured that more surface area of the master cartons was directly accessible for thermal transfer and gas exchange. This type of configuration provides the greatest homogeneity of sterilization conditions across the product load.

Contract EO sterilization is extremely popular today and provides users with "state-of-the-art" systems at reasonable expense. The problem comes not from the sterilizer, but from the logistics involved in transporting the product off-site to the contractor. Pallets are constructed at the product manufacturing site with transportation in mind, not sterilization. Pallets are densely packed because they survive the rigors of overland shipping much better than pallets configured with void spaces (chimneys) for gas

Figure 2 An example of a banded pallet of product providing maximum surface exposure to sterilization vapors. *Note*: Corner protectors on pallet protecting the master cartons.

permeation. Stretch wrap is commonly used to hold the palletized boxes together. Stretch wrap is exceptional for maintaining pallet integrity during shipping, but it may create a tremendous barrier to sterilizing vapor penetration. Stretch wrap manufacturers are now offering a "net" type of wrapping material which significantly increases the surface of the master cartons directly exposed to the sterilizing vapors. The best technique from a sterilization perspective is to use strapping to band the pallets together. This requires the use of corner protectors so as not to crush the outside corners of the master cartons (Fig. 2). An example of uniformly constructed pallets loaded into a sterilizer vessel appears in Figure 3. More pallet configurations can be sterilized successfully of course, but process times may be longer

Figure 3 An example of a uniform load configuration. Two identical pallets side by side. All pallets are exactly the same in construction and product.

and variations of microbial lethality and EO residuals across the load may be greater.

CRITICAL STERILIZATION PROCESS PARAMETERS

There are several major considerations to be aware of in order to structure a validation program that will assure that the sterilization process does what it is intended to do.

These considerations include: (*i*) controlled process parameters and their interaction; (*ii*) an integration of the physical process conditions; (*iii*) the selection of appropriate process conditions; (*iv*) the product design; (*v*) how the product is pretreated prior to exposure; (*vi*) how the product is handled following sterilization; (*vii*) how the process is monitored, including physical, chemical, and biological methods; and (*viii*) the effect of residual EO and its reaction products on the material being sterilized.

There are four critical interactive parameters that must be controlled for EO sterilization process: (*i*) EO gas concentration; (*ii*) moisture; (*iii*) temperature; and (*iv*) time. All these parameters interact to affect the lethality delivered by the process.

EO Gas Concentration

General Use Range of EO Gas Concentration

EO gas concentrations below 300 mg/L and above 1200 mg/L are not commonly used in the industry. EO gas concentrations less than 300 mg/L are not effective in practical process times. Concentrations above 900 to 1200 mg/L do not shorten the process times sufficiently to warrant the additional cost of gas. Sterilization effectiveness is dependent on the molecular collision of the EO molecule and the biological entity that is being sterilized. Therefore, more EO molecules lead to more rapid microbial lethality. A sterilizing process using 600 mg/L of EO delivers approximately twice the lethality as a process using 300 mg/L in the same time. However, considering the cost of EO, processes are generally designed toward the lower concentrations of EO. Concentrations of 400 to 600 mg/L appear to be the more popular conditions today for operations to balance the cost of EO, equipment and throughput time.

EO Gas Concentration Controllers

The EO gas concentration is controlled in one of two ways. The most common method of control is the indirect method through the use of a pressure control system. The EO gas concentration desired is calculated as to the corresponding increase in pressure. The desired pressure settings are then maintained by conventional pressure controllers. The direct control method uses analytical instruments that actually detect the EO gas concentration in the environment inside the sterilizer.

The analytical systems are either gas chromatographic, IR or microwave detectors. These instruments are installed directly to the sterilizer. Periodic gas samples are withdrawn from the sterilizer or gas circulation lines and passed through the detector. Some IR or microwave detectors may be mounted on the exterior chamber wall using an access port or in the gas circulation system. Electronic signals are sent to control valves in the gas supply lines allowing makeup charges to maintain the target gas concentration.

Indirect Methods

There are two approaches for the indirect method of measuring EO gas concentration in the sterilizer—they are weight and pressure. The indirect methods are dependent on using gas cylinders containing certified mixtures of EO. When the chamber is pressurized, it is assumed that the mixture contains the given percentage of EO relative to the change in pressure. Therefore, this change in pressure can be equated to an assumed gas concentration. This system is very easy to monitor using pressure transducers and recorders.

The second indirect method measures the weight of the gas cylinder contents dispensed into the vessel. This method assumes that a uniform mixture of the EO and diluent gas was dispersed into the vessel, yielding an assumed concentration of gas in the sterilizing chamber. This system is easy to monitor using acceptably sensitive scales.

These indirect methods are reasonably good estimates for most gas mixtures. Neither method compensates for absorption of EO by the packaging materials or the product. Different materials absorb EO at different rates than they do diluent gases (13). Furthermore, indirect methods do not consider physical leaks in the sterilization system. Thus the indirect method, at best, provides an approximation of the EO gas concentration in the vessel.

Direct Gas Measurement

Direct analysis of the EO in a sterilizing chamber can be performed by specific analytical instruments. Two of the most common analytical methods are the GC and the IR spectrophotometer.

Gas Chromatography. GC has been the most widely used method for determining the level of EO in the sterilizing environment. Some EO processes operate at atmospheric or positive pressure, making withdrawal of a gas sample easy. Sterilization processes that use 100% EO with a nitrogen blanket may operate at slightly subatmospheric pressures and sampling is slightly more difficult. GC is not used in 100% EO processes with no nitrogen overlay because they operate under a deep vacuum. When dealing with explosive mixtures of EO or pure EO, only intrinsically safe instrumentation must be used.

Sample removal is extremely important in order to assure meaningful data. Sample lines must be heated and insulated upon exiting the sterilizer. If cold spots occur in the sampling lines, the EO and water vapor may condense, yielding false data. These samples may be collected using gas collection bottles or with lines attached directly to the GC if an automatic injection system such as a gas sampling loop is used.

Multiple sample sites also present a problem. Representative sites are generally selected throughout the sterilizing chamber. Small capillary tubes serving as sample delivery lines are fitted to the gas sample ports. Care must be taken to permit these sample tubes to be flushed to assure that the sample being extracted is,

indeed, from the chamber environment and not a residual in the sample delivery line. For this reason, this method is not acceptable for sampling within the product or product packages. The flushing of the sample lines accelerates the gas penetration into these restricted locations and yields data that are not representative of actual load conditions.

Gas samples can be extracted from the gas recirculation system. This provides a good estimate of the gas concentration in the chamber.

The GC unit must be calibrated prior to sample analysis with a certified standard gas. This certified standard may be either a diluted gas mixture or 100% EO. Most laboratories that are established to perform GC analysis are qualified to use 100% EO as the standard for calibration. However, certified mixtures are available from gas suppliers. The GC is calibrated at one point with this standard gas and expressed as mole percent. These calibration results are independent of temperature and pressure. The mole percent concentration of the sterilization chamber is compared to the standard gas and is then converted into mg/L.

$$\frac{\text{mol\%}}{100\%} \times \frac{44.0\text{ g}}{\text{mol}} \times \frac{1000\text{ mg}}{\text{g}} \times \frac{1\text{ mol}}{22.41\text{ L}} \times \frac{(14.7\text{ psia} + Y\text{ psig})}{14.7\text{ psia}} \times \frac{273^\circ\text{C}}{(273^\circ\text{C} + ^\circ\text{C})} = \text{XF}$$

where Y psig, pressure of the sterilizing chamber; °C, temperature in the sterilizing chamber; XF, scaling factor.

Therefore, the EO concentration from the GC data in mole percent multiplied by the scaling factor (XF) yields mg/L.

IR Analysis. Most gases have a characteristic IR spectrum that can be used to identify them. These spectra are usually rather complex; however, each usually contains a small number of strong analytical bands that are used in this analysis.

These IR analyzers incorporate a fixed wave-length filter that corresponds to one of these strong bands. An optical path is also chosen that provides the sensitivity range required for the particular analysis.

The analytical wavelength for 100% EO is 11.8 μm. When HCFC mixtures are used, it has been found that a wavelength of 3.3 μm is more satisfactory and minimizes the interference with the HCFC spectrum. Some systems are theoretically sensitive to 0.4 ppm of EO.

Calibration of these analyzers must also be performed using certified standard gas. Calibration with the standard gas must consider the pressure differential between the calibration gas and the sterilizing chamber. Once the wavelength and path length are set, using the calibration standard, the instrument's response to absorbing the gas is directly correlated to concentration.

EO Gas Monitoring in the Worst Case Location in Product. Gas concentration is generally not monitored inside the product. The reason for this is that the capillary tube necessary to withdraw the gas sample from this location in the sterilizer has sufficient volume to cause erroneous readings in the vicinity of the product. Withdrawing the sample may actually force EO to migrate into the product sample site. If the sample is taken from the environment close to the product, then fewer technical problems are incurred. Samples drawn continuously from the product create a small delta pressure, causing a positive flow of gas from the environment into the sampling locale around the product. If the environment within the sampling area is large and unencumbered, then meaningful gas samples can be withdrawn from the chamber. Samples are generally withdrawn from a spectrum of locations within the chamber, typically warmer as well as cooler than other locations. Samples should be withdrawn from the front, back, top, and bottom of the vessel, so that all geometric areas within the sterilizer are assayed.

EO Gas and Diluents

100% EO No Diluents

The most commonly used form of EO gas for sterilization in the industry is pure EO (100%) with a nitrogen overlay pressure sufficient to reach near-atmospheric pressure within the sterilizing chamber. This process is the most economical and there are no diluent concerns. This process has potentially explosive phases, but the nitrogen blanket minimizes the risk of an explosive mixture with air inside the vessel.

EO Gas Mixtures

Some EO gas mixtures have been created because they are not explosive. Such mixtures do not require expensive safety facilities in which to operate.

Mixtures Diluted with HCFC. The next most commonly used EO is that which has been diluted with halocarbon products, primarily HCFC 124 and HCFC 22. This mixture is normally composed of 10% EO and 63% HCFC 124 and 27% HCFC 22 or 8.6% EO and 91.4% HCFC 124.

Cylinders charged with EO/halocarbon mixtures contain a liquid that is a homogenous mixture of both the EO and the halocarbon. The pressure in these cylinders is low due to the vapor pressure of the liquid at the temperature at which the cylinders are stored. When the sterilizer is charged, a homogeneous blended liquid is drawn off the bottom of the cylinder. The pressure in the cylinder remains virtually constant until the liquid level falls below the level of the cylinder eductor tube. Multiple sterilizer charges can be performed with this mixture yielding consistent EO concentrations.

Mixtures Diluted with Carbon Dioxide. EO may also be mixed with carbon dioxide in concentrations of 10% EO and 90% CO_2, or a 20% EO and 80% CO_2 and 30% EO and 70% CO_2. Since 20% EO and 80% CO_2, 30% EO and 70% CO_2 and 100% EO are explosive, these EO sources must be used only in specially designed sterilizers and buildings that are designed to be intrinsically safe electrically and to withstand potential explosions.

Cylinders charged with EO/carbon dioxide mixtures contain a liquid phase of EO and a gaseous phase consisting mainly of carbon dioxide gas molecules with minor EO. The pressure of these cylinders is much

higher than that of the EO/HCFC mixtures. Because of the biphasic condition of a liquid phase/gas phase mixture, it is virtually impossible to achieve multiple charges that are a consistent molecular blend and a consistent EO concentration. These cylinders are designed with an eductor tube with a fixed orifice that draws the liquid EO from the bottom of the cylinder and much smaller openings at the top of the eductor tube to withdraw the diluent CO_2 gas in the upper portion of the cylinder. These two chemicals are mixed in the eductor tube as they are released from the cylinder. In theory, it should work, provided the orifice openings are free-flowing and cylinder pressure remains constant. In practice the system often does not work as intended. To compound this problem, most sterilizers that use this gas mixture also rely only on a pressure reading as an indirect indication of EO concentration. Gas charges from these cylinders will frequently yield mostly CO_2 with little or no EO present. The approach most users of these mixtures take is to select a cylinder size that is equal to a single (unit dose) charge in the sterilizer. Therefore, inconsistencies that occur during the emptying process of the entire cylinder have little effect on the final concentration of EO in the sterilizer. In large industrial sterilizers it may even take multiple cylinders to charge the sterilizer.

This mixture is relatively inexpensive and is becoming increasingly more popular with small sterilizer users. These users are attempting to deliver multiple charges from the same gas cylinder since smaller "single charge" cylinders may not be readily available. The concentration of EO delivered in multiple charges with EO/CO_2 mixtures tend to have slightly higher than expected EO concentration in the first withdrawals. As the tank approaches empty the concentration of EO then tends to decrease very rapidly until the last few pounds are nearly pure CO_2. This problem is further compounded by the suppliers of these cylinders. It appears that these suppliers are companies who fill cylinders for welding gases, which have similar hazardous properties as EO. The design of these cylinders may vary from supplier to supplier. The types of Quality Control requirements may not be the same as manufacturers who focus on sterilizing gases. Little documentation is available on cylinder and eductor tube design for these "custom fillers" of the EO/CO_2 mixtures.

Calculation of EO Concentration

The calculation of the EO gas concentration in the sterilizer is based on the Ideal Gas Law $PV = nRT$. Several assumptions must be made when applying this law. They include that the pressure rise in the sterilizer is due totally to the EO and its diluent, if used, and that temperature is at equilibrium. It also assumes that the mixture inside the sterilizer, which includes residual air and water vapor along with the EO (mixture), behaves as an ideal gas. It assumes that this mixture of components remains unchanged by molecular activity such as adsorption, absorption, condensation or reaction. It assumes that the label on the gas cylinder is accurate and that this molecular ratio remains constant when delivered into the sterilizer. That said the temperature is not at equilibrium. A temperature variance of 10°C is suggested by ISO 11135 (14), but wider ranges may be encountered in practice. The best analytical approach is to use an average chamber temperature value. EO is an active, highly soluble molecule and this allows it to be absorbed by most product and packaging materials (13).

Water vapor will be absorbed by product and packaging materials. As the vessel pressure is raised there will be a corresponding temperature rise in the water vapor in the vessel. This allows any liquid condensate from the humidification phase of the cycle to vaporize and make an additional contribution to the measured change in pressure that occurs during gas charge.

The EO sterilization process is extremely dynamic at the molecule level. The application of the Ideal Gas Law provides a good estimate of the concentration of the EO gas. Conversion factors useful in performing these calculations appear in Table 1.

The total pressure inside the sterilizer minus the change in pressure due to the addition of EO and its diluent (if used) can be expressed as:

$$P = \frac{nRT}{V} \quad (1)$$

Derivation of the EO Gas Concentration Equation

Most sterilizer operations record the pressure change during EO gas injection; therefore, the following equation was derived to allow the calculation of EO concentration from the pressure rise due to this gas injection, with or

Table 1 Useful Conversion Factors

Pressure	Volume	Weight	Temperature
1 atm = 4.7 psia	1 L = 1,000 cc	1 lb = 454,000 mg	°C = (°F − 32) × (5/9)
1 atm = 760 mmHg	1 L = 0.03532 ft^2	1 lb = 454 gm	K = °C + 273.2
1 atm = 29.92 in Hg	1 ft^3 = 28.32 L		
1 atm = 1.013 bar	1 ft^3 = 28,316.9 cc		
1 atm = 101.3 kPa	1 m^3 = 1,000 L		
1 atm = 1,013 hPa			
1 psi = 6,894.7 Pa			
1 psi = 6.8947 kPa			
1 kPa = 0.145 psi			
1 kPa = 7.5 mmHg			
1 in Hg = 25.4 mmHg			
1 in Hg = 3.387 kPa			
1 hPa = 1 millibar			

Pascal (Pa) is an international standard unit of pressure. The Pascal is a unit of pressure equal to one Newton per square meter, or one kilogram per meter per second. Pressure is most commonly measured in kilopascals (kPa).

without diluent gases such as HCFC or carbon dioxide. The purpose of this equation is to provide a simple and rapid method for calculating EO gas concentration in sterilizers.

The pressure rise can be expressed as in equation (2):

$$P = P_{EO} + P_{DG} = \left(\frac{n}{v}\right)_{EO} RT + \left(\frac{n}{v}\right)_{DG} RT \quad (2)$$

$$P = \left[\left(\frac{n}{v}\right)_{EO} + \left(\frac{n}{v}\right)_{DG}\right] RT \quad (3)$$

The above formula can be expressed in mg/L:

$$\left(\frac{n}{v}\right)_{EO} = \frac{g}{MW_{EO}} L = \frac{10^{-3}}{44}\left(\frac{mg}{L}\right)_{EO} \quad (4)$$

$$\left(\frac{n}{v}\right)_{DG} = \frac{g}{MW_{DG}} L = \frac{10^{-3}}{M}\left(\frac{mg}{L}\right)_{DG} \quad (5)$$

where MW_{EO} = molecular weight of EO = 44.0, MW_{DG} = molecular weight of diluent gas = M.

Then the pressure rise can be rewritten as

$$P = \left[\frac{10^{-3}}{44}\left(\frac{mg}{L}\right)_{EO} + \frac{10^{-3}}{M}\left(\frac{mg}{L}\right)_{DG}\right] RT \quad (6)$$

Since the weight percent EO (wt% EO) is usually known and the sterilizer volume remains constant, the expression derived above can be written as

$$\text{wt\% EO} = \frac{\left(\frac{mg}{L}\right)_{EO}}{\left(\frac{mg}{L}\right)_{EO} + \left(\frac{mg}{L}\right)_{DG}} \times 100 \quad (7)$$

Solving for $(mg/L)_{DG}$:

$$\left(\frac{mg}{L}\right)_{DG} = \frac{\left(\frac{mg}{L}\right)_{EO} 100 - \left(\frac{mg}{L}\right)_{EO} \text{wt\% EO}}{\left(\frac{mg}{L}\right)_{EO}\left(\frac{100 - \text{wt\% EO}}{\text{wt\% EO}}\right)} \quad (8)$$

Substituting the above for $(mg/L)_{DG}$, equation (8) becomes

$$P = \left[\frac{10^{-3}}{44}\left(\frac{mg}{L}\right)_{EO} + \frac{10^{-3}}{M}\left(\frac{mg}{L}\right)_{EO}\left(\frac{100 - \text{wt\% EO}}{\text{wt\% EO}}\right)\right] RT \quad (9)$$

Solving for $(mg/L)_{EO}$ in equation (9):

$$P = RT\left[\frac{10^{-3}}{44} + \frac{10^{-3}}{M}\left(\frac{100 - \text{wt\% EO}}{\text{wt\% EO}}\right)\right]\left(\frac{mg}{L}\right)_{EO} \quad (10)$$

Let wt% EO = E and rewrite:

$$P = RT\left[\frac{10^{-3}}{44} + \frac{10^{-3}}{M}\left(\frac{100 - E}{E}\right)\right]\left(\frac{mg}{L}\right)_{EO} \quad (11)$$

The rewrite:

$$P = 10^{-3} RT\left[\frac{1}{44} + \frac{100 - E}{(M \times E)}\right]\left(\frac{mg}{L}\right)_{EO} \quad (12)$$

$$= 10^{-3} RT\left[\frac{(M \times E) + 44(100 - E)}{44(ME \times E)}\right]\left(\frac{mg}{L}\right)_{EO} \quad (13)$$

Then:

$$\left(\frac{mg}{L}\right)_{EO} = \frac{10^{3} P}{RT}\left[\frac{44(M \times E)}{(M \times E) + 44(100 - E)}\right] \quad (14)$$

Table 2 Gas Constants (R)

Pressure	Volume	Temperatures	R
Atm	cm^3	K	82.057
Atm	L	K	0.08205
Atm	ft^3	K	1.3140
Bar	L	K	0.08314
kg/m^2	L	K	847.80
kg/cm^2	L	K	0.08478
mmHg	L	K	62.631
mmHg	ft^3	K	998.90
in Hg	L	K	2.4549
kPa	L	K	8.312

It is important to maintain the proper units when using the ethylene oxide concentration equation and the gas constants.

This equation can be rearranged to:

$$C = \frac{KP}{RT} \quad (15)$$

C is the EO concentration in mg/L, R is the gas constant (Table 2), P is the difference in total pressure due to EO and its diluent (if used), T is the absolute temperature (K) of the EO diluent gas mixture resulting in Pressure (P), K is the constant for a given diluent (Table 3).

K is calculated using the following formula:

$$K = \frac{4.4 \times 10^4 Mw}{Mw + 44(100 - w)} \quad (16)$$

M is the molecular weight of the diluent or the average molecular weight of the diluent mixture, w is the mass fraction of EO in the diluent.

Moisture

General

Moisture is the most important parameter in the EO sterilization process. Without adequate moisture, the sterilization process is greatly inhibited. When adequate moisture is present, the process will be dependent on the molecular activity of the EO and its interaction with the microbial populations being exposed.

The authors would like to quote Phillips (15) from a 1968 article:

Table 3 Molecular Weights and Gas Constants

Substance	Molecular weight	Gas constants K (mg/g mol)[a]
EO	44.0	4.4×10^4
HCFC 22	86.47	
HCFC 124	136.5	
70% HCFC 124 + 30% HCFC 22	121.49	
CO_2	44.0	
Substance mixtures		
10% EO/27% HCFC 22 and 63% HCFC 124	Diluent MW 121.49	9.989×10^3
8.6% EO/91.4% HCFC 124		9.942×10^3
8.5% EO/91.5% CO_2		3.74×10^3
20% EO/80% CO_2		8.8×10^3
30% EO/70% CO_2		1.32×10^4

[a] Use when calculating mg/L.

Care must be exercised, however, when the objects to be sterilized or, more correctly, the microorganisms contained on them, are equilibrated to lower relative humidities or have been previously exposed to extremely desiccating conditions. Not only is sterilization more difficult at relative humidities below 30%, but once microorganisms have been highly desiccated either chemically or by vacuum, they acquire a resistance that is not completely overcome when the RH is again raised to 30%. Not all of the organisms are resistant, but a few maintain the resistance until they are essentially re-wetted. The phenomenon is not well understood, but it is real.

Moisture is extremely important in making the reactive sites in the microbial cells available to the alkylation action of EO. When cells or spores dry, their proteinaceous and nuclear materials and the active sites are physically withdrawn, making reactions with EO molecules difficult. However, as these materials are hydrated, they swell and expand. This exposes the active sites and makes them available to the alkylation by EO. Without proper humidification, these active sites are protected and impede the lethality of the EO sterilization process.

The moisture take-up of the microorganisms plays an extremely critical role in the sterilization of freeze-dryers used in the pharmaceutical industry. Freeze-drying processes can stabilize organisms in a desiccated state, making them extremely resistant to the EO sterilization process (16). Freeze-dryers are not normally designed as EO sterilizers, and adequate mechanical means for moisturization are not generally supplied. Engineering modifications must be made to these machines in order to sterilize them effectively with EO.

EO sterilization processes must be performed between the adequate levels of moisture. The lower boundary values of 35% to 50% have been referenced (16,17). The upper boundary value appears to be 85%. Once the RH is within the acceptable window for a product and process, increases or decreases in RH within this window do not produce measurable changes in microbial resistance. The boundaries are affected by temperature, load materials, and specific cycle dynamics. Both low RH and dew point conditions during EO exposure phases can produce changes in microbial lethality that are difficult to predict quantitatively. Lower and higher RH levels can produce dramatic and quantum increases in measured microbial resistance. At very low RH conditions, sterilization by EO may not be able to be accomplished in any practical time frame.

The upper limit should be below the conditions where the dew point is reached. Condensed water not only slows down the migration of the EO molecules to the spore, but the EO molecule can react with water or dissolved solutes. Such reactions reduce the available EO for reactions with microbial molecules.

Humidity, Instruments and Controllers

The most problematic parameter to monitor and control in the EO sterilization process is humidity. Humidity is typically measured as RH. The measurement compares the amount of moisture that is present in the air compared to the maximum amount of moisture the air can theoretically hold at that temperature.

$$\%\text{RH} = \frac{\text{Moisture content of the air at the specified temperature}}{\text{Vapor pressure of water at the specified temperature}} \times 100\% \quad (17)$$

Moisture can be measured indirectly using pressure measurements or directly by analytical instruments that measure either absolute water content or dew point.

Indirect Method

The indirect method uses differential pressure measurements. This differential pressure is a valid measurement only when the pressure change is due entirely to the vapor pressure of water. If liquid water is emitted at the same time as steam pressure, an additional pressure rise will occur when the liquid water is vaporized. If there is an air leak into the vessel an erroneously high indication as to the amount of moisture can result. It is also very difficult to get an accurate measurement of this parameter when a product is in the vessel, because of the moisture-absorbing qualities of various products and packages. Packaging materials many times are of greater mass than the product. They typically have a great affinity for moisture and compete with the product for this moisture. This is a process that works extremely well on an empty chamber, but it is very difficult to assess in a practical manner within the loaded chamber because of moisture exchange between the product and chamber gas environment.

If the indirect method is to be applied, measurements can be calculated using the properties of saturated steam found in engineering handbooks (Appendix I) (18). For example, the vapor pressure of saturated steam at 55°C is 117.85 mmHg. If the sterilization cycle is to be run at 55°C and a RH of 50% is desired, a change in pressure due to the addition of steam will be 58.93 mmHg or 50% of 117.85 mmHg (Table 4).

Pressure change required (mmHg)
= (vapor pressure saturated steam in mmHg)T
× desired% RH

where 58.93 mmHg = 117.85 × 50% RH; T, temperature of sterilization process.

Table 4 Comparison of Saturation Moisture Levels and 50% RH

Temperature of air °C	Vapor pressure of saturated moisture (steam) 100% RH (mmHg)	Vapor pressure of moisture at 50% RH (mmHg)
25	23.76	11.88
44	68.26	34.13
50	92.51	46.25
54	112.51	56.25
58	136.08	68.04
64	179.31	89.65

Source: From Ref. 18.

Direct Method

The direct method of assessing moisture uses analytical instrumentation such as an electronic hygrometer, IR analyzer, or GC. Electronic hygrometers can be used inside the vessel. The IR and GC systems require that samples be withdrawn from the sterilizer. When removing samples from the chamber, care must be exercised to assure that sample lines are properly insulated and heated so that the moisture does not condense in the sample lines. Reducing the pressure in the lines is important when using an analytical system that performs only at ambient pressure. If rapid changes in pressure occur, moisture will also be lost from the sample through condensation. These sample lines are usually closed loop-circulating systems. Calibration of these systems is performed with either a saturated water vapor standard or saturated salt solutions that yield very specific headspace water vapor concentration (Table 5).

Moisturization of Load

Product moisturization or humidification generally occur using two distinct operations. Pallets of product may be placed in preconditioning chambers that are typically at ambient pressure. Temperature and moisture are maintained. The pallets are placed into these chambers for a specified time to adequately provide the required moisturization permitting effective sterilization of the load (16,19,20).

The second phase of moisturization occurs within the sterilizer. This phase is generally performed under vacuum. Steam is added to the chamber to create a humid environment for the load.

External Preconditioning of Product Load

A typical controlled preconditioning room will operate at 40°C and a RH of 60%. We find that at 40°C air can hold a pressure of 55.82 mmHg of water vapor (Appendix I). Sixty percent of that value is 60% RH or 33.49 mmHg. If the products are completely equilibrated to that environment, we will have 33.49 mmHg vapor pressure of moisture. Most of the moisture added to the products is done in an external preconditioning room. When product is placed into a sterilizer, operating at 54°C, the air in the sterilizer can hold 112.51 mmHg of moisture (i.e., 100% RH). There is only 33.49 mmHg available, so the RH will drop. The 33.49 mmHg is 29% of the moisture that the air can hold at 54°C. This RH level is dangerously low and as a result the lethality rate produced by EO on the spore may decrease. Re-moisturization of the load in the chamber is thus indicated to ensure that adequate humidity levels are attained.

Table 5 Constant Humidity

Solid phase	*T* °C	% Humidity	Aqueous tension (mmHg)
$LiCl \cdot H_2O$	20	15	2.60
$CaCl_2 \cdot 6H_2O$	20	32.3	5.61
KNO_2	20	45	7.81
$Na_2Cr_2O_7 \cdot 2H_2O$	20	52	9.03
$NaNO_2$	20	66	11.5
NH_4Cl and KNO_3	20	72.6	12.6
NH_4Cl	20	79.5	13.8
$KHSO_4$	20	86	14.9
K_2HPO_4	20	92	16.0
$CuSO_4 \cdot 5H_2O$	20	98	17.0

The % humidity and the aqueous tension at the given temperature within a closed space when an excess of the substance indicated is in contact with a saturated aqueous solution of the given solid phase.
Source: From Ref. 18.

Moisturization Inside the Sterilizer

SAC Cycle. The SAC cycle is most commonly used in sterilizer moisturization. The SAC cycle employs a pre-vacuum phase at the beginning of the cycle and is held static for a specific period of time. During this vacuum hold, moisture is admitted into the sterilizer in the form of steam. This static hold is commonly referred to as a humidity dwell time. The steam vapor moisturizes or humidifies the product to be sterilized during this dwell period. This dwell process is not efficient and takes many hours to moisturize the product adequately enough for sterilization. This process works most efficiently with goods that have been adequately preconditioned at high relative humidities, prior to entering the sterilizer. The SAC cycle effectively replaces the moisture removed during the evacuation process.

Electronic hygrometers placed inside the vessel actually sense the moisture level inside the sterilizer environment and control the desired level as well.

When low humidity levels are sensed, a steam valve is opened and more steam is emitted into the sterilizer. The hygrometer has a minimum and maximum set point. When the high-level reading is indicated, the steam valve is turned off. The humidity can then be controlled when a drop in environmental moisture occurs which reflects moisture absorption by the load. If this type of control is selected, it is always used in the cycle phases prior to the introduction of EO gas. Since some hygrometers are sensitive to EO gas, the control can only be used prior to the introduction of gas. Systems that are compatible with EO should be used throughout all cycle phases.

DEC Cycle. The DEC cycle does not measure or control actual humidity levels. It relies on pressure controls and temperature controls that indirectly control moisture levels. Large amounts of steam are used and the thermal shock to the hygrometer may render it incompatible with this process. If a hygrometer is compatible it would undoubtedly read saturated or 100% RH after the initial steam pulse because of the condensation of steam on the cool hygrometer. As the hygrometer heats up during the cycle, it will begin to give readings less than saturation. The accuracy of hygrometer readings following this saturated condition should be checked by subsequent calibration.

Humidity control in the DEC cycle is built around the laws of physics regarding temperature and pressure of saturated steam of the cycle design. The steam pulses purge the air from the chamber and goods to be sterilized. A temperature control system measures the temperature of the steam condensate. This condensate is indicative of the temperature within the sterilizing chamber. Therefore, when the product in the chamber has been heated to the steam vapor temperature, it has been moisturized by the steam condensate. At this phase of the cycle the goods are at temperature. The next phase of the cycle removes

any excess moisture. Following this steam pulsing phase, EO gas is charged into the sterilizer and a resultant rise in temperature occurs as a result of the rise in pressure in the chamber. This rise in temperature is due to the compression of the steam vapor and causes the condensed moisture to vaporize, thus slightly drying the product.

The DEC cycle is an extremely effective cycle in moisturizing product to be sterilized. The DEC cycle also employs controlled pressure levels based on subatmospheric saturated steam conditions. Humidification and heating, therefore, occur simultaneously. The DEC cycle requires an extremely large vacuum pump for the chamber size and is more popular in smaller sterilizers although it is also used on larger industrial sterilizers.

RH Monitoring in the Worst Case Location in the Product. RH sensors, used to monitor the chamber environment, can also be used to monitor the environment within the package or within the master carton and, in some cases, even within the product. Again, because this is an electronic reading, it does not have the same impact on the parameter that the removal of a gas sample has. However, the humidity element may indeed have an impact on the temperature within that particular environment in the load. Electronic systems may have a mass that has a sufficient heat capacity to cause the water vapor to condense on the sensor, therefore impacting the true environmental conditions within the product. Again, the humidity penetration should be measured at numerous locations within the sterilizer. Specifications should be prepared that detail these specific parameter tolerances. Unfortunately, the degree of biological effectiveness can only be assumed since conditions within the product are not generally measured or monitored.

Temperature

General

Temperature is one of those parameters whose measurement seems quite straight forward. We understand heat and the physics of heat transfer. We use TCs and RTDs to measure temperature in many different pharmaceutical processes. In the case of EO sterilization, we have a very complex situation. The conventional limits on temperature are generally between 20°C (68°F) and 65°C (149°F). Most processes are run between 30°C (86°F) and 54°C (129.2°F). EO gas reactions with cellular molecules correspond to first-order kinetic reactions. First-order reactions are those that proceed at a rate exactly proportional to the concentration of one reactant. Temperature affects the rate of this reaction. An increase in temperature by 10°C (18°F) will approximately double the reaction rate with EO thus affecting sterilization times. This value is expressed as Q_{10}. In a recent publication we empirically derived a Q_{10} for this process as 2.05 (21). A basic doubling of the lethality of the process will occur with this 10°C rise in temperature. The lowest temperature limit is the temperature at which EO is converted from a gas to a liquid, which is 10.4°C (50.5°F). The upper limit is that temperature at which EO gas polymerizes rapidly rendering it biologically inactive.

The laws of physics have created an additional complication in the EO process. Temperature and RH are dependent on each other. If the amount of moisture is fixed, then as temperature rises the percentage RH decreases. The converse is also true.

The international standard for industrial EO sterilization processes (ISO 11135) (14) permits a temperature variance of 10°C in a sterilizer load. The authors believe the temperature spread, if it did exist, would not allow a proper validation program to be performed. This process limit is too large to provide acceptable process controls. The 10°C process temperature variance is very risky with respect to RH conditions. There seems to be a lack of understanding regarding the relationship between temperature and humidity. As stated earlier, RH is the amount of moisture in the air relative to the amount of moisture the air is capable of holding at a specified temperature. Table 4 compares saturated conditions (100% RH) to 50% RH.

Process operating conditions at 54°C and 60% RH are common. At 54°C/60% RH, the vapor pressure of moisture is 67.50 mmHg (Appendix I). If the temperature were to drop to 44°C lower limit which would be allowed by ISO 11135, the moisture level is dangerously close to the dew point (100% RH). The dew point at 44°C (100% RH) is 68.26 mmHg.

Comparing the acceptable high limit allowed by the standard of +10°C or 64°C the vapor pressure (100% RH) is 179.31 mmHg. The moisture level of 67.50 mmHg now drops to 31% which is dangerously low. The rate of lethality decreases significantly below 50%. This change in lethal rate can yield a positive BI spore challenge when it would be expected to be killed.

Temperature increases that lower the RH below 50% are where the real problems occur. The majority of EO sterilization failures are due to insufficient humidification. Thirty years ago it was believed that successful EO sterilization was impossible in the winter months, low ambient humidity, in the northern climates of the U.S.A. and Canada (16,19,22,23). Product warehouses were cold and humidity was very low. Temperature was easily corrected, but humidity was more difficult. The advent of the use of "preconditioning" in rooms external to the sterilizer helped to significantly reduce this problem. Nevertheless, even today "validated" EO cycles yield more positive BIs in the winter or low humidity months than at other times of the year.

Temperature Instruments and Controllers

Process controllers are either the TC-type controllers or RTD controllers. They are compatible with temperature ranges within the sterilization process and give accurate and reliable information. Temperature is controlled primarily by using a jacket around the sterilizer. This jacket may be heated with a hot water/ethylene glycol mixture, or it may be steam heated. The water/glycol mixture operates within a narrower temperature range than steam-heated jackets. Steam heating may be either an atmospheric condition or a subatmospheric condition. The atmospheric steam jackets give the widest spread of temperature, while subatmospheric jackets give the narrowest spread.

The location of the temperature control sensor is much less critical in a glycol-jacketed system. A few degrees of temperature range are generally noted in the glycol system. The temperature controller may even be

located outside the sterilization chamber in a glycol recirculating line that heats the jacket.

Steam-heated jackets, however, are usually monitored within the sterilizing chamber. Placement of the control probe is extremely critical to overall temperature control within the chamber because of the hysteresis effect of the controller. There can also be temperature excursions because the controller calls for heat and puts in excess steam into the jacket due to the thermal lag of the chamber mass.

Temperature Monitoring within the Product

TCs may be very small wires and can actually be mounted into the product. Since the TC is an electronic reading coming from the product, it can be placed well into the product to indicate temperature heat-up of a particular surface or environment within the product. Again, the number of TCs will depend on the complexity of the product and the complexity of the loading configuration. ISO 11135 (14) states that no less than 10 TCs should be used in the chamber. As a general rule, no less than 1% of products should be monitored with TCs when mapping the temperature distribution within the load. There are graphic programs available to use the TC data to provide a lethality map of the temperature distribution within the load (24).

Time

General

The sterilization process time is related to: (*i*) moisturization level; (*ii*) EO gas concentration; and (*iii*) temperature. Process time must account for penetration of these critical elements into the worst case or least lethal locations in the product, load, and packaging barriers around the product. Time must be expressed as "Equivalent Process Time" not clock time. This equivalent process time must integrate the lag factors including the come up to exposure as well as the exhaust time effects on total process lethality (25–27). The selection of the best process parameters will result in adequate EO sterilization process times of less than two hours (28). Process times for manufacturing components that are relatively easy to sterilize may even be less than one hour. With palletized loads these times may exceed 8 to 12 hours.

Establishing Process Equivalent Time

Simple clock time is insufficient as a critical process parameter. Time should be expressed as equivalent process time. This equivalent process time must integrate the lethality delivered during gas charge (come up) exposure (hold time) and exhaust (come down) (26).

Equivalent process time directly relates to process lethality. The dynamic conditions that exist in the dynamic phases of the sterilization process (come up and come-down times) deliver lethality that must be accounted for. Mathematical equations have been described which allow the integration of equivalent lethality similar to the *F*-value concept used in steam sterilization (21,26).

The following classically accepted formulas applied to microbial resistance studies are applied here to calculate the lethality equivalent process time (26):

$$D\text{-value} = \frac{U_t}{\text{Log } N_0 - \text{Log } N_t} \tag{18}$$

where U_t is equivalent exposure time, N_0 is the initial spore population, and N_t is the final spore population.

$$\text{SLR}_{\text{FULL PROCESS}} = \frac{U_{\text{FULL PROCESS}}}{D} \tag{19}$$

where SLR is the spore log reduction and $U_{\text{FULL PROCESS}}$ is the equivalent time for the full sterilization process.

The SAL can then be calculated from these values.

$$\text{SAL} = 10^{\text{Log } N_0 - \text{SLR}} \tag{20}$$

Calculating Equivalent Time

Annexes to ISO standard 11135 (14) identify methods for calculating process *D*-values, which represent the dose or time at steady state required to reduce a microbial population by 90% or 1 $\log_{10}$. (16,29,30). Unfortunately, the document provides little guidance to assist users in actually estimating the equivalent time (U) required for such calculations. In the extreme, use of the actual exposure time (which begins after steady-state pressure has been achieved) rather than equivalent time may lead to a gross underestimation of a process's *D*-value and concomitant overestimation of the SLR and an underestimation of the SAL. It is even questionable whether a true steady-state condition ever exists in densely palletized loads. Whenever equivalent time is underestimated for *D*-value calculations, the result will be the same. Figure 4 demonstrates the relationship between *D*-value, SLR, and SAL at steady state when microbial inactivation follows a straight-line log-linear relationship (26).

EO process *D*-value calculations have been used primarily in BIER vessel studies, where the time to steady state approaches zero and the equivalent exposure time approaches the actual exposure time (31,32). However, applying any *D*-value calculation method to EO systems used for actual production sterilization is inappropriate because standard process chambers do not produce square wave cycles and substantial lethality is generated during both their charge (gas injection) phase and gas evacuation phase (which do not fall within the exposure time). This situation accounts for the popularity of the AAMI overkill validation technique and the equivalent ISO half-cycle method, neither of which require calculations of *D*-value, SLR, or SAL (14).

If the actual exposure time is used in equation (18) rather than equivalent exposure time, then as the exposure time approaches zero when Log N_0 − Log N_t is some positive number, then the *D*-value also approaches zero; subsequently, SLR approaches infinity and SAL approaches $10^{-\infty}$. While no one would suggest that a *D*-value would equal zero, this extreme example demonstrates the dangers of underestimating the equivalent time. Adding some arbitrary number to increase U does not provide the necessary information to determine U and, therefore, the *D*-value, will be underestimated. Overestimating the equivalent time for the full process will similarly result in an overestimation of SLR and an underestimation of SAL, although the percentage error will generally be less than when U is underestimated for *D*-value calculations, because the latter error is multiplicative.

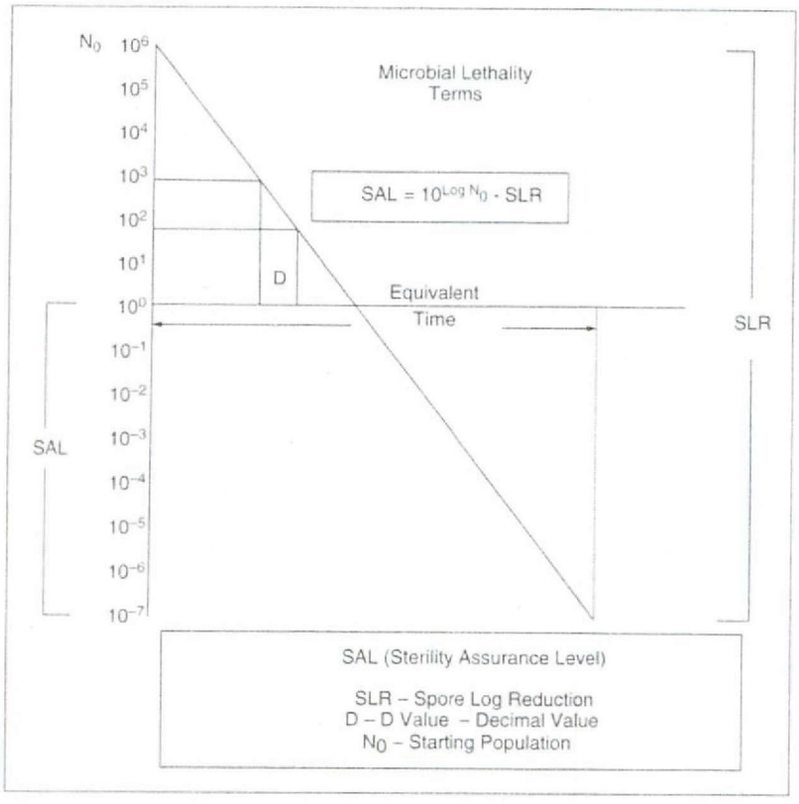


Figure 4 Microbial lethality terms. *Source*: From Ref. 26.

Process Lethality Variations Based on Product Differences. Table 6 represents results from sterilization validations conducted for a variety of medical products (26). These validations used an exposure time of zero minute, yet resulted in few or no positive BIs, which is not surprising if one understands the concept of accumulated lethality. The table also includes estimated equivalent times for these zero-minute exposures, related *D*-values, and full process-cycle SALs. The EO sterilization cycle being validated in most of this testing is depicted in Figure 5.

For this process, the lethality attributed to EO begins with the injection of the gas into the process chamber. Whether pure EO is used, as in the process shown, or a gas mixture (such as or EO/HCFC or EO/CO_2 diluent), lethality increases as the concentration increases, and the concentration increase is proportional to the pressure rise in the chamber (33). For processes with well-controlled pressure ramp-up rates, EO concentration changes also are proportional to time during gas injection and evacuation (exhaust). A cycle's exposure-time phase starts when the control pressure has been achieved, which occurs after gas injection is completed. In practice, absorption, microenvironments, diffusion, and chemical reactions that consume the gas slow the development of steady-state EO concentrations.

The cycle illustrated in Figure 5 indicates that the EO gas injection time is 11 minutes and the exhaust time is 16 minutes, which are common times in EO processing. An 11-minute nitrogen (N_2) overlay immediately follows the EO injection phase; hence EO concentration is at its maximum during that period. Data such as these can be converted to equivalent time for *D*-value, SLR, and SAL calculations using the mathematical model described below (21). The technique is based on lethality rate (L_R), which can be expressed either as a rate function with units of Δ log *N* per minute at specified conditions or as the reciprocal of the *D*-value.

Numerous investigators have shown that microbial *D*-values decline as EO concentration increases (3,22,23,34,35).

Comparative *D*-values are listed in Table 7. Test results are also graphed on a $\log_{10}$/linear plot in Figure 6, which indicates there were reasonable straight-line fits with R^2 values of 0.9695 and 0.9909 for spore strips and the self-contained test, respectively. However, Figure 7, which is a linear/linear plot of both *D*-values and lethality versus EO concentration for spore strips, depicts a more useful relationship.

The Microsoft Excel program for the best fit of data predicts that, when plotted against EO concentration (C), the *D*-value predicts a parabolic curve. As C approaches zero, then *D* will approach infinity. Logically it follows that EO-associated lethality ($1/D$) must approach zero as C approaches zero, creating an intersection on the lethality rate plot at $x=0$, $y=0$; where $D=1/L_R$ approaches zero, then *D* approaches infinity, which is also predicted by the plot of *D*, which is asymptotic in both directions, or hyperbolic. Thus a linear/linear plot of the lethality rate allows a simple approach to calculating equivalent process time if temperature is considered to be constant:

$$L_R \sim C, \quad \text{or} \quad L_R = kC \tag{21}$$

Table 6 Comparison of Different Equivalent Process Times and *D*-Values for Various Products

Product type	Positive BIs/ total BIs	Calculated *U* (min)	Calculated *D*-Value	Calculated full-cycle SAL	Full-cycle process exposure time (hr)
Introducer, delivery, forceps, catheter	1/20 Th[a]	24.65	3.34 Th	1×10^{-66} Th	4
	1/20 SC[b]		3.38 SC	1×10^{-66} SC	
Occluder delivery system	6/20 Th	24.15	3.75 Th	$<1\times10^{-87}$ Th	5
	10/20 SC		3.92 SC	$<1\times10^{-72}$ SC	
Tubing sets and scopes	5/20 Th	24.15	3.69	$<1\times10^{-72}$	4
Cannula	2/20 strips[c]	36.9	5.29	1×10^{-74}	4
Catheters, introducers	22/44 Th	47.75	7.56	1×10^{-32}	2.5
Rotor blade	17/20 Mps[d]	24.95	4.16	$<1\times10^{-51}$	4
Suture anchor	17/20 Mps	25.25	4.00	1×10^{-54}	4
Compass tips and magnets	15/20 Th	25.7	4.07	1×10^{-52}	4
Clamp covers, loops, brush, boots	3/20	24.65	3.63	1×10^{-60}	4
Optical fiber	0/20 SC	24.15	<3.31	$<1\times10^{-74}$	4
Sensor, probe, wire, etc.	0/20 SC	24.65	<3.38	$<1\times10^{-65}$	4
Orthopedic implant product line including bone-harvesting device	0/80 strips	44.9	<6.18 IP	$<1\times10^{-33}$ IP	4
	0/80 SC				
	5/40 IP[e]				
Unassembled bone-harvesting device	0/20 strips	45.15	<6.19 strips	$<1\times10^{-32}$	4
	0/20 SC				
Injectable polymer system	1/19 strips	24.85	4.09	1×10^{-52}	4

[a] Th = 1.5 in. single-strand cotton thread inoculated with $>1\times10^{6}$ *Bacillus subtilis* (SGM Biotech).
[b] SC = Self-contained test, $>1\times10^{6}$ *B. subtilis* (SGM Biotech).
[c] Strips = Paper strips, $>1\times10^{6}$ *B. subtilis* (SGM Biotech).
[d] Mps = Mini paper strip, 2×10 mm, $>1\times10^{6}$ *B. subtilis* (SGM Biotech).
[e] IP = Inoculated product from a spore suspension.

where k is the rate constant. The equation can also be expressed

$$\frac{L_{R_1}}{C_1} = k \quad \text{or} \quad \frac{L_{R_1}}{C_1} = \frac{L_{R_2}}{C_2} \tag{22}$$

and so on. Solving for L_R

$$L_{R_2} = L_{R_1}\left(\frac{C_2}{C_1}\right) \tag{23}$$

Since the *D*-value is a reciprocal of the lethality rate, the equation can also be used to solve for D:

$$\frac{1}{D_2} = \frac{C_2}{C_1 D_1} \tag{24}$$

which simplifies to:

$$D_2 = \frac{C_1 D_1}{C_2} \tag{25}$$

L_R may also be used to derive Δ Log N as a function of time (t) where:

$$L_R = \frac{(\text{Log } N_0 - \text{Log } N_t)}{\Delta t} = kC \tag{26}$$

$$\text{Log } N_0 - \text{Log } N_t = kC\Delta t \tag{27}$$

Calculation of accumulated lethality at a constant temperature (T_1) requires each increment to be multiplied

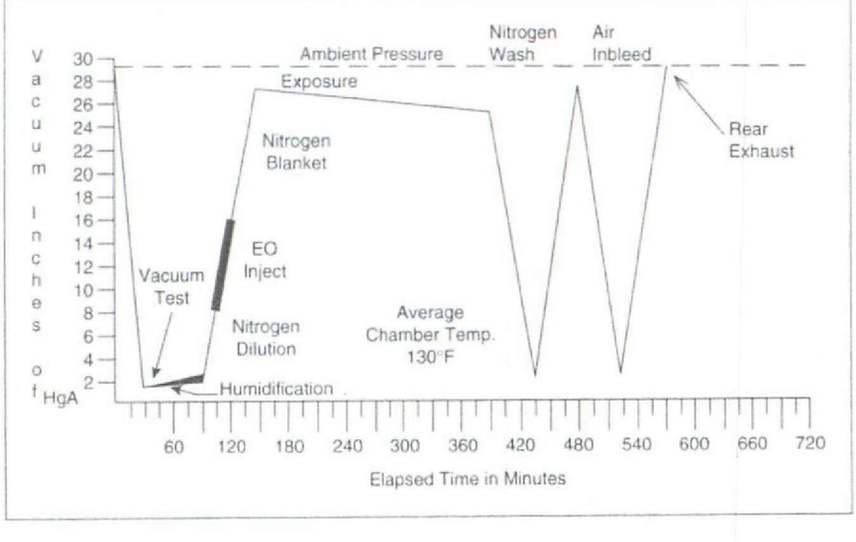


Figure 5 Typical 100% ethylene oxide sterilization cycle with nitrogen overlay. *Source*: From Ref. 26.

Table 7 Comparative *D*-Values at Four EO Concentrations Calculated Using the Holcomb–Spearmen–Karber Method

Lot no.	Log spore population	Biological indicator type	EO concentration (mg/L) 300	450	600	750
			D-value			
G-92P	6.531	Self-contained test	5.8	4.2	3.6	2.8
G-103P	6.322		5.6	4.2	3.2	2.8
G-105	6.255		5.2	4.0	3.2	2.6
Average	NA		5.5	4.1	3.3	2.7
BSUB-235	6.398	Paper strips	6.7	4.3	3.5	2.9
BSUB-244P	7.0		6.2	4.4	3.4	2.8
BSUB-249P	6.398		6.1	4.1	3.4	2.8
Average	NA		6.3	4.3	3.4	2.8

These test results are also shown graphically in Figure 6.
Source: From Ref. 26.

by the time at that increment, which is expressed in the summation formula:

$$\mathrm{SLR} = \sum_{i=1}^{n} L_{R_i} = \sum_{i=1}^{n} t_{T_i}\left(\frac{C_i}{C_{\mathrm{ref}}}\right) L_{\mathrm{ref}} \tag{28}$$

The effect of temperature variations on *D*-values is known as the *Z*-value. This effect has been described for steam and dry-heat applications (25). A number of references in the past have indicated a similar correlation in EO sterilization as in steam and dry heat of a *Z*-value. Ernst (19) reported a theoretical lower limit of $Q_{10} = 1.8$ for EO sterilization, but a consensus seems to have evolved for a nominal Q_{10} value of 2. (This means that a 10°C change would affect lethality by a factor of 2.) Thus a Q_{10} value of 2 was used for a set of temperature-related tests along with a *Z*-value of 33.2°C, which was calculated using the relationship $Z = 10°\mathrm{C}/\log_{10}\mathrm{Q}$. This value was intermediate between a recently suggested *Z*-value of 36°C and an older recommendation of 29.4°C (23,33). Test results of Mosley, Gillis, and Krushefski (21) indicate that the best choice of *Z* to fit the experimental data is 32°C, which is essentially the result for a Q_{10} value of 2.05 and very close to the calculated values of ~29°C suggested in earlier studies (26,27,33).

Because they are independent variables, a reference EO concentration (C_{ref}) and temperature (T_{ref}) can be used to calculate the equivalent process time for various temperatures as follows:

$$U_{C_{\mathrm{ref}},T_{\mathrm{ref}}} = [\mathrm{antilog}(\mathrm{Log}\, t_{T_{\mathrm{ref}}})]\frac{C}{C_{\mathrm{ref}}} \tag{29}$$

where

$$\mathrm{Log}\, t_{T_{\mathrm{ref}}} = \mathrm{Log}\, t_T + \frac{(T - T_{\mathrm{ref}})}{Z} \tag{30}$$

For example, using $Z = 29°\mathrm{C}$, if the exposure time (t) is 40 minutes, the temperature (T) is 40°C, and the concentration (C) is 300 mg/L, the equivalent process time at $C_{\mathrm{ref}} = 600$ mg/L and $T_{\mathrm{ref}} = 50°\mathrm{C}$ is 9 minutes:

$$U_{600\ \mathrm{mg/L},50°\mathrm{C}} = \left\{\mathrm{antilog}\left[\mathrm{Log}\,40 + \frac{1}{29}(40-50)\right]\right\}\frac{300}{600} = 9 \tag{31}$$

In addition, because $D \sim U$, the above equation also can be used to address *D*-value:

$$D_{C_{\mathrm{ref}},T_{\mathrm{ref}}} = \left\{[\mathrm{antilog}(\mathrm{Log}\, D)] + \frac{(T - T_{\mathrm{ref}})}{Z}\right\}\left(\frac{C}{C_{\mathrm{ref}}}\right) \tag{32}$$

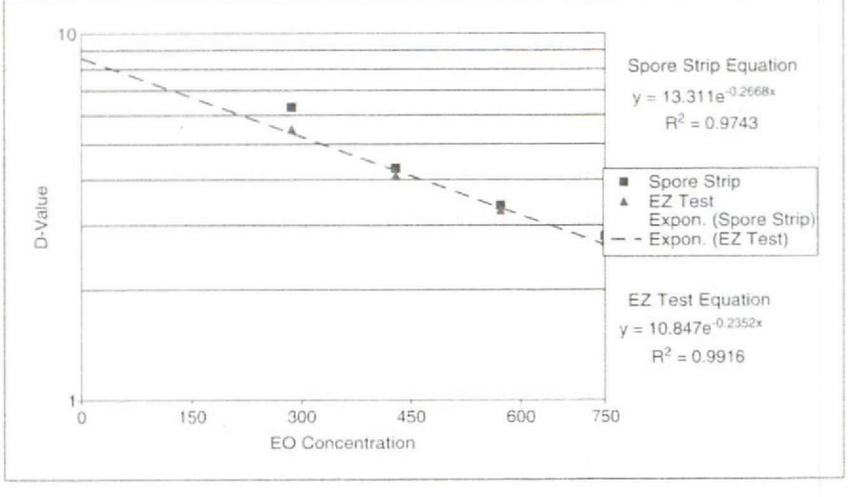


Figure 6 Log_{10}/linear plot of *D*-values versus ethylene oxide. *Source*: From Ref. 26.

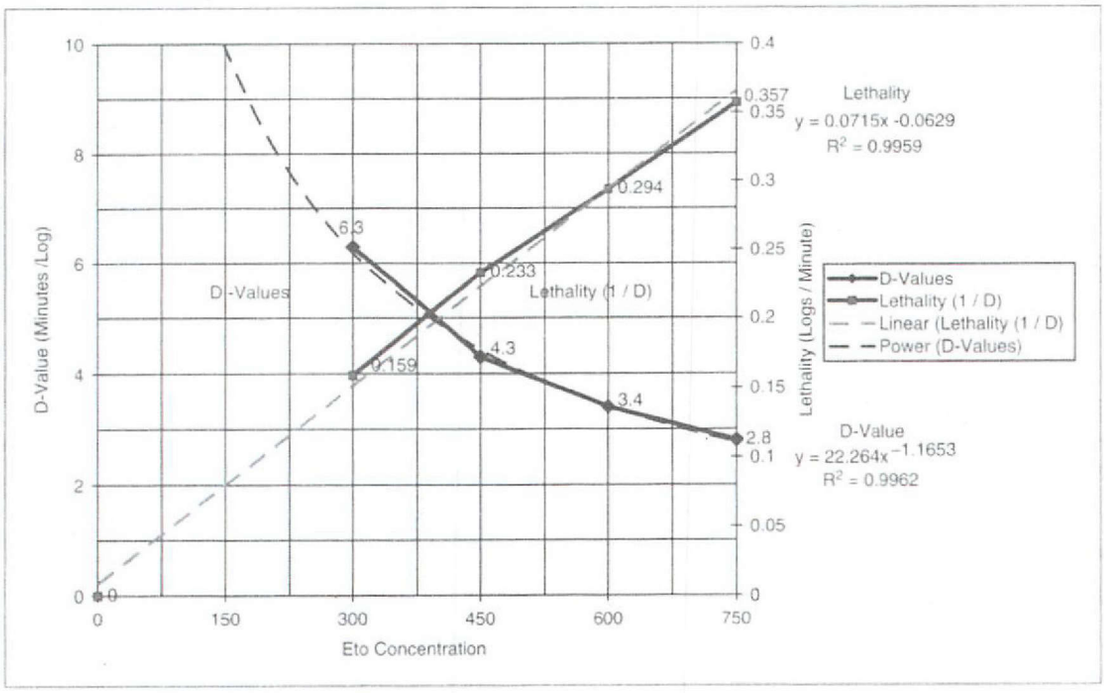


Figure 7 *D*-value and lethality versus ethylene oxide concentration. *Source*: From Ref. 26.

To determine accumulated equivalent process time where conditions are changing for EO concentration and/or temperature, a summation equation can be applied:

$$U = \sum_{i=1}^{n} U_i = \sum_{i=1}^{n} \left\{ \text{antilog}\left[\text{Log } t_T + \frac{1}{Z}(T_i - T_{\text{ref}}) \right] \right\} \frac{C_i}{C_{\text{ref}}} \quad (33)$$

The empirical *D*-value results along with the *D*-values that were calculated from BIER conditions of 54°C, 600 mg/L EO, and 60% RH closely agree.

The *Z*-value is the number of degrees of temperature change required to change the *D*-value by 90% or one $\log_{10}$ cycle. The *Z*-value is not an indicator of the rate of microbial lethality, but rather it is a measurement of the rate of change of microbial lethality with respect to temperature. The *Z*-value is therefore a necessary element in the ability to mathematically express equivalent process time *U*.

$$U = \sum_{i=1}^{n} U_i = \sum_{i=1}^{n} \left\{ \text{antilog}\left[\text{Log } t_T + \frac{1}{z}(T_i - T_{\text{ref}}) \right] \right\} \frac{C_i}{C_{\text{ref}}} \quad (34)$$

The *Z*-value is not linear over a wide range for process temperatures. Casolari (36) has concluded that the linearity of the *Z*-value is theoretically impossible since, in accordance with the Arrhenius relationship "...*Z*-value can not be regarded as being constant, but varies with temperature..." The consistency of *Z*-values obtained by plotting $\text{Log}_{10} D_T$ against temperature is difficult to ascertain in practice, as the evaluation of *D* is not significantly accurate. Several publications reinforce this last assertion of Casolari, particularly at high steam temperatures >132°C (32) where lag factors ensure that *D*-values cannot be accurately determined. It is interesting to note that inaccuracy in *D* due to lag factors at high temperatures was first reported in 1921 by Bigelow (37).

The *Z* is linear over limited temperature ranges and can be appropriately applied to the integration of process lethality. According to the data in Tables 8 and 9 and plotted in Figure 8, the *Z* is quite linear between 40°C and 60°C and EO gas concentration between 300 and 750 mg/L. These limits cover the majority of the commercial EO processes in use today.

These data support the approach that integrated process lethality can be applied to EO sterilization with as much confidence as can be applied to steam processes as long as critical parameters are appropriately controlled. The selection of a universal *Z*-value for EO sterilization appears to be 32°C comparable to the widely accepted value of 10°C for steam. Arguments could be made for *Z*-values ranging from 29°C (21,27,33) to 36°C (23). This range represents a relatively small change in reaction rates when compared to the accepted *Z*-values for steam processes.

BIOLOGICAL MONITORING

Biological Release of Product

Biological monitoring of the sterilization process uses calibrated bacterial spores. The bacterial spores most commonly used are *Bacillus atrophaeus* (28). The *B. atrophaeus* spores are very resistant to the EO sterilization process. These spores are usually placed on a carrier substrate that allows them to be conveniently placed inside product samples (20). The location of choice is the position in the product that is worst-case or least-lethal location. The inoculated product samples

Table 8 *D*-Values of Six New Test Organisms to Various EO Exposure Conditions

Species	EO concentrations (mg/L)	40°C	54°C	60°C
Bacillus atrophaeus ATCC #9372	300	18.11	6.37	4.44
	450		4.30	
	600		3.39	
	750	8.33	2.84	1.94
Bacillus Subtilis "5230" ATCC #35021	300	15.76	6.30	4.44
	450		4.96	
	600		3.98	
	750		3.51	
Bacillus pumilus ATCC #27142	300	13.36	5.40	3.95
	450		4.09	
	600		3.33	
	750	8.29	2.47	1.70
B. subtilis DSM #4181	300	9.26	4.18	3.24
	450		3.11	
	600		2.45	
	750	5.05	2.16	1.50
Bacillus smithii (formerly *coagulans*) ATCC #51232	300	7.69	3.35	2.21
	450		2.55	
	600		2.09	
	750	4.38	1.80	1.19
Geobacillus stearothermophilus ATCC #7953	300	4.09	1.55	1.25
	450		1.11	
	600		0.82	
	750	1.99	0.67	0.56

Source: From Ref. 21.

are then packaged in a similar manner as the product. The samples are placed in positions in the load that also have been identified as worst-case or least-lethal location.

BI systems have been developed using paper strips containing spores. These convenient carriers are placed into least-lethal locations in the product (Figs. 9 and 10). The paper strips may also be packaged in bio-barrier envelopes. Some BIs are packaged in self-contained culture systems. These systems are used in the same manner (Fig. 11). Placement will depend largely on the configuration of the product and package. Sometimes they will not physically fit into the device and must be placed inside the package with the product.

Following the sterilization process, these monitoring systems are removed from the sterilizer and cultured in the laboratory. The *U.S. Pharmacopoeia* recommends culturing in soybean casein digest medium at a temperature of 30°C to 35°C for seven days. Specific culture recommendations may be supplied by the manufacturer of the monitoring system. Some monitoring systems have been challenged using the FDA Reduced Incubation Time protocol with the resulting incubation times of 48 to 72 hours (38).

Manufacturers who intend to run multiple products in the sterilizer load will attempt to define a "master" BI/product combination. Studies must be conducted to demonstrate that the BI/product combination is more resistant when compared to other combinations. For instance, if one has determined that each BI/product combination is a reasonable simulation and that it can be scientifically defended, then it does not matter that they are not directly comparable. The biological challenge becomes the BI/product combination. The type of BI cannot be changed without producing somewhat unpredictable changes in relative resistance. If it has been determined that the BI/product A is the most difficult challenge and a BI strip in glassine is used, then it would be expected that if a second BI lot with a higher *D*-value, of the same type from the same manufacturer was used, then the BI/product combination would yield a higher process *D*-value. However, if, one had decided to use direct product inoculation from a liquid suspension as a replacement for the BI strip, the relative results could not

Table 9 *Z*-Values for Six New Test Organisms at Two EO Concentrations

Species	EO concentrations (mg/L)	°C	Average	Mean ±2 S.D. (±8%)	Mean ±3 S.D. (±12%)
Bacillus subtilis DSN #4181	300	37.93	40.57	37.32–43.82	35.70–45.44
	750	43.20			
Bacillus subtilis "5230" ATCC #35021	300	37.44	36.79	33.85–39.73	32.38–41.20
	750	36.14			
Bacillus smithii (formerly *coagulans*) ATCC #51232	300	35.47	36.37	33.46–39.28	32.01–40.73
	750	37.26			
Geobacillus stearothermophilus ATCC #7953	300	34.94	36.34	33.43–39.25	31.98–40.70
	750	37.73			
Bacillus atrophaeus ATCC #9372	300	32.40	31.89		
	750	31.38			
Bacillus pumilus ATCC #27142	300	28.60	30.18	27.77–32.59	26.56–33.80
	750	31.76			

Source: From Ref. 21.

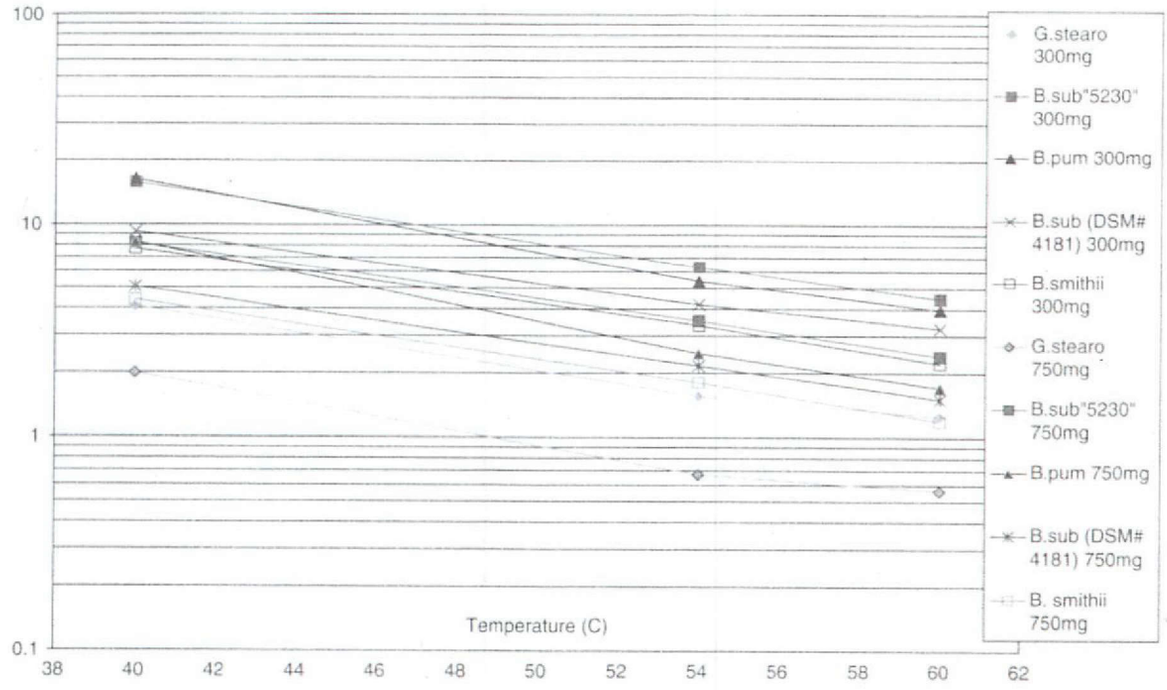


Figure 8 Multispecies composite of *Z*-values—300 and 750 mg/L ethylene oxide and temperatures from 40°C to 60°C. *Source*: From Ref. 21.

be predicted. The new combination could be more or less resistant than the original. The new BI/product A combination might not prove to be the most resistant compared to the other BI/product combinations used in the original study. It is important that the user understand what has been proven and what has not been proven in order that the information can be properly applied. Once a biological master product has been selected, the type of BI used in the BI/product combination cannot be changed without affecting the expected relative resistance. The lot or supplier of the original BI type could be changed, and the overall BI resistance in the supplied BI should create a similar shift in the resistance of the BI/product combination.

BIs are much more convenient than inoculated product or inoculated simulated products (20). A Process Validation program should include product sterility data as well as BI data. Routine process monitoring normally includes the use of BIs only. Normally, a minimum of 10 BIs are used for each sterilization cycle. For extremely large loads up to 1000 ft^3, as many as 30 BIs or more may be tested per cycle. This is dependent on the product application physical size and difficulty to sterilize. The BI data must be integrated into all aspects of the process control program to assure an adequate SAL.

The bacterial spore is the only monitor that can be embedded into the worst case–least lethal location in the product. It is also the only monitor that can integrate all critical process parameters to assess the effectiveness of the sterilization process.

Parametric Release of Product

Details of the current practices for parametric release will not be discussed in this chapter. Parametric release involves accepting or rejecting a load of product from a sterilization cycle based solely on a review of physical and chemical process parameter measurements for the cycle. Once the validation has been completed routine biological testing is not required. This approach has become popular due to potential faster turn around and lower routine sterilization costs. The incubation time for standard BIs has historically been seven days. Although

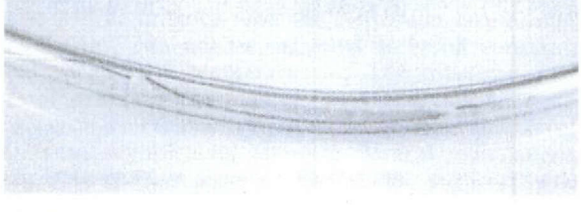


Figure 9 Spore strip biological indicator placed inside process tubing.

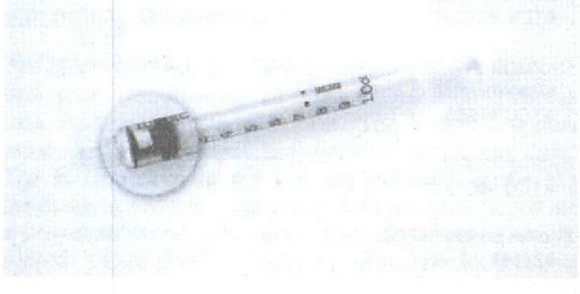


Figure 10 Spore strip biological indicator placed inside syringe.

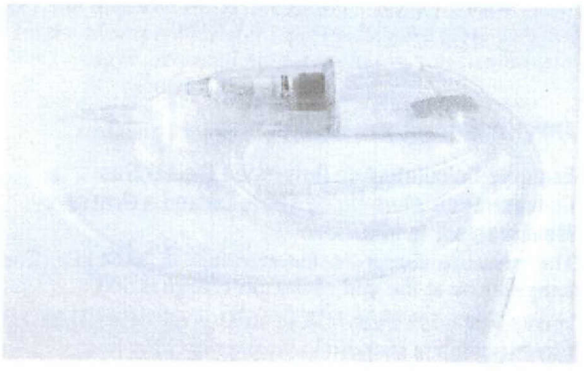


Figure 11 Self-contained ethylene oxide biological indicator placed inside the IV drip chamber of a drug administration set.

the use of BIs with reduced incubation time may reduce the seven-day quarantine time to 5, 3, 2 or less still, there is the cost of BIs and subsequent testing that can be eliminated by a parametric release approach. The cost of a proper validation for parametric release is often significantly more than that of a standard validation because it must be more robust. In addition, the greater amount of routine parametric data may increase review time and associated costs.

However, there are three flaws to the pragmatic implementation of parametric release. First, it is often implemented by companies because they have occasional problems with positive BI results from routine sterilization cycles. Parametric release has been implemented to avoid investigation costs and delays in product flow. This is bad practice and suggests inadequate "root cause" analysis. Since BIs can only detect catastrophic sterilization process failure, a true positive indicates a serious problem. Secondly, most BI positives from routine cycles occur during winter and early spring months, based on our experience. These are the cooler and drier months and suggest problems with material humidification not always detectable using current physical measurements. The complexity of the EO sterilization process should not be underestimated. The Oxborrow et al. (39) report on the AAMI round robin testing of BIER vessels demonstrated significant system bias from one BIER vessel and test lab to another. BIER vessels are designed to operate at control ranges far tighter than routine sterilization systems. However, in the study the unit producing the lowest lethality was 50% less effective than the one with the highest lethality. This suggests that the total variance from calibration, maintenance and routine control for physical measuring systems is greater than often claimed or believed. Total reliance on such controls in the light of empirical evidence seems not to be objectively sound.

Critical process parameters have been discussed extensively in this chapter. The authors know of no instruments that can be placed into the least lethal locations of products to provide meaningful parametric data. Until such instruments are developed, it seems prudent that biological challenge systems should be used to evaluate process delivered lethality.

EO TOXICITY

Residuals

Sterilant residuals and sterilant reaction products must also be considered in the Process Validation program. EO, being a toxic substance, will render a sterile product unusable if excessive amounts remain in the product after sterilization. The EO gas becomes trapped inside product voids. It is also absorbed and adsorbed by the product. Depending on the product material, it is generally easily removed (13). A common approach is to place the post-sterilized product in a heated aeration chamber with very frequent air changes. Ambient storage will also allow the EO gas to dissipate. There are two common EO reaction products that are also considered toxic. The EO gas reacts with chlorine to form ethylene chlorohydrin and with water to form ethylene glycol. The latter compound is much less toxic than the other two chemicals. These reaction products are not easy to remove from materials because their boiling points exceed 100°C. Therefore, it is important to minimize the formation of these reaction products. In the case of ethylene chlorohydrin, product and package materials with chlorinated compounds, such as sodium hypochlorite–bleached paper, are preferably avoided if EO gas is the sterilizing medium. Ethylene glycol formation is dependent on the amount of moisture that is actually present as water. The pH of this water will influence the rate at which the ethylene glycol is formed. The reaction is usually quite slow at neutral pH. The approach is to minimize the EO exposure time and to remove the humidity and EO gas after exposure by evacuation of the chamber and subsequent aeration.

Environmental Exposure

EO is a toxic and hazardous chemical. It is this characteristic that renders it an effective sterilizing agent. Controlling this chemical to minimize and prevent human exposure is an important consideration in the application of EO gas when used to sterilize materials in the pharmaceutical industry. The Occupational Safety and Health Act of 1970 emphasized the need for standards to protect the health and safety of workers (40). The NIOSH has disseminated information about the adverse effects of widely used chemical and physical agents, in an attempt to assist employers in providing protection to employees from exposure to these substances. NIOSH has taken the lead in disseminating information about EO toxicity.

The acute toxic effects of EO in humans and animals include: acute respiratory and eye irritation, skin sensitization, vomiting, and diarrhea.

Known chronic effects consist of respiratory irritation, secondary respiratory infection, and anemia. No definitive epidemiologic studies and no standard long-term study assays are available on which to assess the carcinogenic potential. Limited tests by skin application or subcutaneous injections in mice did not reveal carcinogenicity. However, the alkylating and mutagenic properties of EO are sufficient basis for concern about its potential as a carcinogenic agent. It has since been classified as a carcinogenic agent.

NIOSH is recommending that EO be considered as a carcinogenic agent for humans and that occupational

exposure to it be minimized by eliminating all unnecessary and improper uses of EO. The Federal Register on April 21, 1984, proposed that the worker exposure limit be reduced from 50 to 1 ppm in the worker's environment, based on a TWA. This proposal was finalized on September 9, 1985 (Federal Register 50FR9800—March 12, 1985).

At the time of the proposal to reduce the level from 50 to 1 ppm, little scientific evidence existed to support the contention that 1 ppm was necessary to protect the environmental health of the workers. EO was later classified as a carcinogen and is regulated by OSHA Safety and Health Management Guidelines (Federal Register 54:3904–3916, January 26, 1989). When proper control measures are instituted, the escape of EO into the environment is virtually eliminated. These may include catalytic abator systems or acidified aqueous purge tanks that convert EO to ethylene glycol. Under such control, EO can be used as a gaseous sterilant in pharmaceutical facilities with little risk to the health of exposed workers.

Employee exposure is limited to one part EO per million parts of air (1 ppm) measured as an eight- hour TWA. Employee exposure may not exceed the short-term excursion limit of 5 ppm EO averaged over any 15-minute sampling period. These limits are called PELs.

Systems are typically designed to ensure that employees are protected when handling of products containing EO to ensure that the release of airborne concentrations of EO are at or below the standard action level of 0.5 ppm.

Workplaces are exempt from this standard when objective data shows that processing, use or handling of products containing EO cannot release airborne concentrations of EO at or above the action level or in excess of the excursion limit during normal conditions.

APPENDIX I

Example Calculation to Determine the EO Gas Concentration when Using the 10% EO, 27% HCFC 22, and 63% HCFC 124 Blend of Diluent and a Pressure Measurement in kPa

The EO mixture is 10% EO and 27% HCFC 22 and 63% HCFC 124. The pressure change in the sterilizer as a result of the gas charge is 176.98 kPa. The temperature at the end of the gas charge is 54°C.

$$C_{EO} = \frac{KP}{RT}$$

$K^a = 9.989$ mg/g mol, $P = 176.98$ kPa, $R^b = 8.312$, $T\,K = 54°C + 273.2 = 327.2$ K

$$C_{EO} = \frac{\left(9.989 \times 10^3 \frac{\text{mg}}{\text{g mol}}\right) 176.98 \text{ kPa}}{\left(8.312 \frac{\text{kPa L}}{\text{g mol K}}\right) 327.2 \text{ K}} \tag{35}$$

[a] Refer to Table 3.
[b] Refer to Table 2.

$$C_{EO} = \frac{9989 \times 176.98}{8.312 \times 327.2} = \frac{1767853}{2719.7} = 650 \text{ mg/L} \tag{36}$$

APPENDIX II

Example Calculation to Determine the EO Gas Concentration when Using 100% EO and a Pressure Measurement In kPa

The pressure charge in the sterilizer is 36.64 kPa. The temperature at the end of the gas charge is 50°C.

$$C_{EO} = \frac{KP}{RT}$$

$K = 4.4 \times 10^4$, $P = 36.64$ kPa, $R = 8.312$, $T\,K = 50°C + 273.2 = 323.2$ K

$$C_{EO} = \frac{\left(4.4 \times 10^3 \frac{\text{mg}}{\text{g mol}}\right) 36.64 \text{ kPa}}{\left(8.312 \frac{\text{kPa L}}{\text{g mol K}}\right) 323.2 \text{ K}} \tag{37}$$

$$C_{EO} = \frac{1611720}{2686.4} = 600 \text{ mg/L} \tag{38}$$

APPENDIX III

Example Calculation to Determine the EO Gas Concentration Using 100% EO and a Pressure Measurement In psia

The pressure change in the sterilizer is 5.13 psia. The temperature at the end of the gas charge is 125°F.

$$C_{EO} = \frac{KP}{RT}$$

$K^a = 4.4 \times 10^4$, $P = 5.13$ psia (must convert to atm), $R^b = 0.08205$ (atm L)/(g mol K), $T\,F = 125°F$ (must convert to K)

$$P = \frac{5.13 \text{ psia}}{14.7 \text{psia}} = 0.349 \text{ atm}$$

$$T\,C = \frac{(125 - 32)9}{5} = 51.7°C$$

$$T\,K = 51.7°C + 273.2 = 324.9 \text{ K}$$

$$C_{EO} = \frac{44000 \times 0.349}{0.08025 \times 324.9} = \frac{15356}{26.66} = 576 \text{ mg/L} \tag{39}$$

APPENDIX IV

EO Gas Concentration Determined by Weight of Gas Dispensed

The gas mixture is 10% EO, 27% HCFC 22, and 63% HCFC 124 percentage by weight. The sterilizing chamber is 100 ft^3. The sterilization process requires EO concentration of 475 mg/L. How many pounds of gas mixture must be dispensed?

- Sterilizer volume = 100 ft^3 = 2832 L.
- The percentage of EO in each pound of mixture is 10%.

- The required EO is 475 mg/L.
  - Multiply the sterilizer chamber volume by the mg/L required to determine to total amount of EO required in mg.

$$2832\text{L} \times 475\ \text{mg/L} = 1,345,200\ \text{mg EO}$$

  - Divide the EO mg required by 454,000 mg/lb to determine the lbs of EO required.

$$\frac{1,345,200\ \text{mg}}{454,000\ \text{mg/lb}} = 2.963\ \text{lb of EO}$$

  - Divide the pounds of EO required by the percentage of EO per pound of mixture to determine the total weight of mixture to be added to the chamber.

$$\frac{2.963\ \text{pounds of EO}}{0.10\ \text{pounds of EO/pound of mixture}}$$

$$= 29.63\ \text{lb of mixture} \quad (40)$$

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