⟨1229.7⟩ GASEOUS STERILIZATION

INTRODUCTION

The use of sterilizing gases for the preparation of materials and equipment is commonly used for items that are susceptible to damage by heat or radiation processes. Many polymeric materials, especially medical devices, are surface sterilized in this manner, as is nonpressure-rated process equipment. The sterilization of dry powders using gases is inappropriate due to the inability of gases to penetrate solid materials. The majority of gas sterilization processes employ ethylene oxide (EO), and procedures for use with other gases generally are patterned after EO practices. Ozone, mixed oxides of nitrogen, and chlorine dioxide are some of the other gaseous sterilants used. [Systems that can exist in liquid and gas phase at the operating temperatures (e.g., hydrogen peroxide, peracetic acid, and paraformaldehyde) are excluded from consideration in this chapter.] EO's ability to penetrate through polymers, cellulosics, and other materials allows it to be used for the terminal sterilization of medical devices in their final packaging. The other sterilizing gases may be suitable for similar applications.

Process control for gas sterilization equipment is accomplished by control of sterilant gas concentration, relative humidity, temperature, and system pressure. Mixing of the gas in the sterilization chamber may be beneficial. EO sterilization may be used for parametric release as described in *Terminally Sterilized Pharmaceutical Products—Parametric Release* ⟨1222⟩.

Gas sterilization differs markedly from processes during which the agent used can condense during the operation. Vapor sterilization processes will be addressed separately in *Vapor-Phase Sterilization* ⟨1229.11⟩.

As outlined in *Sterilization of Compendial Articles* ⟨1229⟩, analysts must take care in ensuring sterility and demonstrating that the essential quality attributes of the materials are not adversely affected by the process. With respect to gas processes, key considerations include the immediate effects of sterilizing gas on the materials or equipment being sterilized, residual sterilant, sterilant byproducts, and potential chemical reactions. The common gas processes differ slightly with respect to process execution and material concerns and thus are described individually.

ETHYLENE OXIDE

EO is a powerful alkylating agent that destroys microorganisms by chemical reaction, primarily with cell DNA. The destructive mechanism largely follows first-order kinetics and depends on concentration, humidity, and temperature. The use of EO for medical devices in their final packaging has, to a large extent, shaped EO sterilization processes (and, to a lesser extent, all gas sterilization) for other applications (*2,3*). The usual EO process follows a sequence of prehumidification, air removal, rehumidification in the chamber, gas exposure, gas removal from the chamber, and postexposure aeration. The preexposure steps ensure that adequate moisture is present on and within the items being sterilized. The postexposure steps provide time for the diffusion of EO and its byproducts out of the materials and packaging. When EO is used for nonporous equipment the process can be streamlined, which eliminates many of the pre- and postexposure steps because of the need only for surface sterilization. During EO sterilization the gas is introduced at the beginning, and only minimal additions are necessary later to maintain pressure as the gas is absorbed into the material/sterlization load within the vessel. Humidity adjustment during the process also may be required. In some instances, EO reacts with materials in the load to form ethylene chlorohydrin and ethylene glycol. These compounds, including EO, must be reduced to safe levels before the items can be used by patients (*4*,*5*). EO processing requires strict worker safety and environmental controls because it is associated with carcinogenicity, mutagenicity, and neurotoxicity. In addition, EO is explosive in concentrations of greater than 2.6% by volume in air, therefore, inert gases are often used to minimize flammability. The commonly accepted biological indicator (BI) strain is *Bacillus atrophaeus* (formerly *B. subtilis* var. *niger*).

OZONE

Ozone is a potent oxidizing agent produced by passing a stream of oxygen or air through a high-voltage electrical field. Ozone is an effective biocidal agent for treatment of water supplies and has demonstrated lethality at concentrations from 2%–10% in air. Optimal microbial destruction is accomplished when the relative humidity is above 80% at room temperature. Ozone degrades to oxygen in the presence of moisture and metals and therefore usually is generated in situ. Ozone does not penetrate porous materials to the same extent as EO does. Process systems that use ozone for gas sterilization have the advantage of simplicity. Its generation and destruction (using a catalytic converter) are accomplished without moving parts or consumables other than the supplied oxygen. The sterilization process uses a sequence of humidification, injection, exposure, and ventilation to remove the ozone from the chamber at the end of the cycle. The common BIs identified for ozone are *Geobacillus stearothermophilus* and *Bacillus atrophaeus*.

CHLORINE DIOXIDE

Chlorine dioxide is an effective sterilizing gas. Pure chlorine dioxide is metastable and therefore is generated as needed. Chlorine dioxide is noncarcinogenic, nonflammable, and effective at ambient temperatures. Its ability to penetrate materials may be less than that of EO.

A typical chlorine dioxide sterilization process uses a sequence of preconditioning, conditioning dwell period, charge, and exposure, followed by aeration.

The BI most commonly used is *Bacillus atrophaeus*.

NITROGEN DIOXIDE

Nitrogen dioxide is a sterilizing gas effective at ambient temperature. Liquid nitrogen dioxide is converted to a gas on introduction to the target chamber. Nitrogen dioxide is nonexplosive and its residues are noncarcinogenic, noncytotoxic, and nonteratogenic. It has a limited ability to penetrate polymeric materials in comparison to EO, which makes postcycle aeration more rapid. It is incompatible with cellulosic materials such as paper and cardboard. The suggested BIs for nitrogen dioxide are *G. stearothermophilus* and *B. atrophaeus*.

VALIDATION OF GAS STERILIZATION

The validation of gaseous sterilization generally begins with the establishing of a "minimum lethal process dwell time" through the use of fractional exposure studies. These fractional studies establish that exposure time, under standard process conditions, where the biological indicator is fully inactivated. This minimum exposure time then becomes the basis for the application of the half-cycle approach for validating the sterilization cycle. The absence of information relating the effect of varying gas concentration, humidity, and temperature on microorganisms resulted in a conservative assumption that the bioburden is equal in antimicrobial resistance and population to that of the biological indicator. The half-cycle method can be defined as follows.

The half-cycle validation method requires the destruction of a high concentration (NLT 10^6 spores) of a resistant microorganism under defined, minimum conditions for complete kill. This establishes the minimum lethal process dwell time. In routine operation, the process dwell period is arbitrarily doubled and supports a theoretical reduction of the biological indicator (and thus the bioburden) to a probability of a nonsterile unit (PNSU) of 10^{-6} (for definitions of terms in this chapter, see *Sterilization of Compendial Articles* ⟨1229⟩).

The half-cycle method used for gas sterilization is shown in *Figure 1*.

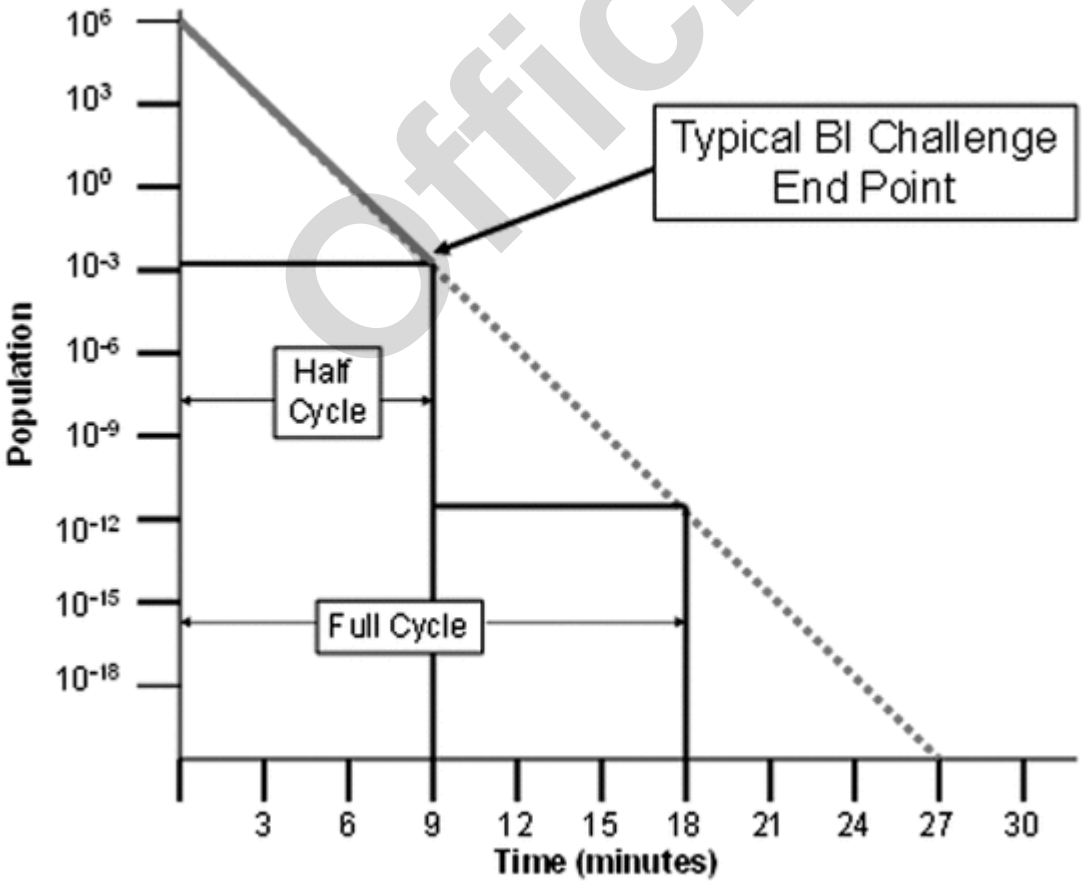

Figure 1. Half-cycle sterilization validation.

Alternative approaches to cycle validation are available. Gillis and Mosley developed a means for parametric evaluation of EO sterilizing conditions that may result in greater use of other validation methods (*6*). A bracketing approach (see *Figure 2*) that better supports the process operating ranges for the critical parameters relative to the half-cycle method has also been used. In the bracketing method, one evaluates conditions that bracket the defined process condition in order to establish parameters for the minimum and maximum effects on the materials and bioburden. The minimum lethal process dwell time (see half-cycle description above) establishes the worst case for microbial kill. Incremental increases in process dwell time beyond the minimum lethal process dwell time are used to establish the routine and maximum exposure periods, the latter of which imparts the greatest effect on materials. In addition, adjustments to agent concentration and relative humidity are utilized to further enhance the bracketing approach. By this method, the routine process conditions may be established between the minimum and maximum process conditions to assure complete microbial kill while maintaining the integrity of the materials.

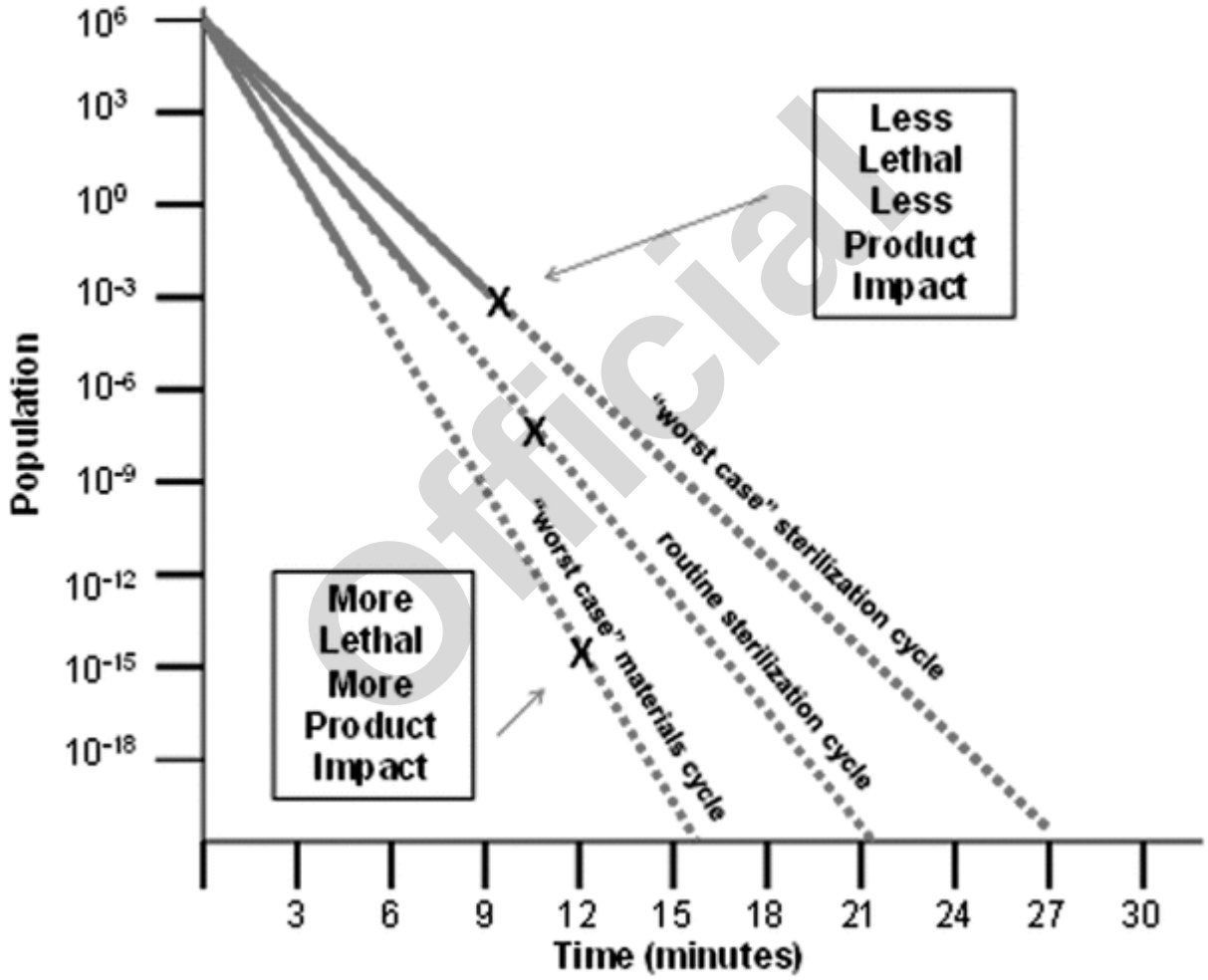

Figure 2. Bracketing method.

Equipment Qualification

The equipment qualification for gas sterilization should include both pre- and postcycle systems to confirm that the equipment has been properly installed and operates as intended.

Empty Chamber Parameter Distribution

Despite the use of true gases, evaluation of parameter uniformity across the chamber is a common activity. This ensures that the gas and humidity introduction methods provide consistency throughout the chamber and can be correlated to the routine monitoring location(s), when present. Biological indicators are not required in the evaluation of the empty chamber uniformity.

Component and Load Mapping

Component and load mapping using invasive sampling are not a part of gas sterilization because sampling systems placed within the load items would alter gas and humidity penetration. Evaluation of lethal conditions with individual items and across loading patterns is best provided by biological indicators or process challenge devices placed within the load items and distributed within the load. Indicators or process control devices are placed within the items and load at locations believed to be hardest for the gas and humidity to penetrate.

Biological Indicators

The biological indicator of choice for gas sterilization varies, as noted above. *B. atrophaeus* (ATCC 9372) is used with EO and chlorine dioxide, and ozone sterilization is monitored with *G. stearothermophilus* (ATCC 12980 or 7953). D-values for the biological indicator can be used to establish exposure periods for the sterilization process to ensure adequate process efficacy. When positioning biological indicators within items it is important to ensure that the placement of the BI does not occlude gas passage or otherwise interfere with the distribution/penetration of the sterilant within the item.

Process Confirmation and Microbiological Challenge

The core of the validation activity is the confirmation of acceptable process parameters with simultaneous physical and chemical measurement and microbial challenge. Sensors are placed in the chamber, or biological indicators are positioned within the load items. Proof of cycle efficacy is provided in replicate studies in which the biological indicators are killed and the physical measurements correspond to the expected values.

ROUTINE PROCESS CONTROL

Gas sterilization is subject to formal controls that maintain a validated state over time. The practices outlined in ⟨1229⟩ include the general requirements appropriate for all sterilization systems. Sterilization is accomplished by a number of related practices that are essential for continued use of the process over an extended period of time. The essential practices to maintain validated status include calibration, physical measurements, ongoing process control, change control, preventive maintenance, periodic reassessment, and training. When parametric release has not been established, biological indicators positioned within the load are used for routine release of each sterilization load, along with a review of documentation from the sterilizer control system.

REFERENCES

1. USP General Chapters—Microbiology Expert Committee. An outline of planned changes to *USP Sterility Assurance* ⟨1211⟩. *Pharmacopeial Forum*. 2012; 38(2).
2. ISO 11135-1:2007 Sterilization of Health Care Products—Ethylene Oxide—Part 1: Requirements for Development, Validation, and Routine Control of a Sterilization Process for Medical Devices. Geneva: International Organization for Standards (ISO); 2007.
3. ISO 11135-2:2008 Sterilization of Health Care Products—Ethylene Oxide—Part 2: Guidance on the Application of ISO 11135-1. Geneva: International Organization for Standards (ISO); 2008.
4. ISO 10993-7 Biological Evaluation of Medical Devices, Part 7: Ethylene Oxide Sterilization Residuals. Geneva: International Organization for Standards (ISO); 2008.
5. Ethylene oxide, ethylene chlorohydrin, and ethylene glycol proposed maximum residue limits and maximum levels of exposure. *Fed Regist.* 1978; 43(122):27474–27483.
6. Gillis J, Mosley G. Validation of ethylene oxide sterilization processes. In: Agalloco J, Carleton FJ, eds. *Validation of Pharmaceutical Processes*. 3rd ed. New York: InformaUSA; 2007.