Moist-Heat Sterilization Principles

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Definition of “sterility”

Absolute *vs* probabilistic (I): European

Ph. Eur. 11.0, 5.1.1, *Methods of preparation of sterile product*s:

“Sterility is the absence of viable microorganisms, as defined by a sterility assurance level equal to or less than 10^{-6}.”

“The achievement of sterility for any one item in a population of items can neither be guaranteed nor demonstrated.”

“Sterilization process conditions are chosen to achieve the highest level of sterility assurance compatible with the drug product…”

“The SAL for a given sterilisation process is expressed as the probability of micro-organisms surviving in a product item after exposure to the process.”

Definition of “sterility”

Absolute *vs* probabilistic (II)

EN ISO 17665-1:2006, 3.49, 3.50 and 3.51:

“sterility: state of being free from viable microorganisms

NOTE In practice, no such absolute statement regarding the absence of microorganisms can be proven.”

“sterility assurance level / SAL: probability of a single viable microorganism occurring on an item after sterilization

NOTE The term SAL takes a quantitative value, generally 10^{-6} or 10^{-3}. When applying this quantitative value to assurance of sterility, an SAL of 10^{-6} has a lower value but provides a greater assurance of sterility than a SAL of 10^{-3}.”

“sterilization: validated process used to render product free from viable microorganisms

NOTE In a sterilization process, the nature of microbial inactivation is exponential and thus, the survival of a microorganism on an individual item can be expressed in terms of probability. While this probability can be reduced to a very low number, it can never be reduced to zero.”

Definition of “sterility”

Absolute *vs* probabilistic (III): USA 1

USP 43, <1211>, *Sterility Assurance - Introduction*:

“…an item is deemed sterile only when it contains no viable microorganisms … **Sterility cannot be demonstrated without the destructive testing of every sterile unit** … microbiological safety is achieved through the implementation of interrelated controls that in combination provide confidence that the items are suitable for use as labeled. It is the controls that provide the desired assurance from microbiological risk rather than the results of any in-process or finished goods testing. **The verification of safety of products labeled sterile is generally known as “sterility assurance”**…

The establishment of an effective sterility assurance program requires information about the material to be sterilized … the appropriate process provides a balance between conditions that are lethal to potential bioburden present in/on the item and those that preserve its essential quality attributes.”

Definition of “sterility”

Absolute *vs* probabilistic (IV): USA 2

USP 43, <1229>
Sterilization of Compendial Articles - Background and Scope:

“Sterility, therefore, is defined in probabilistic terms that establish an acceptable level of risk. Sterility can be accomplished only by the use of a validated sterilization process under appropriate current good manufacturing practices and cannot be demonstrated by reliance on sterility testing alone.”

USP 43, <1229>
Ster. Comp. Art. - Establishing and Justifying Sterilization Processes:

“Articles intended to be sterile must attain a ≤10−6 *probability of a nonsterile unit.*”

Probabilistic definition of “sterility”

PNSU *vs* SAL

American PNSU is referred to the *final product,* this means to the *Probability of a Nonsterile Unit* in a sample-tested lot of product

Theoretically at least, the European concept of *Sterility Assurance Level* is different: it means the *probability of micro-organisms to survive in an item* after a sterilization process and refers to the *trend of the process* of sterilization

But for explanation, the same Ph. Eur. 11.0, 5.1.1 adds:

“An SAL of 10^{-6}, for example, denotes a probability of not more than 1 non-sterile item in 1 x 10^{6} sterilised items of the final product.”

This “example” states the practical equivalence of the two definitions

Sterility and sterilization of medical devices in EN 556-1 (I)

EN 556-1:2001 (amended 2006)

Introduction: "…product items produced under standard manufacturing … may, prior to sterilization, have micro-organisms on them, albeit in low numbers. Such product items are non-sterile. The purpose of sterilization processing is to inactivate the microbiological contaminants and thereby transform the non-sterile items into *sterile* ones ."

Requirement 4.1: "For a terminally-sterilized *medical device* to be designated *"STERILE"*, the theoretical probability of there being a viable micro-organism present on/in the device shall be equal to or less than 1 x 10^{-6}."

Sterility and sterilization of medical devices in EN 556-1 (II)

EN 556-1:2001 (amended 2006)

Requirement 4.2: “Compliance shall be shown by the manufacturer or supplier through provision of documentation and records which demonstrate that the devices have been subjected to a validated sterilization process fulfilling **4.1**.

NOTE 1 Evidence that a medical device is sterile comes from: i) the initial validation of the sterilization process and subsequent revalidations that demonstrate the acceptability of the process; and ii) information gathered during routine control and monitoring which demonstrates that the validated process has been delivered in practice.

NOTE 2 The achievement of sterility is predicted from the bioburden level on products, the resistance of the micro-organisms comprising that bioburden and the extent of treatment imposed during sterilization.”

Basic principles for control of sterilization processes (I)

USP 43, <1229>:

"The basic principles for control of sterilization processes, including method development, validation, and ongoing assurance, are as follows:

1. Sterilization process development that includes evaluation of the stability and compatibility of materials, container integrity, expected pre-sterilization bioburden, equipment method control parameters, etc.
2. Identification of sterilization process parameters that preserve the inherent properties of the materials yet inactivate or remove microorganisms.

Basic principles for control of sterilization processes (II)

USP 43, <1229>:

"The basic principles for control of sterilization processes, including method development, validation, and ongoing assurance, are as follows:

3. Demonstration that the sterilization process and equipment are capable of operating within the prescribed parameters and corresponding to independent measurements of the critical parameters.

4. Performance of replicate studies that represent the operational range of the equipment and employ actual or simulated product. The use of biological indicators for correlation between the measured physical parameters and the expected lethality is recommended wherever possible.

Basic principles for control of sterilization processes (III)

USP 43, <1229>:

"The basic principles for control of sterilization processes, including method development, validation, and ongoing assurance, are as follows:

5. Maintenance and monitoring of the validated process during routine operation.

6. Assurance that the bioburden (number and type) of the materials is acceptable and is maintained within predetermined limits during routine operation."

Methods of sterilization – USP 1

USP 43, <1229> lists several methods:

a) by "destruction of microorganisms":

Steam Sterilization by Direct Contact "with the load items (whether wrapped or unwrapped)" ⟨1229.1⟩

Moist Heat Sterilization of Aqueous Liquids: "the method of choice for aqueous parenteral products, in-process aqueous liquids, laboratory media, and biological waste materials … primarily in closed containers" ⟨1229.2⟩

Dry Heat Sterilization: "a process utilized for heat-stable items (glass, stainless steel, nonaqueous liquids, powders, etc.) that are unsuited for steam sterilization because of either an absence of water (nonaqueous liquids and powders) or requirements for absolute dryness following processing (product contact parts for nonaqueous products)" ⟨1229.8⟩

Methods of sterilization – USP 2

USP 43, <1229> lists several methods:

Radiation Sterilization: "used extensively for the sterilization of medical devices and for a variety of other materials and products" ⟨1229.10⟩

Gaseous Sterilization: "commonly used for items that are susceptible to damage by heat or radiation processes" ⟨1229.7⟩

Liquid Phase Sterilization: "objects to be sterilized are immersed in the solution of a chemical agent *[aldehydes, acids, bases, and strong oxidants]*, after which the agent must be removed in a manner that preserves the sterilized object from recontamination … These agents are highly toxic" ⟨1229.6⟩

Methods of sterilization – USP 3

USP 43, <1229> lists several methods:

Vapor Phase Sterilization: "accomplished using sporicidal agents suspended in air *[vapors of hydrogen peroxide, peracetic acid, formaldehyde, and glutaraldehyde in aqueous solution]*. … They differ from sterilizing gases and liquids in that there are multiple phases within the vessel during sterilization … well suited for heat-sensitive materials and surface sterilization" ⟨1229.11⟩

b) by "physical removal" of microorganisms:

Sterilizing Filtration of Liquids: "The filtration process parameters that influence microbial retention include temperature, flow rate, volume, filtration time, differential pressure, and pressure pulsations" ⟨1229.4⟩

Methods of sterilization – EP 1

Ph. Eur. 11.0, 5.1.1 lists the following methods:

"Steam sterilisation": "achieved by heat transfer during condensation of water"

"Dry heat sterilisation": "a terminal sterilisation method based on the transfer of heat … by means of convection, radiation or direct transfer" — Reference conditions: 2 hrs at 160 °C

"Ionising radiation sterilisation": "may be used for the terminal sterilisation of tissues and cells, or the sterilisation of materials or containers to be employed in aseptic processing" or "the surface sterilisation of materials upon entry to isolators" — Reference absorbed dose: 25 kGy

Methods of sterilization – EP 2

Ph. Eur. 11.0, 5.1.1 lists the following methods:

"Gas sterilisation (Vapor phase sterilisation)": "may be used for the sterilisation of primary packaging materials, equipment and some pharmaceuticals.
It is essential that penetration by gas and moisture into the material to be sterilised is ensured, and that it is followed by a process whereby the gas is eliminated … below concentrations that could give rise to toxic effects"

"Membrane filtration": "used for reduction of viable and non-viable particles in gases and fluid products that are not amenable to sterilisation by heat or irradiation … The principle of membrane filtration is not inactivation but removal of microorganisms … achieved by a combination of sieving and surface interaction"

EP does not list a method equivalent to USP "Liquid Phase Sterilization"

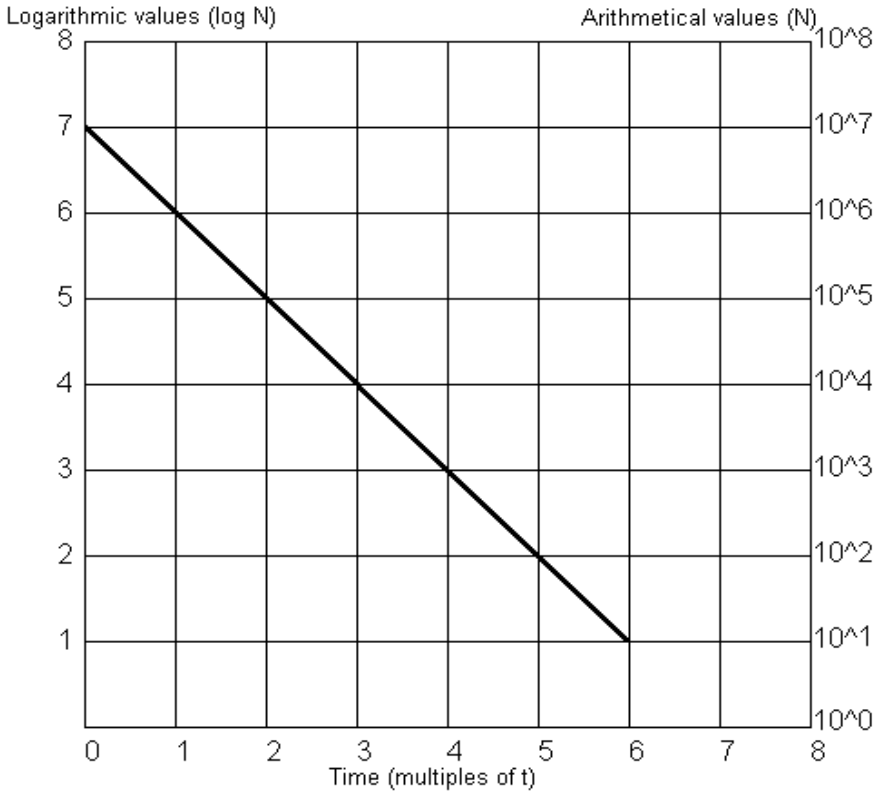
Asymptotic behavior of a sterilization process (I)

It is well known that most sterilization processes (except for filtration) "often approximates to an exponential relationship." (EN 556-1)

Most processes follows a decreasing asymptotic trend, that tends to zero: *in the same time interval* under the same conditions*, the same percentage of the microorganisms present at the beginning of that interval is destroyed, and the same percentage survives, complementary to the destroyed one*

This means that most sterilization processes proceed like a first-order chemical reaction: in this model (often named by Arrhenius), only a sterilization process of infinite duration could assure a complete destruction of all microorganisms initially present

Asymptotic behavior of a sterilization process (II)



D-value: Decimal reduction time (I)

The rate of an asymptotic sterilization reaction is expressed by means of a parameter called *D*-value:

EN ISO 11138-7:2019, Def. 3.4: D-*value is the* **"time or dose required under stated conditions to achieve inactivation of 90 % of a population of the test microorganisms"**

D-value varies according to:

- the microbial "species" considered
- the "substrate" on which it lives
- the culture medium ("matrix")
- the recovery medium
- the test methodology
- the actual conditions of the sterilization process (temperature, concentration of the sterilant, humidity)

For moist-heat sterilization, the essential condition is the presence of liquid water in contact with the microorganisms. The *most common* reference temperature, both for process and D-values, is 121 °C (originally, 250 °F = 121.11 °C)

D-value: Decimal reduction time (II)

USP 43, <1229>, relevant to sterilization in general, defines:

"The D-value is the time (customarily in minutes) or radiation dose (customarily in kGy) required to reduce the microbial population by 90% or 1 log_{10} cycle (i.e., to a surviving fraction of 1/10) and must be associated with the specific lethal conditions at which it was determined."

EP 11.0, 5.1.5, relevant to "steam sterilization of aqueous preparations", defines the *D*-value as follows:

"The D-value (or decimal reduction value) is the time in minutes required at a defined temperature to reduce the number of viable test organisms by 90 per cent. It is only of significance under precisely defined experimental conditions."

So, both these Pharmacopoeias underline that the evaluation of the sterilizing effect of a process starts from a thorough knowledge of the conditions at which the resistance of the microorganisms has been defined

Meaning of *D*-value (I)

When saying that the moist-heat *D*-value at 121 °C of a specific microbial species in a specified culture medium is, for example, D = 1.5 minute, we mean that a population of that microbial species in that culture medium, exposed to moist-heat conditions at 121 °C for 1.5 minute = 90 seconds, is reduced to one tenth of its initial value:

At the beginning: 1,000 microorganisms, i.e. 10^3

after 90" @ 121 °C

➔ *10^2,*

i.e. 100 microorganisms

after further 90"
@ 121 °C

➔ ➔ *10^1,*

i.e. 10 microorganisms

after further 90"
@ 121 °C ➔ ➔ ➔ 10^0,
i.e. only 1 microorganism

What happens after a further 90" @ 121 °C moist-heat?

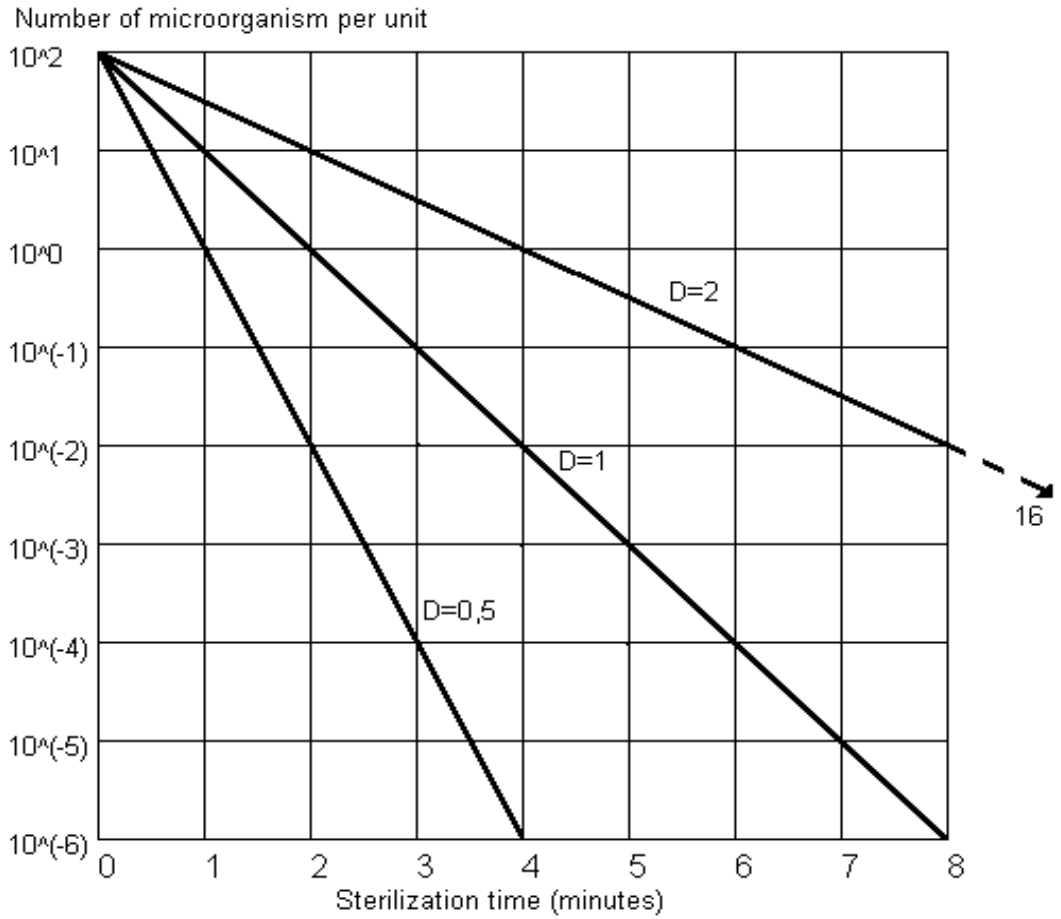
Meaning of *D*-value (II)

According to this model, after the sterilization has continued for additional 90 seconds under the same thermal conditions, the surviving units would be 10^{-1}, i.e., 1/10. It is apparent that the survival of one tenth of microorganism makes no true sense, physical or biological

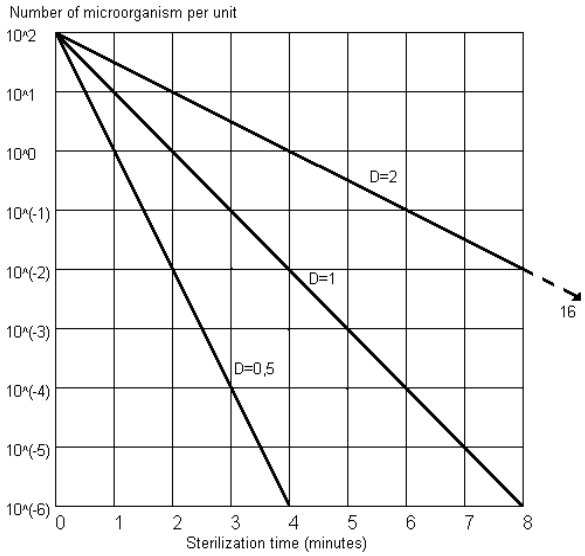
A possible explanation of this paradox could be that the mathematical model of steady decimal reduction is no longer applicable when only few microorganisms survive

But a useful meaning can be attached to this figure by saying that *the probability that the initial microbial population has been completely destroyed is 10 to 1*. If exposure to moist-heat continues for other 5 · D minutes, this probability becomes 1,000,000 to 1, i.e., 10^{-6}, and so on. As already said, the probabilistic definition of "sterile" as "correctly and reliably sterilized" is expressed in Europe by the "SAL" (**S**terility **A**ssurance **L**evel), in the United States by the "PNSU" (**P**robability of **N**on**s**terile **U**nit)

Meaning of *D*-value (III)



Mathematical expressions of *D*-value



$dN / dt = - k N \rightarrow N = N_0 \cdot e^{-kt}$

$D = (\log_e 10) / k = 2.3026 / k$

Any one of the next formulas corresponds to the definition of *D*-value for an asymptotic sterilization:

$$D = t / [\log_{10} (N_0 / N)]$$

$$N = N_0 \cdot 10^{-t/D}$$

$$\log_{10} N = \log_{10} N_0 - t / D$$

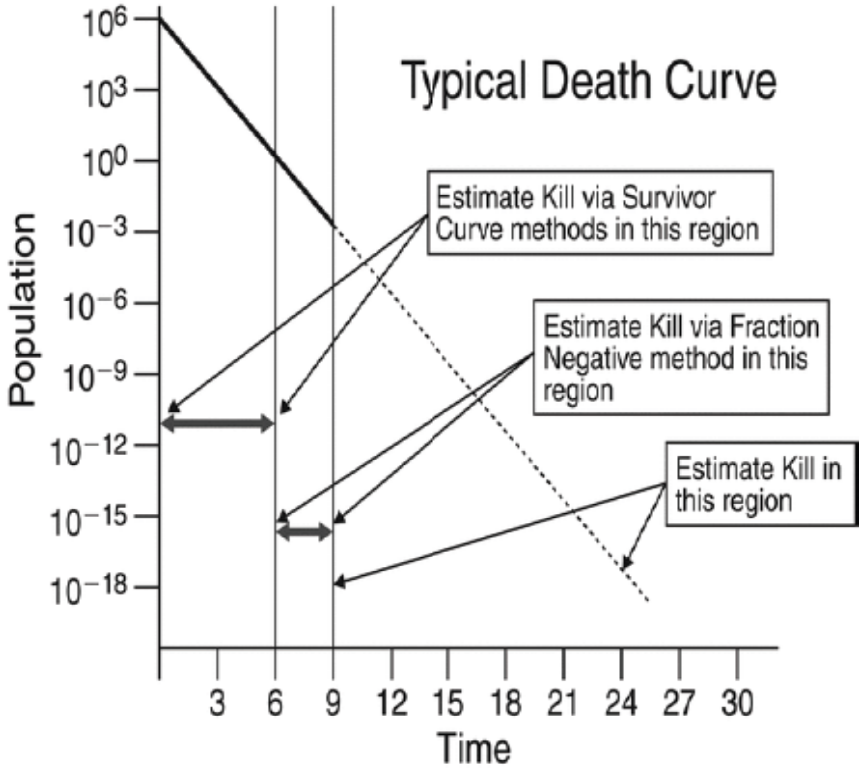
$$t = D (\log_{10} N_0 - \log_{10} N) = D \log_{10} (N_0 / N)$$

In these formulas:

N_0 = initial microbial population
N = actual microbial population after exposure time t

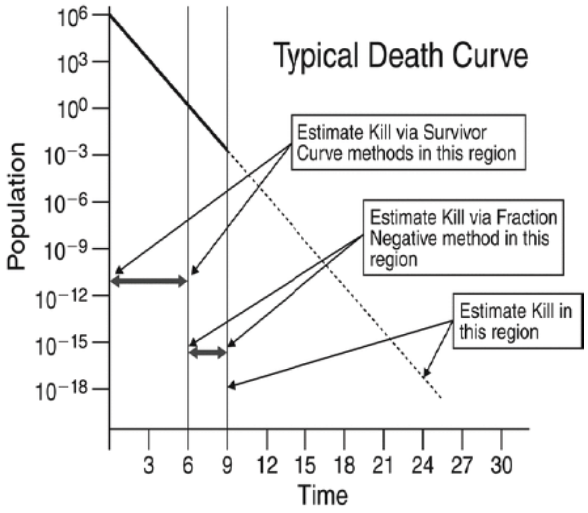
The ratio N_0 / N was called ***IF*** (Inactivation Factor) in EP 9.0, 5.1.5, but this definition has been removed from the current "informative" chapter EP 10.3, 5.1.5

Evaluation of the output of a sterilization process (I)



Source: USP 43, <1229>, Fig. 3

Evaluation of the output of a sterilization process (II)



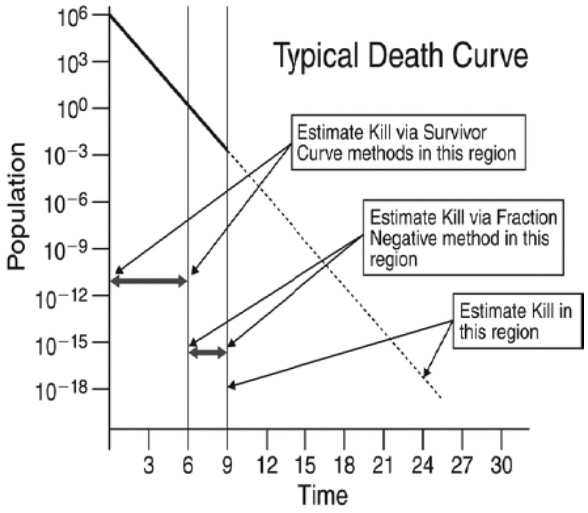
USP 43, <1229>:

"The death curve for microorganisms subjected to a sterilization process is comprised of three distinct regions:

1. *Survivor curve region*—Where viable microorganisms can be recovered and counted to determine the slope of the death curve.

Using survivor counts in short exposure periods, the first section of the death curve can be drawn to where the population is approximately 10 CFU.

Evaluation of the output of a sterilization process (III)



USP 43, <1229>:

The death curve for microorganisms subjected to a sterilization process is comprised of three distinct regions:

2. *Fraction negative region*—Where replicate studies with multiple biological indicators are used to estimate the slope. This can extend the demonstrable portion of the death curve to approximately 10^{-2} to 10^{-3}.

3. *Estimated region*—Where the death rate curve established by either the survivor curve method or fraction negative method is extrapolated to the desired PNSU. Below 10^{-3} the death curve is assumed to be linear and is depicted in *Figure 3* by the dashed line beyond the point assuming that the death of microorganisms continues at the same rate."

z-value: temperature coefficient (I)

Another evidence for heat sterilization is that the *D*-value decreases as the heat sterilization temperature increases, and *vice versa*. This fact is also in accordance with the point of view of chemical reactions involved in the inactivation of microorganisms

For whichever a process, the *reaction temperature coefficient* indicates how many temperature degrees involve a 10-fold change of the process rate. In the case of sterilization by heat, the temperature coefficient is named
z-value and represents *"the temperature difference that leads to a 10-fold change in the* D-*value of the biological indicator."* (EP 11.0, 5.1.2, 3-1-1)

In other words: *"The z-value is the change in temperature in degrees Celsius required to alter the* D-*value by a factor of 10 (the z-value relates the resistance of a micro-organism to changes in temperature)."* (EP 11.0, 5.1.5)

z-value: temperature coefficient (II)

The *z*-value for a microbial species in a temperature range T_1 to T_2 is given by the formula (EP 11.0, 5.1.5):

$$z = (T_2 - T_1) / (\log_{10} D_1 - \log_{10} D_2) = (T_2 - T_1) / (\log_{10} D_1 / D_2)$$

The calculation of a *z*-value demands the knowledge of (at least) two *D*-values D_1 and D_2 at temperatures T_1 and T_2, which will be the limits of the reliability range of the *z*-value so calculated.

Thus, as the *D*-values do, the *z*-value depends on:

- the microbial species considered
- the other factors listed for *D*-value
- the temperature range (this is too often forgotten!)

It would be a meaningless and dangerous extrapolation to utilize a *z*-value outside the range (T2 - T1) of which the *D*-values in the formula are the limits. This is also stated as a rule. (EP 11.0, 5.12, 3-1-1)

Practical meaning of *z*-value

For instance, with the assumption that z = 10 °C in all the temperature range 111 °C to 131 °C, and provided that the sterilizing conditions (in this case the presence of moist heat) are integrally and continuously maintained, one minute of exposure at 121 °C has the same effect of ten minutes at 111 °C, and one minute at 131 °C has the same effect of ten minutes at 121 °C

The above means that the same sterilizing effect of 8 minutes of moist heat at 121 °C would be obtained with only 0.8 minutes at 131 °C, but would require 80 minutes at 111 °C

If z = 10 °C, a change of a mere 1 °C entails a remarkable 26% change of the sterilization rate: in fact, $(1.2589\ldots)^{10} = 10$

Quite differently, the “Arrhenius temperature coefficient” for a tenfold change in rate of chemical reactions is in most cases about 23 °C, thus entailing a mere 10.5% change of rate per 1 °C. This remarkable difference may become very useful when sterilizing heat-sensitive products

Typical *D*-value and *z*-value (I)

In industrial sterilization practice, the *D*-value of microorganisms present in the bioburden to inactivate is usually remarkably lower than 1.0 minute at 121 °C. Likewise, the *z*-values of most microorganisms are included between 5 °C and 12 °C in the temperature range from 110 °C to 130 °C

Thus, if there are no reliable data on the microbial species in the bioburden (the most resistant one, in the common case of heterogeneous contamination), the *D*-value of it for moist-heat sterilization is often assumed to be 1.0 minute at 121 °C, and the z-value of it 10 °C

Typical *D*-value and *z*-value (II)

The combination of a hypothetical D_{121} = 1 minute and a hypothetical z = 10 °C has proven to offer acceptable safety margins, but *the final probability of microbial survival shall be evaluated by challenge with more resistant testing microorganisms* (i.e., by suitable Biological Indicators)

Very clearly, USP 43, <1229.5> states: "It must be established that the BI system provides a challenge to the sterilization process greater than the resistance of the native bioburden."

Microorganisms commonly in use for testing and validation (e.g., *Geobacillus stearothermophilus*) have definitely higher *D*-values than 1.0 minute, and the population of them in commercial BIs is much higher than the usual bioburden

"***Biological Indicator Challenge System (BI)*:** A test system containing viable microorganisms of a pure, specified strain providing a defined resistance to a specified sterilization process." (PDA Technical Report No.1 rev. 2007, Glossary of Terms)

Shared sterilization concepts, targets and requirements

- Sterility means being free of viable microorganisms
- This state can be recognized only as the probability to find a non-sterile item (PNSU) in a lot, or the probability to have a residual microbial contamination in an item (SAL)
- To deem an item “sterile”, both pharma products and medical devices, these probabilities must be not higher than 10^{-6}
- Final tests on samples of finished products do not demonstrate sterility
- Only a documented routine compliance with a pre-validated production process can provide assurance of sterility
- Sterility is predictable on the base of the initial contamination of the product (“bioburden”) and the characteristics of it (*D*-value and *z*-value of the most resistant microorganism expected on the product

Moist-heat sterilization temperature

USP 43, <1229.1> is very clear: "Moist heat sterilization process efficacy is not intrinsically linked to a target temperature of 121°, which is simply the Celsius conversion of 250°F, and other temperatures can be used."

This seems to indicate a complete freedom.

EP 11.0, 5.1.1 keeps valid the concept of a "reference cycle" ("15 min at 121 °C"), for which a less onerous documentation is requested by related guidelines, but states also clearly:
"Product- and load-specific cycles, e.g., applying another combination of time and temperature, may be adopted based on cycle development and validation. The minimum temperature acceptable for a steam sterilisation process is 110 °C."

So, EP prescribes a minimum temperature under which a moist-heat treatment is no longer deemed a method for achieving sterility

CEN ISO/TS 17665-2:2009 includes references to 121 °C only for testing purposes, and exemplification.

No reference to process temperature values can be found in EN ISO 17665-1:2006

The *F* concept: use and misuse in sterilization practice

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Temperature for moist-heat sterilization - USP

USP 43, <1229.1>:

"The process lethality at temperatures other than 121° can be calculated to determine lethality equivalent to that provided at 121°.

Moist heat sterilization process efficacy is not intrinsically linked to a target temperature of 121°, which is simply the Celsius conversion of 250°F, and other temperatures can be used."

Temperature for moist-heat sterilization - EP

EP 11.0, 5.1.2, 3-1-1:

"Sterilisation processes can be operated at temperatures lower than the standard 121 °C (for longer exposure times) or at higher temperatures (for shorter exposure times). The *z*-value (the temperature difference that leads to a 10-fold change of the *D*-value of the biological indicator) is used to compare the efficacy of 2 cycles operated at different temperatures. **For a *z*-value determination, the D-value must be determined at 3 or more temperatures. The intended process temperature should be within the range of the 3 temperatures."**

Equivalent sterilization time *F*

Thus, no doubt that no fixed sterilization temperature is indicated by rules or standards (in Europe, only a lowest limit of 110 °C), and the following question becomes essential:

Which is the lethal effect of the exposure of a microbial population to a variable temperature T *under moist-heat conditions in comparison with a hypothetical sterilization performed at a constant temperature* T_0 *under the same moist-heat conditions for the same time?*

The relationship between the biological effects of moist-heat at different temperatures depends on:

- the variable temperature difference from a constant one T_{ref} assumed as reference
- the reference temperature T_{ref} itself
- the not intrinsically constant temperature coefficient *z*-value

Mathematics provides the theoretical solution of this problem by an algorithm called *F*

F physical

The mathematical algorithm *F* is more properly named the *physical F* and is often indicated as F_{phys}

PDA Technical report No.1 rev. 2007, Glossary of Terms:

"***$F_{Physical}$***: A term used to describe the delivered lethality *calculated based on the physical parameters of the cycle*. The $F_{Physical}$-value is the integration of the lethal rate (*L*) over time. The lethal rate is calculated for a reference temperature (T_{ref}) and *z*-value using the equation: $L = 10^{(T-Tref)/z}$."

According to USP 43, <1229.2>, F_{phys} can be defined:

"the equivalent sterilization time relative to a base temperature"

F biological

F_{phys} is completely different from the *biological F*, that expresses the effectiveness of sterilization from the point of view of microorganisms and is often indicated as F_{bio}

PDA Technical Report No.1 rev. 2007, Glossary of Terms:

"***$F_{Biological}$*: A term used to describe the delivered lethality, *measured in terms of actual kill of microorganisms on or in a BI challenge system*. The $F_{Biological}$-value is calculated as D_T x LR, where D_T is the *D*-value of the BI system at the reference temperature (*T*) and *LR* is the actual logarithmic reduction (log N_0 – log N_F) of the BI population achieved during the cycle."

In the same glossary: "***Biological Indicator Challenge System (BI)*: A test system containing viable microorganisms of a pure, specified strain providing a defined resistance to a specified sterilization process."

The concept of F_{bio} as the expression of delivered lethality measured by biological indicators is present in EP, but not in USP

The *F* algorithm - Mathematical digression

"The z-value is the change in temperature in degrees Celsius required to alter the D-value by a factor of 10 (the z-value relates the resistance of a micro-organism to changes in temperature)" (EP 11.0, 5.1.5)

This ***definition*** may be expressed by a mathematical equation:

$$D_{(T-z)} = 10 \cdot D_T$$

The mathematical ***function*** that satisfies this equation with reference to a known D-value D_R at a "reference" temperature T_R is the solution of a problem of variational calculus. The solution is:

$$D = D_R \cdot 10^{(T_R-T)/z}$$

Let's now remember that the exposure time after which a microbial population N is reduced to a fraction of the initial population N_0 is:

$$t = D \log_{10} (N_0/N)$$

So, the same microbial reduction effect as after a time t_R at temperature T_R will be reached at different temperatures after a time:

$$t = D \cdot t_R / D_R$$

If we consider infinitesimal intervals of exposure time dt_R at a reference temperature T_R equivalent to infinitesimal intervals of exposure time dt at the continuously variable temperature T is thus:

$$\mathbf{dt_R = dt \cdot 10^{(T-T_R)/z}}$$

The *F* algorithm – Practical formula

In practical terms of finite time intervals, the mathematical expression of the equivalent exposure time *F* at a variable *T* with respect to the exposure time at a fixed T_{ref} becomes:

$$F = \Delta t \cdot \Sigma 10^{(T - T_{ref}) / z}$$

where:

Δt = constant time interval between two subsequent temperature measurements

T = mean value of the variable sterilization temperature during each time interval, °C

T_{ref} = fixed reference temperature for F calculation, °C

z = temperature coefficient of the D-value, °C

The shorter the time intervals between two next measurements, the more accurate the calculation of the equivalent exposure time. In moist-heat sterilization practice, intervals of a second or half a second are widely used

At a first glance, *D*-values could seem not involved in the calculation of equivalent time, but in fact, at least two *D*-values (experimental data) are necessary to calculate the z-value, according for instance to EP 11.0, 5.1.5:

$$z = (T_2 - T_1) / (\log_{10} D_1 - \log_{10} D_2) = (T_2 - T_1) / (\log_{10} D_1 / D_2)$$

A graphical method to evaluate z-values by plotting at least two D-values in a semilogarithmic D-T chart, is given by PDA TR#01, rev. 2007, par. 3.1.2

The F_0 "physical": equivalent exposure time

If the reference temperature is assumed equal to 121 °C (originally 250 °F = 121.11 °C, in the past the most common temperature for moist-heat sterilization) and *z*-value equal to 10 °C (originally 18 °F), the physical equivalent time is named F_0 ("F zero" or "F naught"). With the same symbols as in the previous slides, the formula becomes:

$$F_0 = \Delta t \cdot \Sigma 10^{(T - 121) / 10} \qquad (1)$$

"Cycle efficacy for steam sterilization often is measured using F_0, which is defined as the equivalent exposure time at 121 °C. F_0 is a means for quantifying steam sterilization effectiveness by determining the equivalent sterilization time in minutes relative to a base temperature of 121 °C and a *z*-value of 10 °C … The F_0 calculation should begin at 100° and should continue through the end of the dwell period provided that saturated steam conditions are maintained." (USP 43 <1229.1>, relevant to sterilization by direct contact)

The reduction of the reference temperature from 121.11 °C to 121 °C is now universally adopted. A curious exception is Def. 3.17 of EN ISO 17665-1. This reduction causes an overevaluation of almost 2.57% in the resulting F_0, which may be regarded as negligible in practice

F_0 "physical" in Pharmacopoeias

USP 43 provides equations of "physical" equivalent time (as in the previous slide or similar ones) for "direct contact" moist-heat sterilization (<1229.1>), "aqueous liquids" sterilization (<1229.2>), dry-heat sterilization (1229.8), and dry-heat depyrogenation (<1228.1>)

EP 11.0, 5.1.5 simply states that "the total *F* of a process … can be calculated by integration of lethal rates with respect to time at discrete temperature intervals above the minimum temperature" of 110 °C, with z = 10 °C for moist-heat sterilization

To our knowledge, neither EP, nor other European rules or standards provide an equation for what is called "calculated effectiveness from physical parameters (F_{phys})". Apparently, Europeans are expected to know the equation of *Fphys* or "lethal rates" from scientific literature

F_0 "physical": predictive use

The F_0-equation is commonly used also to predict the exposure duration at a constant process temperature other than the reference one and capable to deliver the same lethality dose expressed as expected F_0-target. For this purpose, equation (1) is used in the form:

$$t_T = F_0 \cdot 10^{(121 - T) / 10} \qquad (2)$$

where:

t_T = predicted exposure time to constant temperature *T* under moist-heat conditions for delivering a lethal dose F_0, minutes

T = constant process temperature, °C

F_0 = expected lethal dose expressed as equivalent time at 121 °C, minutes

Some official documents express minimum sterilization requirements as a combination of minimum equivalent lethal dose F_0 and minimum temperature to be attained during the sterilization process. This is the case of EP 11.0, 5.1.1 and the correlated EMA 2019 Guideline, 4.1.1, that demands $F_0 \geq 8'$ at $T \geq 110$ °C for a properly said sterilization. EMA 2019 Guideline, 4.1.1 Table 1 also specifies as "overkill" a process with attained $F_0 \geq 12'$ at $T \geq 121$ °C.

Any lighter treatment is called in Europe "Post-aseptic processing terminal heat treatment"

F or F_0 "biological"
Evaluation of the actually delivered lethality

The biological expression of delivered lethality is given by the equation (PDA TR#01 rev. 2007, 4.4.1.6):

$$F_T = D_T \cdot (\text{Log } N_0 - \text{Log } N_F) \qquad (3)$$

where:

F_T = delivered lethality referred to reference temperature T, as biologically evaluated by the measured inactivation of a BI whose D is known at T, minutes

D_T = *D*-value of the BI at the reference temperature T, minutes

N_0 = initial population of BI, units

N_F = final population of BI, units

It may be worth to note that *z*-value is not involved the calculation of F_{bio}

Only if a D_{121} is used, this biological F_T should properly assume the name the F_{0bio}, as apparently in EMA 2019 Sterilisation Guideline, Table 1 (dated March 6, 2019). This document is strictly related with European rules, but EP 10.0, 5.1.1 (dated July 2019) used the symbol F_{bio} and defines it by an equation $F_{bio} = D_{121} \cdot (\text{Log } N_o - \text{Log } N)$ that is already present in EN ISO 17665-1:2006, D.4.2 for the calculation of the minimum N_0 of BIs to be used in "full cycle approach". To make things plainer, update EP 10.3, 5.1.5 (June 2020) changed this F_{bio} to a mere F but writes it as F_0 in the equation $F_0 = D_{121} \cdot (\text{Log } N_o - \text{Log } N)$ for reference to moist-heat processes in contrast with dry-heat ones…

F or F_0 "biological": predictive use

Regardless to this perhaps unimportant lack of uniformity in symbology, it is recognized (PDA TR#01 rev. 2007, 4.4.1.6) that the F_{bio} equation may be practically used for the actual calculation of delivered lethality *only if the final condition of the BIs is not of complete inactivation;* this limitation, in fact, commands the use of "reduced cycles" for validation

On the contrary, "the BI inactivation requirements of the qualification sterilization cycle are for BIs to be negative; this requires a large F_{PHY}. At this F_{PHY}-delivered condition, it will not be possible to measure an F_{BIO} since this BI condition is outside the measurable quantal area."

In this case, equation (3):

$$F_T = D_T \cdot (\text{Log } N_0 - \text{Log } N_F)$$

can be used "to determine the lethality (F_T) requirement to kill the BI to a probability of non sterility (PNSU)" of a still detectable level (let's say 10^{-2}), which will provide information of the effectiveness of the measured physical parameters

F or F_0 "biological"

Calculation of the lethality to be delivered for an effective process

If the value of $N_F = 10^{-6}$ is used in the F_{bio} equation and both N_0 and D_{121}-values are supposedly known for the actual product, the F_{bio} equation may be used to define the sterilization cycle.

"Regardless of the number and heat resistance of the actual bioburden organisms in the load" (PDA TR#01 rev. 2007,4.1.1.1),

$D_{121} = 1.0'$

and

$N_0 = 10^6$

are assumed for the so-called "overkill design approach", resulting in an F_{0bio}-requirement of 12'. Temperature other than 121 °C may be considered with the additional assumption of z = 10 °C for the mathematical calculation of the delivered lethality F_{0phys}

"This approach assumes both a higher bioburden population and resistance than would be expected … Because worst case assumptions are made for the bioburden population and resistance with this design approach, there is little scientific necessity for routine bioburden monitoring of the load items." (PDA TR#01 rev. 2007, 4.1.1.1)

F or F_0 "biological"

Selection of the challenging BIs

F_{bio}-equation "can be rearranged to determine the minimum starting population of the BI necessary to qualify the delivery of the desired biological lethality … Note that this is a separate exercise from using the model to determine the desired delivered lethality for product safety" (PDA TR#01 rev. 2007, 5.2.1.1):

$$\text{Log } N_0 = \text{Log } N_F + F/D$$

where:

N_0 = initial population of BI ("biological challenge"), units

F = "desired lethality determined during process design"

D = *D*-value of the BI at the same reference temperature at which the desired lethality is referred, minutes

N_F = "the population of the biological challenge after exposure. For calculation purposes, if the biological challenge is killed, then it can be assumed that there is less than one surviving microorganisms, which is depicted as $N_F = 10^0$ in this equation"

Very clear examples of PDA's explain the use of this method.

The F_{bio} equation is also quoted, unfortunately in a rather confusing way but with an explicit reference to PDA now revised Validation Monograph, in EN ISO 17665-1:2006, D.4.2 to calculate, with an additional safety margin of "0,5 x log to the base 10" (i.e., 3.1623 times more), the minimum population of a biological indicator to be used in the "full cycle approach" for "qualifying a sterilization process" of the "overkill" type. This means a desired lethality of 12 equivalent minutes at 121 °C (to be inserted as *F* in the above equation)

Physical and biological *F*-values (I)

F_{phys} can be easily calculated by a process controller and used for control while the sterilization process is going on. On the contrary, F_{bio} can not be calculated ongoing, as it involves biological laboratory measurements to be done after the sterilization process

According to EP 11.0, 5.1.1, "calculated effectiveness from physical parameters (F_{phys}) is correlated with biological effectiveness (F_{bio}). F_{bio} expresses the lethality, in minutes, provided by the process in terms of destruction of the biological indicators used." "In cycle validation … adequate biological effectiveness is verified by exposure of biological indicators" in the "positions in the load that are the most difficult to sterilise."

"The F_{bio} determined for the most-difficult-to-sterilise position is used *to define the parameters necessary to achieve reliably the required SAL* equal to or less than 10^{-6} for the required cycle."

As a "rule of thumb", F_{bio} might be intended for compliance in validation; F_{phys} in the most unfavorite position is expected to routinely exceed F_{bio}

Physical and biological *F*-values (II)

"Validation of F_{0Phys} and F_{0Bio}" is required also by EMA 2019 Sterilisation Guideline, Table 1, which is related to EP. Unfortunately, no practical instructions are provided for complying with this requirement.

"Evaluating the $F_{physical}$ and $F_{biological}$ Agreement" is the title of the already quoted Paragraph 4.4.1.6 of PDA TR#01 rev. 2007, (see Slide No. 13) which provides very useful information, but also summarizes the matter with these challenging words:

While there is no standard approach to designing studies to evaluate the agreement of F_{BIO} and F_{PHY}, several approaches have been detailed in literature. (39, 40) It is important to note that while this evaluation provides a higher degree of process understanding, many successful cycles have been developed and qualified without this evaluation. One of the goals of this technical report is to promote this cycle development objective and to stimulate additional exploration into appropriate methods for its evaluation.

39. Evans, K., Pflug, I.J., Carrying Out Biological Qualification, The Control Operation of Moist-Heat (Steam Sterilization) Processes for Producing Sterile Pharmaceuticals and Medical Devices, *PDA Journal of Pharmaceutical Science and Technology*, 54 (2) (2000) pp 117–135

40. Pflug, I.J., Chapter 17B, Microbial Control in Pharmaceuticals and Medical Devices using Moist Heat (Steam Autoclave), *Microbiology and Engineering of Sterilization Processes*, 12th Edition, Parenteral Drug Association (2007)

F-values: a comparison *phys* vs *bio*

$$F_{phys} = \Delta t \cdot \Sigma 10^{(T - T_{ref}) / z}$$

- independent of initial and final bioburden
- no biological counts directly involved
- dependent on the trend of a *D*-value, expressed by *z*-value
- meaningful only if the sterilization conditions (in our case, condensing steam or liquid water in contact with microorganisms at the measured temperature) are attained and continuously maintained
- evaluable *while* a process runs

$$F_{bio} = D_{T_{ref}} \cdot (\text{Log } N_0 - \text{Log } N_F)$$

- independent of actual temperature, time, *z*-value
- no physical measurements directly involved
- dependent on a *D*-value at a reference temperature
- meaningful independently of the actual compliance of a process with required sterilization conditions
- evaluable only *after* the completion of a process

Cautions for physical F_0

F_0 "physical", or F_{0phys}, is obtained from a mathematical equation for an expected equivalent exposure time at 121 °C with the assumption z = 10 °C.
F_{0phys} assumes a biological meaning only under proper sterilizing conditions, i.e., if there is steady contact of the microorganisms with liquid water, that means inside a water solution or in presence of condensing steam on the contact surface) at the temperature used for calculation: if these conditions are not in compliance, the calculation of F_{0phys} becomes biologically meaningless

The range of validity of *z*-value should always be remembered as well. To use a *z*-value to calculate *D*-values, and consequently F_{0phys}-values, would be improper beyond the experimental range of validity of *z*-value itself (remember EP 11.0, 5.1.2, 3-1-1 quoted in Slide no. 3)

This restriction is consistent both with the minimum temperature of 110 °C required for F_0 calculation by European rules and standards (exceptions shall be justified), and a requirement of EP 11.0, 5.1.2, 3-1-2 on the "establishment of a validation cycle": "A reduced cycle is chosen such that the temperature is not more than 1 *z*-value below the reference sterilization process temperature."

Some correct uses of F_0 (I)

- Calculation of lethality accumulated by *aqueous liquids* at temperatures different from 121 °C, provided that 10 °C are acceptable as *z*-value in the range of calculation.

  This refers to the so-called “liquid loads”.

Average temperatures	104.0	108.5	113.9	118.0	120.0	121.1	120.2	119.3	119.5	119.8	120.0	120.0	120.0	120.0	120.0	120.0	119.8	119.0	114.0	106.0	101.7
Lethality coefficient	.019	.055	.190	.489	.774	.997	.811	.644	.690	.739	.774	.774	.774	.774	.774	.774	.739	.615	.194	.031	.011
Partial F_0	.019	.055	.190	.489	.774	.997	.811	.644	.690	.739	.774	.774	.774	.774	.774	.774	.739	.615	.194	.031	.011

Total F_0 11.64

Here above, lethal rates (here named “lethality coefficients”) are calculated with the old T_{ref} = 121.11 °C. With the actual T_{ref} = 121.00 °C, lethal rates and F_0-values would result almost 2.57% bigger.

Some correct uses of F_0 (II)

- Actual exposure monitoring by the equation F_{0phys} the sterilization target for *liquid loads*

  This allows to directly take in account the lethality accumulated during the heating phase, and, possibly, to validate a reduced target for the exposure phase thanks to the certain, even if small, accumulation of lethality during the cooling phase. The slower are the heating and cooling phases, the more sensible the gain

- Evaluation by the equation F_{0phys} of the lethality delivered by actual contact steam to solids at temperatures different from 121 °C by exposure to temperature, provided that 10 °C are acceptable as *z*-value in the range of calculation

  This refers to the so-called “porous/hard loads”.
  Only time intervals are to consider, while the presence of saturated, i.e., condensing steam on all the items of the solid load may be reasonably expected. Therefore, the calculation of F_0 shall not be started till the removal of air from the chamber and load surfaces has been completed and shall be immediately stopped as soon as the condition of condensing steam fails, typically at the end of the exposure phase, when drying by vacuum, or cooling with air circulation begins. At best, for porous/hard loads, the calculation of F_0 should occur only during the exposure phase, provided that the validated sterilization conditions are complied with

Some correct uses of F_0 (III)

- Standard definition of general requirements for minimum lethality as expected equivalent time at 121 °C

  For instance, the European requirement: "All steam sterilisation processes require a minimum lethality of $F_0 \geq 8$ minutes and a minimum process hold temperature of 110 °C." (EMA 2019 Sterilisation Guideline, 4.1.1)
  Or the European definition: "**Overkill sterilisation**: A process with a lethality of $F_{0BIO} > 12$ minutes." (EMA 2019 Sterilisation Guideline, 6-Definitions)

- Prediction by the equation of F_{0phys} of the exposure time theoretically required to have the same effectiveness of a given sterilization time at 121 °C (see Slide no. 11)

  A European "overkill cycle", defined by $F_{0bio} = 12'$ could theoretically be performed, for instance, at 124 °C with an exposure time of 6 minutes (at least in Europe, lower temperatures than 121 °C are not allowed for overkill cycles): in fact,
  $t = 12 \cdot 10^{(121-124)/10} = 12 \cdot 10^{-0.3} = 12 \cdot 0.5 = 6'$

  A required minimum lethality of $F_{0bio} = 8'$ could be delivered by an exposure of a solid to moist steam, or by a dwell of an aqueous liquid at T = 116 °C for
  $t = 8 \cdot 10^{(121-116)/10} = 8 \cdot 10^{0.5} = 8 \cdot 3.16 = 25.3'$

Some correct uses of F_0 (IV)

- Evaluation of the uniformity of the lethality accumulated by a liquid load or delivered to a porous/hard load.

  This can be regarded as investigational and/or "validational" use of the F_0 as equivalent time. Rather frequently process specifications include monitoring of the maximum discrepancy of F_0 among the load (most and less "favorited" positions)

  Due to the above conditions for the use of F_0 with porous/hard loads, in this case the thermal uniformity can in fact be evaluated only during the exposure phase

Cautions shall be always respected in the selection and use of BIs for the measurement of the F_{bio} delivered by cycles for porous/hard loads

Some frequent misuses of F_0 (I)

All misuses of F_0 derive from forgetting that the F_{0phys} has been introduced, originally by the food industry, for loads that always contain enough water to guarantee the continuous compliance with the basic requirement for most-heat sterilization, i.e., the contact of liquid water with the microorganisms to inactivate.

Therefore, the calculation of F_{0phys} for porous/hard loads after the end of exposure phase is a big mistake. In fact, the saturation conditions required for the condensation of the steam in contact with the load fail immediately if a drying vacuum phase follows and become at least uncertain if a cooling phase begins, that demands the removal of the excess steam by air fed to the autoclave chamber.

Some frequent misuses of F_0 (II)

For the above reasons, it is suggestable to restrict the calculation of F_{0phys} in the cycles for porous/hard loads to the exposure phase itself and to monitor the phase by time. Monitoring by F_{0phys} the sterilization target for porous/hard loads is another typical mistake.

In addition, with porous/hard loads the time between the completion of air removal and the start of the exposure is generally so short, that the contribution of it would make no practical difference for the overall cycle duration.

Thank you

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