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 10.0, 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 10.3, 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 10.3, 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 10.3, 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 my 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 10.0, 5.1.1 and the related EMA 2019 Guideline, 4.2.2 ($F_0 \geq 12'$ at $T \geq 121$ °C for an "overkill" process; $F_0 \geq 8'$ at $T \geq 110$ °C for a properly said sterilization).

Lighter treatments are called "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) uses 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) changes 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., five 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 10.0, 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)

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

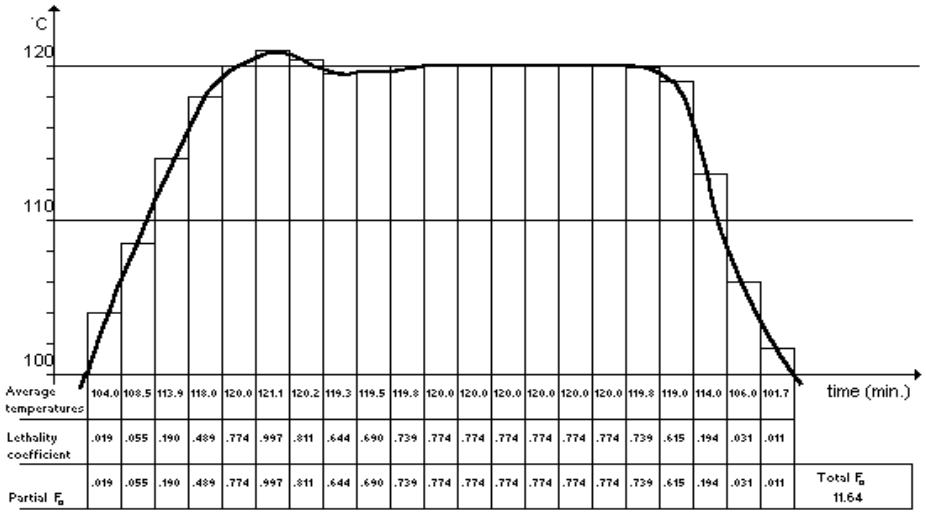
The range of validity of *z*-value should always be remembered as well. To use a *z*-value to calculate *D*-values, and consequently F_{0phys}-values, would be improper beyond the experimental range of validity of *z*-value itself (remember EP 10.0, 5.1.2, 3-1-1 quoted in Slide no. 3)

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 10.0, 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, 4.1.1)

- 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 considered and 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 are based on 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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