The Safety Factor in Preservative Efficacy Testing

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Key words

Preservative system, regulations, efficacy testing, microbial contamination, compliance

Abstract

The authors explain how preservative efficacy testing is done to determine whether a formula is adequately preserved. Product contamination problems are frequently caused by inadequate preservation.

Aqueous consumer products in multiple-use containers need a preservative system to prevent microbial contamination during manufacturing and consumer use, and to comply with governmental regulations. Preservative efficacy testing is performed to determine the type and minimum effective concentration of preservative(s) required for satisfactory preservation of these products.[1]

In its *Cosmetics Handbook* under "Adequacy of Preservation," the FDA recommends that: (a) each batch of a cosmetic which is not self-preserving be tested for microbial contamination before it is released for interstate shipment; and (b) each cosmetic, particularly each eye area cosmetic, be tested during product development for adequacy of preservation against microbial contamination that may occur under reasonably foreseeable conditions of consumer use.[2]

The European Union's Cosmetic Directive states that all cosmetics must have in their product information package ("dossier") the microbiological specifications of the raw materials and the finished product; the dossier must also disclose the purity and the microbiological control criteria of the cosmetic product.[3]

Preservative efficacy test methods include the following:

- Compendial methods, such as the United States Pharmacopoeia (USP) and European Pharmacopoeia (EP) methods;
- Trade association methods, such as the Cosmetic, Toiletry, and Fragrance Association (CTFA) method; and
- Rapid methods, such as the linear regression method.

Several articles have discussed similarities and differences in these test methods. Orth, Delgadillo and Dumatol[4] reported that the slower rates of death allowed by both the USP and CTFA methods may be too lenient.

It is believed that reliance on lenient acceptance criteria may result in products that are inadequately preserved. This can result in sporadic contamination problems and tends to make cosmetic microbiology seem perplexing and governed by factors that we are unable to control. Actually, products that are well preserved kill microorganisms quickly and meet USP and CTFA criteria. Products that are marginally preserved may still meet USP and CTFA criteria, but they may kill Gram negative bacteria so slowly that these bacteria are able to adapt, survive and/or grow.[4,5] This results in product contamination.

Preservative efficacy testing is part of the safety testing of a product. Although aqueous cosmetics and drugs in multiple-use containers are not intended to be sterile, as they are not applied to sterile surfaces, adequately preserved products have a preservative system that renders them self-sterilizing. Such products kill contaminating bacteria quickly enough so that they do not become a health hazard or undergo unacceptable physical changes (color, odor, viscosity, pH, and other factors).

Toxicologists often apply a safety factor, which may be 10-fold or 100-fold greater than toxicity test endpoints observed in animal studies or in vitro test models when extrapolating the data to humans. Although preservative efficacy testing is performed to determine whether the product preservative system can kill microorganisms fast enough to pass test criteria, there is little published information on how to determine whether a preservative system has a satisfactory margin of safety to insure adequate preservation. The goal of this work was to determine the risk factor for bacteria provided by the acceptance criteria of current methods of preservative efficacy testing and to

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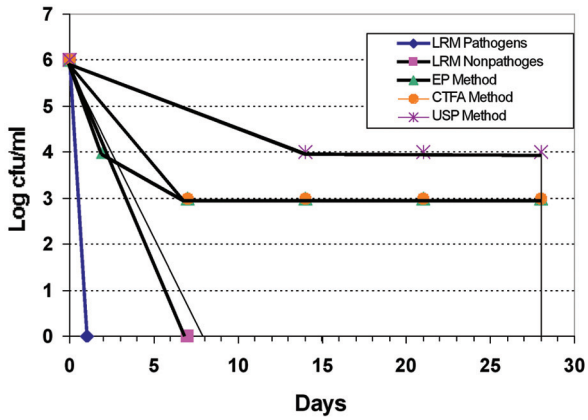

Figure 1. Comparison of slowest rates of death allowed by the USP, EP, CTFA and linear regression methods of preservative efficacy testing. The region where preservative systems may fail (shaded area) shows where the formula risk factor is >1 because the rates of death of bacteria are ≥30 h.

use this factor to determine the preservation safety factor for cosmetic and drug products.

Acceptance Criteria of Test Methods

USP method: The USP acceptance criteria for topical aqueous products is "not less than 2.0 log reduction from the initial count at 14 days, and no increase from the 14 days' count at 28 days."[6]

Although a product that kills microorganisms quickly (e.g., complete kill of 10^6 cfu/g within 24 h) passes USP acceptance criteria, the criteria also allow slow killing. Thus, a 2-log (99%) reduction of bacteria by 14 days actually means that the number of viable bacteria decreases from the initial level of about 10^6 cfu/g to 10^4 cfu/g living organisms at 14 days (and thereafter).

It is evident that these criteria do not require complete kill of the test organisms in 28 days, and it is possible that the survivors will be able to adapt and grow if given the right circumstances.

EP method: The acceptance criteria for topical products using the EP method is a 2-log reduction of bacteria in 48 h and a 3-log reduction by 7 days, with no increase afterwards.[7] This method does not require killing all the bacteria during the 28 day test.

CTFA method: The acceptance criteria of the CTFA method states that there should be greater than 99.9% (3-log) reduction of vegetative bacteria within 7 days following each challenge and continued reduction for the duration of the [28 day] test.[8] This actually means that the number of viable bacteria decreases from the initial level of about 10^6 cfu/g to $<10^3$ cfu/g living organisms at 7 days (and thereafter). Although more stringent than the USP criteria, the CTFA method does not require killing all the bacteria during the 28 day test.

Linear regression method: The rate of inactivation of test organisms in the linear regression method is given by the decimal reduction time (D-value), which is the time required for killing 90% (1-log) of the population of test organisms.

The D-value is calculated by taking the negative reciprocal of the slope of the survival curve, plotted using the log number of surviving microorganisms as a function of the time at which samples were taken, for each test organism. Smaller D-values indicate faster rates of death, and larger D-values indicate slower rates of death.

The target acceptance criteria are a D-value ≤4 h [a ≥6-log reduction in 24 h] for pathogens; a D-value of ≤28 h [a ≥6-log reduction in 7days] for nonpathogenic vegetative bacteria, yeasts and molds; and bacteriostatic or bactericidal for *Bacillus* spp. spores.[9] Adequacy of preservation is indicated by complete kill of at least 10^6 cfu/g pathogens in 24 h, and at least 10^6 cfu/g nonpathogens by 7 days.

Comparison of Test Methods

Figure 1 is a plot of the slowest rates of death allowed by the USP, CTFA and linear regression methods. Although the target criteria of the linear regression method require a complete kill of at least 10^6 cfu/g pathogens in 24 h and at least 10^6 cfu/g nonpathogens by 7 days, the other methods do not require a complete kill of the test organisms during the 28 day test.

Figure 1 shows that the USP and CTFA methods allow microorganisms to persist in products after a 2- or 3-log reduction in viable cell counts. This figure differs from a similar figure in an earlier publication in which the USP criteria then required a ≥3-log reduction by 14 days[10] and initial rates of death were extrapolated to the X-axis.

Orth and co-workers[4] reported the maximum allowable D-value (MA D-value) for Gram negative bacteria routinely used in preservative efficacy testing was <30 h. *Pseudomonas aeruginosa, Burkholderia (Pseudomonas) cepacia* and *Escherichia coli* did not die and/or began to grow after an initial decline if D-values were not <30 h.

Although similar studies have not been reported for Gram positive bacteria, these findings indicate that Gram negative bacteria introduced into products may survive and/or grow unless the preservative system kills them with a D-value of <30 h. This is indicated as the "Region where preservative system may fail" in the shaded area of Figure 1. Here, the survival curves show the slowest rates of death allowed by the USP, EP, CTFA and linear regression methods of preservative efficacy testing. The Region where preservative system may fail (shaded area) shows where the formula risk factor is >1

because the rates of death of bacteria are ≥30 h. The type of contaminating microorganism(s), their physiological state, the nutrients provided by the product, and storage conditions (time, temperature, oxidation/reduction potential, and other factors) determine whether Gram positive or Gram negative bacteria will survive and grow in any given situation.

When discussing rates of bacterial death using %-reductions or log-reductions, a time interval must be included (such as, a 3-log reduction in 7 days). D-values are used in all areas of microbiology to describe rates of death of microorganisms. Conversion of USP, EP and CTFA challenge test acceptance criteria to D-values enables direct comparison of the slowest rates of death allowed by these methods.

The log-reductions (initial rates of death) allowed by the different methods are given in Table 1. Conversion of these log-reductions to D-values gives values of <156 h, ≤24 h, and ≤56 h for USP, EP and CTFA methods, respectively. These D-values give the rates of death without stating the number of hours or days required for a given number of log-reductions.

Table 1. Comparison of acceptance criteria and risk factors provided by the USP, EP, CTFA and linear regression methods

Test Method	Log-Reductions	D-value	MA D-value	Risk Factor*
USP	>2 by 14 days	<156 h	30 h	5.6
EP	≥2 by 2 days	≤24 h	30 h	0.8
CTFA	>3 by 7 days	<56 h	30 h	1.87
LRM-Path.**	≥6 by 1 day	≤4 h	30 h	0.13
LRM-Nonpath.**	≥6 by 7 days	≤28 h	30 h	0.93

* D-value determined from the initial rate of death in USP, EP and CTFA methods

* MA D-value determined for Gram negative bacteria[4]

* Risk Factor = D-value/MA D-value

** LRM-Path. = Linear regression method for pathogens

** LRM-Nonpath. = linear regression method for nonpathogens

Required D-Value

It is possible to specify the rate of death required for test organisms in a product depending on the type of packaging and consumer use. Orth, Barlow and Gregory[5] reported that the preservative efficacy of the formula, the protection provided by packaging (the packaging factor), and the conditions of use (the consumer use/abuse factor) must be considered when determining the adequacy of preservation of a product. Criteria for determining packaging and consumer use/abuse factors are given in Tables 2 and 3.

Orth and co-workers related D-values of a formula with packaging and consumer use/abuse factors in the required D-value (RDV) by the following expression:

$$RDV = D\text{-value}_t / (F_p * F_{cua})$$

where

$D\text{-value}_t$ is the D-value of a target organism,

F_p is the packaging factor of the product, and

F_{cua} is the consumer use/abuse factor.

Application of the RDV enables one to determine what D-value is required by specific (target) organisms when the type of packaging and consumer use are known. The consumer use of a product generally cannot be changed because consumers use shampoos in the shower, apply pressed powders using a pad, and dispense lotions from a tube or bottle. However, the preservative system may need to be improved or packaging modified to achieve the desired RDV and have a product that will be microbiologically safe and stable during consumer use.

Risk Factor of the Preservative System

Experience has shown that Gram negative bacteria generally are more capable of adapting and causing problems in aqueous cosmetic and drug products than are Gram positive bacteria, so use of the MA D-value for Gram negative bacteria in aqueous products provides a more conservative approach to safety assessment than using similar data for Gram positive bacteria.

The contamination risk factor is an expression of the ability of the formulation to prevent growth based on the rate of death of specific test organisms, relative to the rate of death at which bacteria die so slowly that they may adapt and grow (or persist) in the formula.

The risk factor for the preservative system may be determined by dividing the D-value for a specific bacterium in that product by the MA D-value for Gram negative bacteria (30 h). For example, a formula with a D-value of 30 h for Gram negative bacteria would have a risk factor of 30 h/30 h = 1 for these microorganisms. This formula would be expected to prevent growth

Table 2. Criteria for risk assessment of packaging to allow microbial contamination of a product

Packaging Factor	Type of Packaging Closure
1 (low risk)	Single use product/applicator Unit-dose container Hermetically sealed container
5 (moderate risk)	Multiple-use container intended for use where contact with water or wet/soiled fingers is likely • Lotion in pump dispenser • Cream in tube with screw cap or flip-top
10 (high risk)	Multiple-use container intended for use where repeated contact with water or wet/soiled fingers may occur • Shampoo/conditioner in pump dispenser or tube with screw cap or flip-top • Toothpaste tube with screw cap or flip-top • Jar of cream with removable lid • Eye shadow/mascara with reusable applicator (brush/wand)

Table adapted from Orth et. al[5]

Table 3. Criteria for risk assessment of consumer use/abuse that may allow microbial contamination of a product

Consumer Factor	Type of Consumer Use/Abuse
1 (low risk)	Using single-use product/applicator • Shampoo in unit-dose container • Lotion in tear-open packet
5 (moderate risk)	Using multiple-use product where touching product with wet/soiled fingers may occur occasionally • Touching outlet of lotion pump as product is dispensed • Touching tip of tube when dispensing sunscreen onto fingers
10 (high risk)	Using multiple-use product where repeated product touching with wet/soiled fingers occurs or where contact with water occurs • Removing cap from bottle of shampoo while showering • Moistening mascara applicator (sand) and reintroducing it into product • Adding water to the bottle of liquid soap or bath gel to get all of the product out of the bottle, and re-using this diluted product over a period of days

Table adapted from Orth et. al[5]

by unadapted bacteria, but may have no margin of safety. A formula with a D-value of 15 h for *E. coli or B. cepacia* would have a risk factor of 15 h/30 h = 0.5 for these bacteria. If *S. aureus* had a D-value of 20 h in the same formula, the risk factor for *S. aureus* would be 20 h/30 h = 0.67.

Risk factors were calculated using the maximum acceptance criteria for the USP, EP, CTFA and linear regression methods (Table 1). It is believed that products with risk factors >1 may not be adequately preserved, and their preservative system may fail. Formulas that meet EP and linear regression method criteria would be adequately preserved for Gram negative bacteria because all have risk factors ≤1. Note that formulas that just meet the linear regression method acceptance criteria for pathogens (4 h) would have a risk factor of 4 h/30 h = 0.13. It is very important that products be adequately preserved against pathogens that could cause skin or eye infections.

Aqueous formulations that allow microbial growth without the addition of preservatives [i.e., they are not self-preserving due to low water activity (a_w)] should have D-values ≤4 h for opportunistic pathogens such as *P. aeruginosa*. Self-preserving formulas with low a_w should be tested and shown to be bacteriostatic/slowly bactericidal. Although a_w-based formulas may not provide killing of bacteria with D-values of ≤30 h, they are inherently self-preserving, they do not permit microbial growth, and the formula risk factors discussed here do not apply.

Determining the Microbial Safety Factor for a Product

The preservative system of the formula is very important; however, other parameters are required for determining the microbiological safety factor of a product. The microbial safety factor for a product may be calculated by dividing the RDV in hours by the risk factor for a formula, as follows:

Microbial Safety Factor = RDV (in hours) / Risk Factor

Criteria for risk assessment of packaging and consumer use/abuse are presented in Tables 2 and 3.

It is desirable to have a topical lotion with an RDV of 4 h for *P. aeruginosa*. If we have a product with a formula that meets acceptance criteria of the linear regression method (D-value ≤4 h), protective packaging (i.e., a lotion in a small tube that minimizes contamination; packaging risk factor = 1) and good protection from consumer contamination (consumer use/abuse risk factor = 1), we can determine the RDV and microbial safety factor as follows:

RDV = 4 h / (1) * (1) = 4 h

Microbial Safety Factor = 4 / 0.13 = 30

In this example, this product has a 30-fold safety factor, so it is unlikely that it would become contaminated by *P. aeruginosa* during consumer use. If the same formula were to be used as a shower treatment product (consumer use/abuse factor = 10), it would probably have different packaging (i.e., a bottle with a flip-top cap; packaging risk factor = 5). This would change the RDV and microbial safety factors as follows:

RDV = 4 h / (5) * (10) = 0.08 h

Microbial Safety Factor = 0.08 / 0.13

= 0.62

Here, the formula still has a D-value of 4 h for *P. aeruginosa*, but the packaging allows contamination during use in the

shower. This illustrates that the same formula may have a safety factor of 30 when used as lotions are commonly used and that it may have a safety factor of 0.62 when used as a shower product. A product with a safety factor <1 has no margin of safety.

Even though the formula may be adequately preserved for *P. aeruginosa*, it is possible that the use as a shower product will result in contamination because the product will be touched with wet fingers during repeated use in the shower over a period of weeks/months. If the packaging cannot be changed to reduce the packaging factor, it may be necessary to increase the type/concentration of preservatives to decrease the risk factor of the preservative system. This illustrates the necessity for evaluation of both consumer use and the type of packaging prior to final selection of the packaging for any product.

Examples illustrate that the risk factor of the formula and the RDV may be used to determine the safety factor for a product. A formula that just meets USP criteria has a risk factor of 5.6 and a safety factor for *P. aeruginosa* of 4/5.6 = 0.71. There is no margin of safety with a safety factor of <1. Bacterial contamination during consumer use is possible unless the packaging and the manner in which it is used restrict human and environmental contamination. The likelihood of contamination could be reduced by use of small tubes (perhaps 1 oz.) so that the product would be used up in a few weeks. This would reduce repeated exposure and the possibility of microbial adaptation (a consumer use/abuse factor = 1). If the tube had a small orifice to help prevent consumer contamination, the product would have a packaging factor = 1.

A formula that just meets CTFA criteria would need packaging that provides two-times the protection in a formula that just meets linear regression criteria for nonpathogens. When selecting criteria that result in acceptance of formulas in the "Region where preservative systems may fail to prevent bacterial growth" (Figure 1), it is quite possible that the greatest consumer protection would be obtained with unit-dose packaging, or small tubes with narrow orifices (as mentioned earlier).

The linear regression method acceptance criteria provide a product safety factor of just greater than one for nonpathogens and substantially greater than one for pathogens. It appears that this safety factor is adequate for aqueous cosmetic and

drug products because to the best of our knowledge, formulas that meet the acceptance criteria of the linear regression method have never been contaminated with unadapted microorganisms.

On the other hand, USP and CTFA criteria have acceptance criteria that allow microbial safety factors of <1, and it is known that products that are considered to be satisfactorily preserved by these methods may allow microbial growth.[10] Although a safety factor of >1 does not guarantee that a product cannot become contaminated during manufacturing or consumer use, it provides a rational basis for predicting the likelihood of product contamination. Obviously, a product with a safety factor of 10 is much less likely to become contaminated than a formula with a safety factor of <1, if all other conditions remain constant. It is believed that use of microbial safety factor in product development will help take some of the mystery out of cosmetic microbiology.

Conclusions

Preservative efficacy testing is carried out to determine whether a formula is adequately preserved. Product contamination problems seem perplexing, yet they frequently are caused by inadequate preservation – the products do not have a large enough microbial safety factor.

- Lenient acceptance criteria may result in inadequately preserved products.
- Different methods have different acceptance criteria for determining adequacy of preservation. These criteria generally do not consider packaging or consumer use.
- The RDV may be used to determine preservative requirements of a product when the type of packaging and consumer use are known.
- Contamination risk factors for preservative efficacy testing methods may be determined from the slowest rate of death allowed by the acceptance criteria (i.e., the maximum allowable D-value) and the MA D-value for Gram negative bacteria.
- The microbial safety factor for a product may be determined by dividing the RDV by the risk factor.
- Aqueous products with a microbial safety factor of >1 have an excellent history being satisfactorily preserved in manufacturing and during use by consumers.

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