PDA Journal
of Pharmaceutical Science and Technology

Radiation Sterilization of Aseptically Manufactured Products

Barry P. Fairand and Niki Fidopiastis

PDA J Pharm Sci and Tech **2010**, *64* 299-304

RESEARCH

Radiation Sterilization of Aseptically Manufactured Products

BARRY P. FAIRAND[1] and NIKI FIDOPIASTIS[2]

[1]*Senior Consultant, Sterigenics International and* [2]*Sterigenics International*

ABSTRACT: This paper discusses an approach for establishing a sterilization dose for an aseptically processed product after the product is in its final packaged state, in other words, terminal sterilization. It applies to aseptic processes where the fill/finish operation is conducted in a closed system using isolator or restricted access barrier technology, that is, no human intervention. The example that is given in this paper uses gamma radiation as the sterilizing agent. Other forms of radiation such as high-energy electrons or X-rays also could serve as the sterilizing agent. The proposed approach involves irradiation of the aseptically processed product at very low doses of radiation, which is possible due to the extremely low levels of bioburden that may be present on the product following a fill/finish operation. Rather than sacrificing a large number of product units that may be required to obtain a statistically significant sampling of the product for bioburden analysis and other test purposes, the test unit is a surrogate consisting of actual pharmaceutical product that was inoculated with a highly radiation-resistant microorganism. Selection of the microorganism was based on analysis of a library of environmental monitoring data taken from the aseptic area. Because of microbial diversity between different aseptic processing facilities, selection of the test microorganism would depend on the aseptic area under study. The approach that is discussed in this paper addresses selection and preparation of the surrogate, test of sterility of the surrogate following irradiation, determination of the radiation resistance of the test microorganism, and application of the approach to calculate a sterilization dose that is less than 10 kGy. At this low dose, it may be possible to terminally sterilize radiation-sensitive pharmaceutical products, for example, those in liquid form. Additional studies are warranted to determine the general applicability of the proposed approach.

KEYWORDS: Aseptic area, SAL, Radiation sterilization, Gamma irradiator, Surrogate, D_{10} value.

Introduction

Before entering an aseptic manufacturing area, components, closure systems, and packaging are typically sterilized to a sterility assurance level (SAL) of 10^{-6}. Drug products including the active pharmaceutical ingredient and adjuvant or excipient also may be terminally sterilized to an SAL of 10^{-6} before being brought into the aseptic area (1–3). Even though products are terminally sterilized prior to entry into the aseptic area, these products often undergo a fill/finish operation during which they are exposed to the environmental conditions that exist within the aseptic area. Aseptic areas operate under highly controlled and extremely clean conditions, but a post-fill/finish sterilization step after the product is in its final packaged state that confirms an SAL of 10^{-6} may be beneficial. In that regard, it is of interest to note that of 197 Food and Drug Administration (FDA) product recalls of aseptically processed sterile products over the 8-year period from 1998 to 2006, 78% of the recalls were due to lack of sterility assurance (4).

Radiation provides an efficacious method for sterilization of a broad spectrum of products (5, 6). However, high levels of radiation negatively affect some types of pharmaceutical products such as biologically derived drugs, certain types of device materials, and packaging. Therefore, for radiation sterilization of pharmaceutical products that are subjected to a fill/finish operation in an aseptic area, it is desirable to keep the absorbed dose as low as possible. This should be possible, given the fact that the product is manufactured under extremely clean conditions. This is particularly the case for aseptically manufactured prod-

Correspondence author: Barry P. Fairand, 3350 Kendelmarie Way, Dublin, Ohio 43017

ucts where the fill/finish operation is performed in a closed system without human intervention, for example, isolator and restricted access barrier (RAB) technology. For this reason, sterilization of an aseptically manufactured product following a fill/finish operation should only require reduction in the bioburden by at most a few orders of magnitude. Existing methodologies for setting dose to achieve an SAL of 10^{-6} are based on knowledge of the natural bioburden that is present on a product unit and testing of sterility against a challenge population (7). Because any possible bioburden on aseptically manufactured products would necessarily be extremely low, a statistically reliable measurement of bioburden and subsequent test of sterility would require a very large number of product units.

Rather than sacrificing a large matrix of actual product units to establish a dose for sterilization following the tenets of existing methodologies, it is proposed that a properly designed test product or surrogate could be used for this purpose. This approach avoids consumption of a large number of potentially high value product units, which for some batches of aseptically processed products could represent a significant fraction of the entire batch. The surrogate would be used to determine the radiation resistance of the microorganisms that may be present in the actual product, and this information along with a conservative estimate of the initial bioburden could be used to calculate the dose that is required to achieve an SAL of 10^{-6}. The example given in this paper offers a possible approach for terminal sterilization of aseptically processed products at doses less than 10 kGy. Further studies that include different modalities of radiation, analysis of different aseptic areas, and types of pharmaceutical products would help in determining the general applicability of the proposed approach for terminal sterilization of aseptically processed products.

Experimental Method

Selection and Preparation of Test Product

Because possible bioburden on an aseptically manufactured product would be extremely low, an estimate of the effective radiation resistance of possible contaminants would require testing a very large matrix of product samples, which in most cases would not be a practical undertaking. Rather than testing a very large number of product samples, a properly designed surrogate was used for this purpose. The following steps were followed in the selection and preparation of the surrogate.

- A library of information on microbial contaminants obtained from environmental monitoring of an aseptic area was evaluated and based on this information a microorganism was selected for testing. Because the microbiological environment may differ between aseptic areas, this exercise would need to be repeated for each aseptic area.

- An inoculum of the test microorganism was obtained and prepared for the tests.

- Pharmaceutical product was inoculated with the test microorganism. The surrogate consisted of 3-mL, pre-filled, normal saline flush syringes containing sterile, preservative-free 0.9% sodium chloride and USP (United States Pharmacopoeia) water. These syringes were inoculated with the test microorganism. This surrogate is representative of liquid-based pharmaceutical products such as prefilled syringes. Pharmaceutical products in a liquid state are generally more sensitive to the effects of radiation than dry products, which was the basis for the selection of a drug product in a liquid state rather than a dry product. Dry products, for example, lyophilized powders, would require a different formulation for the surrogate. However, drug products in dry formulation are being terminally sterilized without recourse to an aseptic manufacturing process.

Microbiological data from environmental monitoring of an aseptic area was analyzed. The organism chosen from analysis of the data was *Bacillus licheniformis.* This microorganism, which was found as a common contaminant in the aseptic area, is a Gram-positive, spore-forming bacterium that belongs to the *Bacillus subtilis* group of the *Bacillus* genus. Based on these characteristics, *B. licheniformis* is expected to be a radiation-resistant microorganism with a relatively high D_{10} value and thus represents a meaningful challenge to the dose that is required to achieve an SAL of 10^{-6}. An inoculum of *B. licheniformis* was obtained and prepared for the study.

A spore suspension of *B. licheniformis* (ATCC 14580) was used to inoculate the pharmaceutical product samples. The ATCC organism was rehydrated and cultured per manufacturer's instructions. The cells were then harvested to create a suspension. The organism

suspension was heat-shocked (to 60–65 °C) to eliminate the vegetative cells and to produce a spore suspension. The spore suspension was diluted to produce an inoculum sufficient to deliver a minimum population count of 1×10^4 per 100 µL.

Twenty samples were inoculated for each of the test irradiations. The syringes were inoculated with 100 µL of the *B. licheniformis* spore suspension (inoculum level $> 1 \times 10^4$ per 100 µL). Using a sterile needle and syringe, the inoculum was placed into the barrel of the syringe through the syringe tip. After inoculation the tip cap was replaced and the syringe was carefully inverted two to three times to distribute the inoculum throughout the saline within the syringe barrel. After inoculation the syringes were placed in self-seal pouches. To guard against possible temperature excursions during shipment of the samples to and from the irradiator and during storage, the syringes were maintained in a refrigerated state prior to shipment to the irradiator, during shipment to and from the irradiator, and while in storage at the irradiator. All samples were shipped priority mail for overnight delivery to the irradiator and back to the laboratory. Furthermore, the irradiations were scheduled for the day of arrival of the inoculated samples at the irradiator. This minimized the time between inoculation of the samples and the start of the sterility tests. An additional five inoculated samples accompanied the first set of samples that were sent to the irradiator. Upon return to the laboratory, these five control samples, which were not irradiated, were analyzed for the number of recoverable colony-forming units (CFU). The data was used to test for repeatability in the number of recoverable CFU and also served as a check on the stability of the inoculum to environmental conditions between the time of inoculation and the start of the sterility test.

Test of Sterility

Upon completion of each test irradiation, the 20 samples were immediately sent to the laboratory for analysis. The sterility test was conducted using test parameters outlined in the current USP, ISO 11737-1, ISO 11737-2, and internal laboratory procedures (8, 9). Only the contents of the syringes were tested using the direct inoculation sterility test method, as described in USP Chapter 71, Sterility Tests. The saline was injected from the syringe into tryptic soy broth (TSB). The TSB was incubated at 30 ± 2 °C for a period of 14 days, and the results were reported as the number of samples with positive growth and/or the number of samples with negative growth for the 20 verification dose samples. The growth from each of the positive samples was cultured to confirm the presence of the inoculated organism.

Determination of the D_{10} Value

With an increase in dose of radiation, the dose–survivor curve that characterizes the death of microorganisms follows an exponential relationship. This type of relationship is also true for thermal death of microorganisms. The slope of the dose–survivor curve is given by $(-1/D_{10})$, where the D_{10} value is the incremental dose that is required to reduce the surviving number of microorganisms by a factor of ten, or one log. Stumbo, Murphy, and Cochran formulated an expression for thermal death that related time at temperature to the initial number of microorganisms and fraction negative data (10). As noted in reference 5, this same expression can be used to calculate the D_{10} value. The expression for the D_{10} value from exposure to radiation is given by

$$D_{10} = \frac{D_i}{\text{Log } N_0 - \text{Log } N_f} \quad (1)$$

where

D_i = dose delivered to samples

N_o = initial number of microorganisms in CFU

N_f = ln (n/r)

n = total number of samples irradiated at a dose D_i

r = number of samples that show no growth (fraction negative)

Equation 1 was used to calculate the D_{10} value based on the experimental values for N_o, D_i, and r.

Experimental Irradiations

A total of 20 samples were selected for irradiation at each dose. This number was considered sufficient to provide good statistical results while still maintaining a sufficiently small sample package to allow all of the samples to receive approximately the same dose. The irradiations were conducted in a gamma irradiator at Sterigenics International, which is designed to deliver precise doses to validation samples and small batches of product that require special dosing conditions. The 20 syringe samples were arranged in two planar arrays

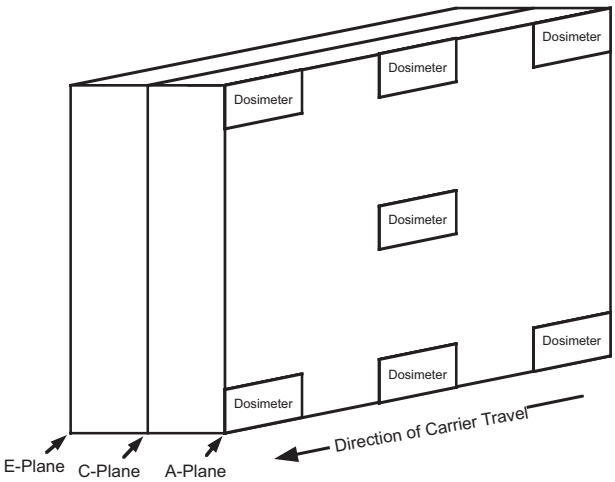

Figure 1

Target geometry and location of dosimeters in A, C, and E planes.

of 10 samples each on cardboard sheets. During irradiation the cardboard sheets were placed back-to-back so that the syringe samples faced outward. The planes that faced outward, which contained the 20 syringe samples, were labeled the A and E planes, and the plane between the cardboard sheets was labeled the C plane. The cardboard sheets were placed in a vertical geometry and centered on the shelf of a carrier in the irradiator. The sheets were oriented so that they were parallel to the direction of travel of the carrier through the irradiator. Doses delivered to the samples were measured with thin film radiochromic dosimeters, which were calibrated at a National Standards Laboratory. The maximum and minimum doses delivered to the 20 syringe samples were determined by placing a grid of 21 dosimeters on each sample package. The grid consisted of seven dosimeters on the outer surfaces of the syringe samples in the A and E planes and seven dosimeters in the C plane between the cardboard sheets. The target geometry and locations of the dosimeters are shown in Figure 1.

The maximum dose was delivered to the surface of the syringe samples that faced outward toward the cobalt-60 source planes. The minimum dose was delivered to the C plane that was located between the two cardboard sheets.

Results and Discussion

Controls

Five controls samples were inoculated at the same time as the 40 samples that were prepared for the first set of two irradiations. These controls accompanied the samples that were dosed at the irradiator. Upon return to the laboratory, the control samples were checked for possible changes in the number of recoverable CFU from the time of inoculation to the start of sterility testing and also to provide information on repeatability in number of recoverable CFU. The control data are given in Table I.

The uncertainty in the average value for the CFU recovered that is given in Table I is expressed at two sample standard deviations or 95% confidence level. The difference between the number used to calculate the D_{10} value, that is, 1.4×10^4, and the average value from the controls is within the estimated uncertainty in the measurement process and therefore is not considered to be statistically significant. Because of the logarithmic relationship between the number of CFU in the inoculum and the D_{10} value, the uncertainty in the number of CFU leads to less than a 0.1 kGy change the resultant value for D_{10}. The stability of the inoculum with time is not surprising given the fact that the inoculum is a spore suspension in sterile water without preservatives.

D_{10} Value for Different Fraction Negatives

The ability of the Stumbo equation to predict a unique D_{10} value was tested by conducting multiple irradiations with different values for N_o and D_i. The input parameters were selected to give a fraction negative that ranged from a few sterile samples up to the majority of the 20 samples being sterile. There were a total of six irradiations. In two of the irradiations, the fraction negative was zero, which suggested that the D_{10} value for the inoculated microorganism was on the order of 2 kGy. This information served as a guide in the selection of the dose conditions for other irradiations. The remaining four irradiations were designed to test the Stumbo equation's ability to predict a

TABLE I
Control Data

Control Number	CFU Recovered
1	1.5×10^4
2	1.8×10^4
3	1.8×10^4
4	1.8×10^4
5	2.0×10^4
Average Value, CFU	$(1.8 \pm 0.4) \times 10^4$

TABLE II
Results of Irradiations and Predicted D_{10} Values

Irradiation	N_o CFU	D_i kGy	r	D_{10} kGy
Number 1	1.4×10^4	7.8 ± 0.3	7	1.9
Number 2	1.2×10^4	9.6 ± 0.4	12	2.2
Number 3	3.5×10^4	8.2 ± 0.4	15	1.6
Number 4	3.5×10^4	10.1 ± 0.3	18	1.8
Average D_{10} Value				1.9 ± 0.5 kGy

unique value for D_{10} when challenged with a range of different values for the fraction negative number.

Results of the irradiations and the predicted value for D_{10} are given in Table II. The data is arranged in order of increasing value for the fraction negative. The doses in Table II represent the average doses that were delivered to the samples. The uncertainty in the average doses, which is given at two sample standard deviations or a 95% confidence level, reflects the variability in dose over the sample package.

In Table II, the uncertainty in the average D_{10} value is given at a 95% confidence level. Uncertainties in the microbiological test method and delivered dose to the samples contribute to the calculated uncertainty of ±0.5 kGy. The additional variability in the calculated values for D_{10} may be due to the fact that tests Number 1 and 2 were prepared from one batch of inoculum and tests Number 3 and 4 were prepared from another batch of inoculum. A D_{10} value of 2.4 kGy, that is, average value plus 95% confidence limit, was selected for calculation of the sterilization dose.

Estimate of Sterilization Dose

The dose that is required to achieve an SAL of 10^{-6} will depend on the radiation resistance of the test microorganism and log reduction in bioburden beginning with an average value (N_i). The sterilization dose can be expressed in the following manner:

$$D = D_{10} (\log N_i - \log 10^{-6}) \quad (2)$$

In estimating the average bioburden that may be present on a pharmaceutical product following a fill/finish operation, it was assumed that the fill/finish area operated as a closed system without human intervention, for example, isolator or RAB technology. This type of environment is highly controlled, with the intent being able to label the drug product as sterile following the fill/finish operation. For these types of aseptic areas, airborne particulates, which are analyzed on an ongoing basis, probably constitute the principal source of potential contamination of the drug product during the fill/finish operation. Given these conditions, it is reasonable to assume that the average bioburden on the drug product would be much less than 0.001 CFU/unit. Results of a media fill consisting of 3000 units could be used to test this hypothesis. If analysis of the 3000 media fill units reveals no contaminated units, the average bioburden (N_i) present on the product should not exceed 0.001. Given an average bioburden of 0.001 and a D_{10} value of 2.4 kGy, it can be seen from eq 2 that the dose to satisfy an SAL of 10^{-6} is

$$D = 2.4 \text{ kGy } (\log 10^{-3} - \log 10^{-6}) = 7.2 \text{ kGy} \quad (3)$$

As seen from the above example, the dose that is required to achieve an SAL of 10^{-6} is significantly less than 10 kGy. This should allow extremely clean products to be radiation sterilized on a commercial basis to an SAL of 10^{-6} while still keeping maximum doses to about 10 kGy or less. At these low doses, many types of pharmaceutical products should be radiation-compatible.

The sterilization dose that is calculated following the above approach should not be less than a sterilization dose that is based on the standard distribution of resistances, which is the challenge population used in Method 1 (7). For a three-log reduction in bioburden, that dose is 5.2 kGy. This sets a lower limit on the sterilization dose regardless of the D_{10} values of the microbial constituents that may be identified in an aseptic area.

Conclusions

An approach that involved the use of a surrogate was developed to establish the radiation dose that is required to sterilize aseptically manufactured products to an SAL of 10^{-6} following a fill/finish operation. The example cited in this paper does not require sacrificing a large number of product samples to establish the dose of radiation that is required to achieve an SAL of 10^{-6}. Rather, a surrogate was developed for this purpose. The surrogate was based on a contaminant that was identified from analysis of a library of

environmental monitoring data from the aseptic area. The test microorganism was a spore-forming, Gram-positive microorganism, which typically has a high D_{10} value and is resistant to radiation. Because of the extremely clean conditions that exist in an aseptic area, it was possible to calculate a sterilization dose significantly less than 10 kGy. At these low doses, it should be possible to use radiation to terminally sterilize many types of radiation-sensitive drug products. Further studies would help in testing the general applicability of the proposed approach for terminal sterilization of aseptically processed products.

Acknowledgments

The authors would like to acknowledge West Pharmaceutical Services for providing the environmental monitoring information that was used in the selection of the challenge microorganism and to thank Sterigenics International for support of this study.

References

1. *Guidance for Industry. Sterile Drug Products Produced by Aseptic Processing—Current Good Manufacturing Practice*. Center for Drug Evaluation and Research, Food and Drug Administration (FDA): Rockville, MD, September 29, 2004.

2. US Department of Health and Human Services, FDA. Use of aseptic processing terminal sterilization in the preparation of sterile pharmaceuticals for human and veterinary use. Federal Register 56: 51354 51358, October 11, 1991.

3. Farrington, J. K. Environmental monitoring in pharmaceutical manufacturing—a product risk issue. *American Pharmaceutical Review* **2005,** *8* (5), 26–30.

4. Jimenez, L. Microbial diversity in pharmaceutical product recalls and environments. *PDA J. Pharm. Sci. Technol.* **2007,** *61* (5), 383–399.

5. Davis, W. K., Strawderman, E. W., Masefield, J. *Proceedings of the International Kilmer Memorial Conference on the Sterilization of Medical Products;* Multiscience Publications Ltd.: Montreal, 1981; Vol. II, p. 34.

6. Sterilization of Health Care Products—Radiation—Part 1: Requirements for Development, Validation, and Routine Control of a Sterilization Process for Medical Devices, International Organization for Standardization, ANSI/AAMI/ISO 11137-1:2006.

7. Sterilization of Health Care Products—Radiation—Part 2: Establishing the Sterilization Dose, International Organization for Standardization, ANSI/AAMI/ISO 11137-2:2006.

8. Sterilization of Medical Devices—Microbiological Methods—Part 1: Determination of a Population of Microorganisms on Products, International Organization for Standardization, ANSI/AAMI/ISO 11737-1.

9. Sterilization of Medical Devices—Microbiological Methods—Part 2: Tests of Sterility Performed in the Validation of a Sterilization Process, International Organization for Standardization, ANSI/AAMI/ISO 11737-2.

10. Stumbo, C. R.; Murphy, J. R.; Cochran, J. Nature of thermal death time curves of PA 3679 and *Clostridium botulinum. Food Technol.* **1950,** *4,* 293–302.

PDA Journal
of Pharmaceutical Science and Technology