Radiation Sterilization of Aseptically Manufactured Products

Barry P. Fairand, Niki Fidopiastis

1 Senior Consultant Sterigenics International, 2 Sterigenics International

ABSTRACT: This paper discusses a method for establishing a sterilization dose for aseptically processed products after these products are in their final packaged state. The process involves gamma 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 to validate the process, 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 environmental monitoring data taken from an aseptic area, which would be unique to the aseptic area under study. The article addresses selection and preparation of the surrogate, test of sterility of the surrogate following irradiation in a gamma irradiator, determination of the radiation resistance of the test microorganism and application of the method to calculate a sterilization dose that is much less than 10 kGy. At this low dose, many types of pharmaceutical products are expected to respond favorably.

KEYWORDS: Aseptic area, SAL (sterility assurance level), Radiation sterilization, Gamma irradiator, Surrogate, D10 value, CFU (colony forming unit), Dose (kGy)

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 API 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 oftentimes 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 validates an SAL of $10^{-6}$ may be beneficial. In that regard, it is of interest to note that of 197 FDA product recalls of sterile product over the eight-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. 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 test of sterility against a challenge population. 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, it is proposed that a properly designed test product or surrogate could be used for this purpose. 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 $10^{-6}$. An approach was developed for radiation sterilization of extremely low bioburden products at doses much less than 10 kGy.

**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.

- Actual 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, USP water. These syringes were inoculated with the test microorganism. This surrogate is representative of liquid based pharmaceutical products such as pre-filled syringes. Pharmaceutical products in a liquid state are generally more sensitive to the effects of radiation than dry products. Other types of pharmaceutical products, e.g., lyophylized powders, may require a different formulation for the surrogate. However, it should be noted that some types of dry pharmaceutical products are being successfully radiation sterilized without recourse to a previous aseptic manufacturing step.
Selection of the Test Microorganism

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, Bacillus licheniformis is expected to be a radiation resistant microorganism with a relatively high D10 value and thus represent a meaningful challenge to the dose that is required to achieve an SAL of 10^-6. An inoculum of Bacillus licheniformis was obtained and prepared for the study.

Preparation of Inoculum

A spore suspension of Bacillus licheniformis (ATCC 14580) was used to inoculate the pharmaceutical product samples. The ATCC organism was re-hydrated 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 x 10^4, per 100µL.

Inoculation of Test Product

A total of twenty samples were inoculated for each of the test irradiations. The syringes were inoculated with 100µL of the Bacillus licheniformis spore suspension (inoculum level > 1 x 10^4 per 100µL). 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 2-3 times to distribute the inoculum throughout the saline within the syringe barrel. After inoculation the syringes were placed in self-seal pouches. 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 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 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 time of inoculation and 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 (7,8). 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 results were reported as the number of samples with positive growth and/or the number of samples with negative growth for the twenty 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/D₁₀), where the D₁₀ 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 (9). As noted in reference 5, this same expression can be used to calculate the D₁₀ value. The expression for the D₁₀ value from exposure to radiation is given by,

\[
D_{10} = \frac{D_i}{\log N_0 - \log N_f}
\]

where:

- \(D_i\) = dose delivered to samples
- \(N_0\) = initial number of microorganisms in colony forming units (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 D₁₀ value based on the experimental values for \(N_0\), \(D_i\) and \(r\).

**Control Samples**

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 change in the number of recoverable CFU from time of inoculation.
to start of sterility testing and also to provide information on repeatability in number of recoverable CFU. The control data are given in Table 1.

Table 1. Control Data

<table>
<thead>
<tr>
<th>Control Number</th>
<th>CFU Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5 x 10⁴</td>
</tr>
<tr>
<td>2</td>
<td>1.8 x 10⁴</td>
</tr>
<tr>
<td>3</td>
<td>1.8 x 10⁴</td>
</tr>
<tr>
<td>4</td>
<td>1.8 x 10⁴</td>
</tr>
<tr>
<td>5</td>
<td>2.0 x 10⁴</td>
</tr>
<tr>
<td>Average Value, CFU</td>
<td>(1.8 ± 0.4) x 10⁴</td>
</tr>
</tbody>
</table>

The uncertainty in the average value for the CFU recovered that is given in Table 1 is expressed at two sample standard deviations or 95% confidence level. The difference between the number used to calculate the D₁₀ value, i.e., 1.4 x 10⁴ and the average value from the controls is within the estimated uncertainty in the measurement process and therefore not considered to be statistically significant. Because of the logarithmic relationship between the number of CFU in the inoculum and D₁₀ value, the uncertainty in the number of CFU leads to less than a 0.1 kGy change the resultant value for D₁₀. 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.

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 an ExCell gamma irradiator at Sterigenics International. The ExCell irradiator is designed to deliver a precise dose to validation samples and small batches of product that require special dosing conditions. The 20 syringe samples were arranged in two planar arrays of 10 samples each on cardboard sheets. During irradiation the cardboard sheets were placed back-to-back so the syringe samples faced outward. The planes that faced outward, which contained the 20 syringe samples, were labeled the A and E planes respectively 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 an ExCell carrier. The sheets were oriented so 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 respectively and seven dosimeters in the C plane between the cardboard sheets. The target geometry and locations of the dosimeters are shown in Figure 1.

Figure 1. Target Geometry and Location of Dosimeters in A, C and E Planes

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.

Irradiations with Different Numbers for Fraction Negative

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 unique value for the $D_{10}$ value 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 2. The data is arranged in order of increasing value for the fraction negative. The doses in Table 2 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.

Table 2. Results of Irradiations and Predicted $D_{10}$ Values

<table>
<thead>
<tr>
<th>Irradiation</th>
<th>$N_0$ CFU</th>
<th>$D_1$ kGy</th>
<th>$r$</th>
<th>$D_{10}$ kGy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number 1</td>
<td>$1.4 \times 10^4$</td>
<td>$7.8 \pm 0.3$</td>
<td>7</td>
<td>1.9</td>
</tr>
<tr>
<td>Number 2</td>
<td>$1.2 \times 10^4$</td>
<td>$9.6 \pm 0.4$</td>
<td>12</td>
<td>2.2</td>
</tr>
<tr>
<td>Number 3</td>
<td>$3.5 \times 10^4$</td>
<td>$8.2 \pm 0.4$</td>
<td>15</td>
<td>1.6</td>
</tr>
<tr>
<td>Number 4</td>
<td>$3.5 \times 10^4$</td>
<td>$10.1 \pm 0.3$</td>
<td>18</td>
<td>1.8</td>
</tr>
<tr>
<td>Average $D_{10}$ Value</td>
<td></td>
<td></td>
<td></td>
<td>1.9</td>
</tr>
</tbody>
</table>

As seen from the data in Table 2, $D_{10}$ values varied by $\pm 0.3$ kGy about an average value of 1.9 kGy. Part of the variability in the calculated values for $D_{10}$, i.e., $\sim 0.1$ kGy, may be attributed to the uncertainty in the delivered dose. The uncertainty in the number of CFU in the inoculum contributes less than 0.1 kGy to the uncertainty in the $D_{10}$ value. 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.

**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,

$$D = D_{10} (\log N_i - \log 10^{-6})$$  \hspace{1cm} (2)
In estimating the average bioburden that may be present on a pharmaceutical product following a fill-finish operation, it is assumed that the aseptic area employs isolator technology without human intervention.

An estimate for the average bioburden ($N_i$) can be based on results of a media fill consisting of 1000 units. If analysis of the 1000 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 1.9 kGy, it can be seen from Equation (2) that the dose required to satisfy an SAL of $10^{-6}$ is,

$$D = 1.8 \text{ kGy} \left( \log 10^{-3} - \log 10^{-6} \right) = 5.7 \text{ kGy}.$$ 

Arguments can be advanced for the case where there may be one contaminated media fill unit in 1000 units. However, this would require an estimate of the maximum number of microorganisms that may be present on the contaminated unit before there is a significant probability of finding more than one contaminated media fill unit. The presence of one contaminated unit would clearly increase the sterilization dose from the value estimated for no contaminated units.

As seen from this 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 less than 10 kGy. At these low doses, many types of pharmaceutical products should be radiation compatible.

**Conclusions**

A test method, which involved 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. This method 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 environmental monitoring of an aseptic manufacturing operation. The test microorganism was a spore forming, Gram-positive microorganism, which is highly resistant to radiation. Because of the extremely clean conditions that exist in an aseptic area, it was possible to establish sterilization doses significantly less than 10 kGy. At these low doses, it should be possible to use radiation to sterilize many types of radiation sensitive drug 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 thank Sterigenics International for support of this study.
References


