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IRRADIATION OF COSMETICS AND HEALTH PRODUCTS AND LABORATORY MEASUREMENTS FOR PRECISE DOSE AS COMPARED TO PHARMACEUTICALS AND DEVICES

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ABSTRACT:

The methods for irradiating drugs, devices, cosmetics and health aids are reviewed. A better understanding of the use of irradiation to control microbial content of health and beauty aids involves a knowledge of the biological and mathematical factors that control the rate and extent of lethality for various types of organisms under various conditions. Dose-survivor curves and calculations of the D-value are presented as well as the differences in the degrees of irradiation required for resistant forms (spores) as opposed to more sensitive forms (the usual pathogens). Two approaches for a rational determination of the minimal dose are described.

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Introduction:

The use of ionizing irradiation is now standard practice in the cosmetic industry as well as the less well defined areas of cosmaceuticals and nutraceuticals. The greatest use of irradiation historically has been in the device industry with the initial guidance promulgated in 1975 (USP 19, MACK, Publishing, Easton, PA) and the AAMI Guidelines of 1984, which have evolved into dose-setting standards that are internationally acceptable (ISO Guidelines 11737-1, 11737-2, 11137). Official guidelines have not been issued for drugs, although the Parenteral Drug Association has promulgated methods of sterilization (Tech. Report No. 11, PDA, Journal Parenteral Science and Technology, 1988:42). The cosmetic industry has made effective use of irradiation for raw materials and finished product. Often the dose of irradiation is merely an estimate that covers a given wide minimum to maximum range, for example, 2 to 10 kGy, or even wider for certain materials of natural origin (8 to 30 kGy). It is the purpose of this paper to describe some of the lethal effects of gamma irradiation on bacteria that occur with the above

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circumstances and how one might more accurately predict minimum doses from (a) better knowledge of the contamination population and (b) the shape of dose survival curves.

A. Organisms: Their Nature And Their Population Dynamics

The population usually consists of spore forming bacteria (genera *Bacillus, Clostridium*) and non-sporing vegetative organisms (*E. coli, Pseudomonas, Staphylococcus,* yeast and mold, etc.). The more "natural" a product or ingredient, the higher and more diverse the microbial flora. The bacterial spores are both dessication-resistant and resistant to irradiation, with a D-value range of 0.6 to 4.0 kGy (Silverman, G.J., in Block, ed., Disinfection, Sterilization and Preservation, 4th ed., Lea and Febiger; 566). The vegetative organisms in general have D-values ranging from 0.06 to 2.8 kGy. In any irradiation study it is important to distinguish between these two populations.

The growth of microorganisms in raw materials and finished products is a function of water content (Aw) and nutrients. An initial concentration of bacteria can decline or increase with age and the magnitude of these changes can offer insight into the nature of the population. The required dose of irradiation is related to this bioburden dynamic. Without routine quality control of pre-irradiated material, especially articles of natural origin, the bioburden is unknown and the dose can be a guess. If batch-by-batch testing is not performed, an audit should be set-up to establish a "worst case" condition for total microbial bioburden. This requires either growth studies or die-off studies, depending upon water content. Die-off rates are described as the dessication D-value and are calculated by the Schmidt and Nank formula, except that the numerator is time (T), for example:

$$D = \frac{T}{\text{Log}N_0 - \text{Log}N_1}$$

Where N_0 (1×10^5) is the initial count of a material and N_1 (1×10^3) is the count 90 days later. Thus, D = 90 ÷ 2 = 45 days

This die-off rate is, of course, temperature dependent. Every 45 days under specified conditions of relative humidity and temperature, the count will decline 90%.

On the other hand, growth and not die-off may occur. In this case, it is possible to determine the "doubling" or generation time of the pure or mixed flora. Here the expression of Cleverdon and Kulp (1955, Kinetics of Bacterial Growth, Graduate Proceedings, University of Connecticut, Storrs, CT) is employed: g = t/n, where g = generation time, t = the incubation time, and n = the number of generations during the incubation time. An example of this is given in one of the earliest irradiation experiments in cosmetics (Prince, H.N. and Welt, M.A., American Perfume and Cosmetics, August 1971, "Studies On The Sterilizing Effect of Gamma Irradiation"). In that work it was shown that a heavily contaminated organic pigment increased in count from 10,000 to 125,000 cfu/gram in 6 weeks at room temperature, giving a generation time of 84 hours. The application of a suitable elevated storage temperature can prevent such multiplication and can act as a "self-sanitizing" mechanism prior to irradiation. Typical organisms encountered in such populations are *E. coli, Proteus, Serratia, Clostridium, Enterobacter, Pseudomonas*, molds, yeasts, and *Salmonella*, members of the genera *Enterococcus, Bacillus, Micrococcus, Streptococcus, Staphylococcus* and various other environmental and/or fecal forms. All of these are usually irradiation sensitive, except for certain gram positive *cocci.*

B. The Heat Shock Experiment

The heat shock experiment can assist in determining an appropriate irradiation dose since it separates the irradiation-sensitive forms from the resistant forms (spores). **Table 1** shows a representative change in count following a heat-shock experiment as applied to materials of natural origin (botanical, animal, marine). In this model, the sample is suspended, dissolved or emulsified in an aqueous medium and the microbial population is determined. The sample is then heated to 60 to 80C for 10 to 15 minutes and the count repeated. The difference in total plate count (TPC) reveals the percentage of the population that are spores. **Table 2** shows the effect of various temperatures on determination of spore population.

Microscopic views of the various bacterial and mold forms are shown in **Figures 1-3.** It should be pointed out that the bacterial spores (**Figure 1**) are more resistant than the mold spores (**Figure 3**). Viruses are poorly studied but can be resistant to irradiation when kept viable and have variable degrees of dessication resistance (Prince et al 1990 Transmission of Viruses in Block, ed., Lea & Febiger, Phil.).

C. The Dose Response Irradiation Experiment

Organisms within or inoculated into a product are exposed to graded doses of irradiation ranging, e.g., from 0.5 to 10 kGy. Replicate samples are then quantitated for survivors and the $\log_{10}$ survivors plotted against dose. Dose-survival curves are then constructed as shown in **Figures 4-7.** From the shape of the curve one can (a) deduce something about the nature of the bioburden and (b) calculate the radiation resistance or D_{10}-value. The D_{10}-value can also be calculated from the Schmidt and Nank formula (Schmidt, C.F. and Nank, W.K., 1960, Food Research 25:321):

$$D_{10} = \frac{\text{kGy}}{\log_{10} N_0 - \log_{10} N_1}$$

where N_0 is the initial population and N_1 the survivors.

Curve #1, (Figure 4), is a biphasic curve and indicates the presence of a population of dual sensitivity. The sensitive population consists of less resistant spores or vegetative organisms and the resistant population consists of more resistant forms. We now know that such resistant forms are primarily members of the genera *Bacillus, Micrococcus* or *Cryptococcus*. One notes that by extending the low-dose portion of the curve at the break point to the X-axis, a sensitive population is depicted. The dual population can consist of variants or dissociates of the same species, different species of the same genus, or different genera.

Curve #2, (Figure 5), is a multi-hit or shoulder curve and usually indicates the presence of clumped bacteria, radio-protective protein, or the need for multiple DNA targeting. A threshold accumulation of ionizations are required for the initiation of death. The number of kGy in this threshold area of the dose-survivor curve are added to the value as calculated from the straight-line portion of the curve. If excluded, the resistivity of the population is underestimated.

Curve #3, (Figure 6), is typical of a relatively sensitive population and **Curve #4 (Figure 7)** depicts the survival characteristics of highly sensitive vegetative forms, such as most of the common pathogens.

Such survival curves usually denote the absence of radio-protective materials and the absence of spores of the genera *Bacillus* or *Clostridium*, or the absence of resistant micrococci or cryptococci.

The dose-survival curves shown in **Figures 4-7** can be constructed by lethality experiments with either pure or mixed cultures isolated from products or by applying graded doses of irradiation to highly contaminated materials themselves. If freedom from pathogens and not sterility is required, pathogen survivals as well as total count are determined at each dose.

D. Calculation Of The Dose From Plate Count Data

Background

In determining microbial content prior to irradiation or other terminal processes (heat, ETO) there are essentially three product-specific techniques.

1. Devices: A Bioburden Approach

We refer to bioburden when we are testing devices and not plate count. The most common approach is the AAMI-Method 1 Bioburden Experiment. It is FDA-sanctioned. The bioburden is determined by elution. A verification dose in kGy promulgated to give a 10^{-2} probability of sterility is read from a standard AAMI table (See **Table 3** for an abbreviated version). One hundred samples are irradiated at this dose and the verification audit is acceptable if no more than 2 of 100 are non-sterile. If this occurs, the product is then dosimetrically released by applying the 10^{-6} dose in kGy (SAL-6)[1] as read from the same table of standard doses. Another method, AAMI-Method 2, is not bioburden-based but provides a sterility assurance based on incremental doses in a dose-response. The AAMI methods are intended for devices but are sometimes employed for drugs or cosmetics. This cannot always be defended. The preliminary validation steps for elution and enumeration of bioburden are specific for devices, articles essentially compounded of plastic, ceramics and metal, products lacking in either water or the potentially radio-protective organic content of drugs and cosmetics.

[1] Sterility Assurance Level 10^{-6}

2. Drugs: A Biological Indicator Approach

Few drugs are sterilized by irradiation. The most detailed discussion appears in the PDA Technical 1988 report mentioned earlier. No official method has appeared. Spore inoculation and spore strip experiments have been performed as mentioned earlier and the D-value determined for *Bacillus pumilis* E-601, or the most resistant spore found either in the product or in the environment. This value (e.g., 1.8 kGy) is then multiplied by the log kill required to achieve an SAL of 10^{-6} kGy). Thus, if the bioburden is 10^2, the dose would be what is required for an 8-log reduction (1.8 x 8 = 14.4 kGy).

3. Health and Beauty Aids: A Composite Approach

Most cosmetics have a low and non-pathogenic flora and are well controlled by GMP and preservatives. When irradiation of creams, lotions, powders, talc, eye products, etc., is employed it is usually accomplished with doses adequate to affect the desired kill but low enough for formulation and package integrity, especially if "actives" are involved. Problems occur more frequently with yeasts and molds than with bacteria, with the exception that (a) bio-film outbreaks, during which encrustation of bacteria never seen before by the preservative are released into the product during manufacture, and/or (b) the use of inadequately tested preservative systems. With such preservative problems, a delayed outgrowth of "wild-type" organisms may not show up for days in a bio-film outbreak, producing the aggravating circumstance of products failing that never failed in the past. With health and beauty aids, including nutraceuticals, high bioburdens are less associated with bio-film than with the filth of the materials themselves. Such materials come from the earth, animals, vegetation, or the sea, areas rich in spores, gram negative rods, pyogenic cocci, anaerobes, pathogenic yeasts and toxigenic fungi.

High bioburden materials or re-work products harboring organisms that potentially put the product (spoilage) or the consumer (infection) at risk ought to receivea validated minimal dose. A recommended approach is a cross between the device method (bioburden) and