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A maximum microbial burden is allowable on spacecraft items, whether parts or subassemblies, prior to final heat sterilization. This report is concerned with the distribution of measurements based on bioassays which would be taken to see if the maximum tolerance is exceeded. The sources of variation involved in such measurements are of particular concern.

A measurement of microbial load, denoted by X, may be represented by the model

$$X = \mu + \alpha + K(\beta + \gamma). \qquad (1)$$

Actually, X is a measurement of mean load for the population from which the item being tested is drawn. In the bioassay of spacecraft components it is not always possible to test a component which will go into the spacecraft. For instance, in order to measure the internal contamination of a small electrical component, the component would be destroyed. Instead of testing items actually used in the spacecraft, items which have been manufactured and handled in the same manner are often assayed. The assayed items and the corresponding item in the spacecraft are assumed to have been randomly selected from the same population. The mean of this population is represented by the term μ in equation (1). The term α in (1) refers to the deviation of the true microbial load for the item assayed from μ. The total load for an assayed item is therefore $\mu + \alpha$.

In addition to variation from one item to another of the same type, there is variation in microbial density over the surface and interior of an item. For some large items, only a small portion would be tested in a bioassay. Let us think of an item as consisting of K units, $K \geq 1$, each of which is of the same size and is small enough to be assayed. If only one unit of an item were selected for assay, its true microbial load would be likely to deviate from the average load per unit for that item, and that deviation is represented in the model given by equation (1) by the symbol β. Moreover, the bioassay measurement of the microbial load for an item would likely deviate from the true load. This deviation is denoted by γ in (1). The total of β and γ is multiplied by K since we are interested in the total load per item, not just the load for a unit of an item selected for assay.

It is reasonable to assume that the expected values, that is, the means over the entire population, of the terms in (1) are given by $E(\alpha) = E(\beta) = E(\gamma) = 0$. The term μ is thought of as a constant, since it is a population parameter which does not vary from one item to another within the population. Therefore, $E(X) = \mu$ and the measurement X we are dealing with is an unbiased estimate of the population mean. Let us also make the reasonable assumption that the sources of variation given in equation (1) are independent of each other. If we let V denote a variance, we may then write

$$V(X) = V(\alpha) + K^2 \, [V(\beta) + V(\gamma)] \ . \qquad (2)$$

The variances in equation (2) for a given type of item could be estimated from experimental data for each type of spacecraft item. A nested, that is, hierarchal, experiment design would be appropriate followed by the analysis of variance for a random effects model, which gives the information needed for the estimation of variance components. Since data from such experiments are not presently available, we shall examine the V(X) as given by (2) on the basis of known information on microbial assays.

If the probability that a microorganism will contaminate a unit is independent of the previous contamination of that unit and is constant for units of equal size, then the distribution of counts of microorganisms per unit would be Poisson. Since the mean and variance of the Poisson are equal and the expected count per unit is μ/K, we may take $V(\beta) = \mu/K$. If these assumptions are not satisfied, clumping of organisms would tend to occur, and the $V(\beta)$ would be greater than μ/K. Furthermore, the sum of Poisson random variables is also Poisson, so $\mu + \alpha$, the total microbial load for an item, would also be expected to have a variance at least as large as its expected value μ. Since μ is constant, we may take $V(\alpha) = \mu$. The other variance, $V(\gamma)$, is for many microbial assays proportional to the square of the mean microbial count per unit. This mean is μ/K for our model. So we shall represent $V(\gamma)$ by $C^2\mu^2/K^2$, where C is positive and C^2 is a proportionality constant. Substitution of these variance expressions in (2) gives, as a minimal variance,

$$V(X) = \mu + K^2[(\mu/K) + C^2(\mu^2/K^2)] \ . \tag{3}$$

Equation (3) would be more easily interpreted in we expressed it in terms of $\lambda = \mu/K$, since if possible assay units are selected so that the mean count per unit is small, certainly less than 1000 and preferably less than 300. This enables separate colonies to be more easily identified. Substituting $K = \mu/\lambda$ in (3) and simplifying, we have

$$V(X) = \mu + \frac{\mu^2}{\lambda}[1 + C^2\lambda] \ . \tag{4}$$

This may be rewritten as

$$V(X) = \mu^2 \left[\frac{1}{\mu} + \frac{1}{\lambda} + C^2 \right]. \tag{5}$$

If both μ and λ are large, equation (5) would lead to the approximation

$$V(X) \doteq C^2\mu^2$$

or in terms of the standard deviation of X, denoted by σ_X,

$$\sigma_X \doteq C\,\mu.$$

The constant C would likely be small, say less than 0.5 or even as small as 0.1. This is the range considered in the December 10, 1965 Avco report to NASA entitled "Sample Size Considerations for Voyager Capsule Sterilization Assays." However, if λ were small, say 1 or 2, and μ were large, minimum values of V(X), based on our Poisson assumptions and the range of C mentioned earlier, could be as high as $1.25\mu^2$, which would mean that σ_X could be greater than $E(X) = \mu$. That is, if an item has a large microbial burden but the expected number of microorganisms per assay unit within that item is small, then σ_X could be greater than E(X), where X is based on a single assay of one unit. This implies that larger values of σ_X should be considered than those studied in the Avco report. The reason that they did not investigate larger values of σ_X relative to μ is that they did not seriously consider sources of variation in the model (1) other than γ, the error introduced by the bioassay, and they did not allow for the multiplication in our model by K, the number of units per item.

In practice, instead of taking our minimum value of V(X) as being correct, it would be better to estimate V(X) and its square root, σ_X, on the basis of experimental evidence in the manner indicated earlier. In using estimated values of μ and σ_X, it should be kept in mind that the population mean, μ, is not of primary interest. At least when the item used in the spacecraft is not the one assayed, μ is not of primary interest.

Instead estimates of the population parameters μ and σ_X would be used to compute an estimate of the probability that another item selected randomly from that same population would have a satisfactorily small microbial load. This probability would be required to be very close to one. The Avco report is only concerned with the probability that the estimate of μ itself is less than the maximum allowable microbial load. This would seem to be the appropriate probability to study only if the item assayed is the one to be used in the spacecraft. This would sometimes be the case. The equations worked out earlier in this paper would still apply in this situation if α were deleted from equation (1), $V(\alpha)$ were deleted from equation (2) and the corresponding variance terms, including $\frac{1}{\mu}$ inside the parenthesis in equation (5), were deleted from the other equations. Also, the discussion of equation (5) would still be pertinent since in that discussion $\frac{1}{\mu}$ was assumed to be negligible, that is, μ was assumed to be large.

If the $V(X)$ were found to be unacceptably large, it could be reduced by assaying several units within an item or by measuring several items of the same type. Then the sample mean of such measurements would be used to estimate μ and the standard error of this sample mean would be of interest instead of σ_X. In this situation the model given by equation (1) would be written as

$$X_{ij} = \mu + \alpha_i + K(\beta_{ij} + \gamma_{ij}), \quad i = 1,2,\ldots,k;\ j = 1,2,\ldots,n$$

where the subscript i refers to the $i^{\underline{th}}$ item measured and the double subscript ij refers to the $j^{\underline{th}}$ unit assayed for the $i^{\underline{th}}$ item. It is assumed that an equal number of units would be assayed for each item.

The mean which would be used to estimate μ would be

$$\overline{X} = \frac{1}{k} \sum_{i=1}^{k} \overline{X}_i$$

where

$$\overline{X}_i = \frac{1}{n} \sum_{j=1}^{n} X_{ij} .$$

The variance of $\overline{X}$ would be given by

$$V(\overline{X}) = \frac{V(\alpha)}{k} + K^2 \left[\frac{V(\beta)}{nk} + \frac{V(\gamma)}{nk} \right] ,$$

assuming mutual independence of and equal distributions for all of the β_{ij}, and of the γ_{ij} terms in equation (6). The standard error of $\overline{X}$, denoted by $\sigma_{\overline{X}}$, would be equal to the square root of $V(\overline{X})$.

If this assay procedure were followed, a decision would have to be made regarding the number of items and units within items to be measured, that is, regarding the sizes of k and n. The relative magnitudes of k and n selected would depend on the relative magnitude of $V(\alpha)$ as compared with $V(\beta) + V(\gamma)$. Under the assumptions which led from equation (2) to equation (5), it would seem reasonable to take n large relative to k. In the Avco report the possibility of taking n greater than one is considered while k is held equal to one since consideration is not given to assaying items other than those in the spacecraft. However, final recommendations on the approximate sizes of k and n should be delayed until experimental evidence is available on the magnitudes of $V(\alpha)$, $V(\beta)$ and $V(\gamma)$. This is contrary to the recommendation in the Avco report that n be approximately equal to 2 with k equal to one.