DILUTION MODEL:

A BAYESIAN APPROACH

By

Andres Petrasovits

Technical Report Number 6

(Preliminary Report)

NASA Grant Number NGR-10-004-029

August 24, 1966

Department of Statistics
Florida State University
Tallahassee, Florida

INTRODUCTION

The dilution method is a means for estimating, without any direct count, the density, λ, of organisms in a liquid. The method consists in taking samples from the liquid, incubating each sample in a suitable culture medium, and observing whether any growth of organisms has taken place.

The probability model behind the dilution method is based on two main assumptions: (i) the organisms are distributed randomly throughout the liquid and (ii) each sample from the liquid when incubated in the culture medium is certain to exhibit growth whenever the sample contains one or more organisms.

Any dilution may be represented by a dose variate, d, such that the density in the dilution is λd per unit volume. Usually, d will be less than unity but values greater than unity (corresponding to concentrations of the original suspension) can be treated with the same theory. Throughout this paper we shall consider values of d between 0 and ∞. It follows then from assumptions (i) and (ii) stated in the first paragraph that the number of organisms per sample, in samples of unit volume with dilution d, follows a Poisson distribution with mean λd.

Typically, samples of size $n_1,\ldots,n_k$ are taken from k preparations corresponding to dilution levels $d_1,\ldots,d_k$. By sample size here we mean the number of plates taken from each preparation to be examined for growth. Denote by $x_1,\ldots,x_k$ the observed numbers of sterile plates corresponding to dilution levels $d_1,\ldots,d_k$. Based on this information, the problem of (a) estimating the bacterial density and (b) planning of the experiment have been copiously treated in the literature. The writings on the subject, however, do not incorporate in a formal manner the previous knowledge which the experimenter may have about the bacterial density. Thus I propose to investigate problems (a) and (b) using a Bayesian approach. In

particular, I plan to organize the work as follows: (1) Bayes estimation of the bacterial density for a given set of dilution levels $d_1,\ldots,d_k$ and sample sizes $n_1,\ldots,n_k$. (2) Design of the experiment and estimation of the bacterial density, making use of the existing prior information about the bacterial density, so as to minimize the expected value of an appropriate cost function.

As future work we plan to insert a discussion of the problems involved in the choice of a prior distribution and cost functions appropriate for the problem under consideration. However in this essay, in order to get some outright idea of the type of algebra and results to be expected, we assume without further discussion that (i) a gamma prior on λ is adequate for a Bayesian analysis of the problem, (ii) the planning of the experiment concerns only the specification of the dilution levels which minimize the Bayes risk corresponding to a quadratic loss function. The cost of the observations is temporarily disregarded here and the sample size is assumed to be fixed. A thorough treatment of problems (i) and (ii) for different cost functions is to be included as part of the future plans.

Section I of this report deals with review of literature. Section II is concerned with problem (1). In Section III, for the case often encountered of successive experimentation, we discuss a method to estimate the parameter λ and design the experiment making use at each instance of all the available information about the bacterial density. Section IV deals with polygamma forms for expressions in Sections II and III. It is hoped that this will help to simplify the computations and will assist in the development of numerical methods when needed. Future plans are discussed in Section V.

SECTION I

REVIEW OF LITERATURE

For many years, bacteriologists have been using dilution methods to give some idea of the number of organisms in the material examined. The problem of estimating the bacterial density, λ, from this type of quantal experiment has long interested bacteriologists and statisticians. McCrady [11] and Greenwood and Yule [6] introduced the "most probable number" method which turns out to be essentially the maximum likelihood procedure. Fisher [5] indicated the method of maximum likelihood and in particular considered the model with expectation

$$E(y) = 1 - \exp(-\rho a^{-w}) , \quad 0 \leq y \leq 1 , \quad \rho > 0 , \quad a > 0 , \tag{1.1}$$

with the values of w equally spaced at unit intervals. Halvorson and Ziegler [7] have given tables from which the maximum likelihood estimate can be read directly for certain assay designs. Finney [3,4] suggested that the maximum likelihood estimate of λ may be obtained by the same type of iterative process as that used in probit analysis. Peto [12] gives tables to facilitate the solution of the maximum likelihood equation and discusses the experimental verification of the hypothesis.

The estimation of the bacterial density in dilution experiments has also been approached from the point of view of Spearman estimation. Johnson and Brown [8] have discussed the model with expectation

$$E(y) = 1 - e^{-\rho d} , \quad 0 \leq y \leq 1 , \quad \rho > 0 \tag{1.2}$$

and with $d = e^{t}$ for equally spaced values of t. The relationship between this model and the one given by Fisher [5] is discussed by Cornell [2].

A Bayesian approach to the bioassay problem is discussed by Kraft and Van Eeden [9]. The authors give a characterization of the class of all prior

distributions, they find the corresponding Bayes estimates for a class of loss functions and they show the completeness of the closure of this type of estimates for a certain topology. A special case is given for which the estimates can be explicitly computed. Thorslund [14] considers explicitly the dilution model, imposes a natural conjugate prior on the quantity $p = e^{-\lambda d}$ and gives the first two moments of $-\ell n\ \hat{p}$ in the form of polygamma functions.

The design problem, which consists of determining optimal (in some sense) dilution levels, has been less copiously treated. From a consideration of the approximate variance of $\hat{\lambda}$ with a single dilution level and sample size one, Finney [4] recommends that d should be chosen so as to satisfy

$$1 < \lambda d < 2 \tag{1.3}$$

Thus, if λ_L and λ_u are believed by the experimenter to be lower and upper bounds respectively for the bacterial density λ, the dilution level should be chosen so as to satisfy

$$\frac{1}{\lambda_u} < d < \frac{2}{\lambda_L} \quad . \tag{1.4}$$

Cochran [1] points out that this rule is satisfactory if a substantial number of samples, say 20 or more, are being taken at each dilution level. With very small numbers of samples per dilution, the rule (1.4) is not quite stringent enough, in that it allows too much risk that all the samples may be fertile.

SECTION II

Bayes estimate of λ with fixed sample sizes and dilution levels.

Let $x_1,\ldots,x_k$ be the number of sterile plates observed in samples of sizes $n_1,\ldots,n_k$ corresponding to dilution levels $d_1,\ldots,d_k$, respectively.

It is intended to find the Bayes estimate, $\hat{\lambda}$, of the bacterial density. We assume that (i) a gamma prior distribution for the parameter λ,

$$g(\lambda) = \frac{1}{\Gamma(\alpha+1)\beta^{\alpha+1}} e^{-\frac{\lambda}{\beta}} \lambda^{\alpha} , \quad 0 < \lambda < \quad , \quad \alpha > -1 , \quad \beta > 0 , \qquad (2.1)$$

is specified based on the experimenter's previous belief, (ii) a quadratic loss function

$$L(\lambda,\hat{\lambda}) = (\lambda-\hat{\lambda})^2 \qquad (2.2)$$

is considered to be an adequate measure of regret for failure to estimate exactly the parameter λ.

It should be noted that the prior distribution $g(\lambda)$ is not a natural conjugate. In fact, following Raiffa and Schlaifer [13], we observe that no procedure to construct a natural conjugate prior is available in this case since the sufficient statistic is $(x_1,\ldots,x_k)$ and it does not admit further reduction.

From a result in Lehmann [10, p. 23], the Bayes estimate of λ, when a quadratic loss function is used, is given by

$$\hat{\lambda} = \int_0^{\infty} \lambda \, p(\lambda|x) \, d\lambda , \qquad (2.3)$$

where $p(\lambda|x)$ is the posterior density of λ.

To obtain $p(\lambda|x)$ we recall that

$$p(\lambda|x) = \frac{\ell(x|\lambda)g(\lambda)}{\int_0^\infty \ell(x|\lambda)g(\lambda)d\lambda} \quad , \tag{2.4}$$

where $\ell(x|\lambda)$ is the likelihood of the observation vector.

From the remarks made in the first paragraph of the Introduction, it follows that

$$\ell(x|\lambda) = \prod_{i=1}^{k} \binom{n_i}{x_i} e^{-\lambda d_i x_i} \left(1-e^{-\lambda d_i}\right)^{n_i - x_i} \quad . \tag{2.5}$$

If we define

$$D_k(\lambda,n,x,d,\beta,\gamma) = \sum_{i_1=0}^{n_1-x_1} \cdots \sum_{i_k=0}^{n_k-x_k} (-1)^{i_1+\ldots+i_k} \frac{e^{-\lambda\left[\sum_{j=1}^{k} d_j i_j + \sum_{j=1}^{k} d_j x_j + \frac{1}{\beta}\right]}}{\binom{n_1-x_1}{i_1}\cdots\binom{n_k-x_k}{i_k}} \lambda^{\gamma}, \tag{2.6}$$

we can write

$$\ell(x|\lambda) = \prod_{i=1}^{k} \binom{n_i}{x_i} D_k(\lambda,n,x,d,\infty,0) \quad . \tag{2.7}$$

It is also convenient to define

$$S_k(n,x,d,\beta,\gamma) = \sum_{i_1=0}^{n_1-x_1} \cdots \sum_{i_k=0}^{n_k-x_k} (-1)^{i_1+\ldots+i_k} \frac{\binom{n_1-x_1}{i_1}\cdots\binom{n_k-x_k}{i_k}}{\left[\sum_{j=1}^{k} d_j i_j + \sum_{j=1}^{k} d_j x_j + \frac{1}{\beta}\right]^{\gamma}} \quad . \tag{2.8}$$

Then it can be shown that the posterior density of λ, given x, is

$$p(\lambda|x) = \frac{D_k(\lambda,n,x,d,\beta,\alpha)}{\Gamma(\alpha+1)S_k(n,x,d,\beta,\alpha)} \tag{2.9}$$

and the Bayes estimate of λ is

$$\hat{\lambda} = (\alpha+1) \frac{S_k(n,x,d,\beta,\alpha+2)}{S_k(n,x,d,\beta,\alpha+1)} \quad . \tag{2.10}$$

The Bayes risk which is equal to

$$R(g,d) = \int_0^\infty E_x(\lambda-\hat{\lambda})^2 \, g(\lambda) \, d\lambda \, , \tag{2.11}$$

reduces to

$$R(g,d) = (\alpha+2)\ (\alpha+1)\ \beta^2 - \frac{(\alpha+1)^2}{\beta^{\alpha+1}} \sum_{x_1=0}^{n_1} \cdots \sum_{x_k=0}^{n_k} \prod_{i=1}^{k} \binom{n_i}{x_i} \frac{\left[S_k(n,x,d,\beta,\alpha+2)\right]^2}{S_k(n,x,d,\beta,\alpha+1)}. \tag{2.12}$$

The expressions obtained for the parameter estimate and the Bayes risk are not easy to compute as it would be desirable for the results to have a practical significance. As future work, we intend to consider asymptotic forms or approximations in order to get the results into a useful form.

SECTION III

Estimation and Design with Successive Experimentation.

The design of the typical dilution experiment involves the determination of the following elements:

(i) Number of dilution levels: k

(ii) Actual dilution levels: $d = (d_1,\ldots,d_k)$

(iii) Number of samples to be observed at each level: $n = (n_1,\ldots,n_k)$.

Thus the design of a dilution experiment can be represented with the notation: $e = (k,d,n)$. It is common laboratory practice to choose d according to a geometric or logarithmic scaling. If we denote by d_i the i^{th} dilution, by geometric scaling we mean that $d_i = a^{-(i-1)}$, for $i = 1,\ldots,k$ and $a > 0$. The experiment can then be represented by $e = (k,a,n)$. By logarithmic scaling we mean that $d_i = e^{w_0+(i-1)s}$, for $i = 1,\ldots,k$ and $-\infty < w_0 < \infty$, $s > 0$. In this case the experiment can be denoted by $e = (k,w_0,s,n)$.

It is our purpose to investigate in the future, for dissertation work, the design of the experiment with respect to all the arguments involved in e, and we intend to do this with a Bayesian approach in order to make use of all the available information, subjective or experimental, in designing the experiment. If the experimenter's subjective knowledge about the bacterial density, λ, is represented by a prior distribution, $g(\lambda)$, and if experimental data, x, from a previous experiment, e, are available, we want to consider the problem of designing the next experiment, e', if one is to be performed, so as to minimize the expected cost associated with an appropriate cost function and incorporating g, x and e in our design procedure.

A treatment of the problem with such a generality will not be attempted here. We shall limit ourselves to report the work which has been done in a simplified version of the design problem which has been described above.

In what follows we assume that

a) a gamma prior distribution, as defined in (2.1), represents the experimenter's convictions about λ,

b) empirical information, $x = (x_1,\ldots,x_k)$, corresponding to a previous experiment, $e = (k,d,n)$, is available. The x's are assumed to be independent.

c) An experiment, e', is to be designed for which we assume:

(c') $k' = 1$

(c") the sample size, n', is fixed

(c"') a quadratic loss function, as defined in (2.2) will be used to compute the Bayes risk.

The aim will be to design an experiment, $e' = (d')$, choosing d' so as to minimize the Bayes risk: $R(g,e,x,d')$. It is hoped that consideration of this simplified case will throw light into the difficulties to be encountered in a general treatment of the design problem.

Let x' be the number of sterile plates observed at dilution level d' and define

$$\begin{aligned} \bar{x} &= (x_1,\ldots,x_k,x') \\ \bar{n} &= (n_1,\ldots,n_k,n') \\ \bar{d} &= (d_1,\ldots,d_k,d') \,. \end{aligned} \tag{3.1}$$

The Bayes risk will be given by

$$R(g,e,x,d') = \int_0^\infty E_{x'}(\lambda-\hat{\lambda})\, p(\lambda|x)\, d\lambda$$

$$= \int_0^\infty \sum_{x'=0}^{n'} (\lambda-\hat{\lambda})^2\, \ell(x'|\lambda)\, p(\lambda|x)\, d\lambda \quad , \tag{3.2}$$

where $p(\lambda|x)$ is given by (2.11),

$$\ell(x'|\lambda) = \binom{n'}{x'} e^{-\lambda d' x'} (1-e^{-\lambda d'})^{n'-x'}$$

$$= \binom{n'}{x'} D_1(\lambda, x', n', d', \infty, 0) \tag{3.3}$$

and $D_1(\lambda,x',n',d',\infty,0)$ is as defined in (2.6). To obtain the estimate, λ, we compute

$$\hat{\lambda} = \int_0^\infty \lambda\, p(\lambda|\bar{x})\, d\lambda \quad , \tag{3.4}$$

where

$$p(\lambda|\bar{x}) = \frac{\ell(x'|\lambda)p(\lambda|x)}{\int_0^\infty \ell(x'|\lambda)p(\lambda|x)d\lambda} \tag{3.5}$$

If we substitute for $p(\lambda|x)$ expression (2.9) into (3.5) we have, since $x_1,\ldots,x_k$, x' are independent,

$$p(\lambda|\bar{x}) = \frac{\ell(\bar{x}|\lambda)g(\lambda)}{\int_0^\infty \ell(\bar{x}|\lambda)g(\lambda)d\lambda} \quad . \tag{3.6}$$

Comparing (3.6) with (2.4) we see that $p(\lambda|\bar{x})$ can be obtained directly from (2.9) using $\bar{x}$, $\bar{n}$ and $\bar{d}$ instead of x, n and d.

From (2.10) we have that

$$\hat{\lambda} = (\alpha+1) \frac{S_{k+1}(\bar{n},\bar{x},\bar{d},\beta,\alpha+2)}{S_{k+1}(\bar{n},\bar{x},\bar{d},\beta,\alpha+1)} . \tag{3.7}$$

If we substitute in (3.2) for λ, $\ell(x'|\lambda)$ and $p(\lambda|x)$ expressions (3.7) and (2.9) respectively we have that the expression for the Bayes risk reduces to

$$R(g,e,x,d') = (\alpha+2)(\alpha+1) \frac{S_k(n_1 x,d,\beta,\alpha+3)}{S_k(n_1 x,d,\beta,\alpha+1)} - \frac{(\alpha+1)^2 C(d')}{S_k(n,x,d,\beta,\alpha+1)} , \tag{3.8}$$

where

$$C(d') = \sum_{x'=0}^{n'} \binom{n'}{x'} \frac{\left[S_{k+1}(\bar{n},\bar{x},\bar{d},\beta,\alpha+2)\right]^2}{S_{k+1}(\bar{n},\bar{x},\bar{d},\beta,\alpha+1)} . \tag{3.9}$$

The experiment will be designed, with respect to the dilution level, if δ' is found such that $R(g,e,x,\delta')$ is a minimum. To prove the existence of a minimum we note that $R(g,e,x,d')$ as given in (3.8), depends on d' only through $C(d')$. Further, $C(d')$ is always a positive quantity because the denominator of each term in the sum (3.9) can be expressed as an integral whose integrand is always positive. In particular we have

$$S_{k+1}(\bar{n},\bar{x},\bar{d},\beta,\alpha+1) = \Gamma^{-1}(\alpha+1) \int_0^\infty D_{k+1}(\lambda,\bar{n},\bar{x},\bar{d},\beta,\alpha)\, d\lambda, \tag{3.10}$$

where $D_{k+1}(\lambda,n,x,d,\beta,\alpha)$ is as defined in (2.6). Thus $C(d')$ is positive and bounded

for $0 < d' < \infty$. $C(d')$ is also a continuous function for $0 < d' < \infty$. Further, it is easy to show that

$$\lim_{d' \to \infty} C(d') = \blacksquare / \beta^{\alpha+3} \tag{3.11}$$

and by repeated use of L' Hopital's rule and of an induction argument it can be shown that

$$\lim_{d' \to 0} C(d') = \blacksquare / \beta^{\alpha+3} \tag{3.12}$$

The above remarks, together with (3.11) and (3.12), imply that there exists some δ' for which $C(d')$ is a maximum, although this maximum may not be unique. Thus the existence of some δ' for which the Bayes risk (3.8) is minimized has been established.

In order to find such a minimum I have utilized without success the conventional calculus method of setting $\frac{\partial}{\partial d'} R(g,e,x,d') = 0$. The resulting expression, however, does not lead to an explicit solution for d'. Thus numerical methods should be contemplated as part of our future work in order to obtain the value δ' which minimizes $R(g,e,x,d')$.

To obtain the estimate of λ, once the observation s' has been taken at dilution level δ', it will suffice to substitute δ' for d' in expression (3.7).

It appears clear that the methods described in this Section for the design problem are no simpler than those of Section II. This fact imposes the need for investigating ways to make the results useful from a practical standpoint. As a means to this end we intend to consider asymptotic theory as a possible avenue to simpler Bayes estimates and hence to a simplification of the design problem. In the next Section we show that, for integral values of the parameter α, it is possible to express the Bayes estimates and Bayes risks obtained in Sections II and III in the form of polygamma functions. It is hoped that this fact will assist in the development of more tractable methods.

If approximate methods can be successfully worked out, a comparison with the existing non-Bayesian estimation and design procedures will be mandatory and is to be considered an integral part of our future work.

SECTION IV

Polygamma form for expressions in Sections II and III.

In this Section it will be shown that the Bayes estimate and the Bayes risk resulting in Sections II and III can be expressed, for integral values of α, in the form of polygamma functions. It is recognized that this restriction is undesirable and we intend to continue to work in order to extend the results of this Section to non-integral values of α. It will suffice to discuss the case where one single dilution level is considered (k=1) in connection with the expressions of Section II.

Note that, by factoring $\frac{1}{d_1}$ from the numerator and denominator on (2.10) and (2.12) and recalling the notation introduced in (2.8), we have that

$$\hat{\lambda} = \frac{\alpha+1}{d_1} \frac{\sum_{i_1=0}^{n_1-x_1} (-1)^{i_1} \binom{n_1-x_1}{i_1} \Big/ [i_1+x_1+\frac{1}{\beta d_1}]^{\alpha+2}}{\sum_{i_1=0}^{n_1-x_1} (-1)^{i_1} \binom{n_1-x_1}{i_1} \Big/ [i_1+x_1+\frac{1}{\beta d_1}]^{\alpha+1}} \tag{4.1}$$

and $$R(g,d) = (\alpha+2)(\alpha+1)\,\beta^2 - \frac{(\alpha+1)^2}{\beta^{\alpha+1}}\, A(d_1)\,,$$

where

$$A(d_1) = \frac{1}{d_1^{\alpha+3}} \sum_{x_1=0}^{n_1} \binom{n_1}{x_1} \frac{\left[\sum_{i_1=0}^{n_1-x_1} (-1)^{i_1} \binom{n_1-x_1}{i_1} \Big/ [i_1+x_1+\frac{1}{\beta d_1}]^{\alpha+2}\right]^2}{\sum_{i_1=0}^{n_1-x_1} (-1)^{i_1} \binom{n_1-x_1}{i_1} \Big/ [i_1+x_1+\frac{1}{\beta d_1}]^{\alpha+1}}\,. \tag{4.2}$$

Set $k = x_1 + \frac{1}{d_1\beta}$ and let m be a positive real number. Thus if we define

$$S_N^{(m)}(k) = \sum_{i=0}^{N} (-1)^i \frac{\binom{N}{i}}{(k+i)^m} \quad , \tag{4.3}$$

we can write from (4.1) that

$$\hat{\lambda} = \frac{(\alpha+1)}{d_1} \frac{S_{n_1-x_1}^{(\alpha+2)}}{S_{n_1-x_1}^{(\alpha+1)}} \quad . \tag{4.4}$$

Also, from (4.2)

$$A(d_1) = \frac{1}{d_1^{\alpha+3}} \sum_{x_1=0}^{n_1} \binom{n_1}{x_1} \frac{\left[S_{n_1-x_1}^{(\alpha+2)}(k)\right]^2}{S_{n_1-x_1}^{\alpha+1}(k)} \quad . \tag{4.5}$$

Next we shall show that if m is a positive integer the sum $S_N^{(m)}(k)$ in (4.3) can be written in the form of polygamma functions. This will be sufficient in order to prove that the Bayes estimate and Bayes risk can be expressed as polygamma functions if we assume that α is a positive integer.

Consider the Beta integral

$$\int_0^1 x^{k-1} (1-x)^N \, dx = \frac{\Gamma(k)\Gamma(N+1)}{\Gamma(k+N+1)} = \frac{N!}{\prod_{i=0}^{N} (k+i)} \tag{4.6}$$

for $k > 0$. Note that (4.6) can also be integrated in the form

$$\int_0^1 x^{k-1}(1-x)^N \, dx = \sum_{i=0}^{N} (-1)^i \binom{N}{i} \int_0^1 x^{k-1+i} \, dx = \sum_{i=0}^{N} (-1)^i \frac{\binom{N}{i}}{k+i} \, . \tag{4.7}$$

From (4.6) and (4.7) we have the equality

$$S_N^{(1)}(k) = \sum_{i=0}^{N} (-1)^i \frac{\binom{N}{i}}{k+i} = \frac{N!}{\prod_{i=0}^{N} (k+i)} \quad . \tag{4.8}$$

Differentiating equation (4.8) with respect to k, we get

$$\frac{\partial S_N^{(1)}(k)}{\partial k} = (-1) \sum_{i=0}^{N} (-1)^i \frac{\binom{N}{i}}{(k+1)^2} = (-1)\, S_N^{(2)}(k) \quad , \tag{4.9}$$

i.e.,

$$S_N^{(2)}(k) = (-1) \frac{\partial S_N^{(1)}(k)}{\partial k} \quad . \tag{4.10}$$

Thus we can obtain $S_N^{(2)}(k)$ by differentiating the right side of (4.8) with respect to k. This yields

$$\frac{\partial S_N^{(1)}(k)}{\partial k} = (-1)\, N! \frac{\sum_{i=0}^{N} \frac{1}{k+i}}{\prod_{i=0}^{N} (k+i)} \quad , \tag{4.11}$$

and from (4.10) and (4.11) we have

$$S_N^{(2)}(k) = N! \frac{\sum_{i=0}^{N} \frac{1}{k+i}}{\prod_{i=0}^{N} (k+i)} \quad . \tag{4.12}$$

Proceeding in a similar fashion we can obtain $S_N^{(3)}(k)$.

Note as before

$$\frac{\partial S_N^{(2)}(k)}{\partial k} = (-2) \sum_{i=0}^{N} (-1)^i \frac{\binom{N}{i}}{(k+i)^3} = -2\, S_N^{(3)}(k) \quad . \tag{4.13}$$

Hence,

$$S_N^{(3)}(k) = (-1)\, \tfrac{1}{2}\, \frac{\partial S_N^{(2)}(k)}{\partial k} \tag{4.14}$$

and to get $S_N^{(3)}(k)$ we differentiate with respect to k the right side of (4.12) which gives

$$\frac{\partial S_N^{(2)}(k)}{\partial k} = (-1)\, N! \, \frac{\sum_{i=0}^{N} \frac{1}{(k+i)^2} + \left[\sum_{i=0}^{N} \frac{1}{k+i}\right]^2}{\prod_{i=0}^{N} (k+i)} \quad . \tag{4.15}$$

Thus from (4.14) and (4.15) we have

$$S_N^3(k) = \frac{N!}{2!} \, \frac{\sum_{i=0}^{N} \frac{1}{(k+i)^2} + \left[\sum_{i=0}^{N} \frac{1}{k+i}\right]^2}{\prod_{i=0}^{N} (k+i)} \quad . \tag{4.16}$$

We can find similar expressions for $S_N^{(m)}(k)$ for other values of m by making use of the recursive relation

$$S_N^{(m)}(k) = (-1)\, \frac{1}{(m-1)} \, \frac{\partial S_N^{(m-1)}(k)}{\partial k} \quad . \tag{4.17}$$

Next we will show that the following result holds:

$$\sum_{i=0}^{N} \frac{1}{(k+i)^m} = \frac{(-1)^{m-1}}{(m-1)!} \left[\psi^{(m)}(k+N+1) - \psi^{(m)}(k) \right], \tag{4.18}$$

where

$$\psi^{(m)}(k) = \frac{d^m}{dk^m} \ell u\ \Gamma(k) \tag{4.19}$$

and

$$\Gamma(n) = \int_0^\infty e^{-x} x^{n-1}\, dx, \quad n > 0 . \tag{4.20}$$

$\psi^{(1)}(k)$ is called the Digamma function, $\psi^{(2)}(k)$ the Trigamma function, $\psi^{(3)}(k)$ the Tetragamma function and so on.

To show (4.18) we first note that

$$\Gamma(k) = \frac{\Gamma(k+N+1)}{K(k+1)\cdots(k+N)} . \tag{4.21}$$

Taking logarithm of both sides of (4.21), we have

$$\ell u\ \Gamma(k) = \ell u\ \Gamma(k+N+1) - \ell u\ k - \ell u(k+1) - \cdots - \ell u(k+N) . \tag{4.22}$$

Then taking the derivative with respect to k of both sides of (4.22), we obtain

$$\frac{d\ \ell u\ \Gamma(k)}{dk} = \psi^{(1)}(k) = \psi^{(1)}(k+N+1) - \frac{1}{k} - \frac{1}{k+1} - \cdots - \frac{1}{k+N} . \tag{4.23}$$

Thus

$$\sum_{i=0}^{N} \frac{1}{k+i} = \psi^{(1)}(k+N+1) - \psi^{(1)}(k) \quad . \tag{4.24}$$

Differentiating now both sides of (4.23) with respect to k and recalling the definition (4.19), we have

$$(-1) \sum_{i=0}^{N} \frac{1}{(k+i)^2} = \psi^{(2)}(k+N+1) - \psi^{(2)}(k) \quad . \tag{4.25}$$

Hence

$$\sum_{i=0}^{N} \frac{1}{(k+i)^2} = (-1)^1 \left[\psi^{(2)}(k+N+1) - \psi^{(2)}(k)\right] \quad . \tag{4.26}$$

Thus (4.18) holds for m = 1,2. If we assume now (4.18) to be true for m and take derivative with respect to k of both sides of (4.18), we have

$$(-m) \sum_{i=0}^{N} \frac{1}{(k+i)^{m+1}} = \frac{(-1)^{m-1}}{(m-1)!} \left[\psi^{(m+1)}(k+N+1) - \psi^{(m+1)}(k)\right] . \tag{4.27}$$

Thus

$$\sum_{i=0}^{N} \frac{1}{(k+i)^{m+1}} = \frac{(-1)^m}{m!} \left[\psi^{(m+1)}(k+N+1) - \psi^{(m+1)}(k)\right] . \tag{4.28}$$

Hence (4.18) holds true for m + 1. Thus by the induction argument (4.18) holds true for all m that are positive integers.

From (4.18), (4.21) and the forms displayed in (4.12) and (4.16) for the sums $S_N^{(2)}(k)$ and $S_N^{(3)}(k)$, it becomes apparent that these forms can be expressed as a

rational combination of polygamma functions. Hence the Bayes estimate $\hat{\lambda}$ in (4.1) and $A(d_1)$ in (4.2) can also be expressed in the form of polygamma functions for integral values of α.

It should be noted that tables for the Digamma, Trigamma, Tetragamma and Pentagamma functions are available in the Handbook of Mathematical Functions edited by the National Bureau of Standards. Another source is H. T. Davis, Tables of Higher Mathematical Functions, Principia Press, Bloomington, Indiana.

Before concluding the Section we shall show that the following holds:

$$S_N^{(m)}(k) = (-1)^m \frac{N!}{(m-1)!} \frac{\partial^{(m-2)}}{\partial k^{(m-2)}} \left[\frac{\psi^{(1)}(k+N+1) - \psi^{(1)}(k)}{\prod_{i=0}^{N} (k+i)} \right], \tag{4.29}$$

for integer values of m greater than one.

For m = 2 we have from (4.12) and (4.24) that

$$S_N^{(2)}(k) = N! \frac{\psi^{(1)}(k+N+1) - \psi^{(1)}(k)}{\prod_{i=0}^{N} (k+i)} . \tag{4.30}$$

Thus (4.29) holds for m = 2. If we next assume (4.29) to be true for m and take the derivative with respect to k of both sides of (4.29), we have using (4.17) that

$$(-m)\, S_N^{(m+1)}(k) = (-1)^m \frac{N!}{(m-1)!} \frac{\partial^{(m-1)}}{\partial k^{(m-1)}} \left[\frac{\psi^{(1)}(k+N+1) - \psi^{(1)}(k)}{\prod_{i=0}^{N} (k+i)} \right]. \tag{4.31}$$

Thus (4.29) holds for m + 1. Hence by the induction argument, (4.29) holds true for m = 2,3,... .

SECTION V

FUTURE PLANS

Throughout the previous sections we have indicated which kind of extensions are thought to be pertinent in order to complete a comprehensive Bayesina treatment of the estimation and design problems involved in dilution experimentation. In this Section we will summarize the suggestions which have been made thus far and will point out other extensions which may be comtemplated in the future.

1. The design problem will be investigated with respect to all the variables involved in a typical dilution experiment: sample size, dilution levels and number of dilution levels to be used. Special attention will be given to the geometric and logarithmic dilution series described in Section III.

2. With regard to cost functions, we plan to restrict outselves to what Raiffa and Schlaifer [13] call additive cost functions. If we denote a terminal act by a, we have that, for these functions,

$$c(e,x,a,\lambda) = C_s(e,x) + C_t(a,\lambda) \tag{5.1}$$

where $C_s(e,x)$ is the sampling cost and $C_t(a,\lambda)$ is the terminal cost. In particular we will assume that the sampling cost is a linear function of the sample size,

$$C_s(e,x) = a + b\ n \tag{5.2}$$

and that

$$C_t(a,\lambda) = p \times L(a,\lambda) \quad , \tag{5.3}$$

where p is a constant that brings C_s and C_t into a common "numeraire" and $L(a,\lambda)$ is the loss function.

Thus far, we have restricted our attention to a quadratic loss function

$$L(a,\lambda) = (\hat{\lambda}-\lambda)^2 \tag{5.4}$$

mostly because the Bayes estimate, $\hat{\lambda}$, is easily obtained as

$$\hat{\lambda} = \int_0^\infty \lambda\, p(\lambda|x)d\lambda\ . \tag{5.5}$$

In future work we are planning to consider

$$L(a,\lambda) = |\hat{\lambda}-\lambda| \tag{5.6}$$

for which $\hat{\lambda}$ is given by the median of the posterior distribution of λ. (See Lehmann [10, p. 23]).

The classical problem of test of hypothesis can also be discussed within this framework. Let $H_0:\lambda \in \Omega_H$ and $H_a:\lambda \in \Omega_k$. Denote by a_0 the statement: accept H_0 and by a_1 the statement: reject H_0. Suppose that the following cost or loss table is given

	H_0	H_a
a_0	0	C_1
a_1	C_2	0

(5.7)

where C_1 and C_2 are costs attached to a wrong decision. Then the design problem can be dealt with in the same manner as with the other loss functions discussed above, namely by minimizing the function

$$C^*(e) = E_{x|e} \min_a \int_0^\infty C(e,x,a,x)\ g(\lambda)\ d\lambda$$

with respect to the elements of e: sample size, dilution levels and number of dilution levels.

3. Tractable expressions for the Bayes estimate and Bayes risk will be sought. It appears from the preliminary results obtained in Sections II and III that in order to make this research useful from a practical standpoint it is of paramount importance to obtain simpler expressions for the parameter estimate and hence for the Bayes risk involved in the design problem. We intend to explore the possibilities which may exist in the polygamma forms derived in Section IV and in this connection we will attempt to extend the results of Section IV to the case where the parameter α of the gamma prior distribution is not an integer value. Approximate forms for the parameter estimate will also be considered as a possible source of simplification for the estimation and design problems.
4. Tables to assist in the computation of the parameter estimate as well as in the design of the experiment will be included as a part of future work.
5. The problem of how much effect the use of prior knowledge has on the parameter estimate and on the design of the experiment will also be considered. This is what Raiffa and Schlaiffer call Sensitivity Analysis.
6. The Bayesian approach suggested in this report for the estimation and design problems in dilution experiments will be extended to other biological assay models including models with the normal and logistic distributions of tolerances. As a further step, an extension to a general class of models, of which the above models would be particular cases, is contemplated.
7. Examples to illustrate procedures and methods will be included.

BIBLIOGRAPHY

[1] Cochran, W. G. [1950]. Estimation of Bacterial Densities by Means of the Most Probable Number. Biometrics 6, 105-116.

[2] Cornell, R. G. [1964]. Spearman Estimation for a Simple Exponential Model. Technical Report M52. Department of Statistics, Florida State University.

[3] Finney, D. J. [1947]. The Principles of Biological Assay. Journal of the Royal Statistical Society, Supplement 9, 46-91.

[4] Finney, D. J. [1952]. The Statistical Method in Biological Assay. New York: Hafner Publishing Company.

[5] Fisher, R. A. [1922]. On the Mathematical Foundations of Theoretical Statistics. Philosophycal Transactions of the Royal Society A222, 309-368.

[6] Greenwood, M. and Yule, G. U. [1917]. On the Statistical Interpretation of Some Bacteriological Methods Employed in Water Analysis. Journal of Hygiene 16, 36-54.

[7] Halvorson, H. O. and Ziegler, N. R. [1933]. Applications of Statistics to Problems in Bacteriology I. A means of Determining Bacterial Population by the Dilution Method. Journal of Bacteriology 25, 101-121.

[8] Johnson, E. A. and Brown, B. W., Jr. [1961]. The Spearman Estimator for Serial Dilution Assays. Biometrics 17, 79-88.

[9] Kraft, Chs. H. and Van Eeden, C. [1963]. Bayesian Bio-Assay. Abstract No. 13. Annals of Mathematical Statistics 34, 1113.

[10] Lehmann, E. L. [1959]. Testing Statistical Hypothesis. New York: John Wiley and Sons, Inc.

[11] McCrady, M. H. [1915]. The Numerical Interpretation of Fermentation Tube Results. Journal of Infections Diseases 17, 183-212.

[12] Peto, S. [1953]. A Dose Response Equation for the Invasion of Micro-organisms. Biometrics 9, 320-335.

[13] Raiffa, H. And Schlaifer, R. [1961]. Applied Statistical Decision Theory. Boston: Division of Research, Graduate School of Business Administration, Harvard University.

[14] Thorslund, T. W. [1965]. Computationally Simple and "Efficient" Estimators for the "One-Hit Curve". Contributed Paper Presented at the Biometric Society, Tallahassee, Florida, April, 1965.