ON LOGARITHMIC EXTRAPOLATION OF MICROBIAL SURVIVOR CURVES FOR PLANETARY QUARANTINE REQUIREMENTS

(Research Note)

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A number of scientists active in planetary quarantine have questioned the logarithmic extrapolation of microbial survivor curves over non-measurable ranges to obtain thermal sterilization cycles for spacecraft applications (DAVIES and HOROWITZ, 1966; GEIGER *et al.*, 1965; JAFFE, 1963). If observed non-logarithmic survival is the result of either sampling errors or population inhomogeneity, then sterilization cycles can safely be set by extrapolation after the curve has entered its final linear phase. If non-logarithmic survival is an intrinsic function of the organism itself, then conceivably gross errors can result from such extrapolation. This communication briefly describes a model based on chemical reaction kinetics in which non-logarithmic survival is inherent in the organism. Results are compared to data for *Bacillus coagulans*.

Assume that microbes die independently, there is no reproduction, and the exposed population is homogeneous. Then, the expected population at time t is given by

$$E[X(t)] = X(0)\, p(t)$$

where $X(0)$ is the initial population and $p(t)$ is the probability of single spore survival to time t. Assume that in a thermal environment, microbial deaths result from chemical reactions which activate one or more of N independent death mechanisms, i.e., the microbe contains N vital systems such that survival depends on all N being functional. Then, the probability of single microbe survival to time t is given by

$$p(t) = \prod_{i=1}^{N} \{1 - [1 - q_i(t)]^{n_i}\}, \tag{1}$$

where n_i is the number of duplicate subsystems in the ith system, $i = 1, \ldots, N$, and $q_i(t)$ is the probability that a subsystem of the ith type is functional at time t. Note that the function carried out by the ith system can be performed provided any one of the n_i duplicates in the system is viable.

Candidates for vital subsystems might include single molecules, say DNA, or perhaps aggregates of protein molecules. GINOZA and ZIMM (1961) have observed that with heat, DNA rapidly loses up to 98% of its activity and then undergoes a deactivation process which obeys simple first-order kinetics. Thus, it seems reasonable to

assume vital subsystems of single molecules deactivated by a reversible reaction competing with a first-order reaction. Since the reactions are monomolecular, it is reasonable to assume the reversible reaction is also of first order. In addition, assume a vital system consisting of an aggregate of protein molecules. It is not expected that deactivation of the entire aggregate is required for microbial death. Thus, for simplicity, assume that the probability that the system is functional is equal to the number of molecules active at time t divided by the initial number. Since protein inactivation is often of higher order (JOHNSON *et al.*, 1954), assume the protein aggregate is degraded by a second-order reaction.

For example, consider only the above two vital subsystems and hypothesize that the system of single molecules has n_1 duplicate subsystems and that the aggregate of protein is not duplicated. Then, if $V_2(t)$ is the number of protein molecules active at time t, $q_2(t)=V_2(t)/V_2(0)$. Thus,

$$\begin{aligned} p(t) &= \{1-[1-q_1(t)]^{n_1}\}\{1-[1-q_2(t)]^1\} \\ &= \{1-[1-q_1(t)]^{n_1}\}\{V_2(t)/V_2(0)\}. \end{aligned} \tag{2}$$

Values for $q_1(t)$ and $V_2(t)$ are assigned via chemical kinetics of small systems. Stochastic processes have been applied to such problems by MCQUARRIE (1963). Briefly, from an initial concentration $Y(0)$ of A molecules in the reversible first-order reaction $A \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} B$, competing with the first-order reaction $A \xrightarrow{k_2} C$, the expected concentration $E\{Y(t)\}$ of A molecules at time t is given by

$$E\{Y(t)\} = e^{-k_2 t}[Y(0)/(k_1+k_{-1})][k_1 e^{-(k_1+k_{-1})t}+k_{-1}].$$

Thus $q_1(t)$ is taken to be

$$q_1(t) = [e^{-k_2 t}/(k_1+k_{-1})][k_1 e^{-(k_1+k_{-1})t}+k_{-1}]. \tag{3}$$

From an initial concentration $Y(0)$ of A molecules the second-order reaction $2A^k B$ yields an expected concentration at time t of approximately

$$E\{Y(t)\} = Y(0)/\{1+Y(0)[e^{kt}-1]\}.$$

Thus $V_2(t)/V_2(0)$ is assumed to be

$$V_2(t)/V_2(0) = 1/\{1+V_2(0)[e^{kt}-1]\}. \tag{4}$$

It should be noted that the familar logarithmic model results from Equation (1) by assuming that $N=1$, $n_1=1$, and the vital subsystem consists of one molecule being deactivated by a first-order reaction.

A comparison of an expected survivor curve generated from Equation (2) with $q_1(t)$ and $V_2(t)$ obtained via Equations (3) and (4) to observed data for *Bacillus coagulans* is given in Figure 1. The reaction-rate constants for the reactions $A \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} B$ and $A \xrightarrow{k_2} C$ are, $k_1=4\ \text{hr}^{-1}$, $k_{-1}=.01\ \text{hr}^{-1}$, and $k_2=2\ \text{hr}^{-1}$. The protein-aggregate system contains 20 molecules inactivated by the second-order reaction $2A \xrightarrow{k} D$ where

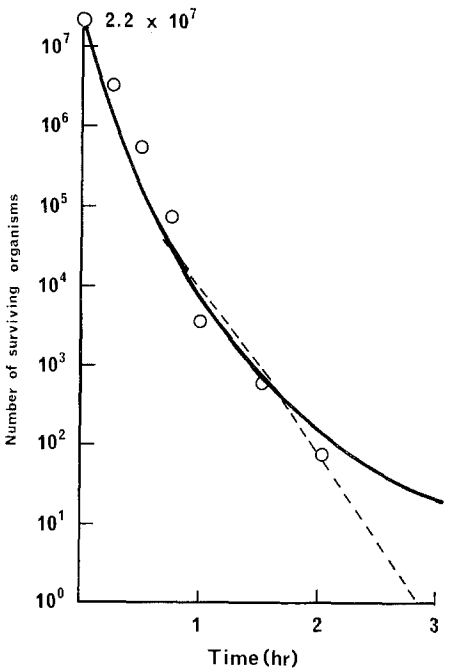

Fig. 1.

the reaction rate constant $k = 10$ molec.$^{-1}$ hr^{-1}. Observed data for *Bacillus coagulans* is taken from DAVIES and HOROWITZ (1966). The dash line represents a linear extrapolation of the observed data after a two-decade population decrease. As can be seen, the questions regarding this extrapolation may indeed have a sound rational basis.

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