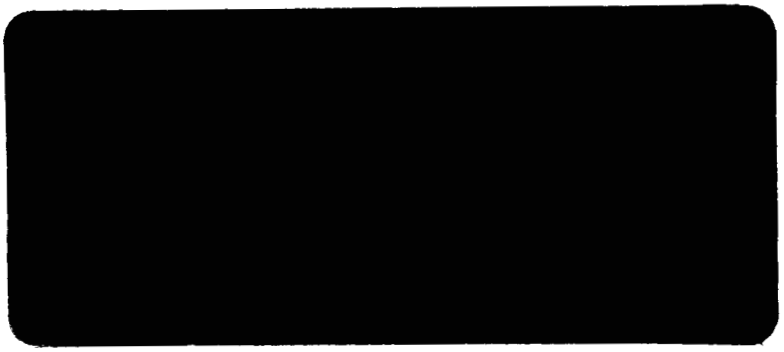

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LOG-NORMAL MODEL FOR MICROBIAL SURVIVAL IN HEAT STERILIZATION

by

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ABSTRACT

An analytical model is developed for the survival times of organisms in heat steriliztion in which the probability of inactivation as a function of exposure time is log-normally distributed. Experimental data is examined relative to this model and it is concluded that the model is valid except during the initial period of heating when an additional interaction between the organisms and their surrounding medium appears to be present. At long heating times, the log-normal model appears more accurate for extrapolating to low survival probabilities than the usual logarithmic survivor curves and is generally more conservative.

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1. INTRODUCTION

Laboratory evaluations of microbial survival times under various destructive environments have been studied for well over half a century. Throughout this time period, including the present, experimental data have been in conflict with the most prevalent theory of microbial survival rates, i.e. that the viable population of micro-organisms exposed to a particular sterilizing environment decreases in number by one decade in equal times of exposure. This theory requires that a plot of the logarithm of survivors vs. exposure time be a straight line. Considering the experimental evidence against this assumption, it is remarkable that the logarithmic assumption should have endured as long as it has. It is even more perplexing to find that even though the theoretical foundations for logarithmic survival of micro-organisms in heat sterilization have also been seriously questioned, e.g. references (1)* and (2), this approach has nevertheless become entrenched as the analytical basis for research as well as application of heat sterilization processes.

In view of the above background it seems pointless to renew the arguments against the logarithmic basis for microbial survival. Arguments for and against it abound in the literature and a small sample of them may be found in references (1) through (9). The idea that a log-normal relationship is a better basis for survivor-time curves has also often been advanced (1) without any significant impact. This approach is revived here but, hopefully, with a difference. Specifically, this report attempts to develop the log-normal basis not only as an improved curve-fitting technique but as a mathematical model for a physical process. Thus, certain assumptions must be made in evolving the mathematical model and these assumptions can have a counterpart in hypotheses concerning the physical process. As a minimum, the assumptions and hypotheses must be mutually consistent. If such a consistent

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* Numbers in parenthesis denote references listed in Section 7.

relationship is maintained it should be possible to interpret the fit (or lack of it) between experimental data and values predicted by the model in terms of the mathematical assumptions of the model and the associated physical parameters.

As a preliminary to the analytical formulation of the problem, it may be useful to consider the principal variables in relation to the current logarithmic model. In general, since the analysis and prediction of sterilization processes is a problem in statistics, we deal with probabilities of an event either in the context of a frequency function or a cumulative distribution function. The frequency function represents the probability that the event will occur at a particular value of the random variable and the cumulative distribution function the probability that the event will occur during the interval up to some chosen value of the random variable. Thus, one event of interest is the inactivation of any one organism with exposure time and $f(\tau)$ will represent the probability per unit time that inactivation will occur at a particular exposure time τ. Similarly, $P_d(t)$ will denote the probability that inactivation will occur in the total time interval $0 \leq \tau \leq t$. Since $P_d(t)$ is the integral of $f(\tau)$ with t the upper limit of integration, only one of these functions needs to be known. (For further detail, see reference (10).)

In the logarithmic model, the entire process is represented by a single parameter, the D-value, contained in the formulation of $P_d(t)$, viz. (10)

$$P_d(t) = 1 - 10^{-t/D}$$

Since $P_d(t)$ is also given by the ratio of inactivated organisms to the original viable population (10), the D-value represents the time needed to reduce the initial population by one decade.

The D-value in the logarithmic model is descriptive of the rate of inactivation in sterilization. In the model to be developed here there will also be such parameters but with two differences. First, these parameters are

believed to be more closely associated with the physical characteristics of the organisms and/or their environment, e.g. a random distribution of resistances in a given environment (a "mortality" table). Second, there will be two parameters to define the rate of inactivation thereby making it possible to fit a wide variety of survivor-curve shapes with the same functional relationship. Specifically, and in contrast to the expression of $P_d(t)$ shown above for the logarithmic model, the log-normal model will define $P_d(t)$ in terms of a normal distribution with a mean value μ and variance σ^2, where μ and σ^2 relate to the physical characteristics of the process rather than the survival curve. As shown in the text, the statement that $P_d(t)$ is log-normally distributed signifies that the probability of inactivation in the time interval t is given by a normal distribution with respect to the logarithm of exposure time.

The principal purpose of the work reported here is not so much to prove the validity of the log-normal model for microbial survival but to examine the extent of its applicability. As will be shown below, this model is found to be useful. However, experimental evidence indicates that microbial survival processes in heat sterilization are sufficiently complex to require more sophisticated analytical techniques than that provided by the simple log-normal model alone. Nevertheless, the log-normal model is a useful starting point: it can provide a workable approximation for many experimental conditions and point the way for the more complex processes.

The work reported herein has been carried out in connection with sterilization requirements of planetary spacecraft for which dry-heat has been selected as the most appropriate agent. Experimental data to be considered here will therefore be restricted to dry-heat. However, some moist-heat data published in the literature have also been examined in the course of this work and the basic analytical approach to be described was found to be applicable. Indeed, the effect of moisture on the physical process and the role it plays in the model is one of the more interesting questions which can be raised within the analytical framework described below.

2. ASSUMPTIONS

The following assumptions underlie the analytical model for microbial survival-time to be developed herein:

(1) A single species of organisms is considered.

(2) The death of one organism is independent of the death of any other organism within the population.

(3) Survival time under a constant sterilizing environment is a function of (a) prior exposure time and (b) a random variable representing pertinent physical characteristics of the organisms and/or their environment.

It will be noted that assumption (3) allows for a random distribution of resistances among organisms in a given species, i.e. resistance can be viewed as one of the physical characteristics under consideration. However, it need not be the only contributor to the random process. This, and the assumption that prior exposure time is a principal factor in determining survival, distinguish the present approach from the analytical basis of the logarithmic model.

3. ANALYTICAL DEVELOPMENT

The assumptions listed above can be used to derive a large variety of models for survival-times and further restrictions will be required to produce the log-normal basis. These will be noted as the need arises.

Consider a population of micro-organisms satisfying assumptions (1) and (2), i.e. they are all of the same species and the "death" of any one of them does not influence the process of rendering others non-viable. As shown in (10), these two assumptions are sufficient to establish a functional relationship between the fraction of survivors and the probability of survival up to time t. Specifically,

$$P_S(t) = \frac{N(t)}{N_0} \qquad (1)$$

where $P_S(t)$ = probability that an organism will survive exposure to time t

N_0 = initial number of viable organisms (at t = 0)

$N(t)$ = number of viable organisms remaining at time t.

Equation (1) is obtained by viewing the exposure time t as consisting of N_0 trials in which survival of an organism is considered a success. The probability of such a success in any one trial is $P_S(t)$ and the frequency of successes in N_0 trials is obtained from the binomial distribution. The expected number of successes is then given by $N_0 \cdot P_S(t)$, which leads to equation (1) provided it is understood that N(t) is obtained by statistical estimation from a number of experiments on N_0 organisms in which exposure was maintained for the same time interval t. (The alternative of graphically estimating N(t), which is the conventional procedure for generating survival-time curves, is discussed in Reference (10).)

If $P_d(t)$ denotes the probability that any one organism will die in the time interval t, then clearly

$$P_d(t) = 1 - P_S(t). \qquad (2)$$

$P_S(t)$ is a cumulative probability function and is related to the frequency function of survival times through the conventional definition of cumulative probabilities, viz.

$$P_d(t) = \int_0^t f(\tau)d\tau \qquad (3)$$

where τ is the time of death. Thus $f(\tau)d\tau$ expresses the probability that death will occur in the interval between τ and $\tau + d\tau$.

Survival-time curves are generally obtained in the laboratory by observing $N(t)$ with respect to N_0. These data, when plotted against exposure time t in the form of equation (1), yield the survival probability function $P_S(t)$. (The alternative experimental approach in which "end point" data is obtained by observing sterility of multiple samples is discussed in (10) and its relationship to equation (1) defined.)

In a curve-fitting approach to survival-time functions the procedure would be to use any one of a number of cumulative probability functions for $P_S(t)$, i.e. the logarithmic, gamma distribution, Weibull distribution etc., and determine which gives the best fit for all the curve shapes obtained by experiment, allowing the parameters of the distribution to change with changing experimental conditions. This is a workable approach but its usefulness is limited when empirical distribution functions are its basis. We therefore restrict ourselves to distribution functions whose evolution can more closely be related to the physical process which they are intended to model. On this basis we have selected the log-normal distribution as a starting point for modeling microbial survival-time functions in heat sterilization.

The log-normal model for $P_S(t)$ can be formulated in three steps. Refering to assumption (3), it is first necessary to define the variables which will enter the functional relationship between survival time and exposure time. These variables are intimately related to the physical characteristics of the sterilization process and a detailed definition would be desirable. However,

little is known about these physical characteristics to permit a meaningful functional formulation. It is therefore assumed that their total effect on survival time can be represented by a random variable, ϵ, which can take on different values as a function of exposure time. No assumptions are made concerning the explicit form of the distribution function of this random variable except that it has some mean value μ and a variance σ^2. As a minimum, this random variable can reflect the variable resistances of organisms of a given species to a particular sterilization environment. In addition, should other characteristics of the environment in an assumed model of the physical process also be random, they can be viewed as being a part of ϵ and contributing to its mean value and variance.

The second step in formulating the log-normal model consists of specifying the explicit functional relationship between survival time and prior exposure time. For this purpose we visualize a population of N_o initially viable organisms ranked in order of their death times. Thus, τ_1 denotes the time of death of the first organism and τ_j defines the time of death of the j-th organism. We then define the time dependence as

$$\tau_j - \tau_{j-1} = \epsilon_j \tau_{j-1} \tag{4}$$

i.e. the incremental time $\tau_j - \tau_{j-1}$ which must elapse before the j-th organism will die is proportional to the prior exposure time τ_{j-1}. The parameter of proportionality is the random variable ϵ described above. The particular value which it assumes in the time interval $(\tau_j - \tau_{j-1})$ is ϵ_j.

It should be noted that equation (4) is a hypotheses concerning the dependence of survival times upon prior exposure time and the justification for its choice is, for the present, the fact that it leads to a log-normal model for the probability of death as a function of exposure time. The derivation of the log-normal model, the third and final step in the analytical development, is contained in Appendix A.

Before proceeding with an evaluation of the validity of the above hypothesis, general features of the log-normal model will be described.

It will be useful to distinguish between three groups of variables. The first consists of μ and σ and they will be referred to as the independent variables or parameters. They are of prime interest since they relate directly to the causes of heat inactivation. However, they are not directly observable in heat sterilization experiments and must be inferred from the observed parameters through the analytical model. The second group, therefore, are the observed variables. They include: (1) the number of survivors as a function of exposure time, N(t), and (2) the initial number of viable organisms, N_o, for which N(t) has been observed. The probability of an organism surviving to time t, $P_s(t)$, and the complementary probability $P_d(t)$ will be viewed as equivalent forms of the observed variables in accordance with equations (1) and (2).

The third group of variables may be viewed as providing a transition between the independent variables μ and σ on the one hand and the observed variables N(t) and N_o on the other. Since this transition must be made through the analytical model, this group contains variables or parameters of the model. In the case of the log-normal model (see Appendix A), it consists of the following:

(1) $f(\tau)$ - probability (or frequency) of death at a particular exposure time τ

$$f(\tau) = \frac{1}{2\pi\tau\sigma} \exp\left[-\frac{1}{2\sigma^2}(\ln\tau - \mu)^2\right] \qquad (5)$$

(2) μ_τ - mean value of $f(\tau)$

$$\mu_\tau = \exp\left[\mu + \frac{1}{2}\sigma^2\right] \qquad (6)$$

(3) σ_τ^2 - variance of $f(\tau)$

$$\sigma_\tau^2 = \left[\exp(2\mu + \sigma^2)\right]\left[\exp(\sigma^2) - 1\right] \tag{7}$$

(4) η_τ - coefficient of variation of $f(\tau)$

$$\eta_\tau = \left[\exp(\sigma^2) - 1\right]^{1/2} \tag{8}$$

(Note that η_τ for the log-normal model is independent of μ.)

With $f(\tau)$ defined, as in (1) above, the transition from the observed variables to the independent variables is achieved through equation (3).

The effect of μ on $f(\tau)$ for a constant σ is illustrated in Figure 1 whereas Figure 2 shows the effect of σ for a constant μ. The wide range of skewness obtainable through changes in μ and σ is evident from these illustrative graphs.

Of greater interest than the shape of $f(\tau)$ is the shape of survivor curves which would result from the log-normal model. This is illustrated in Figures 3 and 4 for a range of values of μ and two values of σ. These plots have been made in semi-log form to permit comparison with experimentally obtained curves which are normally plotted in this manner. It is readily evident from Figure 3 that when the data spans only a few decades of reduction in N_O, sections of the log-normal curves could be approximated by straight lines, particularly for the less resistant cases, i.e. μ small. However, the straight line fit on a semi-log plot becomes a poor approximation to the log-normal when extended over more than about four decades of reduction in the viable population.

The sigmoid nature of the log-normal curves is also evident from Figures 3 and 4, including the initial lag observed in experimental data. Qualitatively, therefore, the log-normal model would appear to be a good basis for interpreting experimental data. A more quantitative assessment is, however, needed, as discussed below.

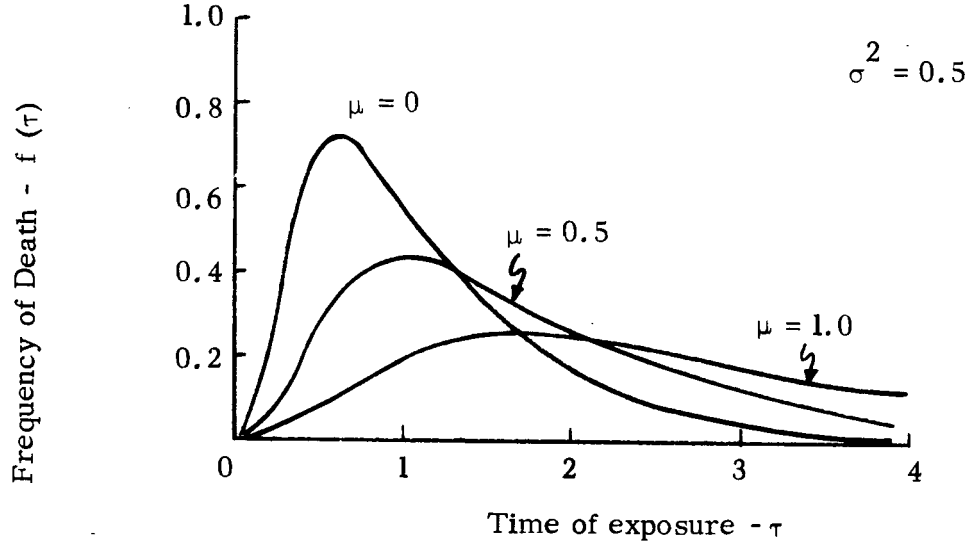

FIGURE 1: Effect of Mean-Value μ on Frequency Curve

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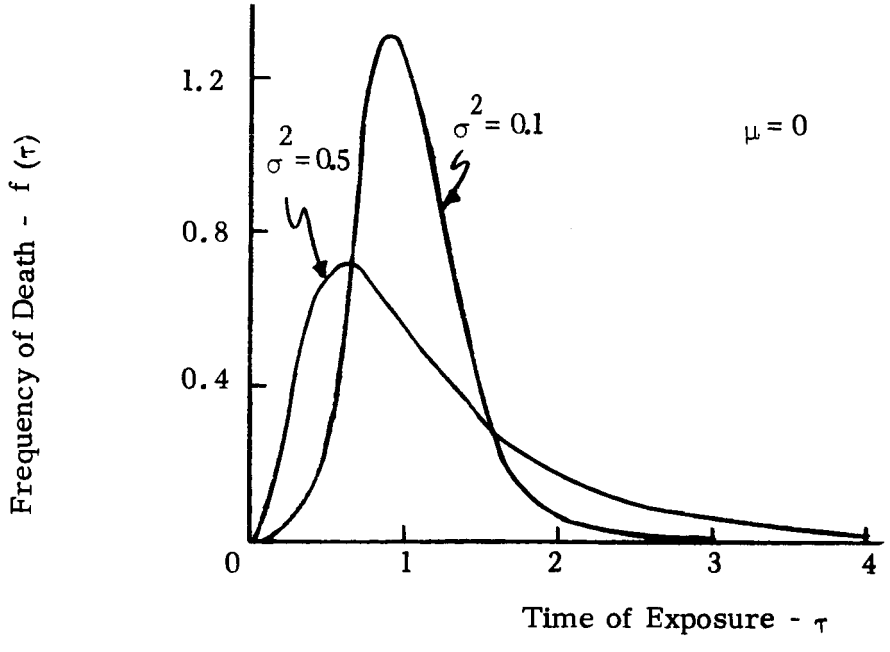

FIGURE 2: Effect of Variance σ^2 on Frequency Curve

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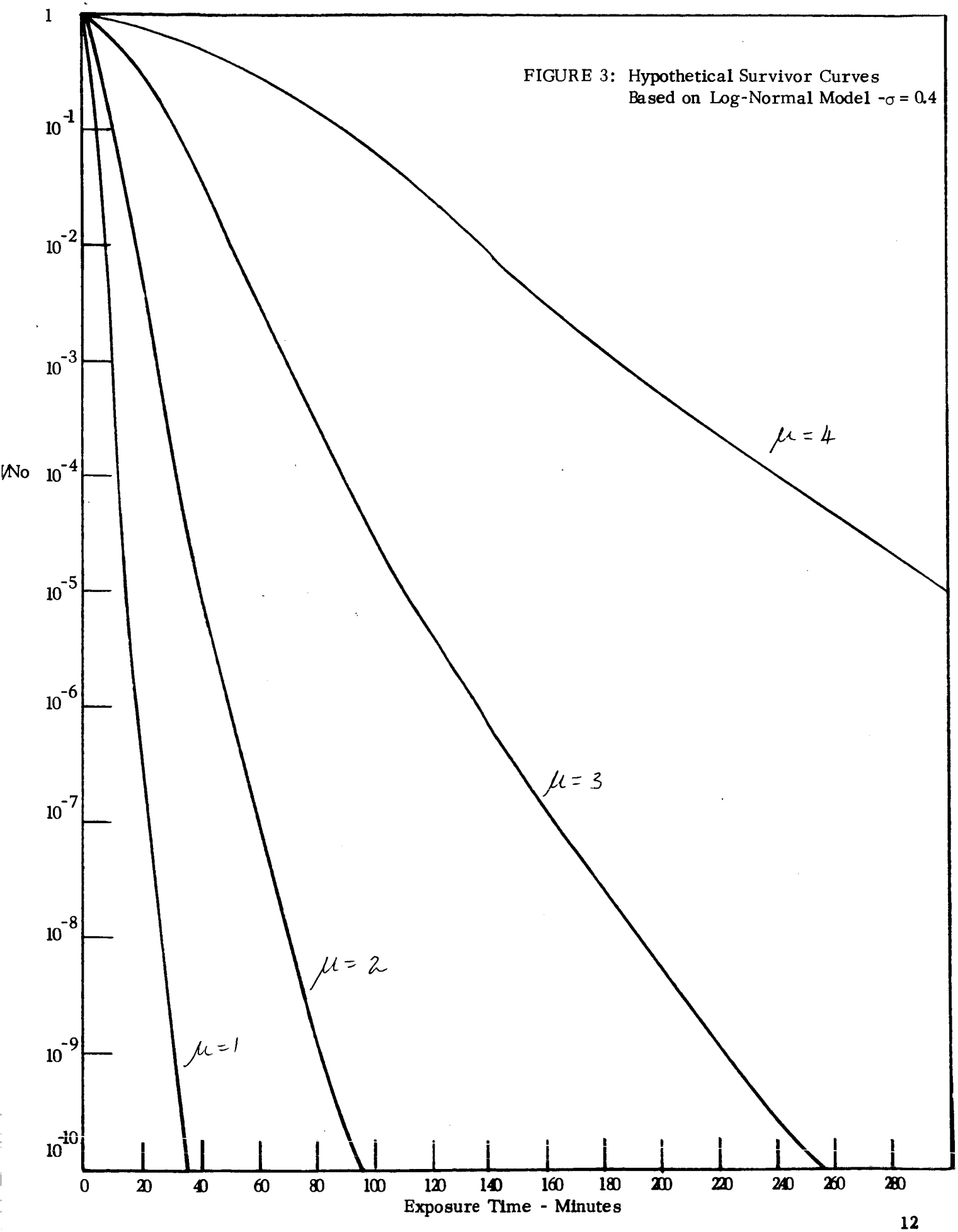
FIGURE 3: Hypothetical Survivor Curves
Based on Log-Normal Model -σ = 0.4
1
10-1
10-2
10-3
10-4
10-5
10-6
10-7
10-8
10-9
10-10
N/No
μ = 4
μ = 3
μ = 2
μ = 1
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
Exposure Time - Minutes

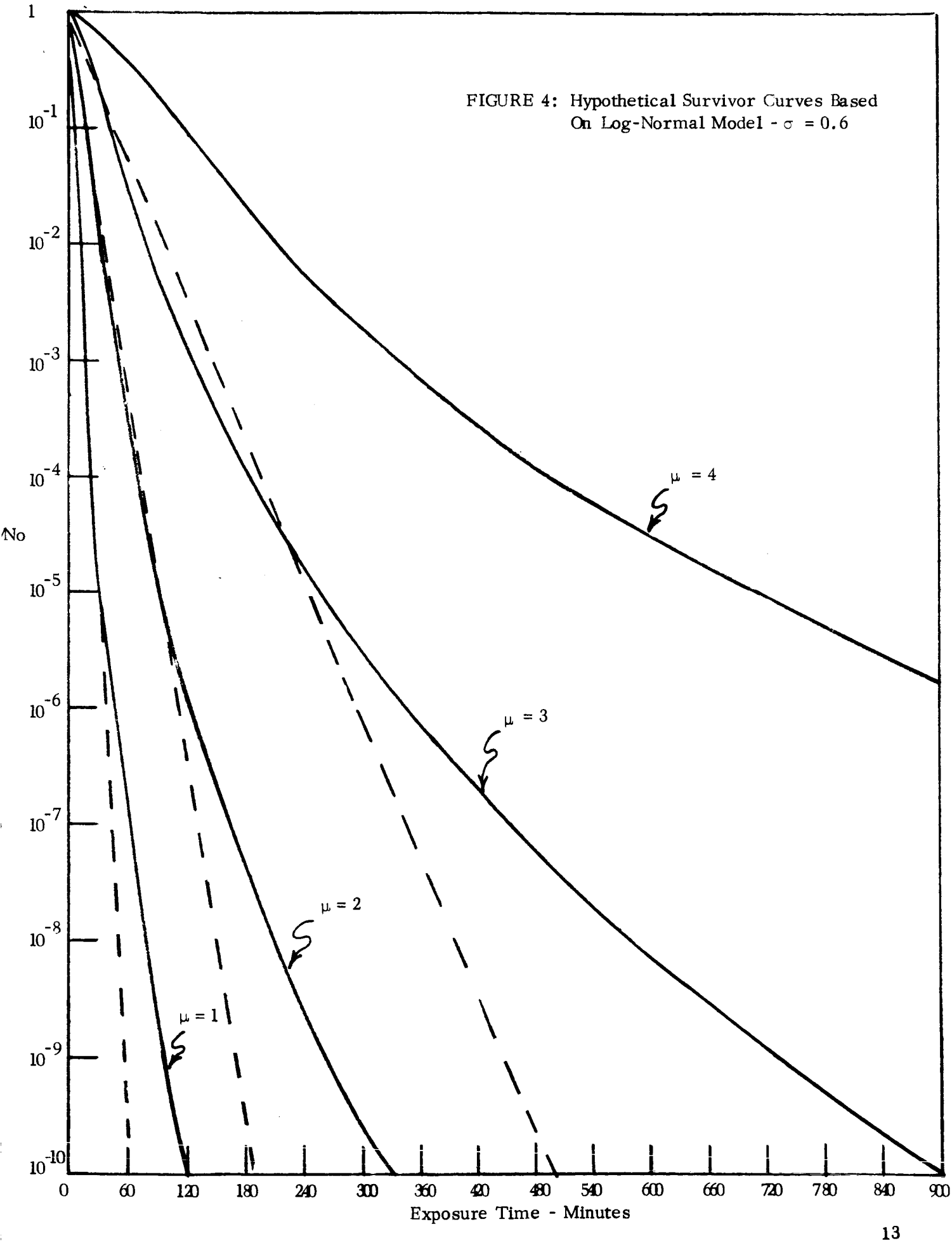

FIGURE 4: Hypothetical Survivor Curves Based On Log-Normal Model - $\sigma = 0.6$

4. COMPARISON WITH EXPERIMENTAL DATA

Experimental data on the heat inactivation of organisms was gathered from a variety of sources and evaluated with respect to the log-normal model described above. The basic procedure was as follows. First, the values of N and N_0 were read off the graphs or tables, whichever were available, along with the corresponding exposure times. For any one experiment, i.e. a particular organism on paper strips or embedded in plastic and subjected to a fixed sterilization temperature, the data were arranged in the form $N(t)/N_0$. These data points were then plotted on log-normal paper to test for conformance to the model. For the present purposes, a graphical approach to testing log-normality is adequate. Thus, the degree to which the data points approximate a straight line on log-normal graph paper is a measure of the validity of the log-normal model for the heat inactivation process of the experiment.

A large number of experiments were evaluated in the above manner, including moist-heat sterilization data. The data presented herein are representative of experiments which fit the log-normal model as well as of experiments which do not. The criterion for inclusion in this report has been the adequacy of data points over a sufficiently wide range of N/N_0, i.e. $10^{-6} < N/N_0 < 1$.

Table I summarizes the data shown in Figures 5 through 14. In these figures a "log-normal plot" refers to a plot on log-normal graph paper and a "log-normal fit" shows the conventional semi-log plot of N/N_0 as a function of exposure time but with the fitted curve obtained from the log-normal plot, i.e. the straight line drawn on the log-normal plot through the data points has been transferred to the semi-log plot. The latter has been done to indicate the degree to which a log-normal fit suits the variety of shapes encountered, including those cases where the experimenter has concluded

TABLE I

SUMMARY OF EXPERIMENTAL DATA

Reference	Experiment	Fig. No's.	Experimental Parameter	MINUTES					Log-normal Model valid for $N/N_o <$
				μ	σ	μ_T	σ_T	η_T	
Koesterer(11)	B. Subtilis v. Niger in Air, Helium and vacuum environments at 120°C	5, 6, 7	Air	3.69	0.45	44.7	21.00	0.47	0.4
			Helium	2.79		18.0	8.45		10^{-1}
			Vacuum	1.72		6.2	2.9		10^{-2}
Koesterer(12)	Soil Isolate 541 Spores in Dry FG Soil	8, 9, 10	125°C	5.78	0.57	379.0	235.0	0.62	$0.5\text{-}10^{-1}$
			135°C	4.87		153.0	94.7		
			145°C	4.03		66.2	41.0		
			160°C	2.58		15.5	9.6		
Angelotti (13)	Bacillus Globigii* Spores on Paper Strips	11,12,13	125°C	4.46	0.41	86.5	61.3	0.71	10^{-1}
			135°C	2.77		16.0	11.35		
Angelotti (13) (14)	Bacillus Globigii* Spores in Lucite	14	115°C	5.7	0.57	300.0	185.5	0.62	10^{-3}
			125°C	4.43		84.0	52.0		
			135°C	3.28		26.6	16.5		

* Alternate name for B. Subtilis v. Niger

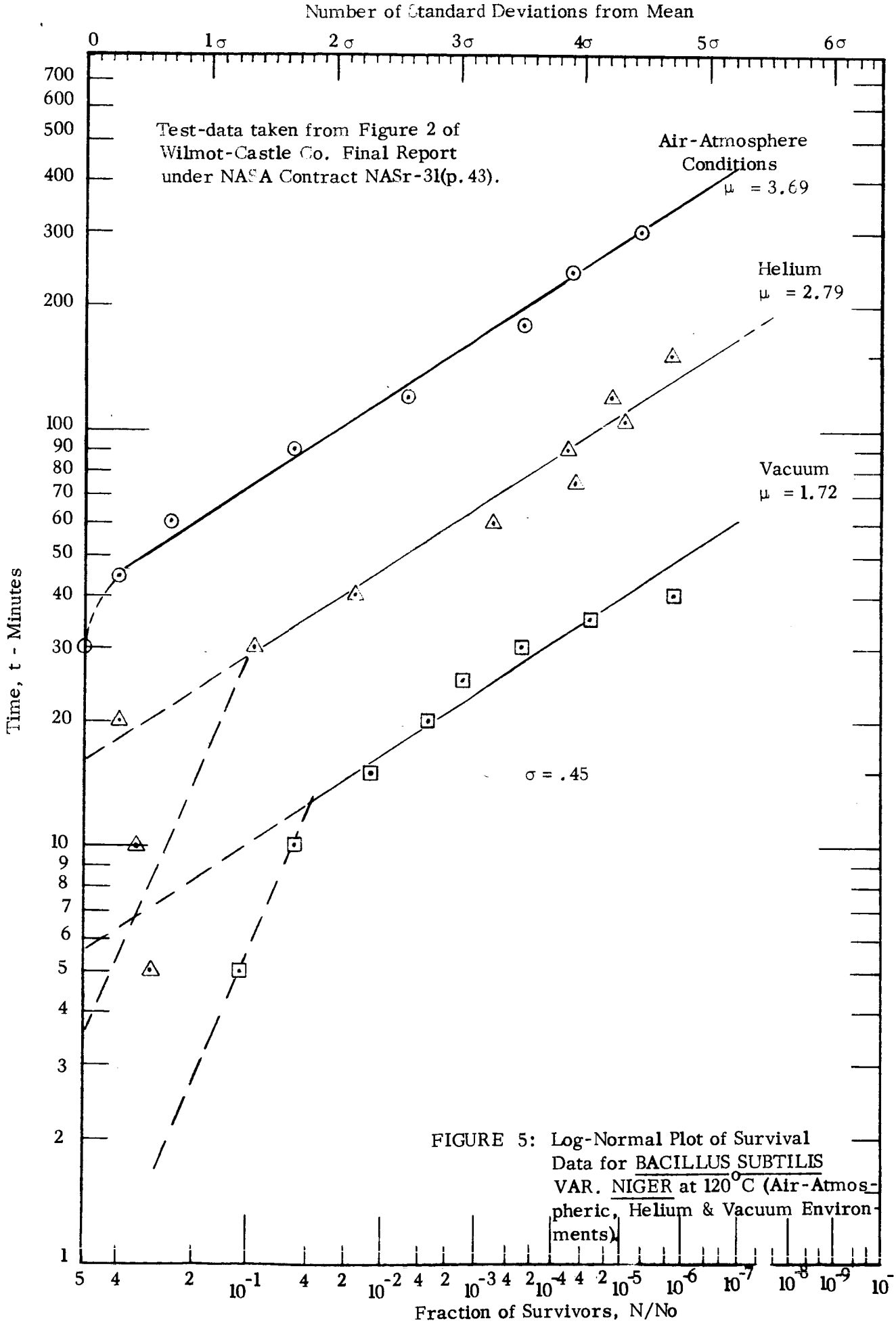

FIGURE 5: Log-Normal Plot of Survival Data for BACILLUS SUBTILIS VAR. NIGER at 120°C (Air-Atmospheric, Helium & Vacuum Environments)

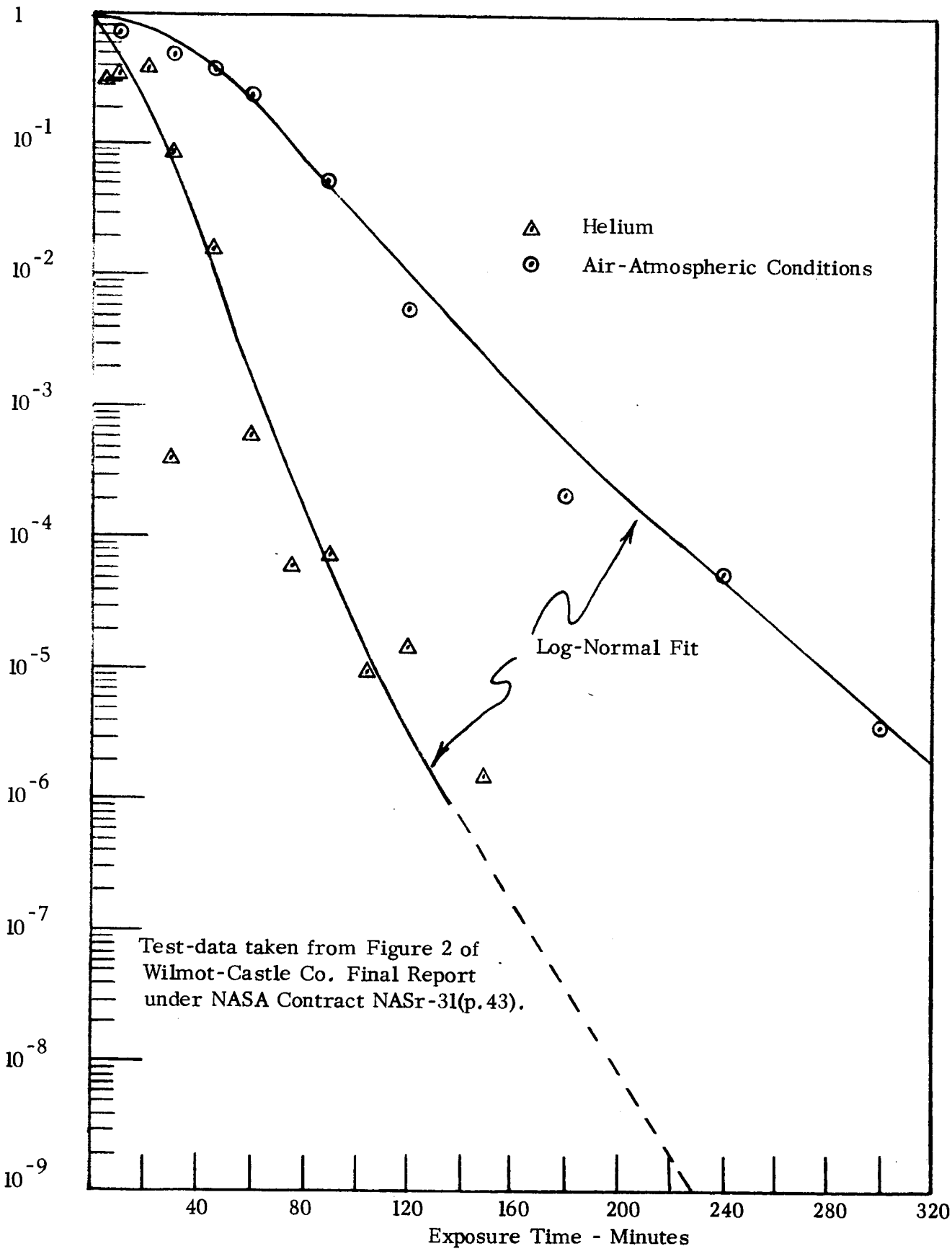

FIGURE 6: Log-Normal Fit of Survival Data for BACILLUS SUBTILIS VAR. NIGER at 120°C - Air Atmospheric and Helium Environments

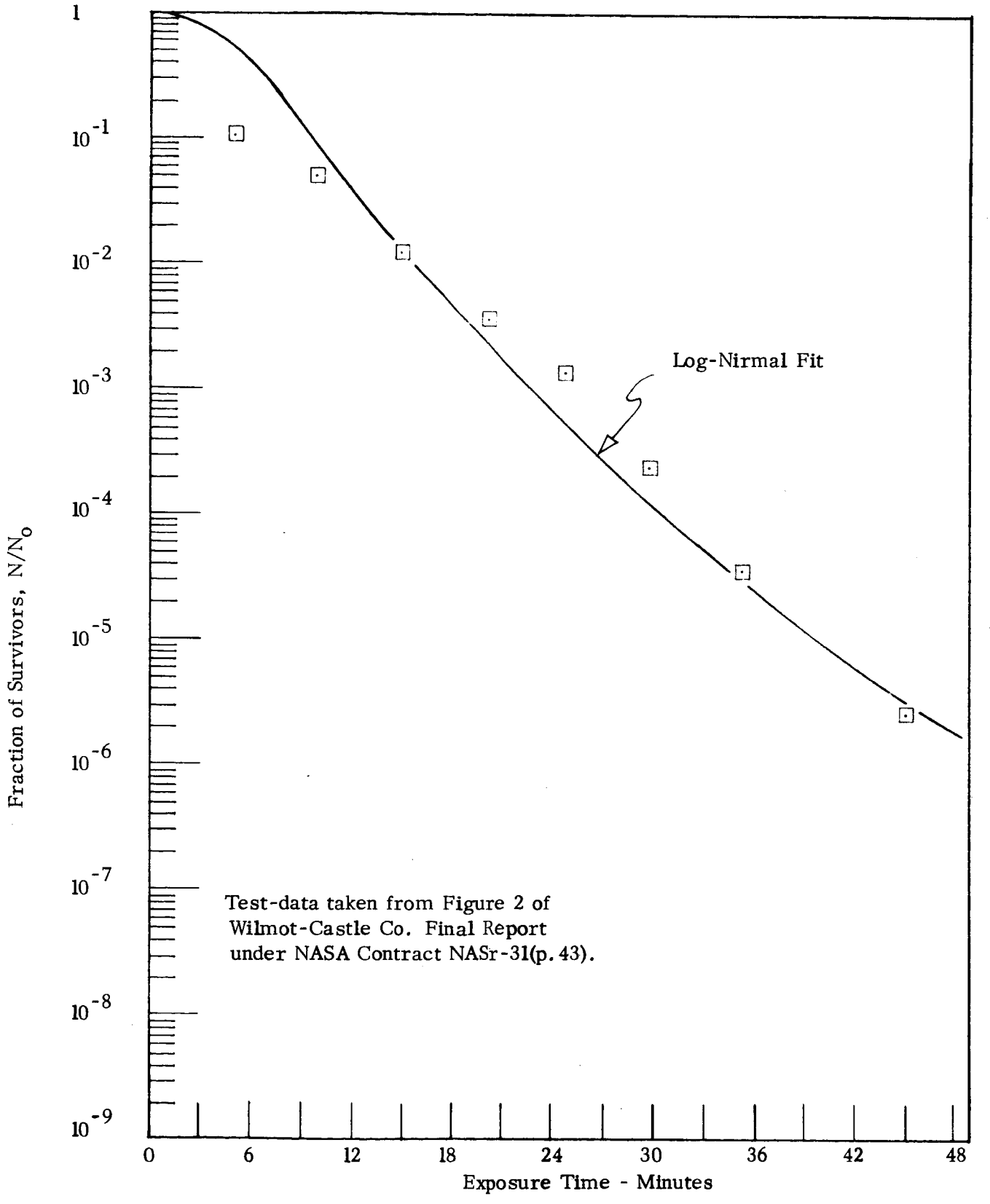

FIGURE 7: Log-Normal Fit of Survival Data for BACILLUS SUBTILIS VAR. NIGER at 120°C and Vacuum Environment

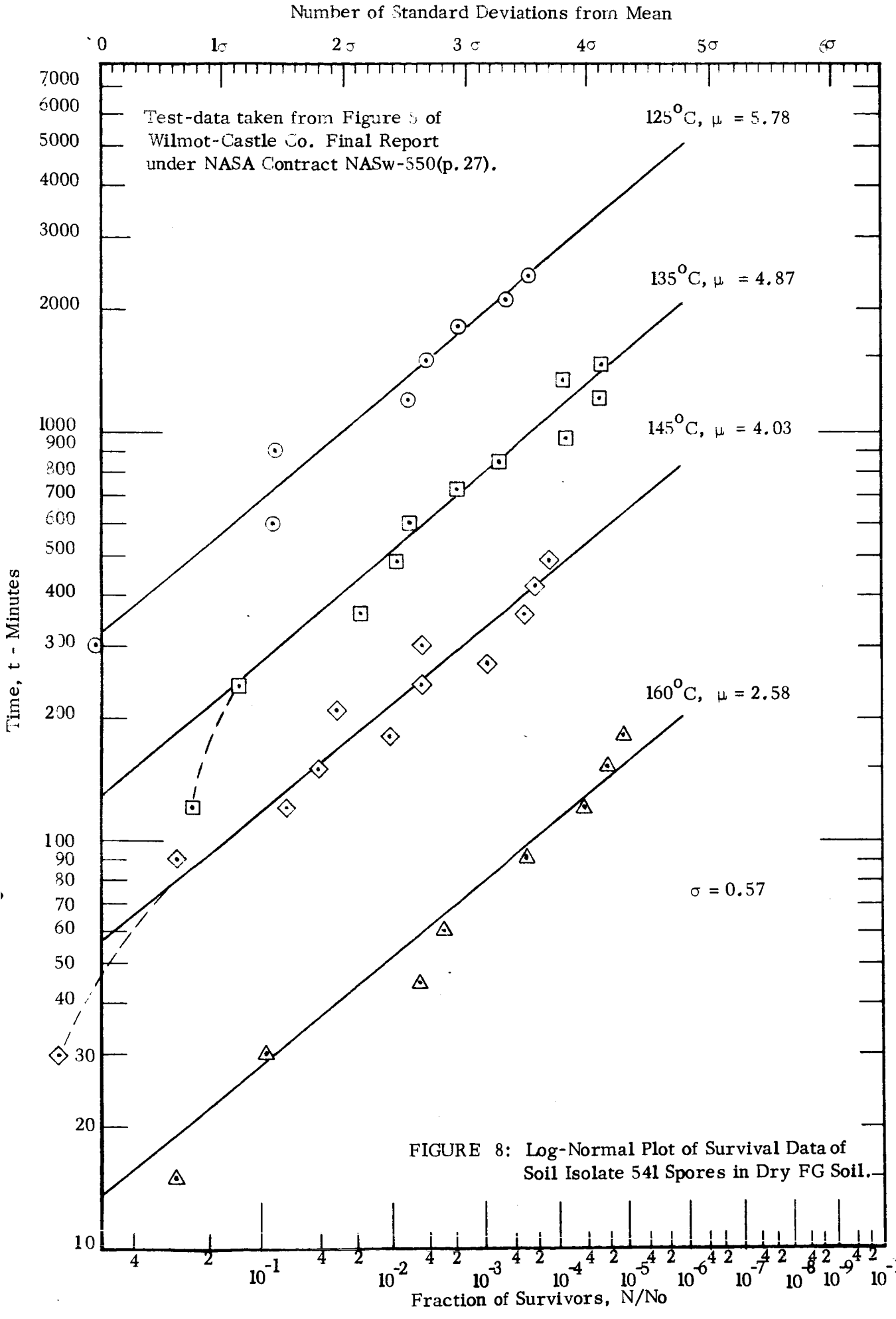

FIGURE 8: Log-Normal Plot of Survival Data of Soil Isolate 541 Spores in Dry FG Soil.

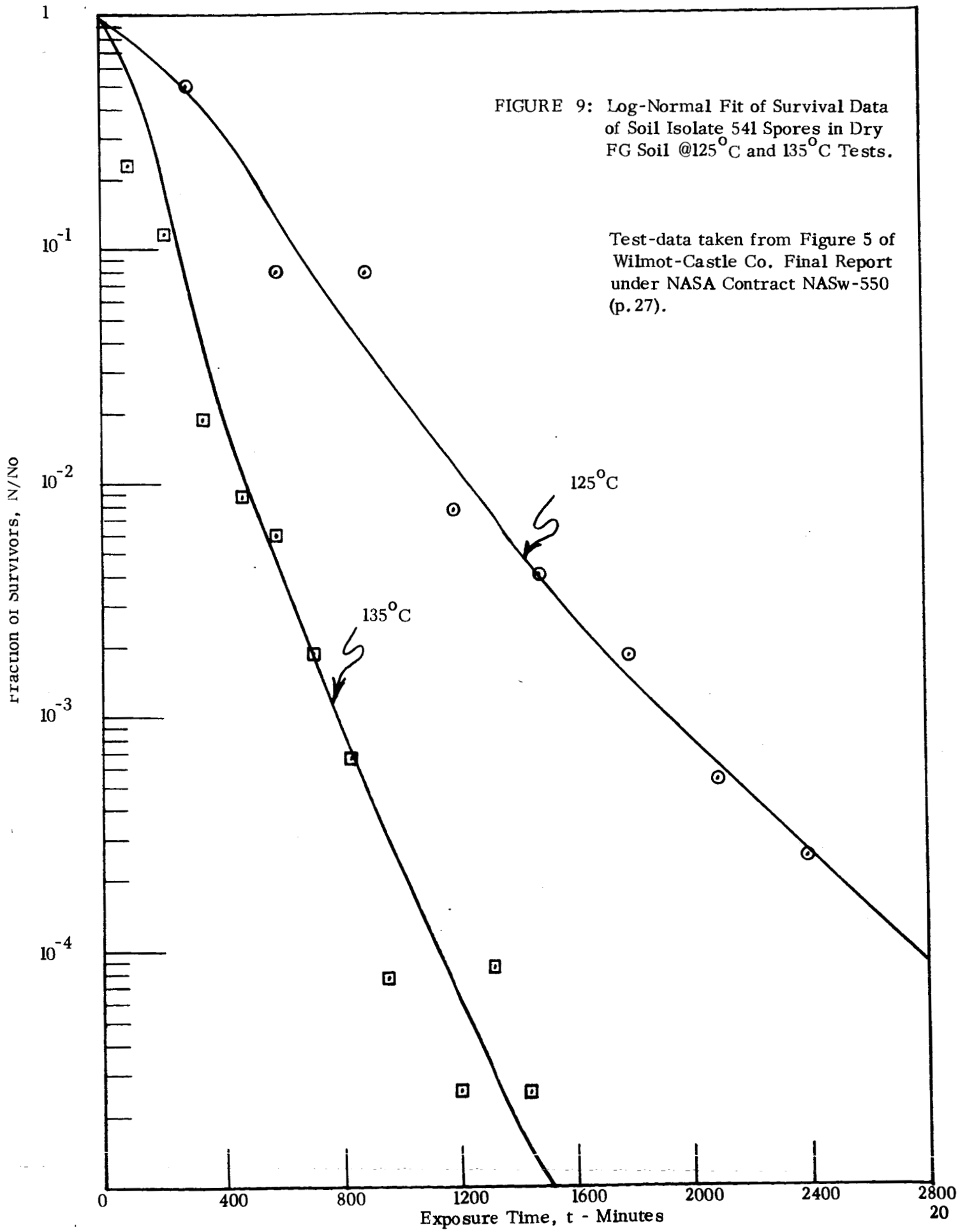

FIGURE 9: Log-Normal Fit of Survival Data of Soil Isolate 541 Spores in Dry FG Soil @125°C and 135°C Tests.

Test-data taken from Figure 5 of Wilmot-Castle Co. Final Report under NASA Contract NASw-550 (p. 27).

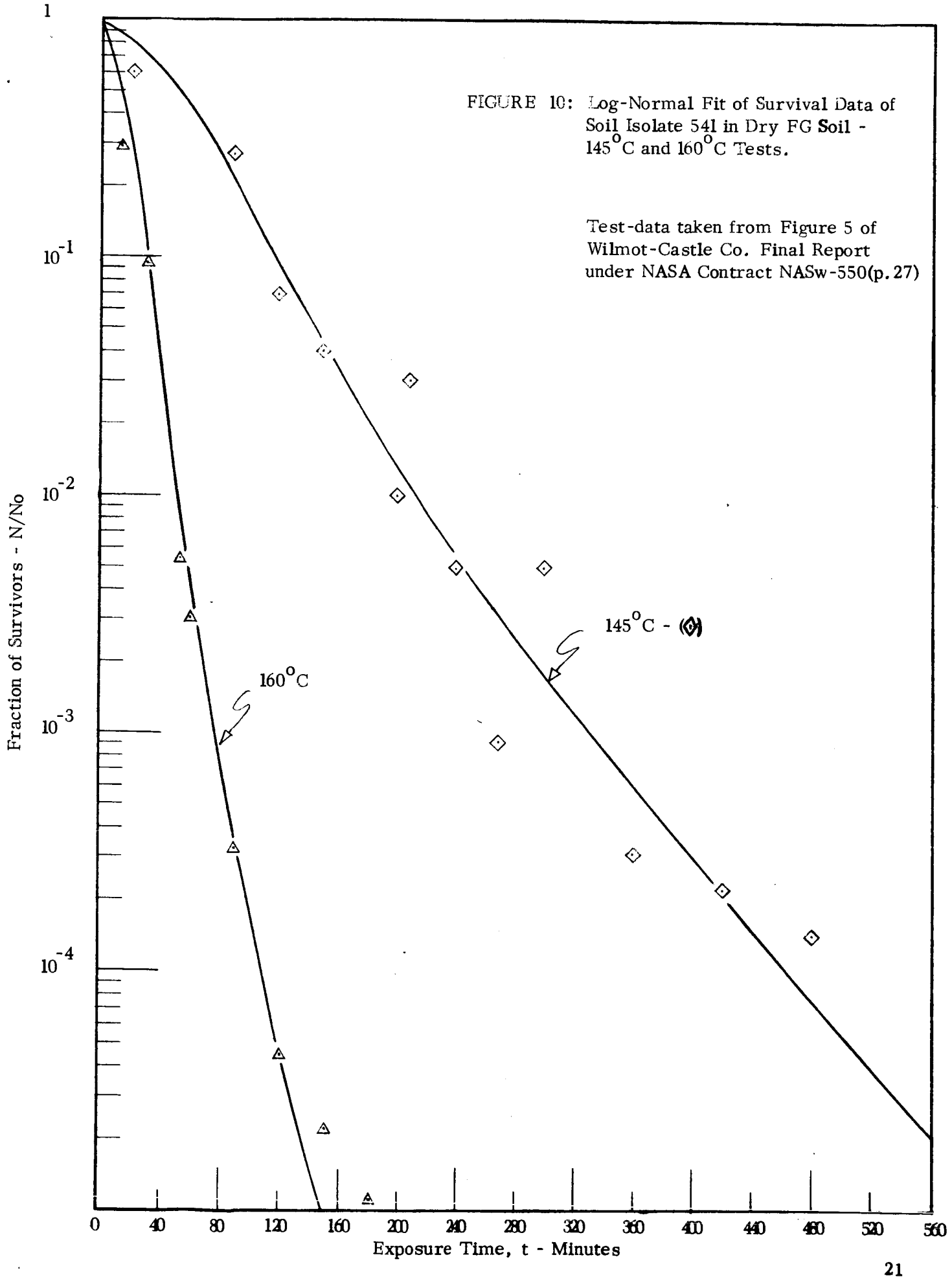

FIGURE 10: Log-Normal Fit of Survival Data of Soil Isolate 541 in Dry FG Soil - 145°C and 160°C Tests.

Test-data taken from Figure 5 of Wilmot-Castle Co. Final Report under NASA Contract NASw-550(p. 27)

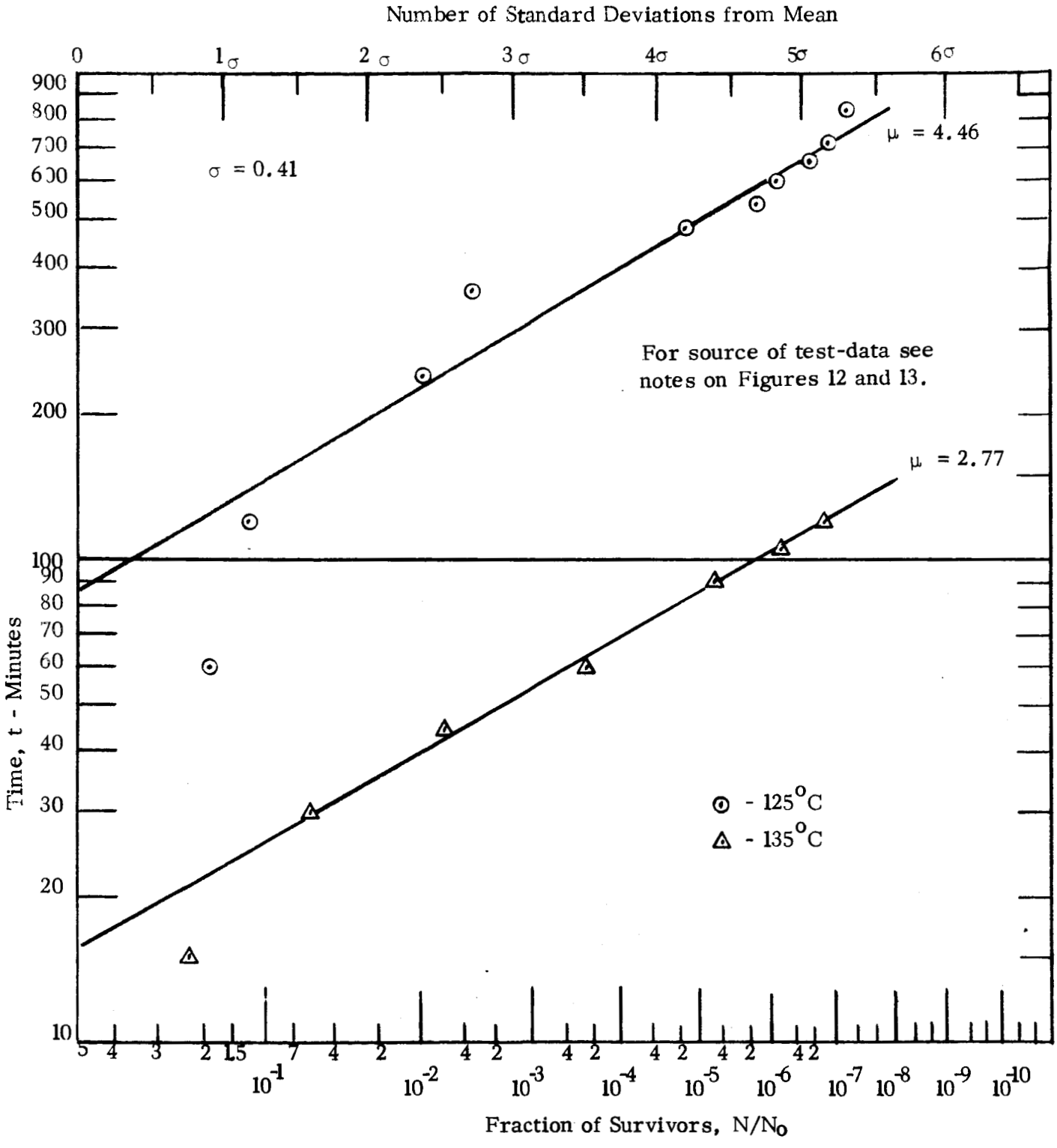

FIGURE 11: Log-Normal Plot for Survival Data of BACILLUS GLOBIGII Spores on Paper Strips.

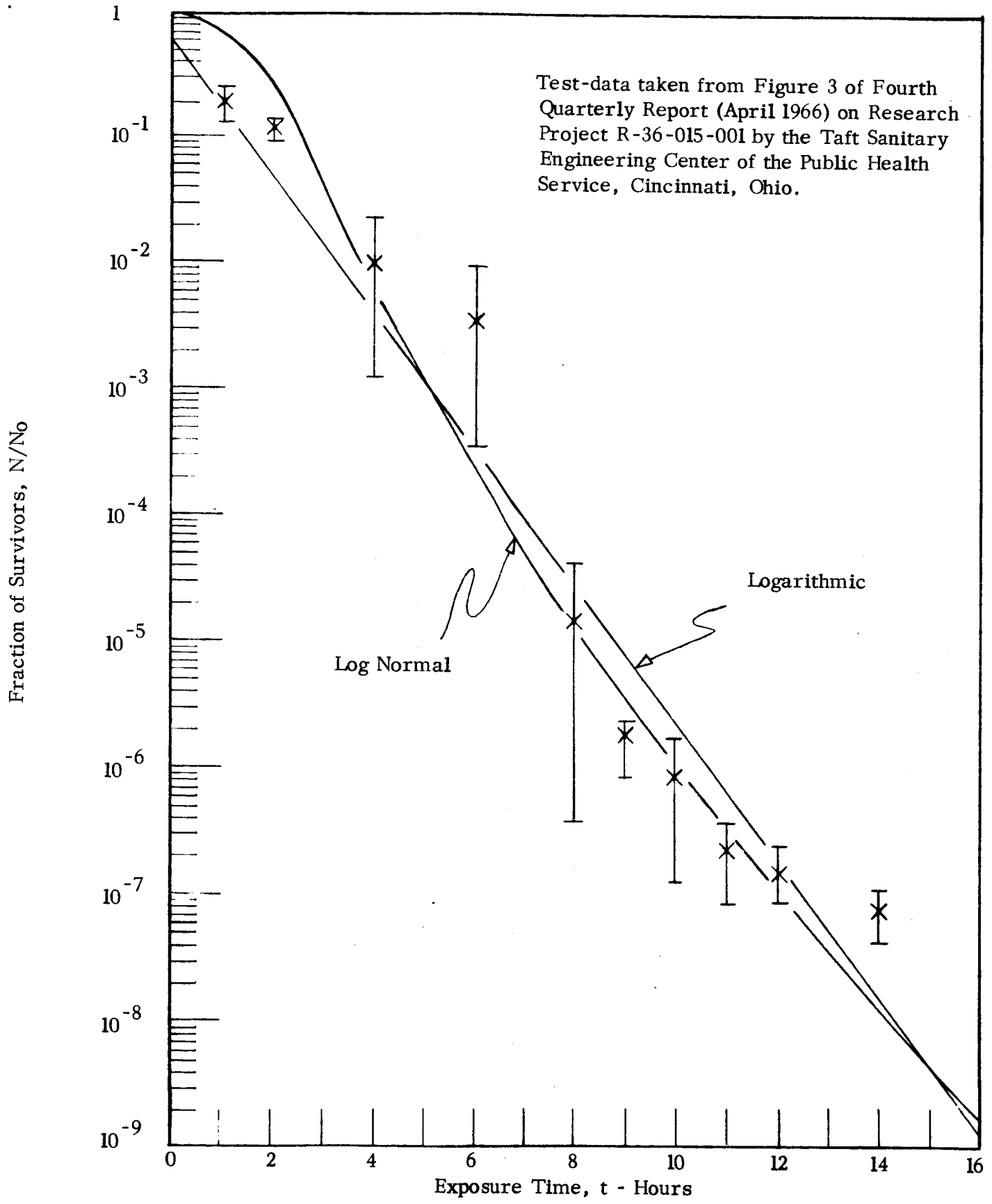

FIGURE 12: Comparison of Log-Normal and Logarithmic Fits for Survival Data at 125°C of BACILLUS GLOBIGII Spores on Paper Strips.

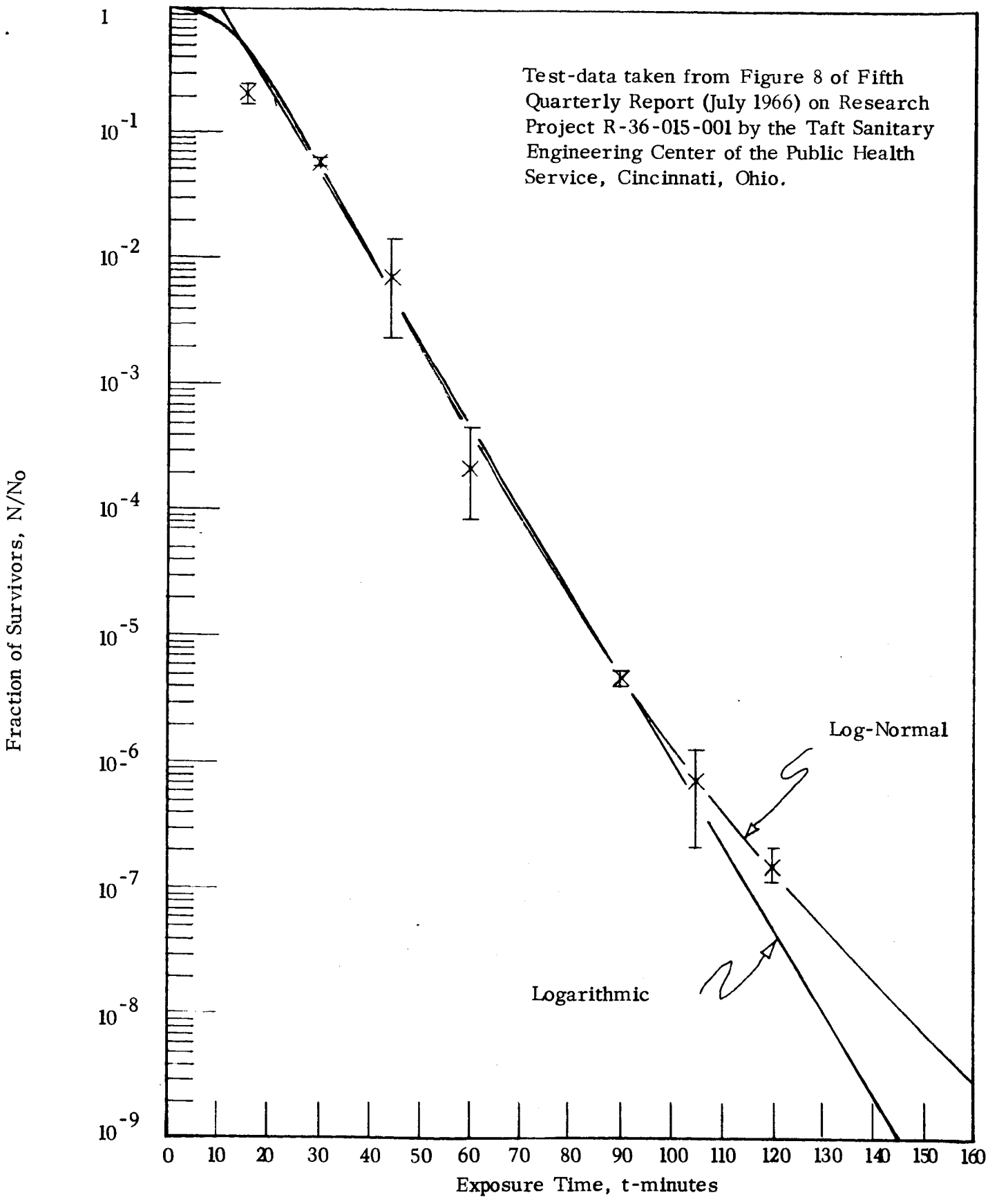

FIGURE 13: Comparison of Log-Normal and Logarithmic Fits for Survival Data at 135°C of BACILLUS GLOBIGII Spores on Paper Strips.

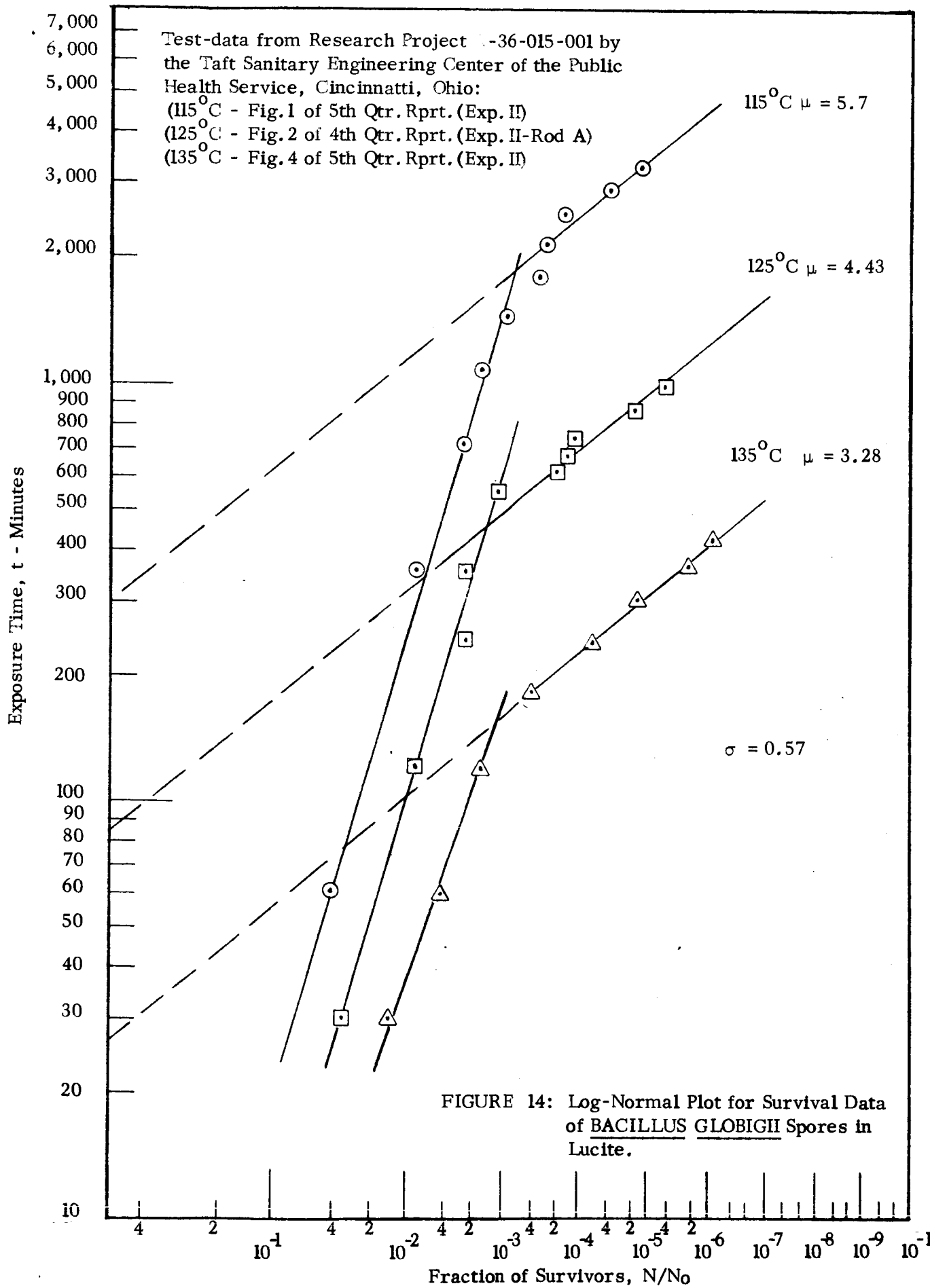

FIGURE 14: Log-Normal Plot for Survival Data of BACILLUS GLOBIGII Spores in Lucite.

that a straight line, i.e. a logarithmic model, is appropriate. Examples of this are shown in Figures 12 and 13, where the straight lines represent the experimenters choice, even though they do not start at $N/N_0 = 1$.

Our major interest is in the log-normal plots of each of the four experiment categories shown in Table I, i.e. Figures 5, 8, 11 and 14. In these plots each straight line represents a particular experimental condition and each line has a value of μ and σ. μ is obtained from the intersection of the straight line with the time axis, i.e. μ is equal to ln t when $N/N_0 = 0.5$. σ is obtained from the slope of the straight line and it will be noted that the family of lines in any one of the above figures have the same slope. This appeared to give a reasonably straight-line fit for each set of data points in the group of experiments and results in the same value of σ for the group. There is however, some variation in the values of σ among the four groups (see Table I).

Scrutiny of the log-normal plots leads to the conclusion that the log-normal model is valid only beyond some initial time period of exposure. (The corresponding value of N/N_0 below which the model holds are indicated in Table I.) However, the nature of the deviations from log-normality are also worth noting since they are the basis for future modifications of the model. These may be summarized as follows:

(a) The deviations occur during the initial heating period and consistently show an accelerated inactivation rate relative to the subsequent log-normal phase.

(b) The range of the accelerated inactivation phase appears to depend upon the medium surrounding the spores. Thus, for spores on paper strips and in air-atmospheric conditions only 50-90% become inactivated during this initial phase, regardless of the sterilization temperature. However, in a vacuum environment 99% become inactivated before the log-normal phase, and when embedded in lucite 99.9% of the initially viable population is inactivated during the accelerated phase. viz. the data points during this initial period consistently fall below the straight line.

In three of the four experiment groups discussed herein, the parameter which is changed between experiments is the sterilization temperature. Although the range of temperatures covered is relatively small, there is nevertheless an opportunity to explore the form of temperature dependence in the log-normal model.

Since within an experiment group σ was found to be essentially constant, only μ appears to be a function of sterilization temperature. (The dependence of the transition parameters, e.g. μ_τ or σ_τ, upon temperature was not considered since they are not directly related to the physical process of heat inactivation.) The possibilities for $\mu(t)$ considered included a linear relationship between either μ or $1/\mu$ and (1) T (2) $T^{1/2}$ (3) $T^{3/2}$ and (4) $e^{k/T}$. The last case represents an Arrhenius form and T is, in all cases, the sterilization temperature in degrees Kelvin. A plot of μ and $1/\mu$ vs. the above four forms of T was made using the data from Figures 8 and 14. It was thus found that only powers of T yield a linear relationship. Figure 15 shows the plots for

$$\mu = \mu_R - K_1 T^{1/2} \tag{9}$$

and

$$\mu = \mu_R - K_2 T^{3/2} \tag{10}$$

where K_1 and K_2 are the slopes of the respective curves and μ_R is some reference value of μ on the curve.

Equation (9) suggests relationships in the kinetic theory of gases, whereas equation (10) contains T in the form encountered in self-diffusion. Needless to say, however, equations (9) and (10) cannot both be valid and the fact that a linear relationship is observed for T, $T^{1/2}$ and $T^{3/2}$ merely reflects the inadequacy of the data with respect to the range of temperatures covered.

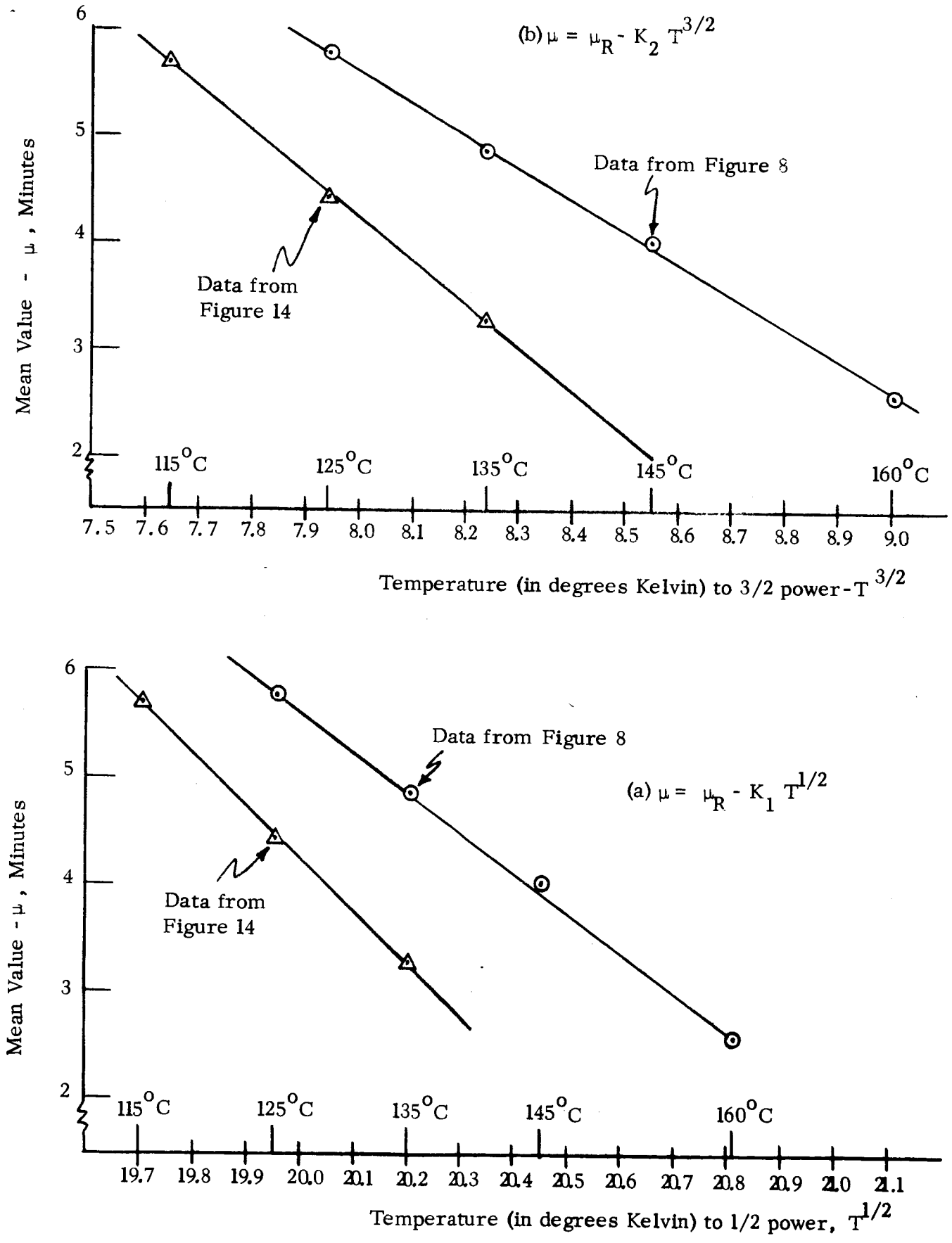

FIGURE 15: Temperature Dependence of Mean-Value Parameter μ

5. DISCUSSION

As noted in the introduction, a major objective of this study is the evolution of a model which will represent the physical inactivation of organisms during heat sterilization. Although this objective is not fully attained in the study reported herein, the results are believed to be of immediate practical utility. This can be demonstrated by assuming that the log-normal model is used to set sterilization requirements based on experimental data and the resulting requirements compared with requirements based on a logarithmic (D-value) interpretation of the same data.

For the purposes of this discussion, assume a constant temperature requirement and fixed environmental conditions, e.g. spores encapsulated in lucite and sterilized in air-atmospheric conditions at a constant temperature. The requirement itself is viewed as a specification on the time of exposure to heat such that the initial viable population will be reduced by 10 decades, i.e. to $N/N_0 = 10^{-10}$. It is further assumed that the experimental data is obtained for only five or six decades of reduction, i.e. to $N/N_0 = 10^{-5}$. The comparison will therefore consist of extrapolating the experimental data to $N/N_0 = 10^{-10}$ based upon (1) the logarithmic model and (2) the log-normal model.

First, consideration is given to data which might appear to faithfully follow a logarithmic model, i.e. it can be fitted with a straight line on a semi-log plot and the line would start at $N/N_0 = 1$. This would, for example, be true for the curves on Figure 4 when $\mu = 1$, $\mu = 2$ and, to a lesser degree, when $\mu = 3$. The straight lines which might thus be obtained based on data for $1 < N/N_0 < 10^{-5}$ are shown as dashed lines, and these lines are continued down to $N/N_0 = 10^{-10}$. The solid curves are similarly interpreted as the log-normal fits to the same data and their extrapolation to $N/N_0 = 10^{-10}$.

Table II, below, compares the required sterilization times based on the above two interpretations of the data.

TABLE II

		Sterilization Time - Minutes		
		t_2	t_1	
μ - Minutes	D-Value, Minutes	Logarithmic	Log-Normal	t_1/t_2
1	12	66	120	1.8
2	33	190	330	1.7
3	90	496	900	1.8

The log-normal model would thus require about twice as long a sterilization time compared to the logarithmic model and, as previously noted, this applies to cases where there might be the least hesitation in using the logarithmic model.

For the most resistant organisms it is difficult, if not impossible, to attempt a meaningful straight line fit starting at $N/N_0 = 1$. What is sometimes done, e.g. Figure 12, 13 and References (13), (14), is to fit a straight line without requiring that it start at $N/N_0 = 1$. This is, of necessity, a rather arbitrary procedure since the D-value thus obtained depends entirely on the range of data to which it is fitted and the choice of the experimenter as to which portion of the data is to dominate the fit. It is therefore not surprising to find a great divergence in D-values reported for essentially identical experimental conditions.

For reasons similar to the above it would not be very meaningful to make numerical comparisons between the logarithmic and log-normal models when highly sigmoid curves are encountered in a semi-log plot of experimental data. For, as shown herein, the log-normal model will produce a

consistently good fit to all or most of these data points, whereas the choice of a D-value is largely arbitrary.

It might appear that the deviation from log-normality during initial heating, as noted herein, should also enter the above comparison. This would be true only if sterilization requirements were considered in terms of very small reductions in N/N_0, i.e. to values of $N/N_0 > 10^{-2}$. Such reductions are seldom the objective of sterilization processes. Since the parameters of the log-normal distribution are obtained from data beyond the initial die-off period, the extrapolations to small values of N/N_0 based on these parameters are therefore unaffected. This is true for the constant temperature case considered above. When lethality is integrated over temperature transients, e.g. heat-up and cool-down periods, the initial die-off phase would enter the estimate of sterility versus exposure time. However, in this case, use of the log-normal model will produce a conservative sterility estimate because the die-off predicted by it, ignoring the die-off phase, will be smaller than the actual die-off during the initial phase.

The data presented herein suggest a number of directions for further study which might shed some light on the physical process of heat sterilization. Some of these are briefly discussed below.

The constancy of σ in a given set of experimental conditions, i.e. when only one parameter is varied, leads to speculation concerning the degree to which the values of μ and σ are determined by characteristics of the environment surrounding the organisms as opposed to the resistance characteristics of the organisms. For purposes of illustration, assume that the inactivation mechanism is due to the thermal motion of molecules surrounding the organisms. The distribution of molecular velocities at a given temperature are well known, as is the temperature dependence of this distribution function.

One might therefore seek a distribution function for the resistance of micro-organisms (and a normal distribution would certainly be a good candidate) such that the compound distribution will result in a constant σ with temperature but μ will vary linearly with T^n, where n is to be evaluated on the basis of more extensive experimental data.

The above will require use of experimentally obtained values of μ and σ based upon the log-normal model. However, it was shown that the log-normal model does not hold over the initial phase of heating which, although of short time duration, can cause the inactivation of a large part of the viable population. Furthermore, this initial phase appears to be characterized by a different process in that it is particularly dependent upon the nature of the medium surrounding the organisms. The use of experimentally obtained values of μ and σ, ignoring the initial phase, is therefore not a good basis from which to proceed.

A more fruitful approach would be to extend the model so as to account for the initial, accelerated die-off phase. As noted in the analytical development of the log-normal model, the hypothesis that the incremental change in death-time is proportional to prior exposure time, i.e. equation (4), has been chosen only because it was known to lead to a simple log-normal model. This hypothesis should therefore be replaced by others which will give a more complex formulation but will reduce to the log-normal model for long exposure times and/or small values of N/N_0. This is best done in conjunction with more detailed experimental data covering the entire range of interest.

6. CONCLUSIONS

Although the log-normal model does not fully describe the process of heat sterilization where a significant accelerated phase is present during initial heat application, it has considerable value for the following reasons:

(a) The major concern in sterilization is the inactivation of the entire viable population and the terminal phase of the process, encompassing the last few remaining organisms, is of particular interest. The log-normal model appears to be particularly valid for this latter phase and is therefore a useful basis for sterility estimation.

(b) In many instances the initial phase is not significant and the log-normal model could be expected to represent the entire process with more accuracy and greater repeatability than is currently obtained on the basis of the logarithmic model (D-value approach). In general, use of the log-normal model in sterility prediction would represent a conservative approach since neglecting the initial phase (as was done here in obtaining the values of μ and σ) will lead to conservative process requirements.

(c) The log-normal model provides a meaningful reference with respect to which the validity of basic assumptions may be judged. It thus offers guidelines for extensions of the model which, ultimately, may lead to more generally applicable analytical formulations and a better understanding of the physical processes causing microbial heat inactivation.

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7. REFERENCES

1. Withell, E.R., "The Significance of the Variation in Shape of Time-Survivor Curves", J. Hy. 42, p. 124-183 (1942).

2. Clark, A.J., The Mode of Action of Drugs on Cells, 296p. London: Edward Arnold and Co., 1933.

3. Tischer, R.G. and Hurwicz, H., "Thermal Characteristics of Bacterial Populations", Food Research 19, 80 (1954).

4. Shull, J.J., Cargo, G.T. and Ernst, R.R., "Kinetics of Heat Activation and of Thermal Death of Bacterial Spores", App. Microbiology 11, 6, p485 (Nov. 1963).

5. Licciardello, J.J. and Nickerson, J.J.R., "Some Observations on Bacterial Thermal Death Time Curves", App. Microbiology 11, 6, p476 (Nov. 1963).

6. Levine, S., "Determination of the Thermal Death Rate of Bacteria", Food Research 21, 3, p295 (May-June 1956).

7. Rahm, O., "The Problem of the Logarithmic Order of Death in Bacteria", Biodynamica 4, 81 (1943).

8. Ball, C.O. and Olson, F.C.W., Sterilization in Food Technology, McGraw Hill, 1957.

9. Schmidt, C.F. in Disinfectants, Fungicides and Chemical Physical Sterilization, (edited by G.F. Reddish), Lea and Febiger 1957, Philadelphia, Pa.

10. Schalkowsky, S. and Wiederkehr, R., "A Stochastic Sterilization Model", Report TR-13 by Exotech, Inc., Washington, D.C. under NASA contract NASw-1340, May 1966.

11. Koesterer, M., Final Report by Wilmet Castle Co. under NASA contract NASr-31.

12. Koesterer, M., Final Report by Wilmet Castle Co. under NASA contract NASw-550.

13. Angelotti, R., Fourth Quarterly Report (April 1966) on Research Project R-36-015-001 by the Taft Sanitary Engineering Center of the Public Health Service, Cincinnati, Ohio.

14. Angelotti, R., Fifth Quarterly Report (July 1966) on Research Project R-36-015-001 by the Taft Sanitary Engineering Center of the Public Health Service, Cincinnati, Ohio.

15. Aitchison, J. and Brown, J.A.C., The Lognormal Distribution, Cambridge University Press, 1963.

APPENDIX A

DERIVATION OF LOG-NORMAL MODEL FOR MICROBIAL SURVIVAL IN HEAT STERILIZATION

The derivation described below follows the general patterns described in reference (15). To develop the log-normal basis for heat sterilization it will be convenient to visualize a population of N_o organisms ranked in chronological order of their death times. Thus, when subjected to a constant heat environment, we can define the following discrete death-times:

τ_1 - time of death of first organism

τ_2 - time of death of second organism

. . .

. . .

. . .

. . .

τ_{j-1} - time of death of (j-1) organism

τ_j - time of death of j-th organism

. . .

. . .

τ_k - time of death of k-th organism

. . .

. . .

τ_{N_o} - time of death of last organism

As discussed in the body of the report, the assumption leading to a log-normal distribution is that the incremental time between successive deaths is proportional to the prior exposure time, viz.

$$\tau_j - \tau_{j-1} = \epsilon_j \tau_{j-1} \qquad \text{(A-1)}$$

where (ϵ_j) is a set of independent, distributed random variables. Equation (A-1) can be written as

$$\frac{\tau_j - \tau_{j-1}}{\tau_{j-1}} = \epsilon_j \qquad \text{(A-2)}$$

and the two sides summed between j=2 and j=k, i.e.

$$\sum_{j=2}^{j=k} \frac{\tau_j - \tau_{j-1}}{\tau_{j-1}} = \sum_{j=2}^{j=k} \epsilon_j \qquad \text{(A-3)}$$

It will be noted that the j=1 term has been excluded from the summation since its use would give $\tau_1 = 0$ (see Equation A-1). τ_1 will however be accounted for in the following development.

The left-hand side of A-3 can be approximated by an integral if N_o, and k, are sufficiently large. This is generally the case and we can use

$$\sum_{j=2}^{j=k} \frac{\tau_j - \tau_{j-1}}{\tau_{j-1}} \simeq \int_{\tau_1}^{\tau_k} \frac{d\tau}{\tau} = \ln \tau_k - \ln \tau_1 \qquad \text{(A-4)}$$

We could dispose of τ_1, the time of death of the first organism, in one of two ways. Thus, we might simply say that because we generally look at death times much longer than τ_1, $\ln \tau_k \gg \ln \tau_1$ and $\ln \tau_1$ could be neglected. An alternative approach is to incorporate $\ln \tau_1$ into the summation on the right-hand side of A-3, i.e. let

$$\ln \tau_1 = \epsilon_1 \qquad \text{(A-5)}$$

Thus, ϵ_1 is also viewed as a random variable, independent of $\epsilon_2, \epsilon_3 \ldots\ldots \epsilon_k$.

From A-3, A-4 and A-5 we have

$$\ln \tau_k = \sum_{j=1}^{j=k} \epsilon_j \qquad \text{(A-6)}$$

Although we do not know the distribution of (ϵ_j), we can associate with it a mean μ and σ variance. Under the general regularity conditions of the central limit theorem, and with k sufficiently large, the summation of ϵ_j, i.e. the right-hand side of A-6, is normally distributed with mean μ and variance σ. Hence $\ln \tau_k$ is normally distributed and τ_k is log-normally distributed.

The subscript k derives from the ranking according to death-times which was used to facilitate the analytical development. Needless to say, such ranking is not possible in practice nor is it needed in the application of the analytical results. For when we consider the death time of the k-th organism, this is equivalent to saying that k out of N_o initially viable organisms have died. Thus k corresponds to N_o-N(t), where N(t) is the number of survivors, and k can therefore be supressed. The statement that $\ln \tau_k$ is normally distributed can therefore be defined as (see Equation 3 in body of report):

$$P_d(t) = 1 - \frac{N(t)}{N_o} = \int_o^t f(\tau)d\tau =$$

$$\frac{1}{2\pi\sigma} \int_o^t \exp\left[-\frac{1}{2\sigma^2}(\ln \tau - \mu)\right] d(\ln \tau) \qquad \text{(A-7)}$$

It will be noted that although the lower limit of the last integral is $-\infty$ for the normal distribution, the fact that the variable in the integrand is $\ln \tau$ serves to confine the limits of integration to positive values of τ only, since for $\ln \tau = -\infty$ $\tau = 0$ and the lower limit becomes t = o.

Since τ is the running time of heat application, it is useful to define the frequency function $f(\tau)$. From equation A-7

$$f(\tau) = \frac{1}{\tau} \quad \frac{1}{2\pi\sigma} \quad \exp\left[-\frac{1}{2\sigma^2} \ln(\tau - \mu)\right] \qquad \text{(A-8)}$$

Equation A-8 gives the frequency function of deaths, i.e. the fraction of the original viable population which die at time τ. This frequency function differs from the bell-shape of the normal frequency function not only because the logarithm of time is the variable in the exponent, but also because of the additional factor $1/\tau$. This is therefore a highly skew frequency curve with frequencies of death becoming relatively smaller with time compared to the normal distribution. And as previously noted, the frequency curve is restricted to positive values of time.

A detailed discussion of the log-normal distribution will be found in Reference (15). For our purposes we note the following parameters of $f(\tau)$:

$$\text{mean value of } f(\tau): \quad \mu_\tau = \exp\left[\mu + \frac{1}{2}\sigma^2\right] \qquad \text{(A-9)}$$

$$\text{variance of } f(\sigma): \quad \sigma_\tau^2 = \left[\exp(2\mu + \sigma^2)\right]\left[\exp(\sigma^2) - 1\right] \qquad \text{(A-10)}$$

$$\text{Coefficient of variation of } f(\tau): \quad \eta_\tau = \left[\exp(\sigma^2) - 1\right]^{1/2} \qquad \text{(A-11)}$$

Although both μ_τ and σ_τ appear to be dimensionless quantities, this is not the case and both have the same units of time as used for τ and t. That this must be so can be demonstrated by noting that τ, μ and σ in the exponent of equation A-7 must all be dimensionless quantities. To achieve this we should have written each of them as $\tau/\bar{\tau}$, $\mu/\bar{\tau}$, $\sigma/\bar{\tau}$ where $\bar{\tau}$ is unity in magnitude and of the same dimension as τ, i.e. seconds, minutes or hours. Carrying $\bar{\tau}$ through the analytical development would then serve to carry the units of τ into μ_τ (as well as μ and σ). It will also make $f(\tau)$, equation A-8, dimensionless, as required.