ANNEX G

Pasteurization of Liquid Egg Products and Shell Eggs

CONTENTS

INTRODUCTION

The presence of viable *Salmonella* in eggs and egg products is a public health threat. A common method used to eliminate or reduce the number of viable cells is pasteurization, a process in which liquid egg products are heated at below boiling to kill vegetative microbial cells. Consequently, in order to define performance standards for *Salmonella* in eggs and egg products, it is necessary to determine the probabilities that viable *Salmonella* in eggs or egg products will remain following pasteurization. This annex describes the derivation of inactivation models used for determining the distribution of the number of *Salmonella* cells that survive the pasteurization processes. To do this, inactivation data for (i) liquid egg white product at various pH levels, (ii) 10% added salt to whole and yolk egg product, (iii) 10% added sugar to whole and yolk egg product, (iv) 5 formulated liquid egg products (see Attachment 1 for formulations), and (v) shell eggs. In addition, information from published literature was used to derive an inactivation model for plain whole egg and yolk products. A short discussion of inactivation models and their use in the risk assessment is presented, followed by a statistical methods subsection that presents the functions used to describe the models and a subsection that presents the data analysis used for selecting the distribution functions for these lethality models.

To model inactivation, there are two sets of equations, in a hierarchal relationship. The first level is called the primary equations, which models the survival of *Salmonella* for known temperature. These equations are functions of certain parameters, with values that depend upon the temperature. The second set of equations (secondary equations) consists of equations that relate the parameter values as a function of temperature. These equations are usually linear or quadratic. A brief discussion of the primary equations to be used is given below.

Primary Equations for modeling survival of *Salmonella*

For modeling inactivation of cells subjected to a lethality treatment, the primary assumption made is that the events of inactivation are mutually independent This assumption may not be innocuous: if the cells were "clustered" or somehow bound together to provide some degree of mutual protection, then this assumption would not be true. However, as far as is known, there have not been studies investigating the occurrence of cell clustering in egg products and the effect that such clustering would have on inactivation kinetics. Consequently, this risk assessment is based on the assumption that events of cell inactivation are independent.

This assumption implies that the distribution of the number of cells at time, *t*, subjected to some lethal treatment is a binomial distribution with parameters N_0, the initial number of cells, and $p(t)$, the probability that a single cell would survive at time t.[1] If, from a liquid product, a constant density or level, *r*, is assumed throughout a large volume, then it can be shown that the distribution of the number of surviving cells in a small volume, *v*, of product is Poisson with parameter $rvp(t)$. The values of *r* are derived from the distribution of levels in the pre-pasteurized product. The volumes represent the consumed amount of pasteurized product. Thus, to model lethality, it is necessary and sufficient to model or estimate $p(t)$. The purpose of this annex is to present the data analysis that was used for modeling $p(t)$.

Rather than modeling $p(t)$ directly, the natural logarithm of $p(t)$ was modeled. The graph of $\ln(p(t))$ versus t is termed the survival curve. If the survival curve is known, $p(t)$ can be determined directly by taking the anti-logarithm. The typical experimental design for determining survival curves is to fix environments (pasteurization temperatures) for a series of experiments, and determine survival curves for each experiment. From results of these experiments, secondary equations are developed that permit derivation of survival curves for any environmental condition within the range of those studied. It is assumed the derived survival curves are valid for a constant temperature (or otherwise a constant environment).

The difficulty is in extending results of such experiments to scenarios when temperatures are changing. To combine results of survival curves derived from experiments with fixed environments, the slopes of the survival curves are used, based on the following mathematical feature: the slope or derivative of the survival curve is the "hazard" function, $h(t) = p\text{N}(t)/p(t)$, which can be considered as the instantaneous probability of cell inactivation at time t. Conversely, if a hazard function $h(t)$ is known, then a survival curve is computed as $\ln(p(t)) = \text{I}h(t)\,dt$. That is to say, that if the instantaneous probabilities of inactivation are known then the survival curve can be constructed, by integration. For example, if $h(t|T)$, designating a hazard

$$p'(t|T) = h(t|T)p(t) \tag{G1}$$

function at a fixed temperature T, is constant ($= -k$) then $\ln(p(t) = -kt$; so that $p(t) = e^{-kt}$; a constant hazard function implies a linear survival curve for T. However, when temperatures are changing with time, the above relationship of $h(t|T)$ and $p(t)$ may not hold. Specifically, the relationship for all fixed temperatures, T, does not imply that, if temperatures are changing with time such that $T = T(t)$, then

$$p'(t) = h(t, T(t))p(t), \tag{G2}$$

where $h(t, T(t))$ represents the hazard function where temperature is a function of time. This is because the instantaneous probability of cells being inactivated at time t, given that they have survived up to time t most likely depends upon what has happened to the cells at times before t. For liquid egg products, since the product is heated relatively fast, it is not necessary to rely on Equation 2, because it can be assumed for most cells, inactivation would take place at the maximum temperature. Thus, for liquid products, lethalities are calculated at an assumed fixed (maximum) temperature. However, for pasteurization of shell eggs, because the temperature of the eggs changes slowly there may be a significant amount of inactivation before the temperatures each the maximum (equilibrium) temperature. If Equation 2 were valid, then the survival curve would be:

$$\ln(p(t)) = \int_0^t h(\tau, T(\tau))d\tau \tag{G3}$$

In the simplest case of linear survival curves and thermal death curves $(\ln(k(T))$ versus $T)$, $h(t|T) = -k(T)$, and $\ln(k(T)) = a+bT$, so that Equation 3 becomes,

$$\ln(p(t)) = -\int_0^t e^{a+bT(\tau)}d\tau \qquad (G4)$$

Thus, if $T(t)$ is known, then, from Equation G4, the parameters, a and b, can be estimated from an observed survival curve. (Note that a and b are assumed to be constants, not dependent upon temperature.) Then the negative of the inverse of b $(-1/b)$ is proportional to the z-value (which is the temperature change necessary to affect one $\log_{10}$ change in the D-value - the time needed to reduce the population of viable cells by 90% or one $\log_{10}$).

Strain variability

An issue addressed in this risk assessment is strain variability of survival kinetic parameters. Comparisons of D-values have been given[2] in which estimates of D-values for 17 strains of *Salmonella* Enteritidis (SE) at 57 and 60°C in liquid egg white product were described (Table G1).

TABLE G1 REPORTED D-VALUES (SECONDS) FROM SHAH ET AL.[2] FOR 17 DIFFERENT SE STRAINS. THE PHAGE TYPES AND THE SOURCES OF THE STRAINS ARE ALSO GIVEN.

Source	Phage Type	D-value at 57°C	D-value at 60°C
Egg yolk	.	109.4	18.9
Cloacal swab	8	76.6	18.6
Human	8	90.1	24.2
Human	8	90.5	18.8
Ice cream	8	99.6	23.5
Human	8	83.8	14.8
Human	8	102.7	15.3
Cracked egg	13	76.0	18.1
Human	13	84.4	13.5
Ice cream	8	105.0	11.8
Human	8	113.4	12.0
Egg yolk	8	168.5	24.1
Chicken	4	136.2	20.4
Chicken ovary	8	154.6	21.8
Egg	8	72.6	13.7
Egg slurry	8	93.8	23.0
Egg slurry	8	91.6	31.3

The coefficient of variation (CV) of the D-values for both temperatures was approximately 27%. From an analysis of variance of the data that were provided in the article (based on the ln(D-values)), there is a marginal significant effect for the phage type (P-value

Definition of Lethality

The term 'lethality (at time t)' is defined to be '$-\log_{10}(p(t))$' (or, negative value of $\log_{10}(p(t))$), where $p(t)$ is the individual cell-specific probability of survival at time t.

= 0.094) and a statistically significant source effect (P-value = 0.04). It is not possible to say that the source variable implies a meaningful stratification of the population of serotypes that exists in eggs. Thus including the source effect in an analysis of variance does not necessarily provide "better" estimates of the between–strain variance from the populations. Rather, the 17 strains are considered as a random sample of strains from the existing populations, and thus, the between strain variance is computed, ignoring source and phage designation. Performing the analysis of variance, considering temperature and a fixed factor, the between–strain variance is equal to 0.017, implying a between–strain standard deviation of 0.13. Thus, using the approximation, for CV values less than 25%, that the standard deviation of the natural logarithm of the variable is approximately the CV divided by 100% of that variable, the between-strain CV is estimated to be 13%. In another article,[3] a comparison of 5 serotypes of *Salmonella* indicated an approximate 20% between serotype CV for asymptotic *D*-values determined in liquid medium. A simple adjustment was suggested for accounting for the between-serotype CV when developing lethality models for risk assessments. The adjustment assumes the studied serotypes are a random sample from a population of serotypes and knowledge of the between-serotypes CV of lethalities among the serotypes of the population.

It is not clear that this assumption of randomness would be valid for the serotypes chosen to be studied: *Salmonella* Blockley, for example, was selected to be part of the five-serotype cocktail for the FSIS-approved protocol for lethality studies in egg products that were conducted under contract with the United Egg Association.[4] The reason was that *S*. Blockley exhibits higher heat resistance than other serotypes. Yet because there seems to be a between-serotype variance of heat resistance, it is possible that there could be serotypes that would be more heat resistant than any of the five serotypes selected for inclusion in the cocktail. This possibility was accounted for in the risk assessment by including parameters that reflect the between-serotype variation. In addition, an adjustment was needed to account for the bias that arises when using strain cocktails, as far as the asymptotic behavior of the survival curves reflects that of the most heat-resistant strain or serotype of the cocktail. Thus, in the risk assessment an adjustment to the computed lethalities that accounts for the bias introduced by the between serotype variability, based on the assumption that the five studied serotypes are a random sample from some population. The parameter values are determined using a simple notion[3]: let $x_{(n)}$ be the maximum observed value among a set of n sample values $\{x_j, j = 1, ..., n\}$, where each x_j is a value of a random variable X_j, where $X_j, j = 1, \ldots, n$ are assumed to be independent, identically distributed normal variables with unknown mean, μ, and known standard deviation, σ. Then there exist a constant, g_n, dependent on n, such that the expected value of $x_{(n)} - g_n\sigma$ is μ. In application, $x_{(n)}$ is the estimated value of $\ln(p(t))$ and n is the number of serotypes in the cocktail. For the data from Froning et al.[4] $n = 5$; thus, as determined by simulation, $g_{(5)} = 1.163$ (based on 2 million simulated results of the maximum of 5 generated values from a standard normal distribution (SAS[®])). The value of σ is assumed to be 0.13, corresponding to the estimate derived above. Thus the expected value of $\ln(p(t))$ would be lower by an amount 0.13(1.163) = 0.1512. For the risk assessment, a standardized random normal variate, ζ, is generated and the quantity 0.13(ζ - 1.163) is added to derived value of $\ln(p(t))$.

Statistical methods

Primary survival curves

Pasteurization of shell eggs

Equation G4 was assumed for determining lethality of *Salmonella* in shell eggs. Data available for estimating parameters *a* and *b* of Equation 4 consisted of observed levels of colony-forming units (cfu) and temperatures over time.

Pasteurization of liquid egg products

Experimental data consisted of observed survival curves for fixed temperatures.[4] Five strains of *Salmonella* were inoculated into egg products as a mixture or cocktail (*S*. Enteritidis PT4 and PT 13; *S*. Typhimurium TM-1; *S*. Blockley; and *S*. Heidelberg). The products selected for the study included: egg whites with pH values of 7.8, 8.2, 8.8, and 9.3; whole and yolk egg products with 10% added salt; and whole and yolk egg products with 10% added sugar; and 5 formulated products (3 consisting of whole eggs, 1 of egg yolks, and one of egg white). The specific formulations are given in Attachment G1 of this Annex.

An examination of all the data from the study indicated that the survival curves were not linear. In much of the literature, the primary kinetic parameter for which estimated values are reported are the *D*-values, which implies that the survival curves from which the *D*-values were derived are linear. However, many studies report nonlinear survival curves for *Salmonella*. For example, Blackburn et al.[5] described non-linear inactivation of SE in culture broth. Based one the University of Nebraska data and the results from Blackburn et al.,[5] the use of *D*-values to characterize the survival curves could lead to biases in the estimation of inactivation probabilities. Therefore, the *D*-values reported for egg products in the literature were not used directly. While the raw data generated by Blackburn et al.[5] are not available, the reported results are credible for use in this risk assessment.

Many functions have been used to fit nonlinear survival curves.[5-9] The first step in selecting a function is to examine the plots of the observed survival curves. The general shape of the survival curve is usually self-evident[10] and is often characterized by the shape of the curves for a short period and for times approaching infinity. The more common types are: (i) initially concave (negative second derivative) and approaching a straight line – often this type of curve is referred to as having a "shoulder"; (ii) a straight line; (iii) initially convex and approaching a straight line with non-zero slope; (iv) convex such that the probability of survival approaches zero, so that the lethality has no upper bound; and (v) initially concave and becoming convex (sigmoidal). These latter curves are sometimes referred to as having a tail, or as 'tailing". Other functions implying that the lethality as time approaches infinity has an upper bound (e.g., Blackburn et al.[5]), were not used in the risk assessment.

It often difficult to determine for sigmoidal or convex survival curves whether they are tailing.[3] For the data from Froning et al.[4], this is particularly true because the individual observed survival curves often included only four or five data points and in a few of these the observed lethality ($\log_{10}$ reduction of the measured levels) were less than 4 or 5 $\log_{10}$. Consequently, when convex shaped survival curves are fit to functions for which tailing is observed in plots of the data, the assumption implicit is that the asymptotic *D*-value approaches zero. Such functions are derived by either assuming that (i) the hazard function (or the instantaneous inactivation rate) is

constant for a given cell and that over the population of cells the cell-specific instantaneous inactivation rates are distributed as some designated distribution such as the gamma or normal distributions,[11] or (ii) a survival distribution for $p(t)$[8] with the appropriate asymptotic behavior. For the former assumption, gamma or normal distributions are mathematically convenient to use because the *Laplace* transform of these distributions has a simple closed form. Very simply, for survival curves that display tailing, it is assumed that each cell has a linear survival function with instantaneous inactivation rate of k, so that $p_k(t) = e^{-kt}$, and, over the population of cells, k is a random variable, distributed with cumulative distribution function (cdf), F, described by the parameter, θ. Integrating $p_k(t)$ with respect to $F(k)$, and taking the logarithm, the survival curve can be described as

$$\ln(p(t)) = \ln(\phi_F(t)) \tag{G5}$$

where $\phi_F(t)$ is the *Laplace* transform of F. If there is an initial shoulder, then there would be additional terms,[9;12] but asymptotically, the above equation would hold. For a normal distribution, the *Laplace* transform is quadratic in t, and for sufficiently large t, becomes an increasing function, implying a decreasing lethality, which is contrary to normal expectations. Therefore, the normal distribution was not considered further.

A gamma distribution has a particularly simple *Laplace* transform function: $-\alpha \ln(1+\beta t)$, where α and β are the parameters that characterize the gamma distributions: $x^{\alpha-1} e^{-t/\beta}/(\Gamma(\alpha)\beta^{\alpha})$. As t approaches infinity, the derivative of $\log_{10}(p(t))$ approaches 0, and as t approaches 0^+, the derivative approaches $\alpha\beta/\ln(10)$. Based on the gamma assumption, the derivative is proportional to the mean of the exponential inactivation rates. The coefficient of variation (CV) divided by 100% is equal to $\alpha^{-1/2}$. If $\alpha<1$, there is no mode, and the distribution of k would be highly skewed with a CV greater than 100%. In this analysis, the gamma distribution is one of the functions considered, and all estimated values of α are greater than 1.

Another function considered for convex or sigmoidal survival curves, based on the logistic probability distribution, [3;6] is

$$\log_{10}(p(t)) = -\log_{10}(1 + \exp(a\ln(t) + b)) \tag{G6}$$

where a and b are parameters. This function provides the flexibility to fit a variety of shaped survival curves that have asymptotic convex behavior. The derivative of the right side of Equation 6 approaches $-e^b a t^{a-1}/\ln(10)$, as t approaches 0. Thus, if $a>1$, then the slope at zero is zero, and if $a<1$, then the limiting slope at zero is minus infinity.

For convex curves with no shoulder or sigmoidal shape, if functions defined by Equation G5 for the gamma distribution and by Equation G6 fit the observed lethalities equally well, then, provided $a<\alpha$, the asymptotic lethality (as t approaches infinity) for the gamma function is larger. This is seen by noting that the ratio of the limit as t approaches infinity of the function of Equation G6 to that of the function of Equation G5 for the gamma distribution is equal to a/α,

which is less than 1 when $a<\alpha$. Because the curves are assumed to be convex, $a<1$, and, as discussed above, it is reasonable to assume that $\alpha>1$. In other words, it appears that for convex survival curves such as for egg products with 10% added salt, the use of the logistic function implies a greater degree of tailing and cell heterogeneity regarding their ability to resistant heat, than would be the implication if the gamma function were used. In the case for 10% added salt product where these two functions provide equally adequate fits to the data, model uncertainty becomes an issue. One function is not clearly better to use in the risk assessment than the other is. The differences of predicted times needed to obtain specified lethalities that are outside the range of the observed data can be profound. For example, to achieve 8-$\log_{10}$ lethality, the predicted times using the logistic assumptions are up 33% and often 10-15% larger than the predicted times using the gamma assumption. However, for the proposed regulation, the lethalities being considered are less than 7 logs and the difference that arises when using one function or the other is not large. For the risk assessment, the gamma function was used.

A third function considered, based on assuming a Weibull distribution for the survival function[8] is

$$\ln(p(t)) = -at^{b} \tag{G7}$$

where a and b are parameters. The shape of this curve depends on the value of b: (i) if $b>1$, then the curve is convex; (ii) if $b = 1$, then the curve is linear; and (iii) if $b<1$, then the curve is concave. This function does not approach a straight line (unless $b = 1$) and would imply a decreasing or increasing hazard function. For convex curves, the asymptotic lethalities obtained from Equation G7 are greater than those from Equation G5 and G6 are when assuming F is the gamma distribution.

For the 10% added sugar in whole egg products experiments with concave survival curves, this Weibull function was used because the function provided a good fit and the maximum observed lethalities were between 6 and 7 $\log_{10}$. This range of lethality is considered large enough in order to validate the asymptotic behavior of the model, since the proposed lethalities are within this range. However, for the concave survival functions of egg white products, the Weibull function was not considered because the maximum lethalities observed for many curves were only 3 $\log_{10}$, insufficient for validating asymptotic behavior that would imply increasing rates (derivative with respect to t) of lethality for large t (rather than an asymptotic D–value, for which the derivative asymptotically would be constant). Therefore, for egg white products with pH less not more than 8.8, a different concave function was considered, motivated by assuming a two-stage process[9] as follows. Assume that a cell to be inactivated needs to pass through one stage before inactivation, so that a cell is in one of three stages: (i) initial stage; (ii) second stage, on the verge of inactivation; or (iii) inactivated stage, I. Further assume that the times of transfer from one stage to the next are distributed exponentially, with parameters k_j, $j = 1, 2$. Thus, the probability of being in the second stage at time t is $1-\exp(-k_1 t)$, and the probability of being inactivated, given that the cell is in the second stage at time t_1, is $1-\exp(-k_2(t-t_1))$. Unconditionally, the probability of being in the inactivation stage, I, at time t is:

$$P_t(I) = \int_0^t (1 - e^{-k_2(t-\tau)})k_1 e^{-k_1\tau}d\tau \tag{G8}$$

The survival function, $p(t) = 1 - P_t(I)$, as time t is derived to be

$$p(t) = \frac{k_2 e^{-k_1 t} - k_1 e^{-k_2 t}}{k_2 - k_1} \tag{G9}$$

Put $k = \min(k_1, k_2)$ and $w = |k_1 - k_2|$, then $\ln(p(t))$ can be written

$$\ln(p(t)) = -kt + \ln\left(1 + \frac{k}{w}(1 - e^{-wt})\right) \tag{G10}$$

As t approaches infinity, the derivative of $\ln(p(t))$ approaches $-k$, and as t approaches 0^+, the derivative $\ln(p(t))$ approaches 0. Thus, Equation G10 describes a survival curve with an asymptotic D-value and curved "shoulders" with initial slope equal to 0.

Secondary equations

Once a function for estimating the survival curves is selected, it is necessary to estimate the values of the parameters that characterize the function. For fixed environmental conditions, estimates of the parameter values are derived using regression of the log of the observed levels versus time. Because environmental conditions such as temperature and pH vary, in order to derive equations for survival curves for any given set of environmental conditions within the range of those studied, regression analyses are performed by fitting the survival curve-specific estimated parameter values derived at particular environmental conditions studied, as a function of those conditions. A complete model for prediction is developed by considering mixed effects models where the parameters of the selected function need not be assumed constants, but can be assumed random error variables, reflecting the experimental error structure that would cause correlations between observations. Procedures used to determine models follow those as given by Pinheiro and Bates[13] using S-plus 6 or PC - SAS® (release 8.0) nonlinear mixed effects procedures. Initial data analyses were performed to assist in model selection. Fixed and random effect parameters were included in the model if statistically significant. The Restricted (or residual) Maximum Likelihood (REML) estimation method was used for estimating parameters,

but for testing the significance of including factors, the Maximum Likelihood Estimation (MLE) method was used.[13]

The lethality models used in the risk assessment include only the fixed effect parameters; it is assumed that the existence of random effect parameters are due to experimental errors and thus do not represent an inherent lethality variation associated with the specified set of environmental conditions. The presence of the random effect parameters affects the standard errors of the estimates. Following the notation and nomenclature used by Pinheiro and Bates,[13] when a variable, for example, a, is referred to as a random variable, this means that the variable a can be written as a constant (the expected value of a) plus sums of random errors terms with zero expected values. The expected value of a would be the fixed parameter that is used in the model. In the tables that specify the values of the parameters in the model, given below, the entry for a, under "value" refer to the expected value of a, and the entry under "standard error" refers to the standard error of the estimated value. The degrees of freedom assigned by S-plus 6 associated with the estimates are generally large so that normal approximation theory can be used. Exceptions are noted.

Plain whole and yolk egg products

FSIS has no lethality data for whole egg and yolk products without additives. FSIS thus used graphical data from a lethality study for SE PT4 (P167807) in tryptone soy broth (TSB) at various pH and salt levels using a submerged-coil heating apparatus.[5] Non-linear ties for the SE survival curves were reported. To fit survival curves, the authors used a function referred to as the log-logistic,

$$\log_{10}(\mathrm{p(t)}) = -\frac{a}{1 + e^{-b(\ln(t)-c)}} \tag{G11}$$

where a, b, and c are parameters. This function has the property that the lethality approaches an upper bound, a, as t approaches infinity. As discussed above, no upper bound is assumed for the lethalities in this risk assessment, and thus the log-logistic function is not used.

The authors did not state why the log-logistic function was selected. It is reasonable to assume that the authors examined the data and selected this function based on their observations of SE survival curves, when not linear, that were consistent with the asymptotic behavior of the log-logistic of approaching a horizontal asymptote or "flattening" out. Thus, it is assumed that the survival curves have this property, and, consequently, for this risk assessment, it is assumed that the survival curves could be described by a logistic (Equation G6) function. The selection of the logistic function is, asymptotically, more conservative than the selection of the gamma function (Equation G5), and may reflect somewhat better the assumed "flat" asymptotic behavior of the log-logistic curve. The authors presented two graphs (Figures 1a and 2a of Blackburn et al.[5]) showing the predictions of times needed to obtain 3- and 5-log lethalities when using their nonlinear equation versus their predicted D-values. Consequently, using these two graphs, if a two-parameter survival curve is assumed, the parameters can be determined, as a function of an assumed D-value, as follows. Let $L(t|a,b)$ be the predicted lethality, as a function of the time and the values of the parameters, a and b, based on Equation G6. The data points of the graphs that compare the predicted times for a 3 $\log_{10}$ lethality fall around a line with slope of 1, implying

that, on average, $L(3D|a,b) = 3$. The data points for the 5 $\log_{10}$ lethality graph fall around a line with a slope of about 2, implying that $L(10D|a,b) = 5$. From these two relationships, a and b can be solved for in terms of D. Specifically, the solutions for a and b are:

$$a = \frac{\ln(10^5\text{-}1) - \ln(10^3\text{-}1)}{\ln(10) - \ln(3)} = 3.8258 \tag{G12}$$

$$b = \ln(\frac{10^3\text{-}1}{(3D)^a}) \tag{G13}$$

Further, the predicted D-values derived by the authors for experimental studies conducted in food matrices, including whole and yolk egg products, are reported (Table 6 in Blackburn et al.[5]). A portion of this table, corresponding to the whole and yolk egg products, is presented below in the subsection titled *Whole and yolk egg products*. Of course, because the survival curves are nonlinear, the values of the predicted D-values cannot be thought of as D-values in the usual sense. Rather, these values are considered calculated numbers for use in the formulae given above in order to predict lethalities. In other words, the relationships of the predicted times from the nonlinear model and the predicted times using the predicted D-values are assumed accurate.

Four attachments to this Annex are given: (i) the product formulation of the 5 mixture products studied by Froning et al.[4]; (ii) detailed results from the FSIS plant survey that collected information on times and temperatures that are being used for specified products; (iii) technical details for calculating average lethality, given times and temperatures that were reported in the FSIS plant survey; and (iv) the data used by Froning et al.[4]

Design of University Nebraska lethality study

The protocol for the University of Nebraska study,[4] approved by FSIS, is briefly described below. For egg white, three trials were conducted at each of four tested temperatures. Fresh shell eggs were used to prepare egg white samples at pH targets of 7.8 to 8.2. Shells were disinfected with hypochlorite before aseptic separation of contents. Egg whites were pooled for final volumes of 25-50 ml and homogenized at a speed to prohibit foaming of the product. Egg whites of higher pH values were prepared by storage at 5°C for one week (pH 8.8) and two weeks (pH 9.3). Egg whites were inoculated at approximately 9-log10 cfu *Salmonella* spp./ml, mixed, and filtered through sterile gauze prior to dispensing 0.05 ml aliquots into sterile capillary tubes (implying an almost instantaneous time to reach equilibrium temperatures). Inoculated tubes were sealed and submerged in a heated circulating water bath. Temperatures were recorded using hypodermic thermocouples within the capillary tubes and verified by a calibrating thermometer. Four replicate tubes were removed per time interval and immersed in an ice-water bath. After a 2-minute incubation at room temperature, the sealed capillary tubes were placed in test tubes

containing 5 ml buffered peptone with yeast extract and sodium deoxycholate for enrichment of heat-injured *Salmonella*. Enrichment cultures were refrigerated until sampling at all four time intervals per experiment was completed. Capillary tubes were then aseptically crushed and serial dilutions were spread onto non-selective tryptic soy agar (TSA) plates and incubated at 35°C along with the enrichment tubes for 48 hours. TSA plates were replicated on *Salmonella*-selective xylose lysine tergitol (XLT) plates. One ml of the lowest dilution for plating was spread on triplicate TSA plates (0.33 ml dilution/plate). The sum of the *Salmonella* colonies on triplicate TSA plates was then recorded. For each experiment, at a given temperature and pH value, 4 or 5 observations were made. Each replicate consisted of a set of 4 experiments at different temperatures for a given pH. For each pH there were 3 replicates, so in total, for the 4 pH values studied, there were 12 replicates. The entries are means of three measurements (data not shown).

The protocol for whole eggs and yolks was identical to the previous description for egg whites with the following exceptions. Egg white was added back to isolated yolk to achieve the 43.3% solids content needed in commercial egg breaking operations. Salt or sugar was added on a 10% weight basis for testing at day 0. Generally 3 independent replicates were performed, with one exception: for 10% added sugar to liquid whole product, 4 replicates were performed. Replicates consist of experiments, generated survival curves at 4 temperatures. Consequently for each replicate an estimate of the lethality for a given time and temperature was made, and from these 3 or 4 replicate-specific estimates, a mean lethality and a standard error of the mean were computed. The mean value is reported as the expected lethality and confidence bounds were determined using the *t*-distribution with 2 or 3 degrees of freedom, as appropriate.

Analysis of data used for estimating lethalities based on time and temperature given in FSIS Plant Survey

For each egg product type, except for 10% added sugar in egg yolk product, three replicates were conducted. For the exception, 4 replicates were conducted. In each replicate, survival curves for four temperatures were measured. Thus, the design is nested, with 3 levels: replicate, experiments within replicate, and actual measured levels within experiments.

For the Basic Model: let $N(t)$ be the observed counts at time t, for an experiment at a fixed temperature T and $r(t) = N(t)/N(0)$. The basic model for a survival curve is: $\log_{10}(r(t)) = f(t|\tau(T))$+error, where $f(t|\tau(T))$ is some function of t that represents the expected or predicted lethality, and $\tau(T)$ is a set of parameters, that are functions of temperature. Statistical analysis is performed to determine estimates of the function $\tau(T)$. A simple way of determining the uncertainty of an estimated predicted lethality value is to consider the three or four replicates, as appropriate, as mutually independent events; compute a replicate-specific predicted value for each replicate; use the average of these as the estimated predicted value with standard error determined from the three replicate–specific estimates; and determine confidence bounds using a t-distribution with 2 degrees of freedom for all products except for 10% added sugar egg yolk product, where 3 degrees of freedom are used.

Ten percent added salt in liquid whole egg and egg yolk

For this product, the survival curves were convex or tailing. Nonlinear regressions for each temperature, product type within replicate, were performed for the 3 convex functions, defined in Equations G5-7 (using the gamma function for Equation G5: $f(t) = -\log_{10}(1+\beta t)$,) with $\log_{10}$ of the observed relative reduction as the dependent variable and the values at $t = 0$ were of course deleted. Table G2 provides pooled root mean square errors for the two types of products.

TABLE G2 POOLED ROOT MEAN SQUARE ERRORS (RMSE) FROM NONLINEAR REGRESSIONS FOR YOLK AND WHOLE EGG PRODUCTS.

Product	RMSE Logistic	RMSE Gamma	RMSE Weibull
Whole	0.1885	0.1981	0.2616
Yolk	0.1383	0.1274	0.1224

Based on these results, the gamma function is used because the RMSE is lower or about the same as those for the other functions. The following function, based on Equation G5 assuming F is the gamma distribution with two parameters: α and $\beta > 0$, $f(t; \alpha, \beta) = -\alpha\log_{10}(1+\beta t)+\varepsilon$, where ε is an error term. For each experiment, nonlinear regressions were done. The mean of gamma distribution, using $f(t)$ is: mean $= m = \alpha\beta$; and the variance is $= s^2 = \alpha\beta^2$. Table G3 gives estimates of α, β, mean, and standard deviation of the estimated gamma distribution.

TABLE G3 RESULTS OF NONLINEAR REGRESSION: $\text{LOG}_{10}(P(T)) = -A\text{LOG}_{10}(1+BT)$

Product Type	Temp (°C)	Replicate No.	α	β	Mean	Stdev	CV(%)
Yolk	63.3	1	4.986	3.715	18.52	8.30	44.8
Yolk	63.3	2	7.230	1.471	10.63	3.95	37.2
Yolk	63.3	3	7.010	1.848	12.95	4.89	37.8
Yolk	65.5	1	5.769	4.803	27.71	11.54	41.6
Yolk	65.5	2	4.818	8.778	42.29	19.27	45.6
Yolk	65.5	3	5.652	6.799	38.43	16.17	42.1
Yolk	67.8	1	7.607	6.700	50.96	18.48	36.3
Yolk	67.8	2	6.575	10.029	65.94	25.72	39.0
Yolk	67.8	3	12.241	3.823	46.80	13.38	28.6
Yolk	70.0	1	9.278	12.480	115.79	38.01	32.8
Yolk	70.0	2	6.603	19.971	131.88	51.32	38.9
Yolk	70.0	3	12.706	7.159	90.96	25.52	28.1
Whole	61.1	1	2.907	8.346	24.26	14.23	58.7
Whole	61.1	2	2.741	12.255	33.60	20.29	60.4
Whole	61.1	3	2.590	14.599	37.81	23.49	62.1
Whole	63.3	1	3.624	8.816	31.95	16.78	52.5
Whole	63.3	2	3.743	10.260	38.40	19.85	51.7
Whole	63.3	3	3.634	12.008	43.64	22.89	52.5
Whole	65.5	1	6.006	8.410	50.51	20.61	40.8
Whole	65.5	2	4.699	12.944	60.82	28.06	46.1
Whole	65.5	3	5.448	9.577	52.18	22.35	42.8
Whole	67.8	1	4.700	31.341	147.31	67.95	46.1
Whole	67.8	2	5.480	28.973	158.76	67.82	42.7
Whole	67.8	3	5.452	19.679	107.30	45.95	42.8

Ten percent added salt in whole egg product

Figures G1 and G2 are plots of the $\ln(m)$ and $\ln(s)$ versus temperature, with the replicate as a label. The graphs indicate a nonlinear relationship between the log of the mean and standard deviations versus temperature. For each replicate, a quadratic polynomial was fit to both the $\ln(m)$ and $\ln(s)$ versus temperature, (actually temperature minus 60ºC) and from these fitted curves, predicted lethality for any given temperature and time can be computed. Table G4 gives the values of the coefficient parameters for each replicate.

Ten percent added salt in egg yolk product

The survival curves for this product were also convex, similarly shaped as the ones for the 10% added salt in whole egg product. Figures G3 and G4 are similar plots as Figures G1 and G2 except for the 10% added salt egg yolk product. The relationships appear more linear, though are not consistent. Consequently, a linear model fitting $\ln(m)$ and $\ln(s)$ versus temperature was used. Table G4 provides an estimate of the parameter values for the equations.

TABLE G4 VALUES OF PARAMETERS USED FOR SECONDARY EQUATIONS FOR PREDICTING LN(M) AND LN(S), FOR 10% ADDED SALT TO LIQUID WHOLE EGGS AND EGG YOLKS, WHERE M AND S ARE THE MEAN AND STANDARD DEVIATION RESPECTIVELY FOR THE GAMMA FUNCTION USED TO PREDICT LETHALITIES: $A\text{LOG}_{10}(1+BT)$, WHERE $M = AB$, AND $S^2 = AB^2$. QUADRATIC EQUATIONS FOR WHOLE AND LINEAR EQUATIONS FOR EGG YOLK, WITH INDEPENDENT VARIABLE TEMPERATURE, T, MINUS 60°C.

		Coefficients for Predicting ln(m)			Coefficients for Predicting ln(v)		
Type	Replicate	Constant	T-60°C	(T-60°C)^2	Constant	T-60°C	(T-60°C)^2
Whole	1	3.25	-0.0832	0.0389	2.90	-0.2341	0.0510
Whole	2	3.61	-0.1256	0.0399	3.20	-0.2144	0.0441
Whole	3	3.74	-0.1059	0.0287	3.42	-0.2438	0.0375
Yolk	1	1.91	0.2742	-	1.28	0.2258	-
Yolk	2	1.42	0.3590	-	0.51	0.3583	-
Yolk	3	1.86	0.2686	-	1.15	0.2112	-

FIGURE G1 ADDED SALT IN WHOLE EGG PRODUCTS. PLOT OF LN OF ESTIMATED MEAN, M, FOR SURVIVAL CURVES VERSUS TEMPERATURE, TOGETHER WITH FITTED QUADRATIC POLYNOMIAL, BY REPLICATE.

FIGURE G2 ADDED SALT IN WHOLE EGG PRODUCTS. PLOT OF LN OF ESTIMATED STANDARD DEVIATION, *S*, FOR SURVIVAL CURVES VERSUS TEMPERATURE, TOGETHER WITH FITTED QUADRATIC POLYNOMIAL, BY REPLICATE.

FIGURE G3 ADDED SALT IN EGG YOLK PRODUCTS. PLOT OF LN OF ESTIMATED MEAN, *M*, FOR SURVIVAL CURVES VERSUS TEMPERATURE, TOGETHER WITH FITTED QUADRATIC POLYNOMIAL, BY REPLICATE.

FIGURE G4 ADDED SALT IN EGG YOLK PRODUCTS. PLOT OF LN OF ESTIMATED STANDARD DEVIATION, *S*, FOR SURVIVAL CURVES VERSUS TEMPERATURE, TOGETHER WITH FITTED QUADRATIC POLYNOMIAL, BY REPLICATE.
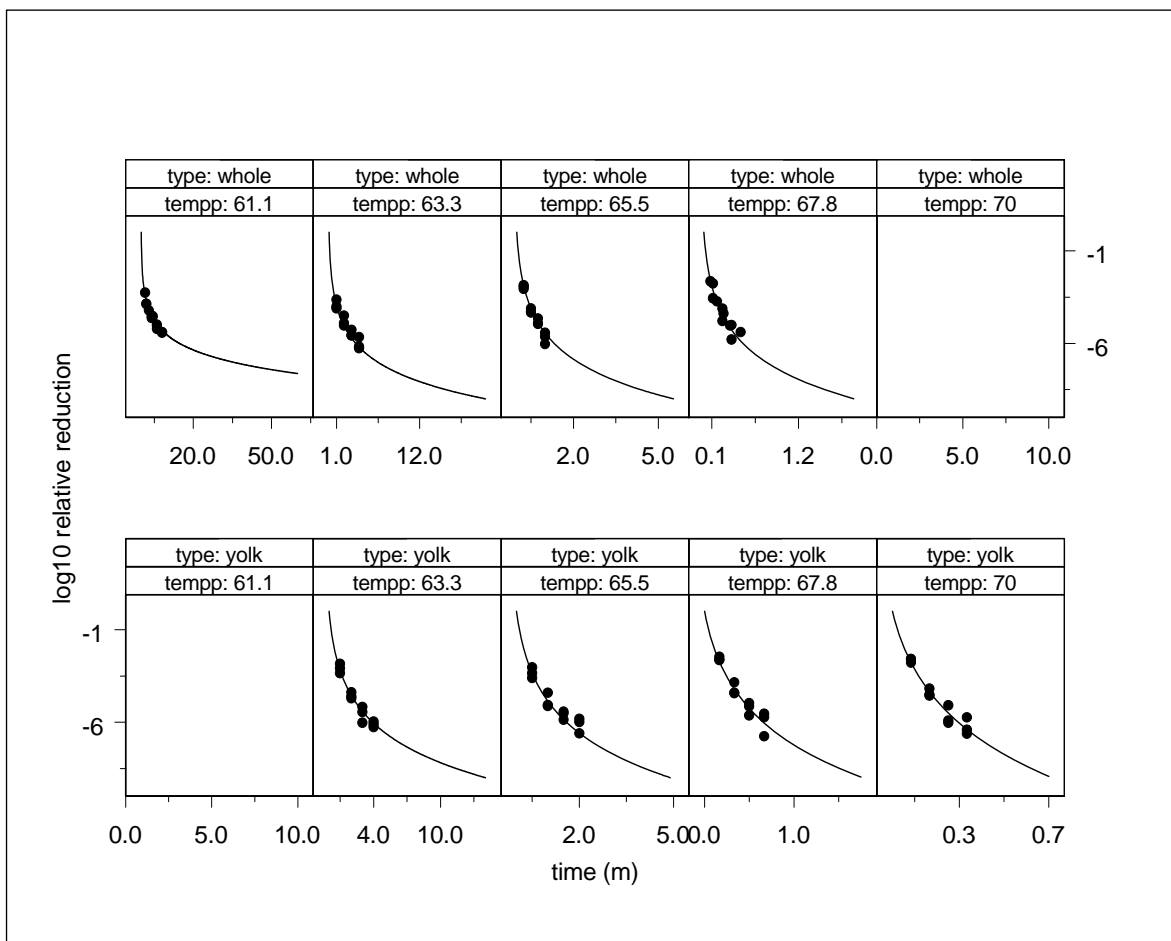
FIGURE G5 PLOTS OF OBSERVED DATA POINTS AND FITTED SURVIVAL CURVES, LOG$_{10}$(RELATIVE REDUCTION THE Y-AXIS) VERSUS TIME (MIN)- THE X-AXIS, ASSUMING THE GAMMA ASSUMPTION FOR 10% ADDED SALT IN WHOLE AND YOLK EGG PRODUCT AND GIVEN TEMPERATURES, BASED ON AVERAGE VALUES OF ESTIMATED LETHALITIES.
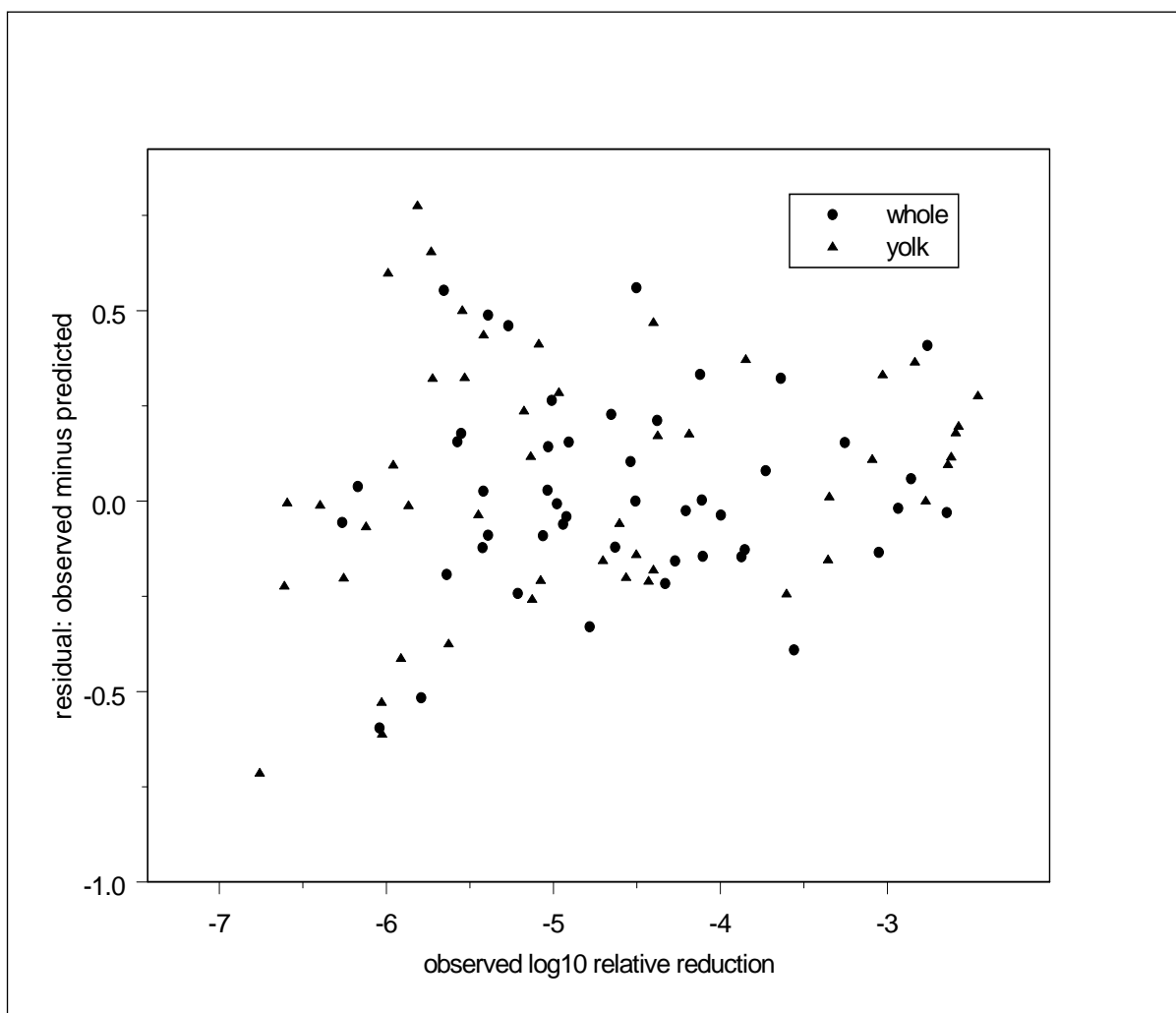
FIGURE G6 PLOT OF RESIDUAL: OBSERVED MINUS PREDICTED LOG RELATIVE REDUCTION.

Ten percent added sugar in whole egg product

The observed data indicated concave survival curves with an initial shoulder. There were three replicate sets of experiments, where each replicate set consisted of experiments at the 4 different temperatures of 60, 62.2, 64.4, and 66.7°C. In total, there were 12 survival curves. Data recorded as non-detect (less than 100 cfu *Salmonella* spp./ml) were excluded. In addition, the data at the temperature of 66.7°C for the first replicate (for which two of the three repeated measurements were recorded as non-detect and the other one at 100 CFU/mL), were also excluded.

The Weibull function defined by Equation G7 with parameters $k = b$ and $w = \log_{10}(e)a > 0$, was considered for modeling $\log_{10}(p(t)) = -wt^k$. Equation G7 describes a survival curve without an asymptotic D-value and with curved "shoulders" with initial slope equal to 0. For these data, the range of the observed lethality was close to 6-7 $\log_{10}$ thus permitting the use of this model for predicting lethality in ranges of concern of approximately 7-9 $\log_{10}$. This function provided

better fit to the data than the function defined by Equation 10, which was used for egg white product, though that function provided a good fit with the possible exception for data at 66.7°C. Table G5 provides the estimates of w and k from nonlinear regression of Equation G7 for each temperature and replicate.

TABLE G5 RESULTS OF REGRESSION OF NATURAL LOGARITHM OF LOG$_{10}$ RELATIVE REDUCTIONS.

Temp. (°C)	Replicate	w	ln(w)	k
60.0	1	0.971	-0.030	1.132
60.0	2	0.804	-0.218	1.206
60.0	3	0.980	-0.020	1.131
62.2	1	3.892	1.359	1.241
62.2	2	3.313	1.198	1.201
62.2	3	4.015	1.390	1.241
64.4	1	18.419	2.913	1.483
64.4	2	14.860	2.699	1.412
64.4	3	18.861	2.937	1.520
66.7	1	63.007	4.143	1.582
66.7	2	74.708	4.314	1.740
66.7	3	55.476	4.016	1.523

Figures G7 and G8 are plots of ln(k) and ln(w) versus temperature respectively, by replicate. It can be seen the inconsistent of the shape of the fits of the quadratic polynomials in temperature for ln(k) thus is assumed that ln(k) is linear with temperature. For ln(w), the polynomials are nearly linear, so that also ln(w) is assumed linear with temperature. Table G6 presents the estimated values of the parameters for each replicate used to predict w and k, as a function of the temperature. Figures G9 and G10 are plots of the residuals using the average estimated lethalities, over the three replicates, and the residuals from those estimates, respectively.

Table G6 Values of parameters used for secondary equations for predicting ln(k) and ln(w), for 10% added sugar to liquid whole eggs, where k and w parameters for the Weibull equations: -wt^k. Quadratic equations for whole and linear equations for egg yolk, with independent variable temperature, T, minus 60ºC.

Replicate	Coefficients for ln(k)		Coefficients for ln(k)	
	Constant	T-60°C	Constant	T-60°C
1	-0.143	0.0529	-3.155	0.631
2	-0.155	0.0568	-3.638	0.677
3	-0.113	0.0490	-3.012	0.612
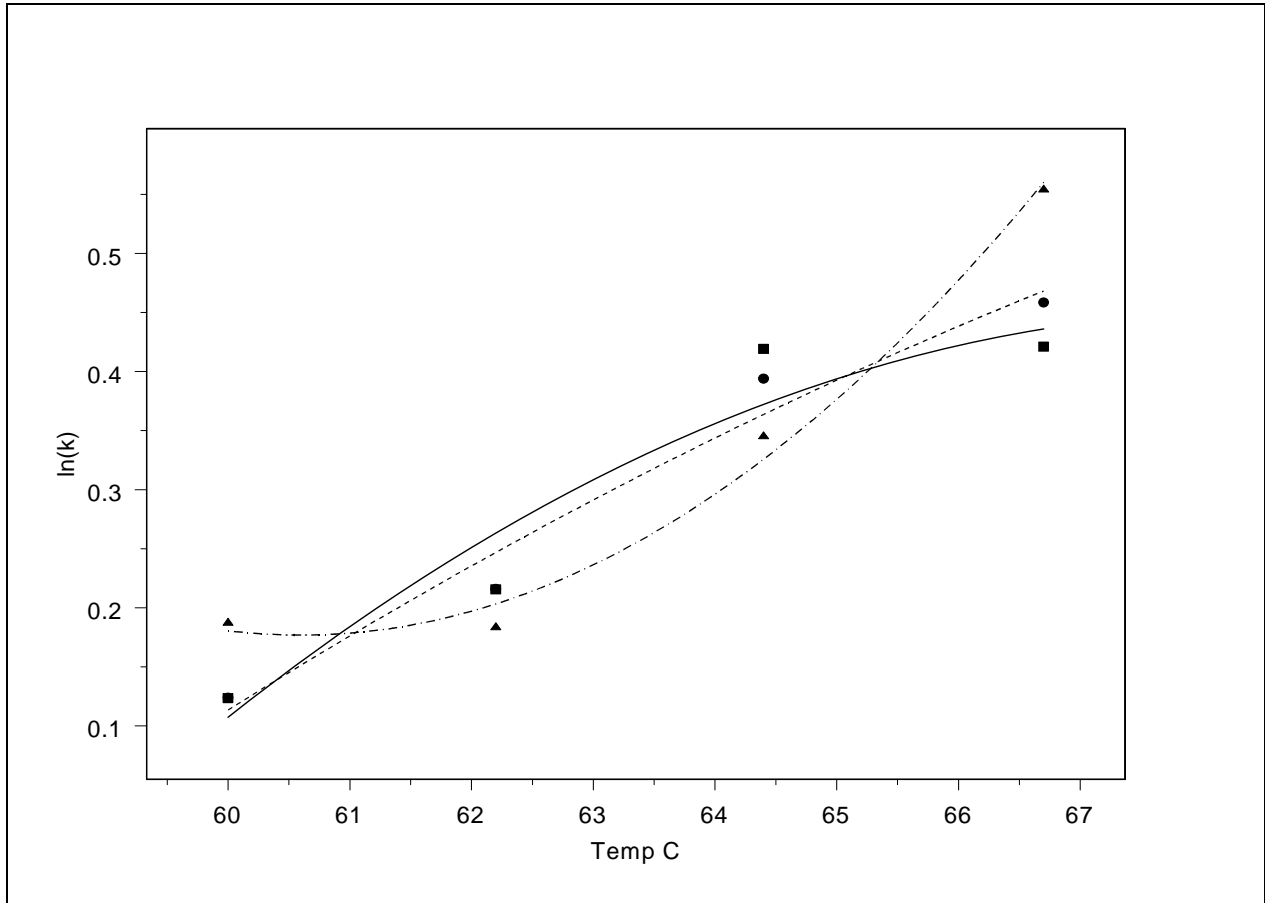
FIGURE G7 ADDED SUGAR IN WHOLE EGG PRODUCTS. SURVIVAL CURVE: $LOG_{10}(P(T)) = -WT^K$. PLOT OF LN OF ESTIMATED MEAN, K, FOR SURVIVAL CURVES VERSUS TEMPERATURE, TOGETHER WITH FITTED QUADRATIC POLYNOMIAL, BY REPLICATE.
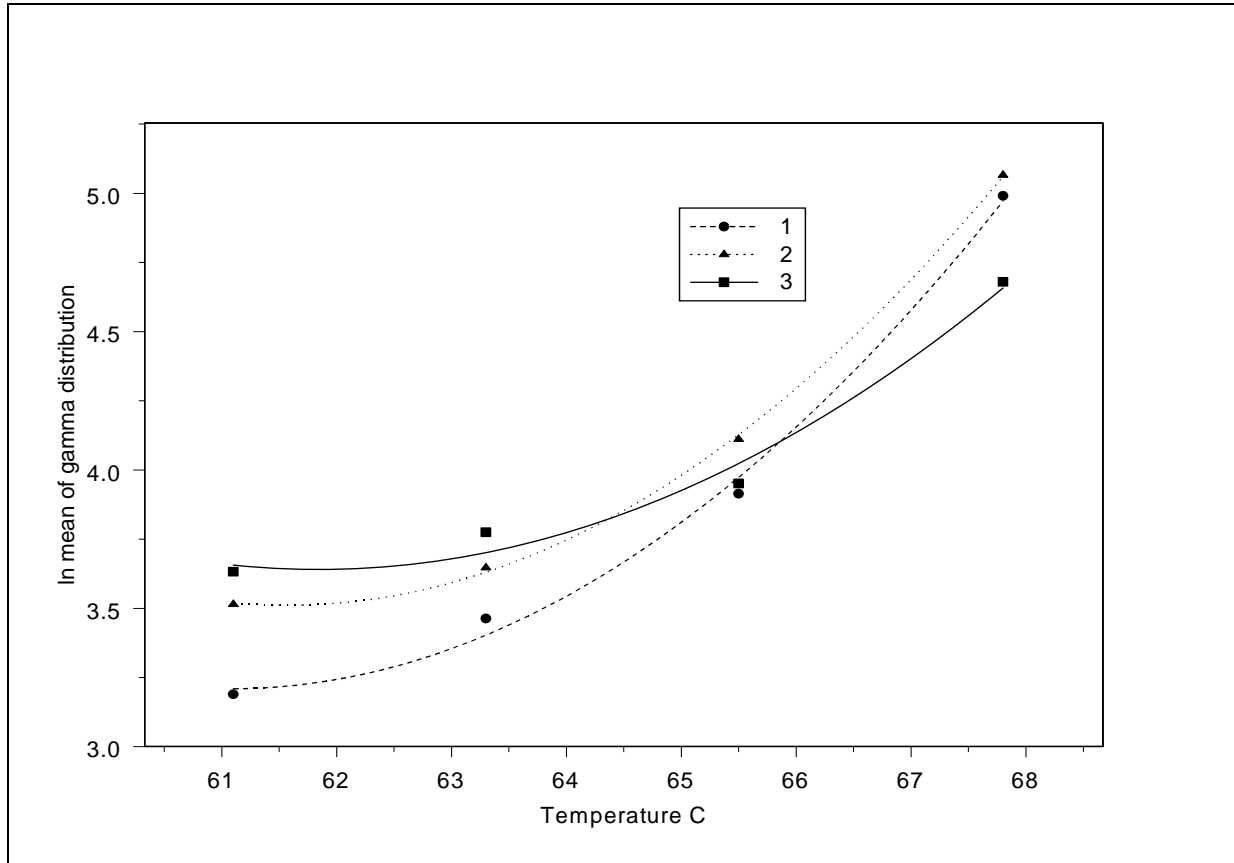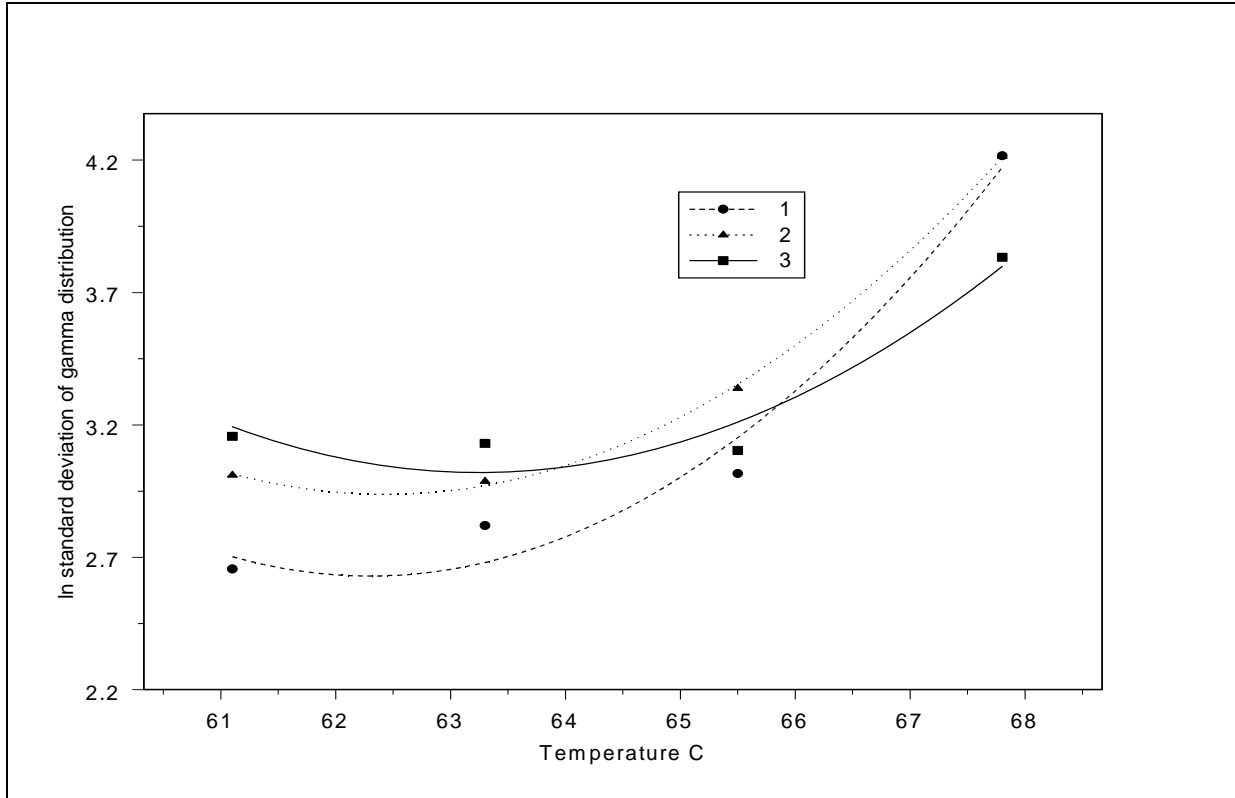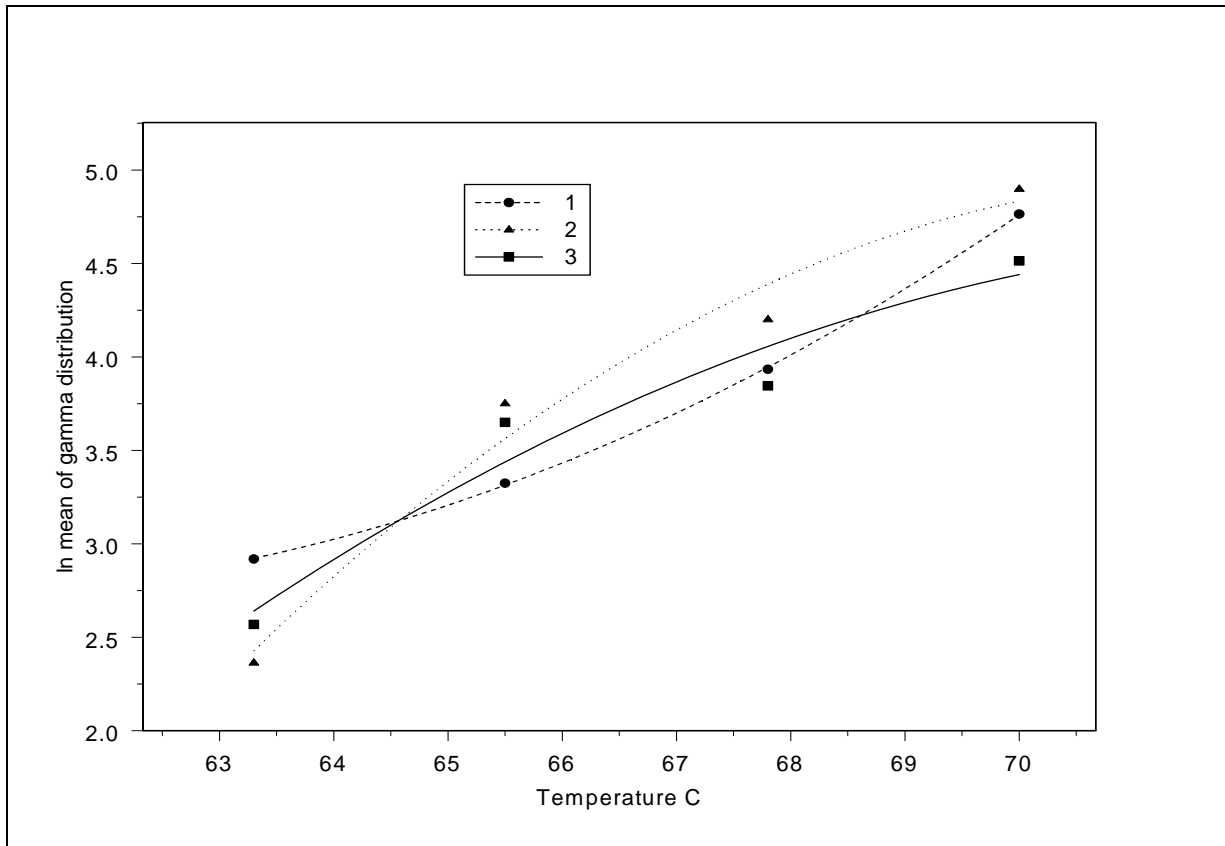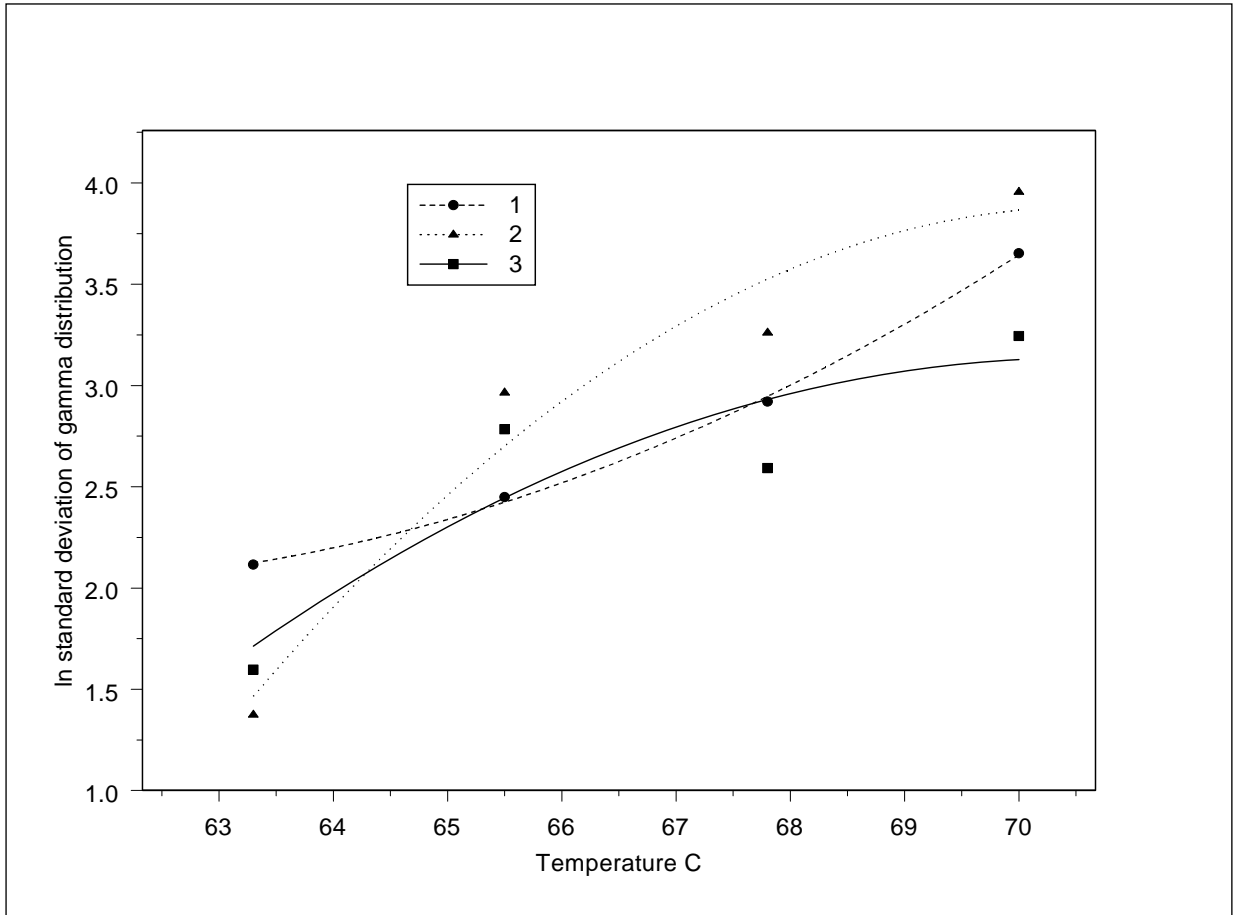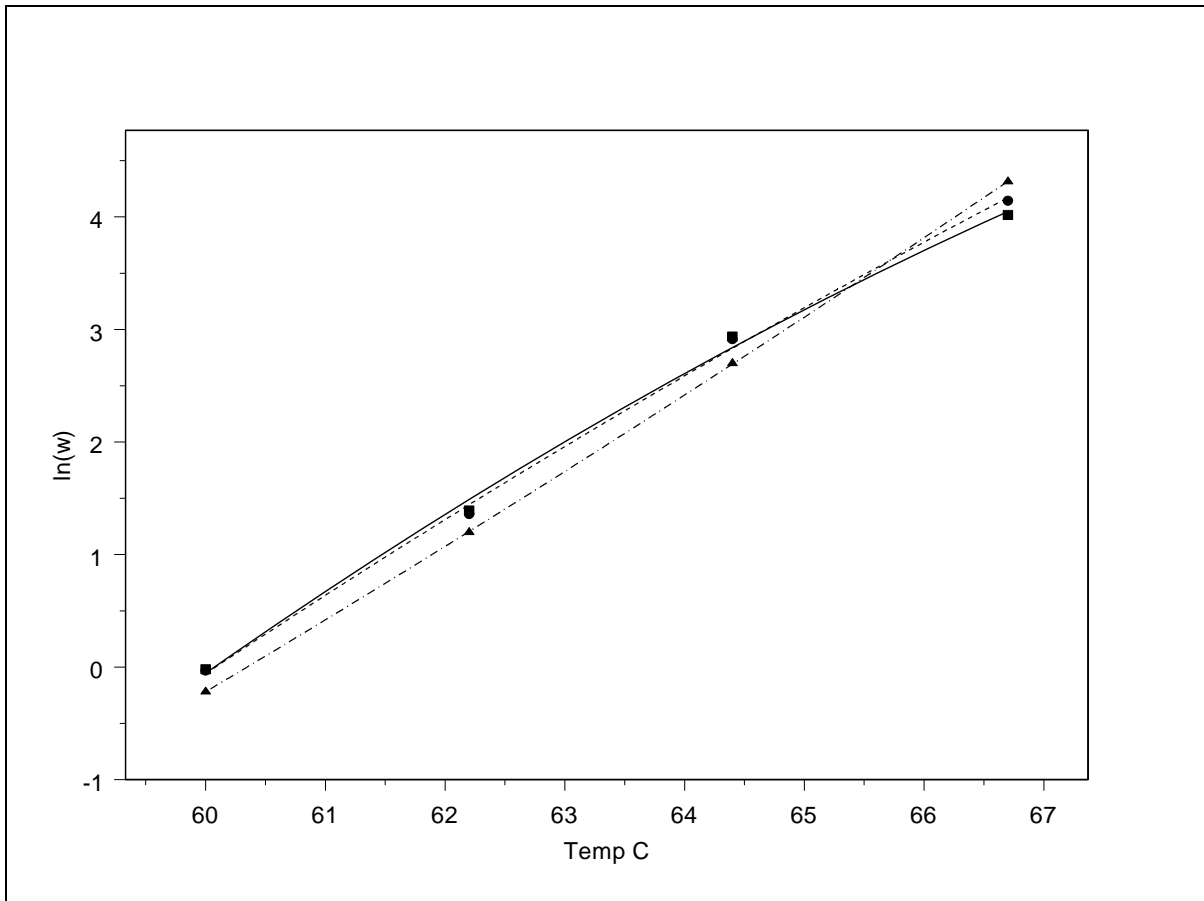
FIGURE G8 ADDED SALT IN WHOLE EGG PRODUCTS. PLOT OF LN(*w*) FOR SURVIVAL CURVES VERSUS TEMPERATURE, TOGETHER WITH FITTED QUADRATIC POLYNOMIAL, BY REPLICATE.
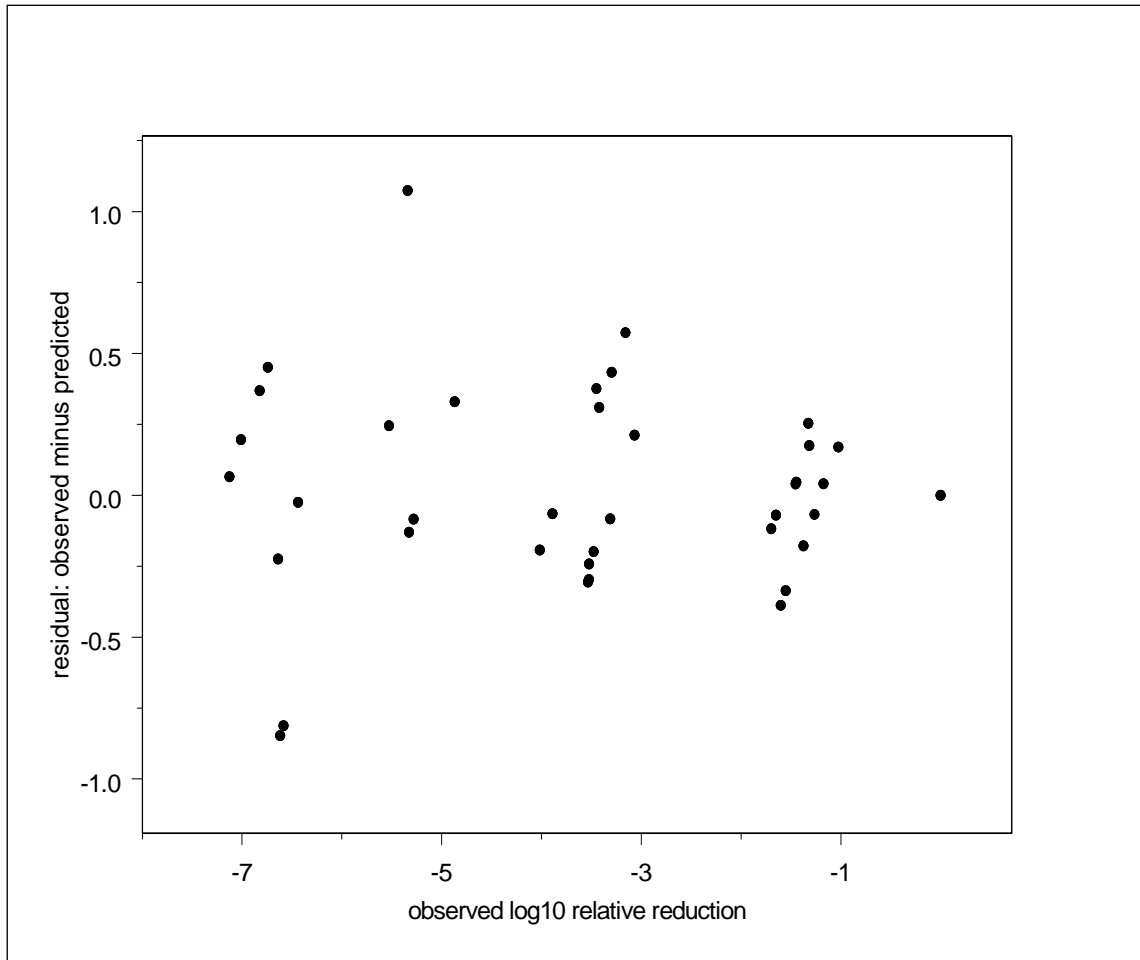
FIGURE G9 PLOT OF RESIDUALS VERSUS OBSERVED LOG$_{10}$ RELATIVE REDUCTIONS FOR 10% ADDED SUGAR IN WHOLE EGG PRODUCTS. DATA POINTS OF THE SAME SYMBOL ARE FROM THE SAME REPLICATES.

FIGURE G10 PLOTS OF OBSERVED AND FITTED SURVIVAL CURVES (LOG$_{10}$(RELATIVE REDUCTION) VERSUS TIME, *M*, FOR 10% ADDED SUGAR IN WHOLE EGG PRODUCT FOR GIVEN TEMPERATURES.

Ten percent added sugar in egg yolk product

For this product there were four replicate sets of experiments, where each replicate set consisted of experiments at the 4 different temperatures of 61.1, 63.3, 65.5, and 67.8°C so that in total, there were 16 survival curves. Data recorded as less than 100 cfu/mL were excluded. In addition, at the temperature 61.1°C for the second replicate, one of the three repeated measurements was changed from <100 cfu/mL to 100 cfu/mL because the other two measurements were >100 cfu/ml. The observed data indicated asymptotic convexity of the survival curves and in some cases an initial shoulder. The function used to model the survival curve in the risk assessment is the logistic:

$$\log_{10}(r(t)) = -\log(1 + \exp(a\ln(t) + b))$$

where a and b are constants. Nonlinear regression for experiment using the above equation was performed and the estimates of a and b are presented in Table G7.

TABLE G7 ESTIMATED VALUES OF A AND B FOR THE ABOVE LOGISTIC EQUATION FOR EACH EXPERIMENT OF 10% ADDED SUGAR TO YOLK EGG PRODUCT.

Temp (°C)	Replicate	Number of Observations	a	b
61.1	1	4	11.388	-2.824
61.1	2	5	9.921	-1.290
61.1	3	5	9.576	-0.359
61.1	4	5	9.281	0.375
63.3	1	4	8.410	9.660
63.3	2	5	9.794	10.299
63.3	3	4	12.132	10.933
63.3	4	4	10.147	10.321
65.5	1	4	8.048	18.320
65.5	2	4	6.588	17.400
65.5	3	4	7.808	18.272
65.5	4	5	8.720	19.140
67.8	1	4	11.556	30.076
67.8	2	5	8.833	22.858
67.8	3	5	5.628	21.847
67.8	4	5	9.566	27.730

An examination of Table G7 reveals that the values of a appear not to be related strongly with temperature whereas the values of b appear to be related with temperature. Figures G11 and G12 are plots of a and b versus temperature. The graphs show inconsistencies with respect to the relationships of the parameter values with temperatures for the different replicates. The values of a display no consistent pattern, thus a is assumed to be a constant that varies with the experiment, within each replicate. The values of b increase with temperature. A quadratic polynomial in temperature was used to fit b. Table G8 provides the estimates of the parameter values for predicting a and b, as a function of temperature, for each replicate.

Table G8 Values of parameters used for secondary equations for predicting a and b for 10% added sugar to liquid egg yolks, where a and b are parameters for the logistic equation: $-\log_{10}(1 + \exp(a\ln(t)) + b)$. The parameter a was assumed to be a constant, and b a quadratic equations with independent variable temperature, T, minus 55 °C.

Replicate	Value of a	Constant	Coefficient of T-55	Coefficient of $(T\text{-}55)^2$
1	9.851	-36.41	5.916	-0.058
2	8.784	-48.12	9.672	-0.323
3	8.786	-51.95	10.910	-0.402
4	9.429	-31.32	5.736	-0.088
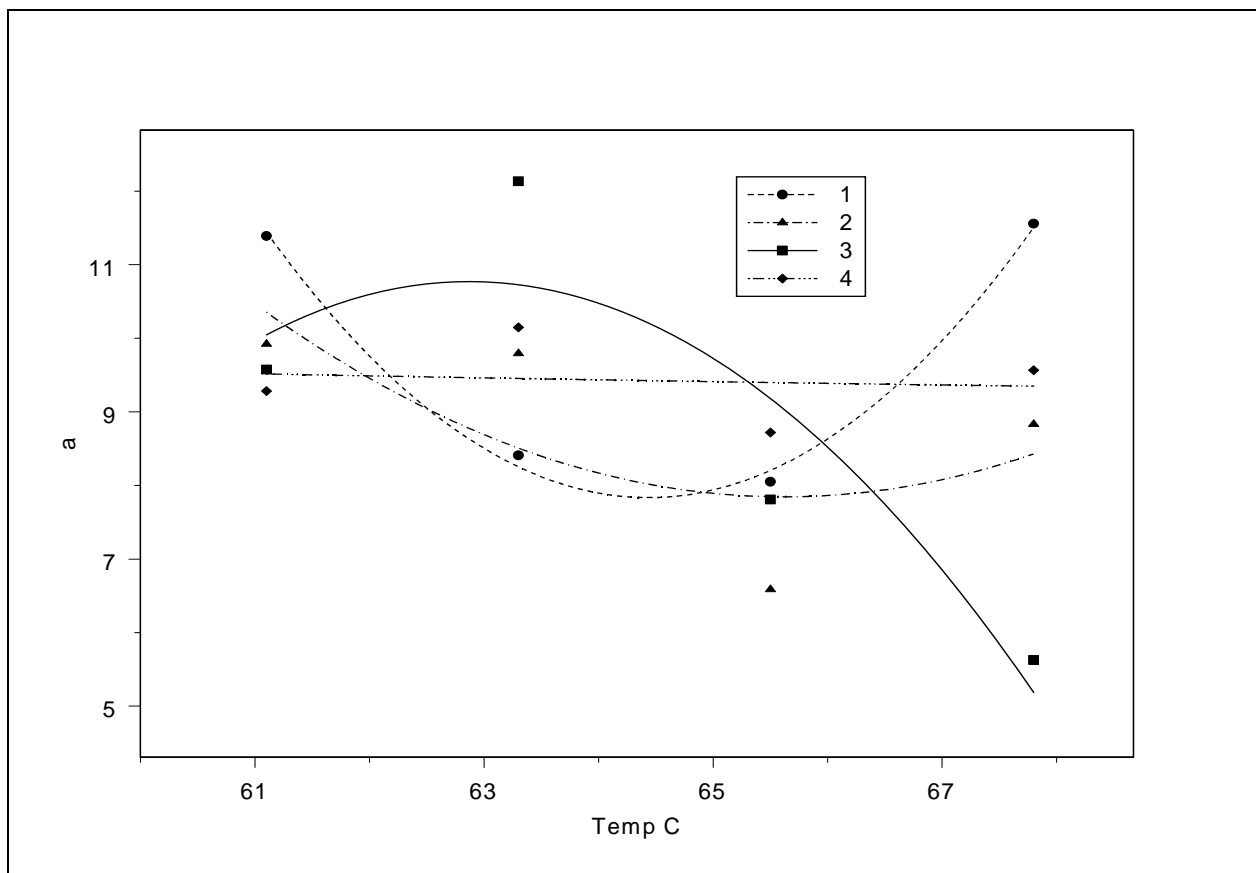
FIGURE G11 TEN PERCENT ADDED SUGAR IN EGG YOLK PRODUCTS. SURVIVAL CURVE: $LOG_{10}(R(T)) = -LOG_{10}(1+ EXP(A+ BLN(T)))$ PLOT OF A VERSUS TEMPERATURE, TOGETHER WITH FITTED QUADRATIC POLYNOMIAL, BY REPLICATE.
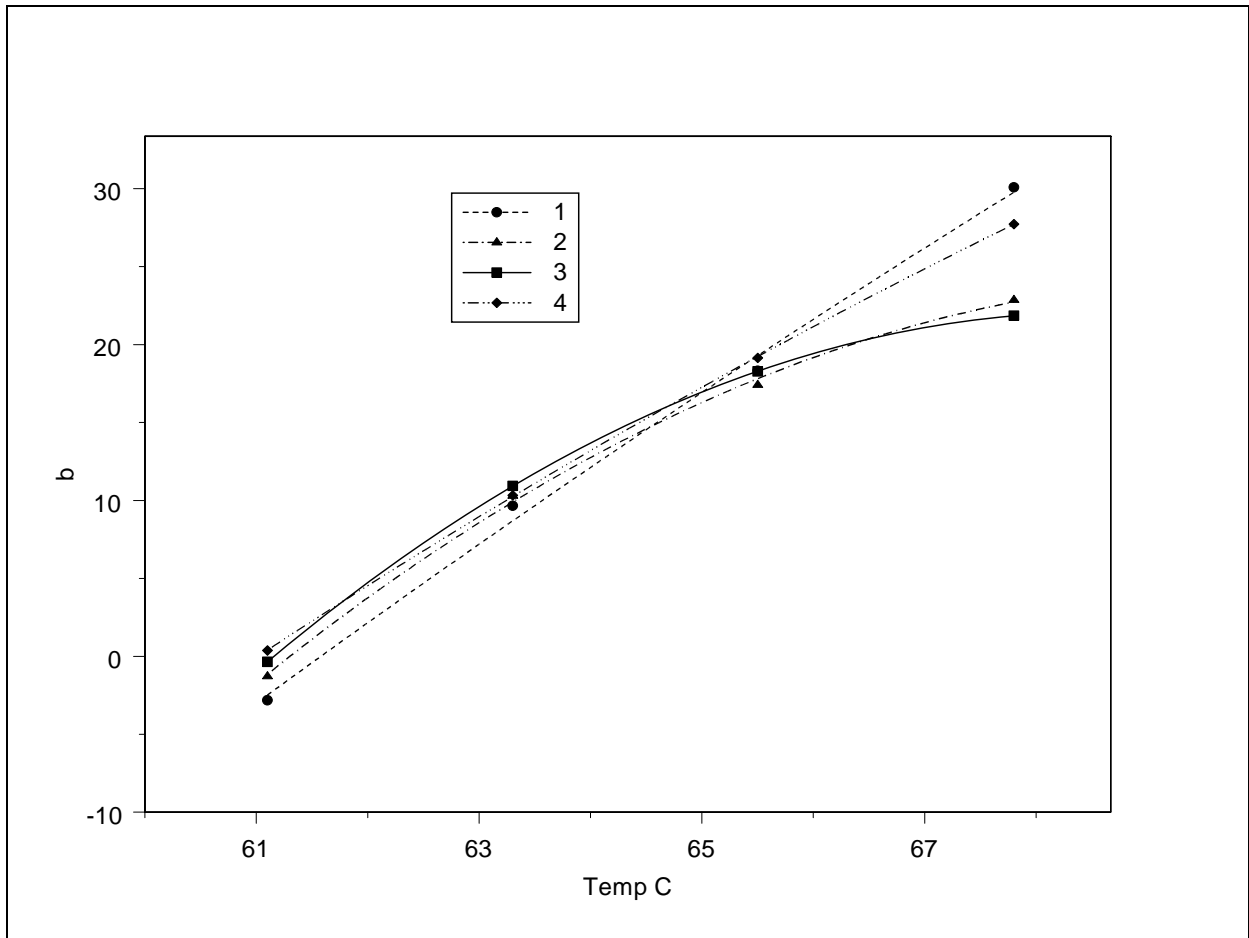
FIGURE G12 ADDED SUGAR IN EGG YOLK PRODUCTS. PLOT OF *B* VERSUS TEMPERATURE, TOGETHER WITH FITTED QUADRATIC POLYNOMIAL, BY REPLICATE.
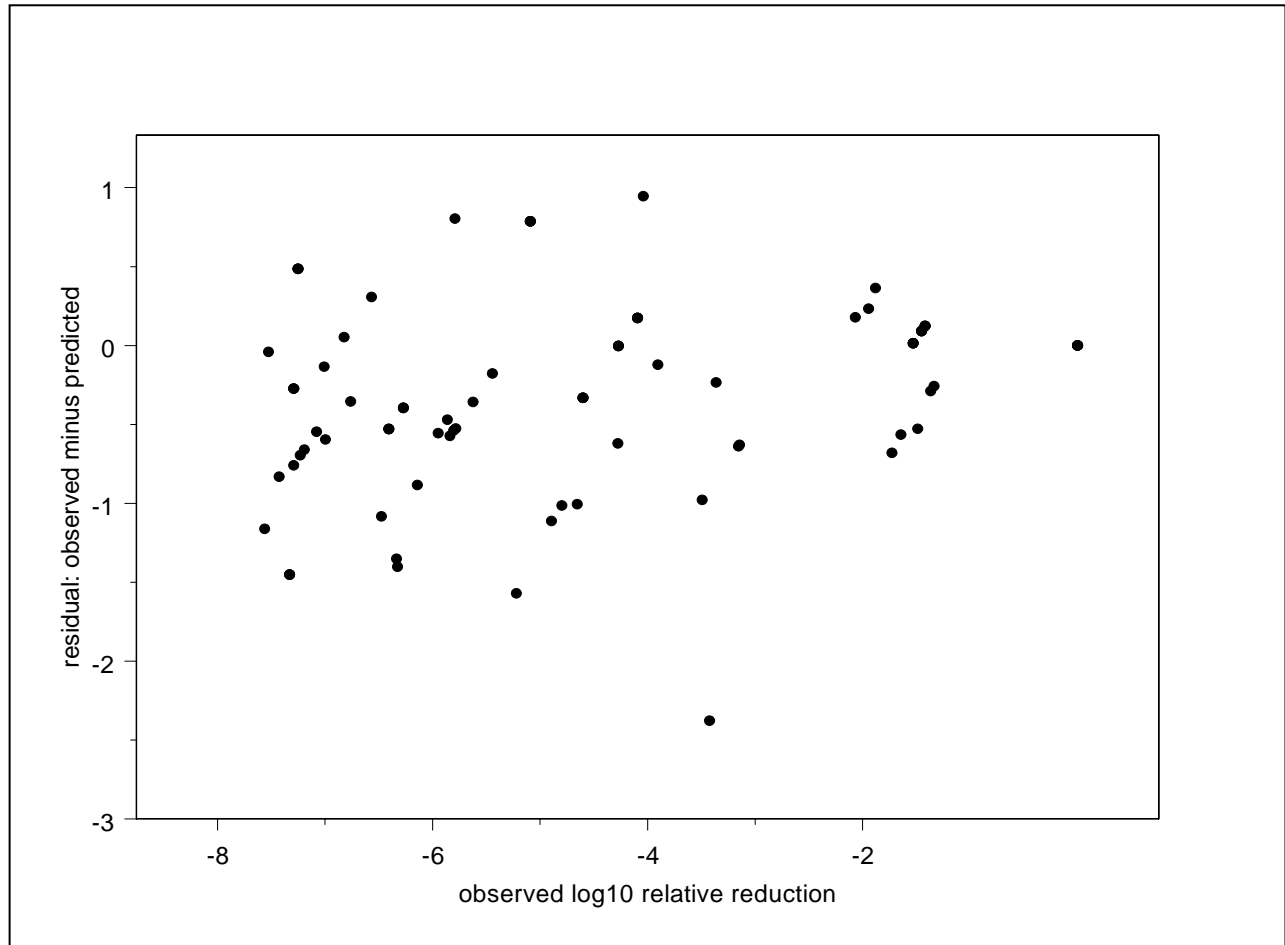
FIGURE G13 PLOT OF RESIDUALS VERSUS OBSERVED LOG$_{10}$ RELATIVE REDUCTIONS FOR 10% ADDED SUGAR TO EGG YOLK PRODUCT.
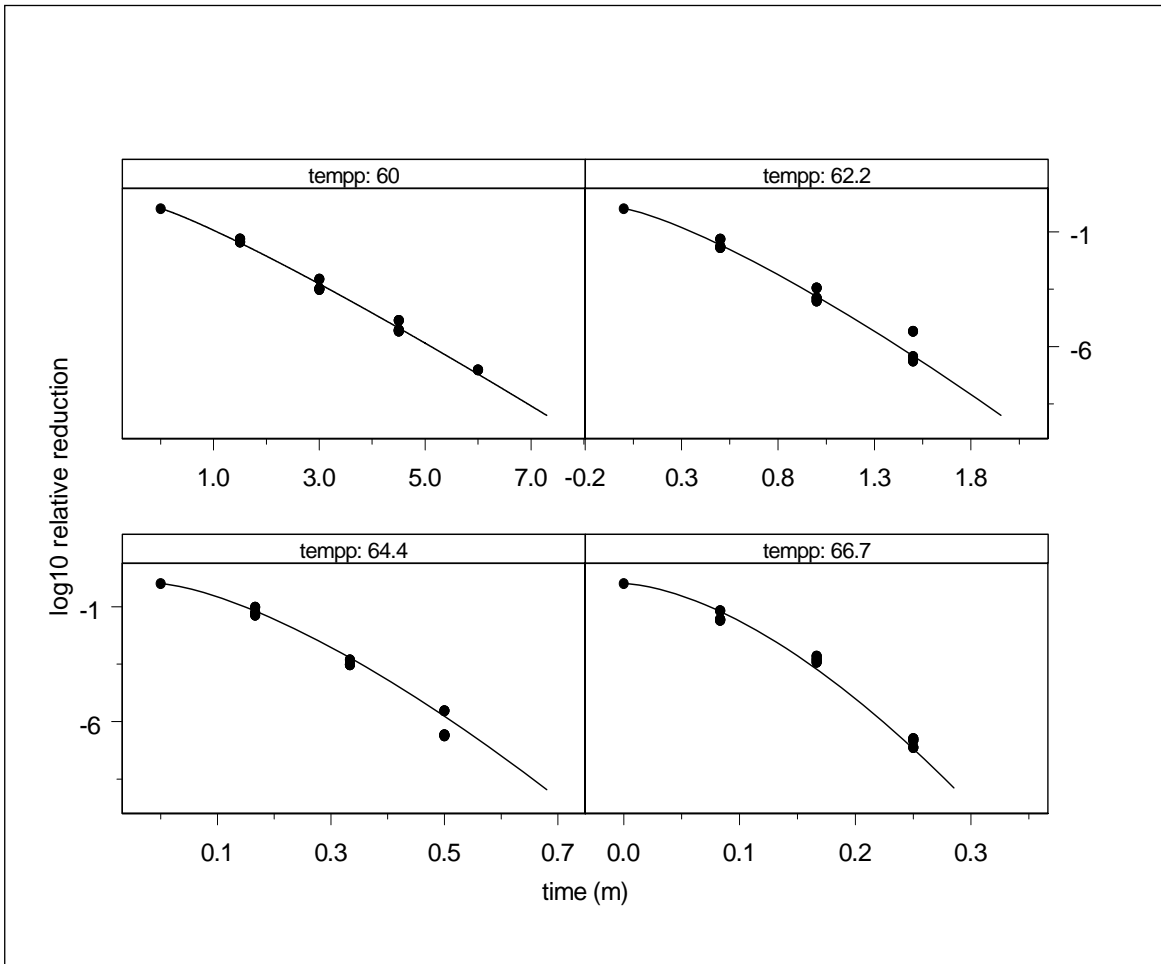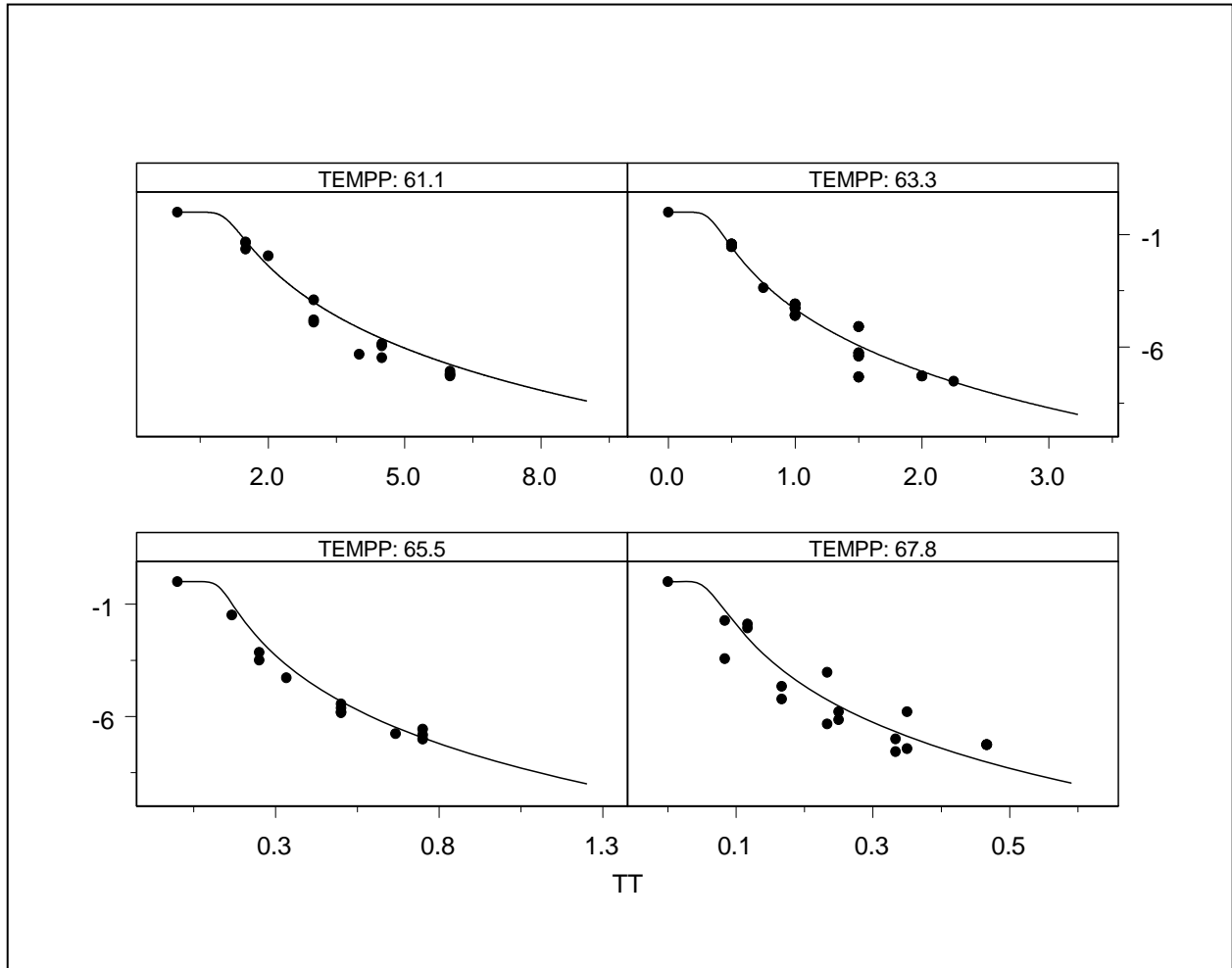
FIGURE G14 PLOTS OF OBSERVED AND FITTED SURVIVAL CURVES (LOG$_{10}$(RELATIVE REDUCTION) VERSUS TIME (*M*) FOR 10% ADDED SUGAR IN YOLK EGG PRODUCT FOR GIVEN TEMPERATURES.

Lethality in Eggs Mixture Products

Five product formulations (1, 2, 4, 6, and 7) were studied, each at four temperatures (not necessarily the same ones for each product). Products 1, 2, and 4 are whole egg mixtures; product 6 is an egg yolk product with 16% corn syrup, and product 7 is an egg white product. For each product, there were three replicates, where each replicate consists of 4 experiments, one at each temperature.

From a visual examination of the survival curves ($\log_{10}(N(t))$ versus t, where t is time in minutes, and $N(t)$ is the observed counts (cfu/ml), the survival curves appear to be concave, possibly with asymptotic D-values, with the exception of the product formulation labeled 6, for which linear survival curves are apparent. Figure G16 presents, by product, graphs of the residuals obtained from a linear regression of $\log_{10}(N(t))$ on t, versus the observed $\log_{10}(N(t))$.

Included in the graphs are fitted quadratic curves. As is seen the quadratic curve for product 6 is nearly flat, and the residuals are nearly centered about zero over the range of the x-axis, suggesting that the linear fit provides a good fit of the data for product 6. For the other products, this pattern does not exist.
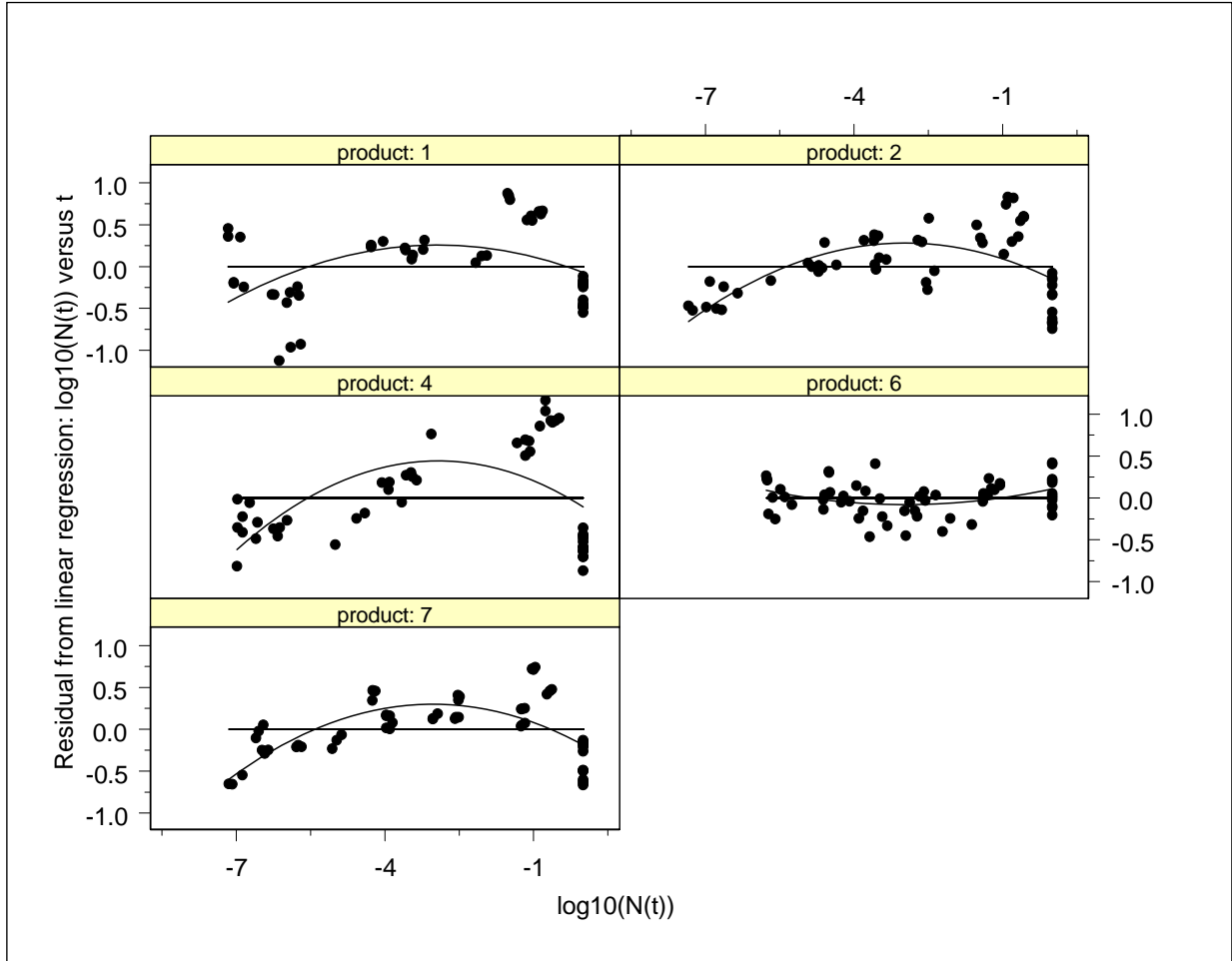
FIGURE G15 RESIDUAL PLOTS OF LINEAR REGRESSION: RESIDUAL VERSUS LOG$_{10}$($N(T)$), TOGETHER WITH FITTED QUADRATIC POLYNOMIAL AND HORIZONTAL LINE AT ZERO, FOR EACH EXPERIMENT, FOR EACH OF THE 5 PRODUCT FORMULATIONS.

Besides the linear model, two other models were considered. The first one is the Weibull lethality model, and the second one is a spline function:

$$\log_{10}(r(t)) = \min(0, -k(t-t_0)) \tag{G14}$$

where k and t_0 are parameters whose values are to be estimated.

Table G8 provides mean values of the root mean square error (RMSE) for the linear regression and the nonlinear regressions for each product type, except product 6, and

temperature. The RMSE for the nonlinear regression has one less degree of freedom for each experiment than that for the linear regression.

TABLE G8 MEAN VALUES OF ROOT MEAN SQUARE ERRORS (RMSE) FOR LINEAR AND NONLINEAR REGRESSIONS USING THE WEIBULL MODEL AND SPLINE MODEL.

Product	Temp (°C)	RMSE Linear	RMSE Weibull	RMSE Spline
1	60.0	0.62	0.25	0.09
1	62.2	0.60	0.24	0.06
1	64.4	0.98	1.39	1.32
1	66.7	0.27	0.18	0.26
2	57.8	0.20	0.24	0.20
2	60.0	0.40	0.14	0.13
2	62.2	0.62	0.26	0.16
2	64.4	0.85	0.27	0.17
4	60.0	0.60	0.19	0.13
4	62.2	0.68	0.45	0.16
4	64.4	0.96	0.42	0.30
4	66.7	0.93	0.86	0.48
7	54.4	0.19	0.11	0.17
7	56.7	0.23	0.19	0.13
7	58.9	0.65	0.45	0.16
7	61.1	0.66	0.21	0.39

From Table G8, it is seen that the means of the RMSE for the spline regressions are generally less than those for the Weibull model. This analysis indicates that the spline model provides generally a better fit than the Weibull model and thus is used in the risk assessment for estimating lethalities for these products (with the exception of product 6, for which a linear regression was used).

Figures G16 and G17, respectively, are graphs of the $\ln(k)$ and $\ln(t_0)$ versus temperature for each product types, 1, 2, 4 and 7, together with linear regression lines. As seen, the regression lines provide reasonable fits, with only perhaps a slight curvature seen for the $\ln(k)$ versus temperature for some of the products.

Seemingly unrelated regressions (SUR) were performed for each product type and replicate, fitting the equations:

$$\ln(k) = a+b\mathrm{T}$$
$$\ln(t_0) = c+d\mathrm{T} \qquad (G15)$$

where a, b, c, and d are parameters and T is temperature in °C. Figure G18 presents the observed and fitted survival curves based on estimates for parameter values identified in Equation G15. From the estimated values of the parameters, for each replicate, within a product type, computed were estimates of lethalities. Table G9 provides estimates of the parameters of Equation G15, by replicate.

TABLE G9 ESTIMATED VALUES OF *A, B, C,* AND *D* OF EQUATION G15, USED TO PREDICT LETHALITIES OF *SALMONELLA* WITHIN FORMULATED LIQUID EGG PRODUCTS, FOR A GIVEN TEMPERATURE, BY REPLICATE. FOR PRODUCT 6, *Z*-VALUES AND INTERCEPT FOR THERMAL DEATH CURVE (ASSUMING LINEAR SURVIVAL CURVES) ARE GIVEN.

Product	Replicate	Coefficients for ln(k)		Coefficients for ln(t_0)	
		Constant	T	Constant	T
1	1	-19.09	0.3298	29.37	-0.4913
1	2	-18.58	0.3222	33.70	-0.5614
1	3	-19.54	0.3371	29.18	-0.4905
2	2	-34.45	0.5876	17.71	-0.3118
2	3	-34.44	0.5863	19.18	-0.3356
2	4	-35.25	0.5992	12.95	-0.2370
4	1	-27.79	0.4747	23.62	-0.3952
4	2	-25.85	0.4422	21.81	-0.3698
4	3	-27.28	0.4655	24.52	-0.4122
7	2	-28.05	0.5016	14.79	-0.2734
7	3	-28.02	0.5010	13.74	-0.2566
7	4	-27.95	0.4995	12.60	-0.2381
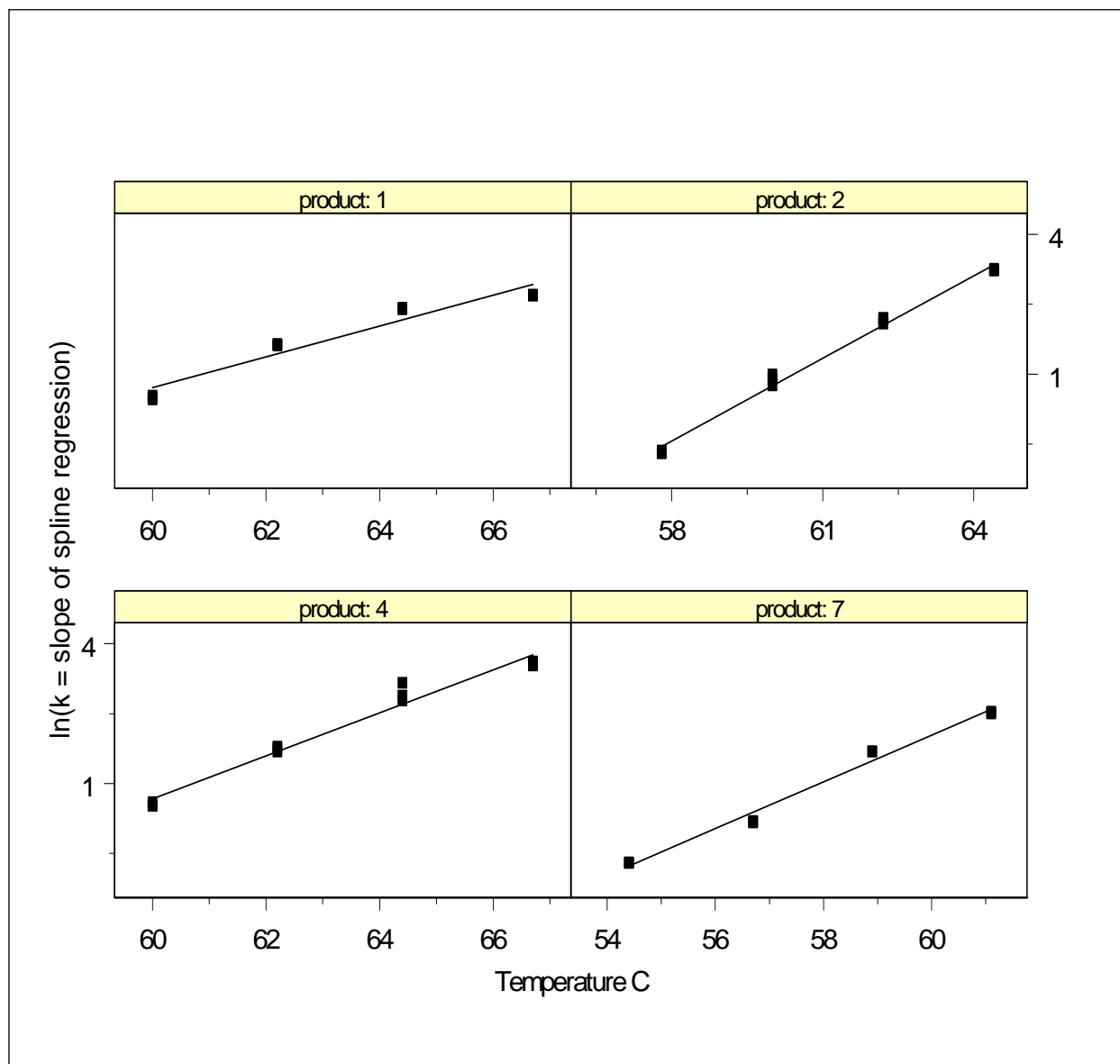
FIGURE G16 PLOT OF LN(κ), WHERE K IS THE SLOPE PARAMETER FOR THE SPLINE SURVIVAL CURVE (EQUATION G14), VERSUS TEMPERATURE ºC, TOGETHER WITH LINEAR REGRESSION LINES FOR EACH PRODUCT TYPE.
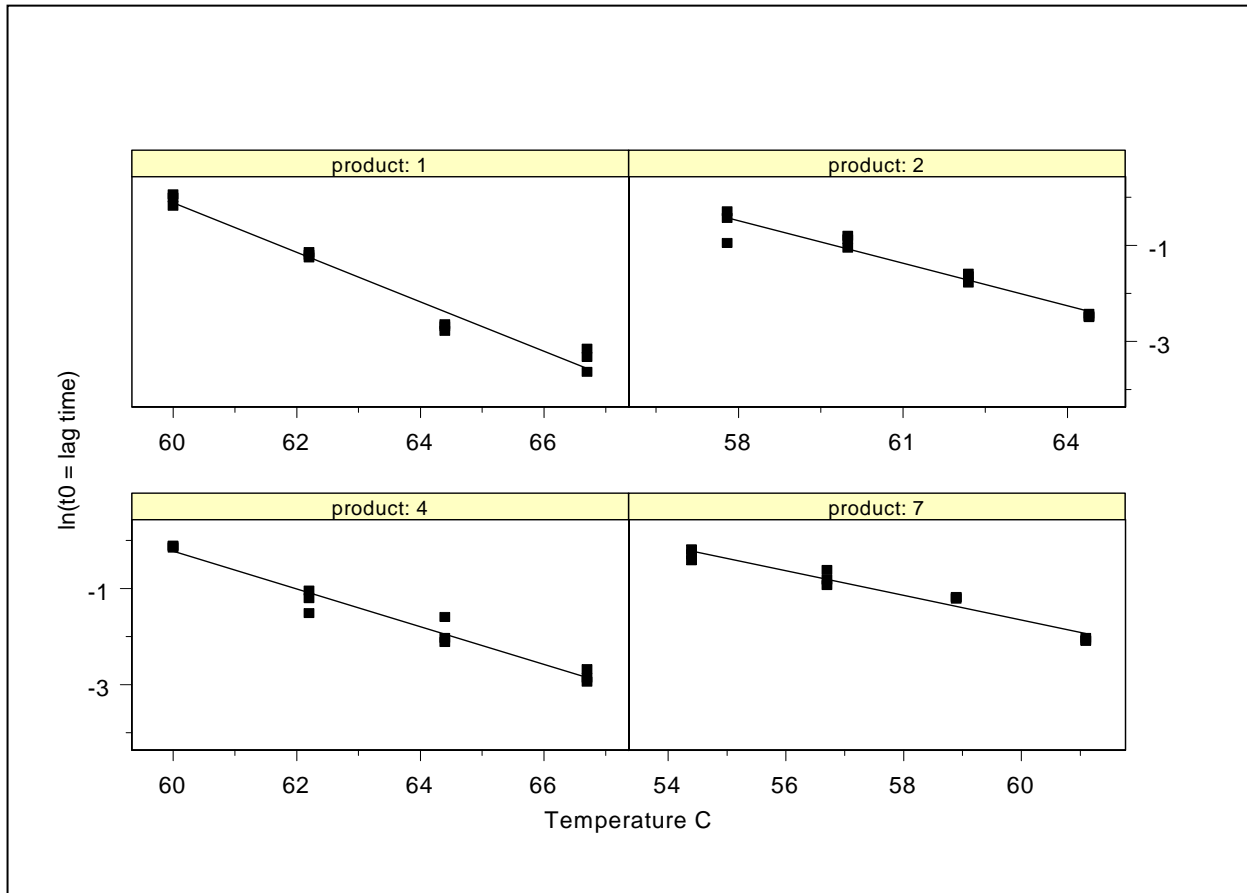
FIGURE G17 PLOT OF LN(T_0) WHERE T_0 IS THE LAG TIME BEFORE LINEAR INACTIVATION, VERSUS TEMPERATURE °C FOR PRODUCTS 1, 2, 4, AND 7, WITH LINEAR REGRESSION LINES FOR EACH PRODUCT TYPE.
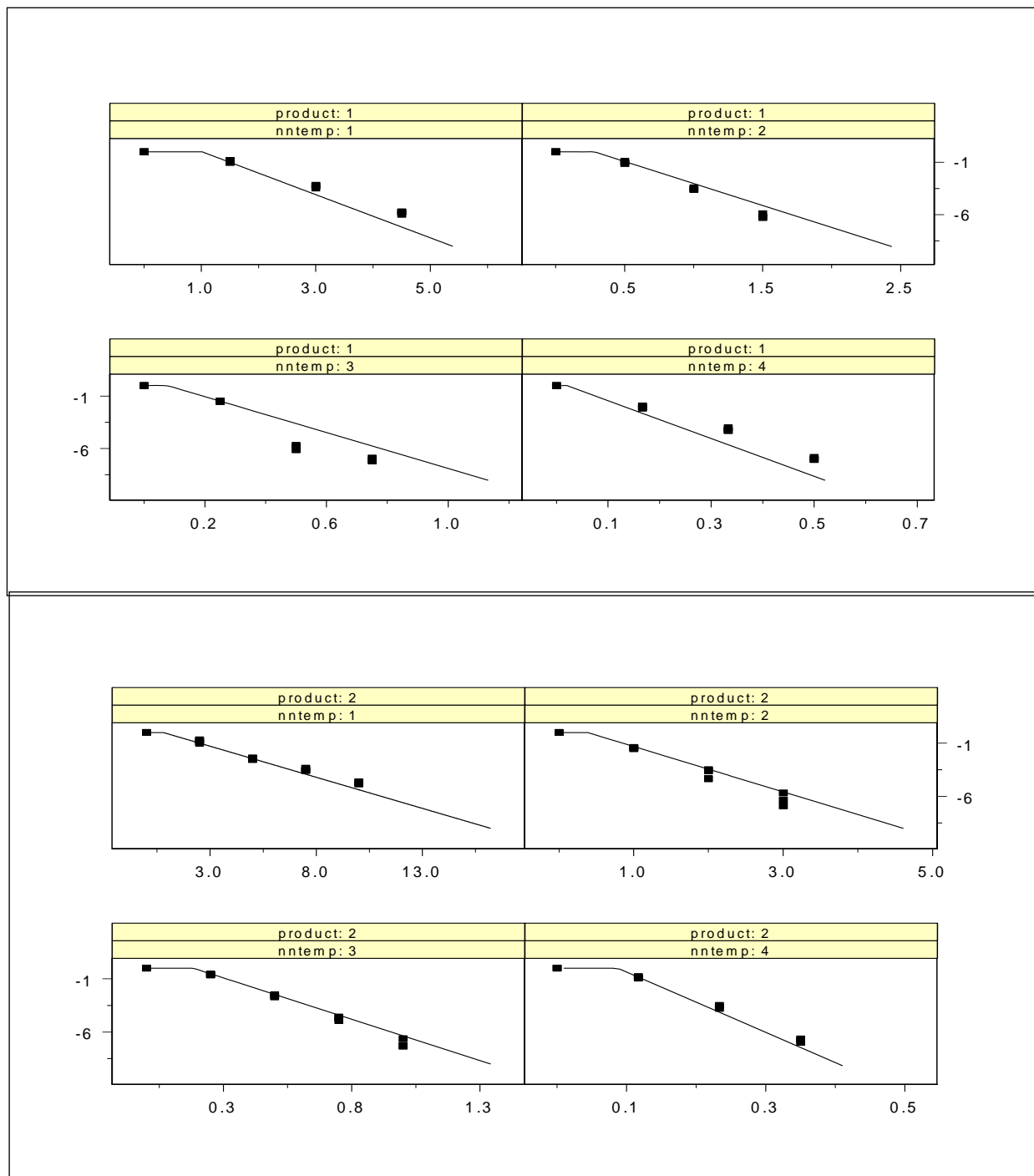
FIGURE G18 FITTED SPLINE AND OBSERVED SURVIVAL CURVES: PRODUCTS 1 AND 2.
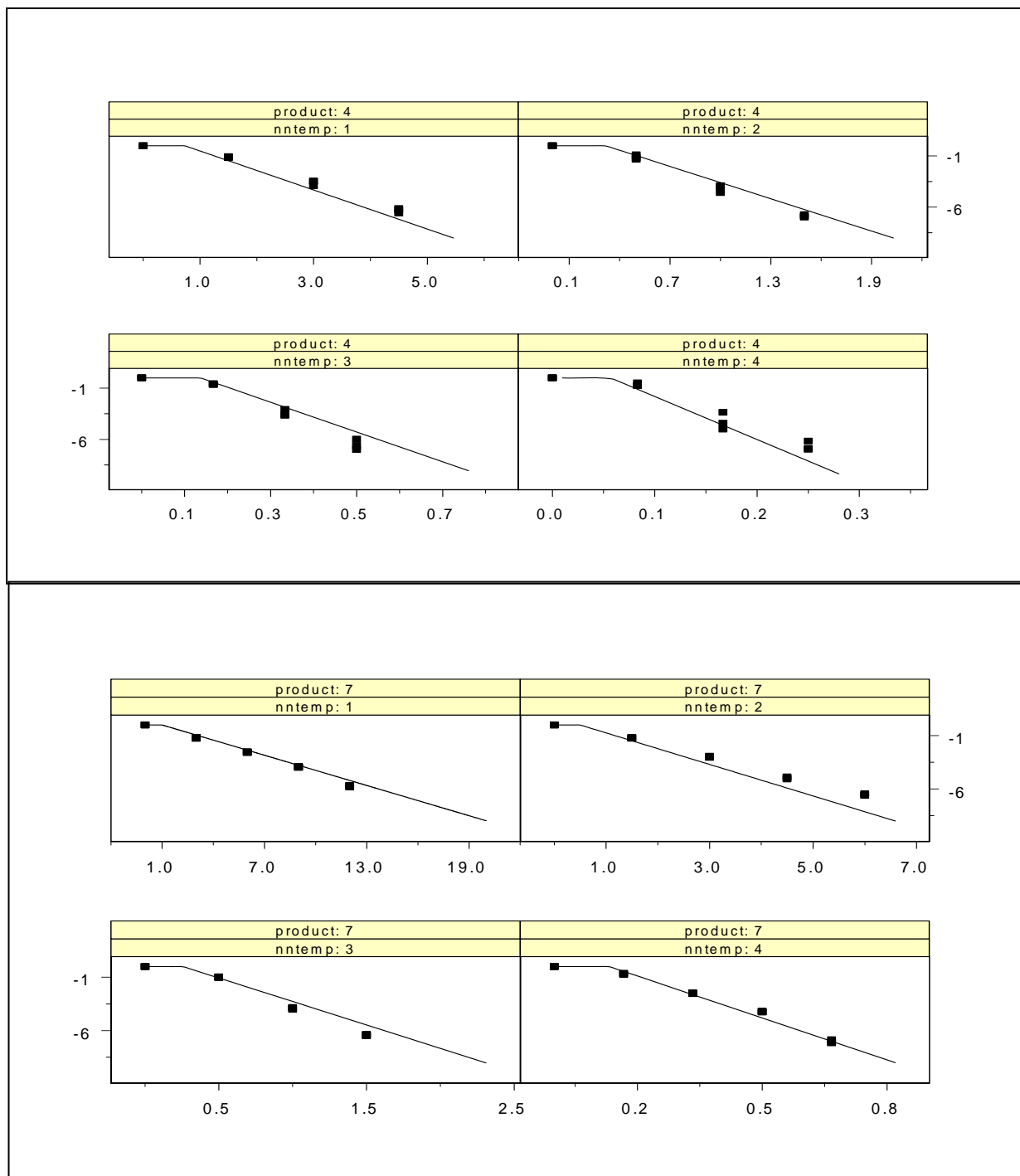
FIGURE G18 (CONTINUED): PRODUCTS 4 AND 7.

Plain Whole and yolk egg products

Data were not available for these products. However, the lethality model of Blackburn et. al.[5] with SE phage type 4 (P167807) in tryptone soy broth (TSB) was assumed to represent these products. The 88 survival curves generated in the Blackburn et al.[5] study were generated for the following conditions: temperatures of 54.5, 59.5, 62.5, and 64.5°C; pH values of 4.2, 4.6, 5.2, 7.0, 8.0, 8.7, and 9.5; and salt levels of 0.5, 3.5, and 8.5%. Broth samples of SE were heated in a submerged-coil heating apparatus. Samples were removed and rapidly cooled to room temperature, then stored at room temperature for 90 minutes to permit recovery of heat-injured SE. Surviving cells were enumerated following serial dilutions and inoculation of duplicate tryptone soy agar plates incubated at 37°C for 48 hours.

As discussed in the *Statistical methods* section, the model for estimating the lethality for these products is assumed to be based on the logistic function (Equation G6), where the parameters are determined from Equations G12 and G13, based on "predicted *D*-values" that are given by Blackburn et al.[5] The predicted values for the above egg products are given in Table G10.

TABLE G10 PREDICTED D-VALUES FOR WHOLE AND YOLK EGG PRODUCTS, TAKEN FROM TABLE 6 OF BLACKBURN ET AL.[5] THE PH VALUES FOR THE WHOLE PRODUCT WERE 7.7-7.8 WITH ONE NOTED EXCEPTION; FOR THE YOLK PRODUCT, THE PH WAS 6.5.

Product	Strain	Temperature(°C)	Predicted *D*-values (min)
Whole	SE	55.0	4.46
Whole	various	58.0	1.12
Whole	various	60.0	0.41
Whole	SE	57.2	1.64
Whole	SE	60.0	0.43
Whole	SE	60.0	0.41
Whole	*S.* Typhimurium	60.0	0.41
Whole (pH = 5.5)	*S.* Typhimurium	57.8	1.86
Whole	Various	60.0	0.41
Yolk	Various	62.2	0.36
Yolk	*S.* Typhimurium	59.5	0.85

Whole egg products

Figure G19 shows the thermal death curve ($\log_{10}$(*D*-value) versus temperature) for whole egg product. The data value that is farthest from the linear regression line is the data point associated with the 5.5 pH. Excluding this data point, the regression of the $\log_{10}$(*D*-value) versus temperature (*T*) yields the following equation:

$$\log_{10}(\text{D-value}) = 12.1199 - 0.20834 \, T \tag{G16}$$

To determine the survival curve, values of *a* and *b* are determined from Equations G12 and G13, which are used in Equation 6. The uncertainty associated with the above procedure is accounted for by generating random variables *f* and *g* such that the standardized variables, $(f\text{-}12.1199)/0.13879$ and $(g\text{-}0.20834)/0.00236$, are distributed as bivariate *t*-distribution with 3 degrees of freedom and correlation -0.999551.
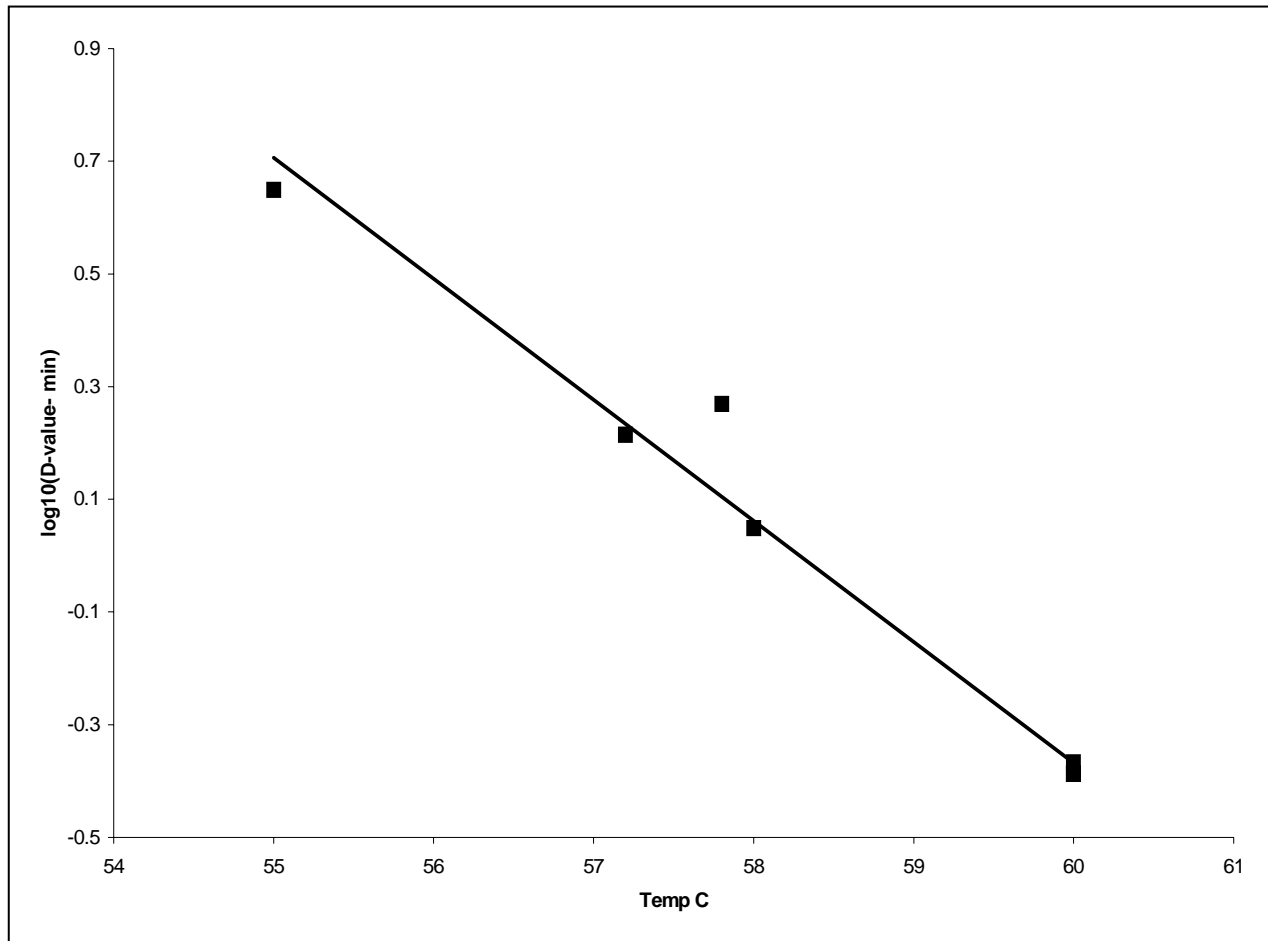
FIGURE G19 THERMAL DEATH CURVE DERIVED FROM D-VALUES REPORTED IN BROTH USED FOR PREDICTING LETHALITIES IN WHOLE EGG PRODUCTS.[5]

Yolk egg product

There were only two observations; hence, it is not possible using these data alone to determine the magnitude of error. Rather, to estimate the magnitude of error associated with these estimates, these data were pooled with the whole egg data. The regression for the yolk egg product is

$$\log_{10}(\text{D-value}) = 8.1518 - 0.1382\ T \tag{G17}$$

and the standard error for the intercept is 0.3750, and for the slope, it is 0.006161. The correlation between the two is -0.999754. The uncertainty is determined as above, namely through generation of random variables f and g such that the standardized variables $(f\text{-}8.1518)/$ 0.3750 and $(g\text{-}0.1382)/0.00616$ are distributed as bivariate t-distribution with 3 degrees of freedom and correlation -0.999754.

Plain egg white at selected pH levels

The observed data indicated survival curves with an initial shoulder, followed by an asymptotic linear line for pH values of 7.8, 8.2, and 8.8. For pH = 9.3 then survival curves seemed nearly linear. In the risk assessment lethalities were predicted assuming a pH = 9.3. This was assumed because the pH increases once the eggs are broken, and by the end of one day could be in excess of pH of 9. Survival curves were fit for pH = 9.3. However, a model for pH between 7.8 and 8.8, using the data for the egg white product for pH = 7.8, 8.2, and 8.8 is also provided below. The observed and estimated lethalities using this latter model are low, and were thought unrealistic.

A preliminary analysis was performed where for each experiment the asymptotic D-values were estimated using a linear regression in which the observed values at time = 0 were excluded. This analysis indicated that the common logarithm of the asymptotic D-values, $\log_{10}(asymD)$, were linearly related with the temperature for a given pH. However, for a given temperature, the logarithms of the asymptotic D-values seemed linear for the three lowest pH values of 7.8, 8.2 and 8.8, but, when projecting the regression lines at a pH of 9.3, the projected values were substantially higher than the observed values. Thus, the model for all pH values could not be reliably constructed.

The survival curves for pH not more than 8.8 were fit to the function defined above in Equation G10, where ε represents an error term, with two parameters: k and $w>0$, assuming k and w are functions of the pH and temperature, was used to fit the data. To avoid boundary problems, the dependent variables considered were $\ln(k)$ and $\ln(w)$, which were assumed at most quadratic polynomial functions of pH and temperature. From the above analysis of the asymptotic D-values, $\ln(k)$ would not contain quadratic terms of temperature and pH, and it would contain an interaction term between them. Consequently,

$$\ln(k) = a + b(T\text{-}50) + c(\text{pH-}7) + d(t\text{-}50)(\text{pH-}7) \tag{G15}$$

$$\ln(w) = e + f(T\text{-}50) + g(pH\text{-}7) + h(T\text{-}50)(pH\text{-}7) + i(T\text{-}50)^2 + j(pH\text{-}7)^2 \qquad (G16)$$

where T is temperature and a, b, c, and d are parameters. The values of 50 and 7 were subtracted from the temperature and pH respectively, to provide coefficients that are more manageable. For $\ln(w)$, a similar function,
is considered.

To account for possible replicate and experiment nested within replicate effects, it was initially assumed that the variables a and e are correlated random variables. However, convergence often was not obtained when assuming both a and e random; thus the variable a, reflecting the experimental error of the asymptotic D-value, is assumed to be random and the other parameters are assumed to be fixed. Specifically, a is assumed to be equal to a constant plus an error term, which possibly has a nested error structure, $\varepsilon_r + \varepsilon_{e(r)}$, where the first term represents the replicate effect and the second term represents the experimental within replicate effect. Assuming that the coefficients of the second order terms are not zero (h, i, and j) in Equation G16, the replicate effect is statistically significant, at the 0.004 significance. With only three pH values used, the presence of the quadratic term for pH would seem like an example of "over fitting." Assuming a replicate effect, the coefficients of the square terms of temperature and pH, taken together, were not statistically significant (P-value = 0.14). Assuming that the coefficients of the square terms (i and j) are zero, the term f (coefficient of temperature) was the only term not significantly different from zero; the significance, based on the likelihood ratio test, is 0.17, suggesting that this term can be assumed zero. For this model ($f = i = j = 0$) the other terms (h, g) are not, individually, statistically significant from zero. The model: $j = i = h = g = 0$, compared to the model: $j = i = 0$ – testing for significance of adding nonzero terms g and h, given that i and j are zero- has a P-value of 0.05, which is (only) marginally significant. The loglikelihood value of the model that has $f = i = j = 0$, and g and h not equal to zero, is larger by 4 than the loglikelihood value for the model that has f is not zero and $j = i = h = g = 0$. Consequently, the former model is favored. Using this model, the root mean square of the residuals (RMSE) is 0.2889 and the average standard error of the predicted lethality for the conditions (times, temperature, and pH) of the experiment is 0.0938; the largest is 0.2439 (for a predicted lethality of 6.6). The coefficient of variation (CV) is a decreasing function of the predicted lethalities, so that, for example, the CVs for predicted lethalities of about 7 $\log_{10}$ at a pH = 8.8 are about equal to, or slightly less than, 5%.

The estimated values, standard errors, and the correlation matrix of the estimated parameters, copied from the S-plus output, and plots of the residuals versus the observed $\log_{10}$ relative reductions are given in Table G11. Figures G20 and G21 give, respectively, plots of residuals versus observed log reductions, and plots of the fitted survival curves and the observed ones.

TABLE G11 ESTIMATED VALUES OF PARAMETERS USED FOR MODELING LETHALITY IN EGG WHITE PRODUCTS FOR PH NOT MORE THAN 8.8 AND NOT LESS THAN 7.8.

	Value	Std. Error	DF	*t*-value	*p*-value
a	-4.76610	0.22143	102	-21.52417	<0.0001
b	0.71335	0.03135	102	22.75718	<0.0001
c	0.52728	0.17373	102	3.03508	0.0031
d	-0.05284	0.02453	102	-2.15367	0.0336
e	-10.99275	6.99251	102	-1.57207	0.1190
g	14.46086	12.60939	102	1.14683	0.2541
h	-1.69467	1.58304	102	-1.07052	0.2869

Correlation	*a*	*b*	*c*	*d*	*e*	*g*
b	-0.955					
c	-0.965	0.920				
d	0.927	-0.965	-0.959			
e	-0.713	0.704	0.720	-0.718		
g	0.675	-0.667	-0.699	0.698	-0.985	
h	-0.644	0.637	0.672	-0.673	0.964	-0.995
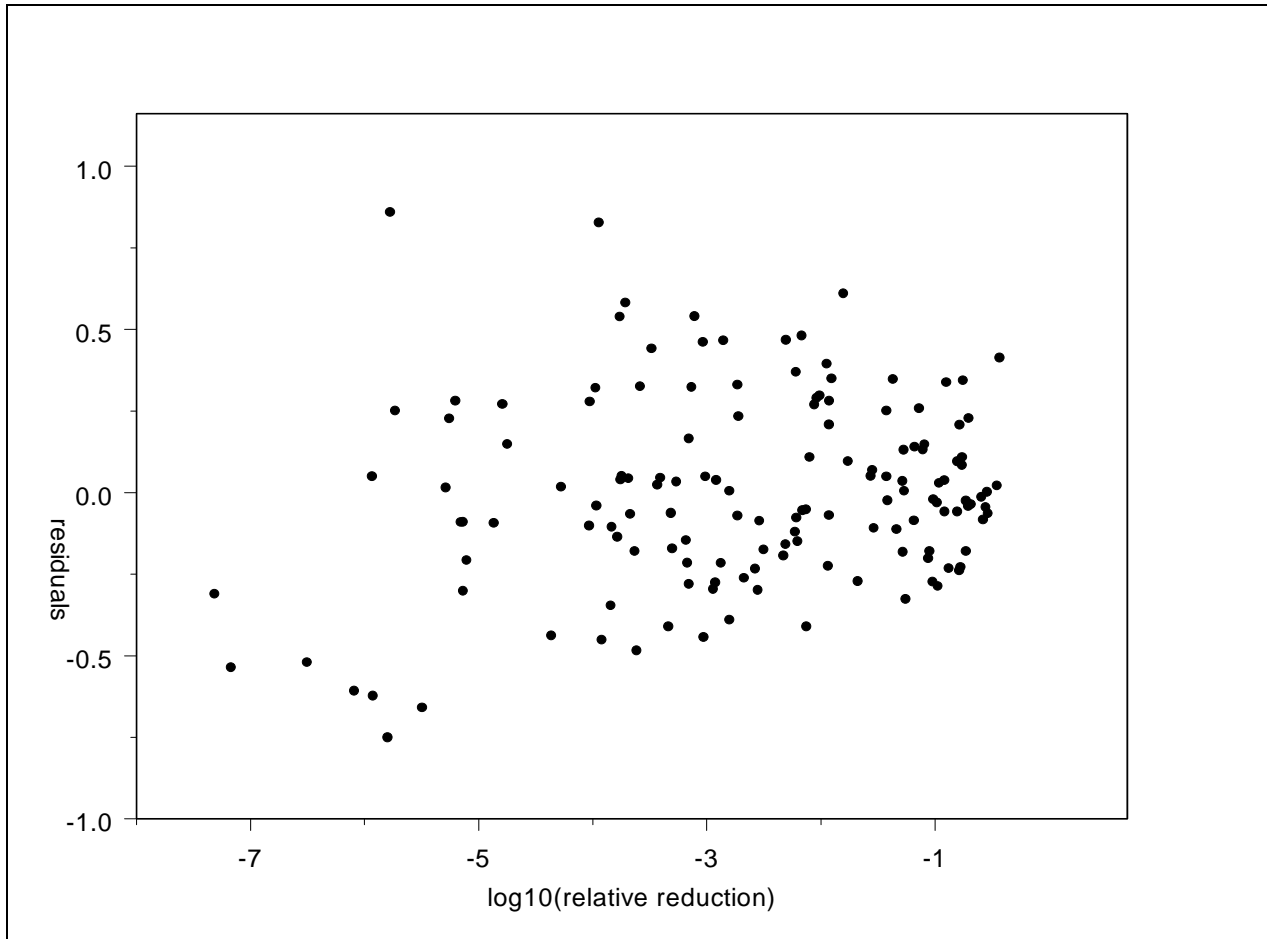
FIGURE G20 PLOT OF RESIDUALS VERSUS OBSERVED LOG$_{10}$ RELATIVE REDUCTIONS FOR SELECTED MODEL USED FOR MODELING LETHALITY *SALMONELLA* SPP. FOR EGG WHITE PRODUCTS WITH NOT MORE THAN PH OF 8.8.
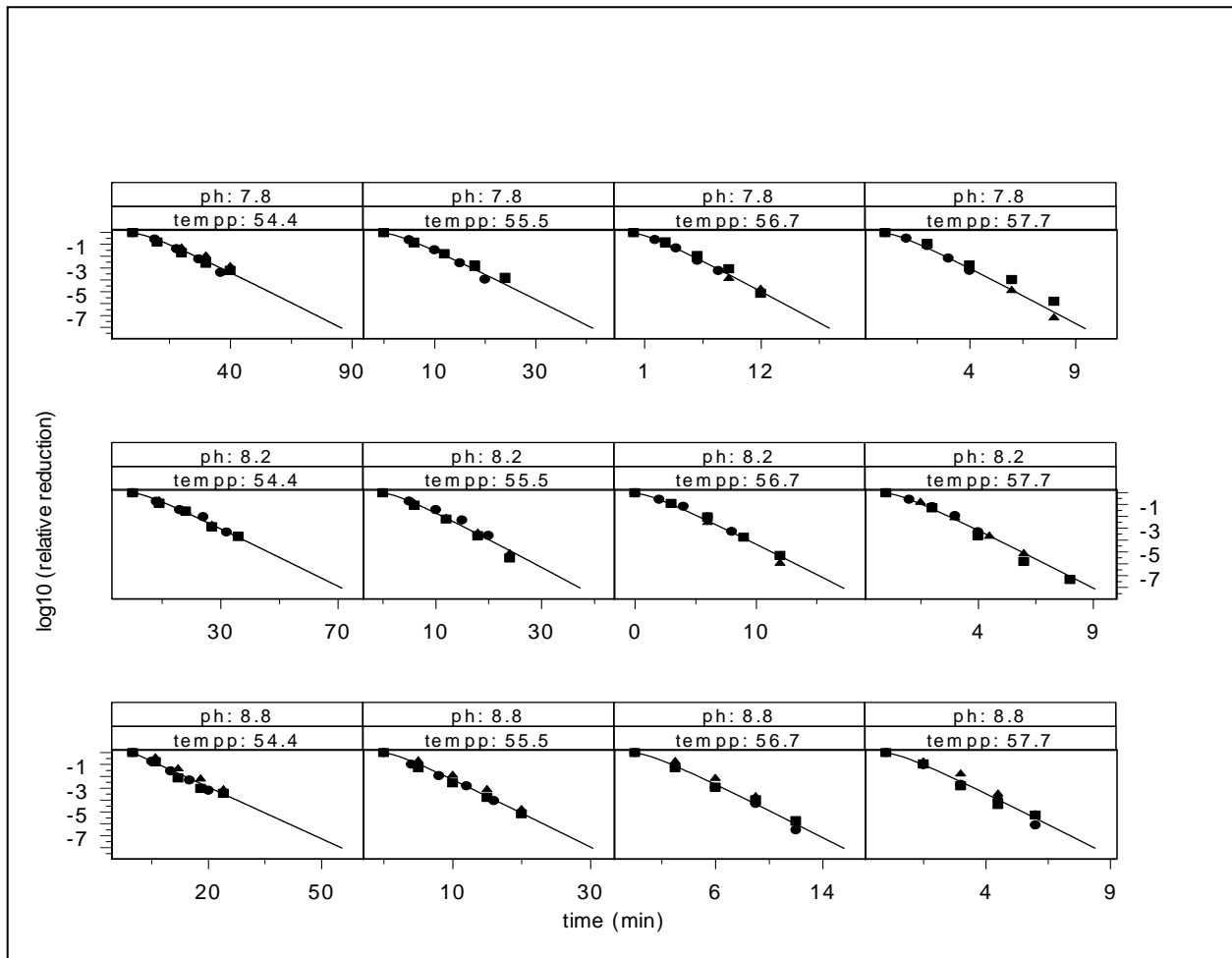
FIGURE G21 PLOTS OF OBSERVED DATA POINTS AND FITTED SURVIVAL CURVES (LOG$_{10}$(RELATIVE REDUCTION) VERSUS TIME (MIN) FOR EGG WHITE PRODUCT FOR GIVEN TEMPERATURES AND PH VALUES.

Estimate of model for egg white with pH = 9.3

The above nonlinear model was fit for data for pH = 9.3. The specific equations were:

$$\ln(k) = a+b(T\text{-}50) \tag{G17}$$

where T is temperature and a, b, and

$$\ln(w) = c+\sigma\varepsilon \tag{G18}$$

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where c and σ are constants, and ε is a random error, assumed distributed as a standard normal distribution (with zero expected value and standard deviation = 1), associated with the survival experiment. Figure G22 provides plots of the fitted curves and observed log reductions.
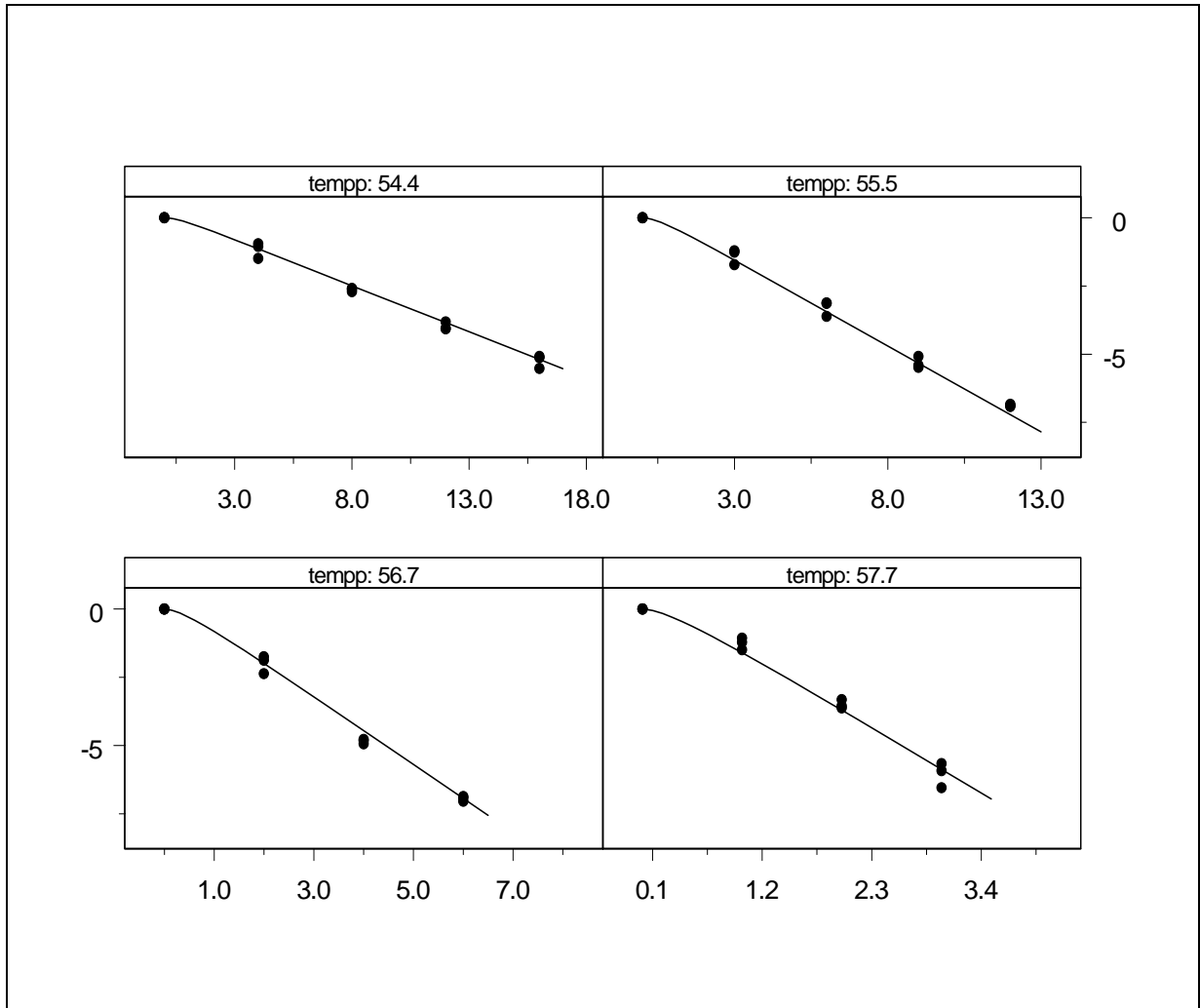
FIGURE G22 PLOTS OF FITTED CURVES AND OBSERVED LOG REDUCTIONS FOR EGG WHITE WITH PH = 9.3.

The parameter values are:

	a	b	c	σ
Parameter Estimate	-2.7427	0.5662	0.2298	0.4783
Standard Error	0.0567	0.0092	0.4376	0.2710

The above equations are difficult to work with, for example for determining values of time, given target lethality and determining confidence intervals. Froning et al.[4] fit linear survival curves with only a slight bias, but with a greater considerable ease of determining estimates. A linear survival model thus was also estimated for each replicate. Figure 23 provides graphs of the

thermal death curves for each replicate. As is seen the thermal death curves appear linear, corresponding to Equation G17 above. Table G12 provides estimates of the temperature-specific *D*-values for each replicate and the corresponding *z*-value.
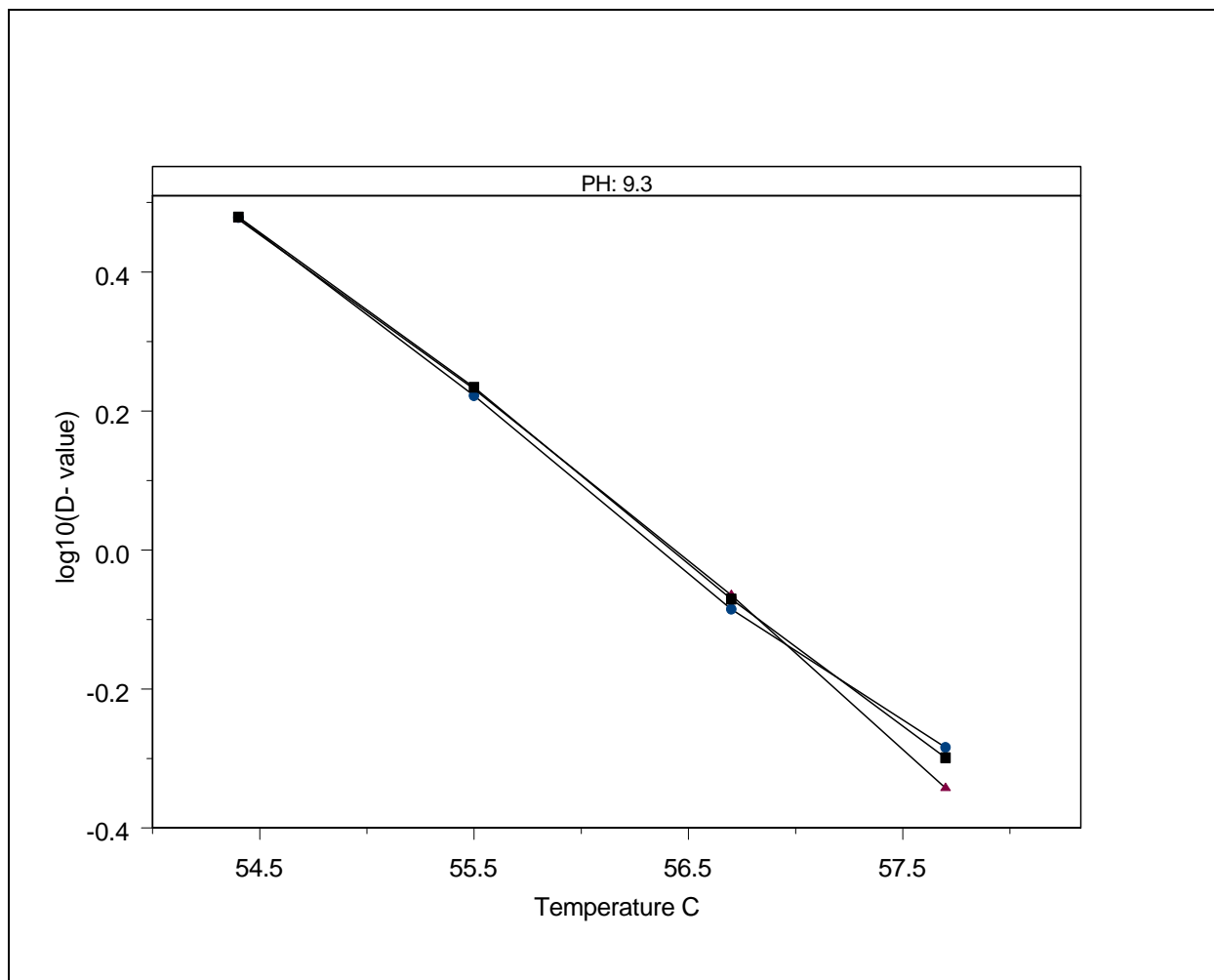
FIGURE G23 THERMAL DEATH CURVES (PLOT OF LOG$_{10}$ D-VALUE VERSUS TEMPERATURE ^{o}C) FOR SURVIVAL OF SALMONELLA IN PLAIN LIQUID EGG WHITE PRODUCT (PH = 9.3).

TABLE G12 *D*-VALUES (MIN), *Z*-VALUES (^{o}C) AND INTERCEPT OF THERMAL DEATH CURVE: LOG$_{10}$(*D*-VALUE) = INTERCEPT $- T/Z$, WHERE *T* IS TEMPERATURE AND *Z* IS THE *Z*-VALUE.

Temp (°C)	Replicate 1	Replicate 2	Replicate 3
54.4	3.01	2.99	3.01
55.5	1.67	1.70	1.71
56.7	0.82	0.86	0.85
57.7	0.52	0.45	0.50
z value	4.27	4.04	4.20
intercept	13.22	13.96	13.43

The bias in the predictions of the log reductions when the observed log reductions are greater than 3 is generally less than 0.2 $\log_{10}$, with the linear model predicting slightly larger lethalities. In the risk assessment, the linear model was used following conventional practice.

Pasteurization Within Eggs

Schuman et al. [14] conducted a study consisting of 4 experiments for which measured levels of SE and temperatures over time were made. Two of the experiments had a water-bath temperature of 57°C and the other two had a water-bath temperature of 58°C. SE was inoculated near the geometric center of the yolk (with SE level ca 8.5 $\log_{10}$ cfu of concentrated, pooled SE cells in 50ul of sterile peptone water). The initial SE level at time = 0 ranged from 6.67-6.80 log cfu/g. Although the temperature profile with time was not shown in the paper, the raw data was given in a graph by the authors (personal communication). Details of the study are provided at the end of this subsection.

In the analysis, it is assumed that the 4 data sets from the experiments are statistically independent. Tables G13 and G14 provides the data that were used for estimating the parameters of Equation G4. For fitting the curves, only 4 data points of the total of 7 data points per experiment were used; values recorded as "<1 $\log_{10}$" were not used. The temperature versus time curve, after an initial "lag" period, displayed the log-linear relation:

$$T(t) = T_w + (T_i - T_w)\, e^{-kt} \tag{G19}$$

where k is the exponential heating rate, T_i is the initial temperature, and T_w is the water-bath temperature. Figures G24 and G25 provide the temperature profiles for the two temperatures.

Estimates of k are given in Table G15. To estimate $T(t)$ in lag period in the temperature profiles (Figures G24 and G25), linear interpolation was used. The actual equation used to fit the data, derived from Equation G4 is,

$$\log_{10}(N(t)) = \log_{10}(N(0)) - \log_{10}(e)\int_0^t e^{a+bT(\tau)}d\tau \tag{G20}$$

where $\log_{10}(N(0))$ is considered a parameter whose value is to be estimated and which corresponds to the initial level of SE cells at time equal to 0. The parameters in Equation G20 were estimated by minimizing the mean square errors of the predicted values using Microsoft Excel® 2000_Solver. Figures G26 and G27 display the observed data and the predicted survival curves. As is evident, the fitted curves fit well with the observed data that were used in this analysis. This is not surprising because there are only 4 data points and 3 parameters for each curve.

TABLE G13 THERMAL INACTIVATION OF SE IN SHELL EGGS SUBJECTED TO LOW-TEMPERATURE, LONG-TIME IMMERSION HEATING 58°C.

Dwell time in 58°C water-bath (min)	Mean egg center temperature (°C)		Survivors (log cfu/g)		Samples *Salmonella*-positive by enrichment testing	
	Trial 1	*Trial 2*	*Trial 1*	*Trial 2*	*Trial 1*	*Trial 2*
0.0	21.2	20.6	6.67	6.73	3/3	3/3
24.0	55.9	55.9	4.88	5.35	3/3	3/3
35.0	57.0	57.6	2.81	1.77	3/3	3/3
42.5	57.2	57.0	1.10	0.87	3/3	1/3
50.0	57.1	57.5	<1.00	<1.00	1/3	0/3
57.5	57.1	57.5	<1.00	<1.00	0/3	0/3
65.0	57.6	57.6	<1.00	<1.00	0/3	0/3

TABLE G14 THERMAL INACTIVATION OF SE IN SHELL EGGS SUBJECTED TO LOW-TEMPERATURE, LONG-TIME IMMERSION HEATING 57°C.

Dwell time in 58°C water-bath (min)	Mean egg center temperature (°C)		Survivors (log cfu/g)		Samples *Salmonella*-positive by enrichment testing	
	Trial 1	*Trial 2*	*Trial 1*	*Trial 2*	*Trial 1*	*Trial 2*
0.0	21.1	19.6	6.77	6.80	3/3	3/3
35.0	55.3	56.2	4.30	4.83	3/3	3/3
45.0	55.5	56.3	2.83	3.62	3/3	3/3
55.0	56.8	56.6	1.00	1.41	2/3	3/3
65.0	56.2	56.4	<1.00	<1.00	1/3	0/3
75.0	56.2	56.9	<1.00	<1.00	0/3	0/3
85.0	56.2	57.0	<1.00	<1.00	0/3	0/3
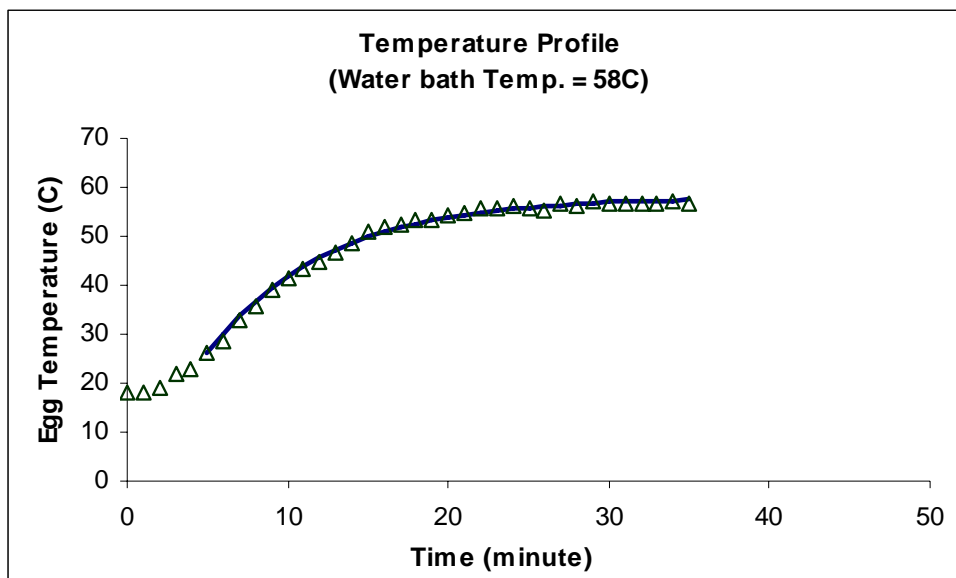
FIGURE G24 TEMPERATURE PROFILE FOR SHELL EGG PASTEURIZATION AT 58°C.
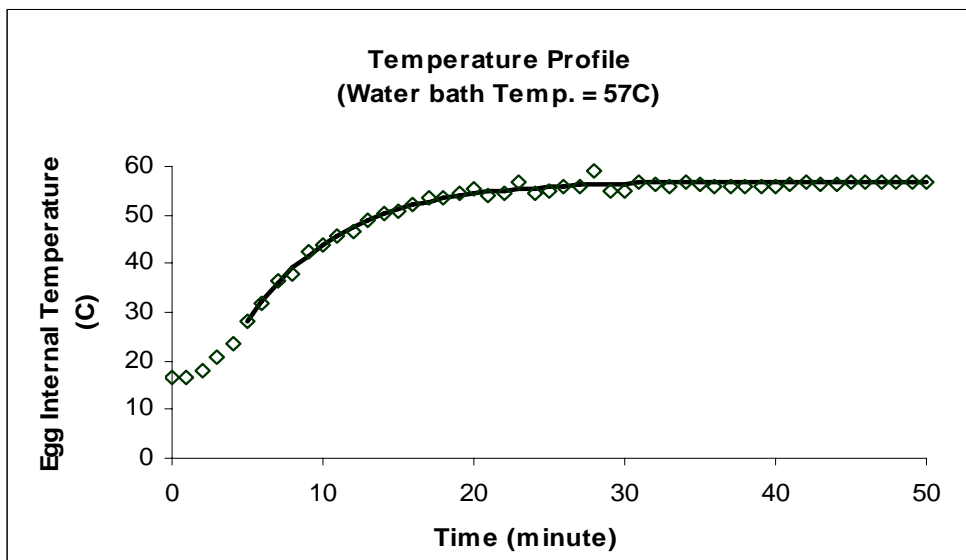
FIGURE G25 TEMPERATURE PROFILE FOR SHELL EGG PASTEURIZATION AT 57°C.
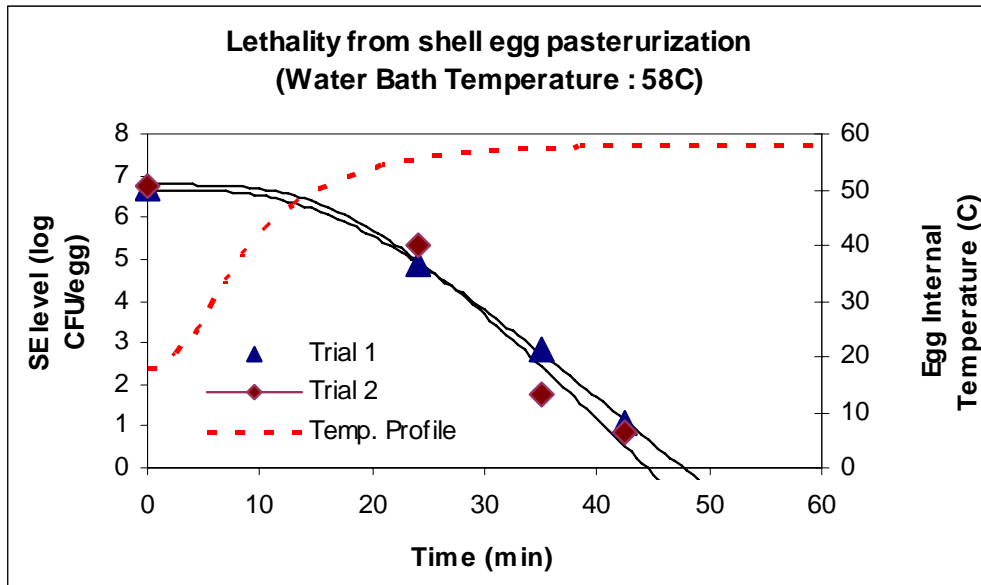
FIGURE G26 THE LOG-REDUCTION OF SE DURING THE SHELL EGG PASTEURIZATION AT 58°C.
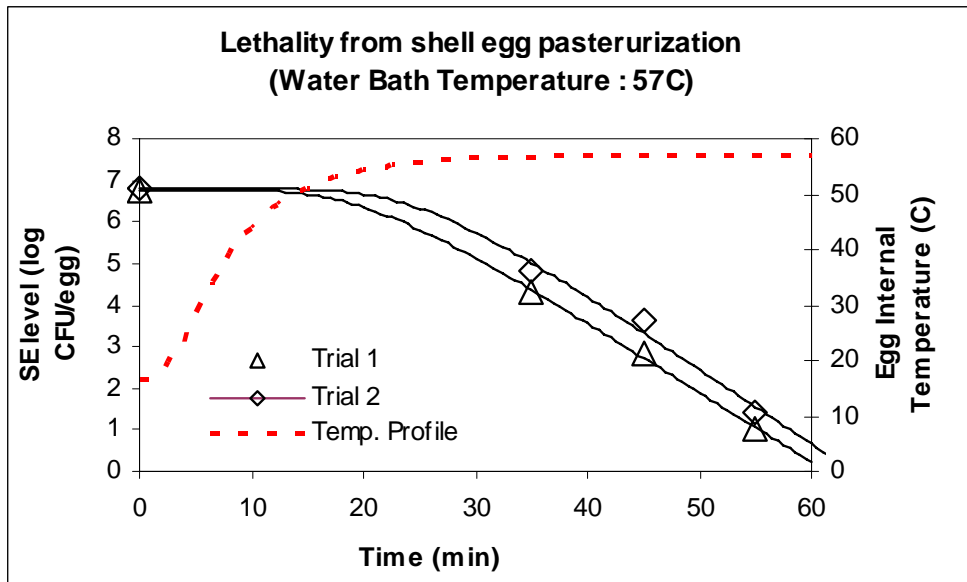
FIGURE G27 THE LOG-REDUCTION OF SE DURING THE SHELL EGG PASTEURIZATION AT 57°C.

Table G15 gives the estimated values of *a* and *b*.

TABLE G15 THE INITIAL SE LEVEL BEFORE PASTEURIZATION AND THE ESTIMATE OF PARAMETERS OF EQUATION 25.

Experiment		Initial SE level (log cfu/g)	k	a	b
58°C	Trial 1	6.67	0.136	-7.22	0.101
	Trial 2	6.79		-8.25	0.122
57°C	Trial 1	6.77	0.161	-15.90	0.250
	Trial 2	6.79		-32.34	0.540

The values of *a* and *b* appear dependent on the water-bath temperature, which is contrary to the theory used to develop the above equations. This creates a problem in interpreting the results and using them in the risk assessment. To generalize for the risk assessment, it is necessary to find two simple functions of these variables, which give quantities that are, or are assumed, not to be dependent upon the water-bath temperature. The first of these is the ratio of *b* to *a*, which seems nearly constant. The mean of the values of the quantities $\ln(-a) - \ln(b)$ is, $R = 4.1821$, with a standard error of 0.03827. The second function is determined from a linear regression of $\ln(b)$ versus temperature. The slope is, $\alpha = -1.1969$, with standard error of $s_\alpha = 0.3965$; the intercept is, $\beta = 67.225$, with standard error of, $s_\beta = 22.798$; and the correlation is, $\rho = -0.9056$, based on two degrees of freedom.

Assumptions for modeling

For a given water-bath temperature, T_w, if $T(t)$ is the temperature profile with an exponential heating rate between 0.10 and 0.20, then, from Equation G4, the probability of an SE cell surviving up to time t, $p(t)$, is given by,

$$\ln(p(t \mid T_w)) = -\int_0^t e^{a(T_w)+b(T_w)T(\tau)}d\tau \tag{G21}$$

where $a(T_w)$ and $b(T_w)$ are functions of the assumed water-bath temperature. The values of $a(T_w)$ and $b(T_w)$ are determined such that $R = \ln(-a(T_w))-\ln(b(T_w) = 4.18$ and $\ln(b(T(_w)) = \alpha+\beta T_w$, where $\alpha = 0.3965$ and $\beta = -1.1969$.

Uncertainty is determined by generating random variables RN, αN, βN, such that, the quantity $r = (RN-R)/0.03827$ is distributed as a t-distribution with 2 degrees of freedom, independently, and the vector, $v = (v_\alpha, v_\beta) = ((\alpha N-\alpha)/s_\alpha, (\beta N-\beta)/s_\beta)$, is distributed as a multivariate t-distribution with correlation $\rho = -0.9056$ and 2 degrees of freedom. This is done using the following transformed variables: $t_1 = (v_\alpha-\rho v_\beta)/(1-\rho^2)$ and $t_2 = v_\beta$. Two independent random normal distributed variables, z_1 and z_2, are generated, and a random chi-square variable, χ, with two degrees of freedom is generated. Put $t_j = z_j/(\chi/2)^{0.5}$, for $j = 1, 2$, and then solve for αN and βN.

Features of the protocol used to generate data

For the analysis of pasteurization of shell eggs, the data studied by Schuman et al.[14] were used to model the lethality of SE from shell egg pasteurization. Features of the protocol are: (i) A pooled, six-strain inoculum was used to compensate for strain-to-strain variations in thermal resistance. For a risk assessment, between-strain variability might be important to know. The six SE strains in the cocktail were: Benson-1(human clinical); ATCC 4931(human clinical, type strain); ME-14 (poultry manure); ME-15(shell egg transfer belt); ME-16(shell egg transfer belt); and ME-18 (live poultry); (ii) Stationary-phase cells of SE were used in the inoculum suspension. Cells in stationary phase are generally several-fold more heat-resistant than cells harvested in the log or exponential phase of growth;[15] (iii)[16] The inoculation site is at or near the geometric center of the egg within the yolk, which is the slowest-heating point during the pasteurization, and is expected to provide a conservative estimate for the lethality of SE within eggs in general.[15;16]

Methodology of Schuman et al.[14]

Shell eggs

Nest-run brown shell egg (62±2 g per egg) within 1d of laying from a single flock. Eggs were washed with warm soapy tap water, rinsed twice in sterile deionized water and air dried at room temperature for 2 hours.

Bacteria and culture conditions

Six SE isolates were maintained on tryptic soy agar slants at 4°C. Stationary-phase cultures containing 9.1-9.6 log cfu/ml were obtained (transferring 10 ml of tryptic soy broth → working stock culture → transferring 60ul from each working stock culture to centrifuge tube containing 30 ml of TSB)

Inoculation protocol:
1. Affixation of septum to egg
   Place a droplet of glue (Duro Super Glue) on the approximate geometric centre along the equatorial axis of each shell eggs. → Affix a 9.5 mm diameter rubber septum to each egg at the glue droplet site and dry at room temperature for 2 hours.
2. Warming up egg to room temperature:
   The eggs were held overnight at 4°C and allowed to warm to room temperature before inoculation
3. Inoculation of SE in the center of yolk:
   a. Inoculated SE in near the geometric centre of the yolk with ca 8.5 $\log_{10}$ cfu of concentrated, pooled SE cells in 50 μl of sterile PW.
   b. Injection of SE- perforating the septum and the egg using a 2.54cm/23 gauge sterile needle coupled to a calibrated 50 μl repeating syringe.
4. Keeping eggs at room temperature:
   For ≤ 1 hour prior to immersion heating.

* Test for the location of inoculation:

Inoculated 50 μl of aqueous tracer dye into the yolk → a standard hard-cooling procedure demonstrated : this inoculation procedure provided consistent placement of the dye near the centre of the yolk with no detectable inoculum drift.

Immersion hearing apparatus:

1. Temperature monitoring:

By perforating the septum and shell with the thermocouples, the internal temperature at the geometric centre of control eggs was monitored at every 5 seconds using a personal computer (LabTech Notebook software)

2. Heating trials:

By submerging the Plexiglas egg try apparatus into a preheated circulating water-bath containing 16.8 liters of deionized water.

The water-bath was equipped with a calibrated temperature control module accurate to ±0.05°C

3. Calibration of temperature:

Using a standardized mercury-in-glass thermometer

4. The location of immersion:

The upper surface of each shell egg was approximately 2.5 cm below the surface of the water in the bath.

Salmonella Inactivation trials:

- Two trials for SE inactivation
    1. Submersion in a pre-heated 58°C water-bath for up to 65 minutes
    2. Submersion in a pre-heated 57°C water-bath for up to 85 minutes
- 3 eggs for each sampling time (randomly): removed from egg tray
  3 eggs x 7 sampling time/trial/temperature x 2 trials x 2 different temp (57 and 58°C) = 84 eggs.
- Transferred to beaker containing 1 liter of water (22°C sterile deionized water) → cool for 5 minutes.
- Series of dilution in PW and 0.1-0.33 ml were surface-plated onto pre-poured TSA plates. → held at room temperature for 3 hours → overlaid with tempered (45°C) xylose lysine deoxycholate agar.
- Solidification at room temperature → incubation at 37°C for 48 hours.
- Enrichment at 37°C for 24 hours: using the remaining blended egg/lactose broth (ca. 520 ml) at the time of plating onto TSA.
- Confirmation of *Salmonella* isolates: 1 ml of enrichment broth was transferred to 10 ml of selenite cysteine broth (37°C, 24h) → A loopful of each SC enrichment was then streaked for isolation onto a prepoured XLD plate, and presumptive *Salmonella* isolates were selected and confirmed on TSI slants.

Attachment G1: Product formulation for University of Nebraska's 5 egg mixture products

1. Scrambled Egg Mix-USDA

Ingredient	Percentage	Solids
Whole Egg- 24.2% solid	66.30	16.05
Nonfat Dry Milk- 95 % solid	9.60	9.12
Vegetable Oil	4.80	4.80
Salt	0.03	0.30
Water	19.00	
Total	**100.00**	**30.27**

2. Scrambled Egg Mix Solids – pH 6.5 to 6.8

Ingredients	Percentage	Solids
Whole Egg – 24.2 % solid	81.20	19.65
Nonfat Dry Milk – 95 % solid	2.60	2.47
Xanthan Gum	0.18	0.18
Citric Acid	0.13	0.13
Water	15.89	
Total	**100.00**	**22.43**

4. Fortified Whole Eggs – "Tex" Product

Ingredients	Percentage	Solids
Whole Egg – 24.2% solid	71.68	17.35
Egg Yolk - 43% solid	20.00	8.60
36 DE Corn Syrup Solids	6.46	6.14
Salt	0.40	0.40
Water	1.46	
Total	**100.00**	**32.49**

6. Fortified Egg Yolk "Tex " Product

Ingredients	Percentage	Solids
Egg Yolk – 43 % solid	82.00	35.26
Corn Syrup – 80% solid	16.00	12.80
Salt	0.80	0.78
Water	1.20	
Total	**100.00**	**48.84**

7. Imitation Egg Products

Ingredients	Percent by total weight of liquid formulation
Egg Whites	95.50
Nonfat Dry Milk	3.59
Soybean Oil	0.50
Modified Food Starch	0.20
Xanthan Gum	0.20
Color (beta carotene)	0.01
Total	**100.00**

Attachment G2: Results of the FSIS Liquid Egg Plant Survey

This attachment consists of tables for which the times and temperatures that were recorded from the FSIS plant survey are given, together with the estimated lethalities using one or more of the models described above, representing the lethalities for the fastest particle. The first row of results in each table, with the exception of the 5 mixture products, corresponds to the present regulatory minimal time and temperatures (based on the 1971 regulation), when the time is equal to 3.5 m. The other rows of the table are times and temperatures that were provided in the survey for products that were matched to the product specified by the table. Each table also includes a "weighted" lethality, which is the negative of the weighted average of the probabilities of individual cell survival, where the weight for each time and temperature combination is the number of times it was identified.

Estimates of lethality are provided for the reported times and temperatures. When the University of Nebraska data were used, 99% upper and low confidence bounds are computed, by means of a t-distribution with 2 degrees of freedom with the exception of 10% added sugar to liquid whole egg products, for which 3 degrees of freedom were used because in this case there were 4 replicates instead of 3 replicates In addition, for some products, estimates were given for a nonlinear model and for a linear model. This was done when the nonlinear model was concave, and, as a result, would provide larger estimates of lethalities for large times, but smaller estimates for small times. Often in these cases, to be conservative for large times, linear models would be used; thus in the tables below, both estimates are given. The conclusions drawn from either set of estimates are the same.

For the plain LWE and egg yolk products that were not studied by the University of Nebraska,[4] no confidence bounds are given. All reported lethalities are for the fastest particle speed.

Ten percent added salt in liquid whole egg product
Convex survival curves were used for estimating lethalities.

TABLE G2-1 SUMMARY OF INFORMATION FOR 10% ADDED SALT IN LIQUID WHOLE EGG PRODUCT (PROPOSED LETHALITY = 6.0; WEIGTHED LETHALITY = 6.3).

Temp (°C)	No. Entries	Reported Time (m)	Expected Lethality Reported Time	Upper 99% Confidence Bound	Lower 99% Confidence Bound
*63.3	7	3.5	6.0	6.6	5.4
64.4	1	3.9	7.2	8.0	6.4
64.4	1	3.7	7.1	7.9	6.3
64.4	1	5.8	8.0	8.9	7.0
65.6	1	3.8	8.3	8.8	7.7
65.6	1	3.9	8.3	8.9	7.7
66.1	1	3.8	8.8	9.0	8.6
66.7	1	4.5	9.8	10.1	9.5
67.8	1	3.8	10.5	12.7	8.3

*Present minimal requirement

Ten percent added salt in liquid egg yolk product
Convex survival curves were used for estimating lethalities.

TABLE G2-2 SUMMARY OF INFORMATION FOR 10% ADDED SALT IN LIQUID EGG YOLK PRODUCT (PROPOSED LETHALITY = 6.2; WEIGHTED LETHALITY = 6.1).

Temp (°C)	No. Entries	Reported Time (m)	Expected Lethality Reported Time	Upper 99% Confidence Bound	Lower 99% Confidence Bound
*63.3	8	3.5	5.8	7.0	4.6
63.3	2	4.0	6.1	7.4	4.7
63.9	1	4.5	6.6	8.2	5.1
64.4	1	3.7	7.0	8.8	5.2
64.4	1	5.8	8.1	10.4	5.9
65.0	1	3.9	7.7	10.0	5.5
65.6	1	4.5	8.8	11.8	5.8
65.6	1	6.8	10.0	13.6	6.3
66.1	1	4.2	9.4	12.9	5.8
66.1	1	6.0	10.4	14.6	6.1
67.8	1	3.8	11.4	17.8	5.0

*Present minimal requirement

Ten percent added sugar in liquid egg yolk product
Convex survival curves were used.

TABLE G2-3 SUMMARY OF INFORMATION FOR 10% ADDED SUGAR IN LIQUID EGG YOLK PRODUCT (PROPOSED LETHALITY = 6.2; WEIGHTED LETHALITY = 9.8).

Temp (°C)	No. Entries	Reported Time (m)	Expected Lethality Reported Time	Upper 99% Confidence Bound	Lower 99% Confidence Bound
*63.3	6	3.5	9.4	10.3	8.5
63.3	2	4.0	9.9	10.8	9.0
63.3	1	5.8	11.4	12.3	10.4
63.9	1	3.9	10.8	11.6	10.0
64.4	1	4.5	11.9	12.9	11.0
64.4	1	3.5	11.4	12.4	10.5
64.4	1	3.7	11.6	12.6	10.6
64.4	1	3.8	11.7	12.7	10.7
65.6	1	4.5	14.2	16.5	11.9
66.1	1	4.1	13.8	16.1	11.6
66.1	1	4.4	14.9	18.1	11.7

*Present minimum requirements

<u>Ten percent added sugar in liquid whole egg product</u>
Concave survival curves were used for estimating lethalities.

TABLE G2-4 SUMMARY OF INFORMATION FOR 10% ADDED SUGAR IN LIQUID WHOLE EGG PRODUCT (PROPOSED LETHALITY = 6.0; WEIGHTED LETHALITY = 9.1).

Temp (°C	No. Entries	Reported Time (m)	Expected Lethality Reported Time	Upper 99% Confidence Bound	Lower 99% Confidence Bound
*61.1	1	3.5	8.6	12.6	4.7
61.1	1	4.0	10.1	14.8	5.5
61.1	1	3.7	105.1	117.3	93.0

*Present minimum requirement

<u>Egg mixture products</u>
Concave survival curves were used for estimating lethalities for all but product number 6.

TABLE G2-5 SUMMARY OF INFORMATION FOR UNL PRODUCT CODE = 1 (PROPOSED LETHALITY = 6.0; WEIGHTED LETHALITY = 6.1). NONLINEAR MODEL.

Temp (°C)	No. Entries	Reported Time (m)	Expected Lethality Reported Time	Upper 99% Confidence Bound	Lower 99% Confidence Bound
60.0	1	3.5	5.3	5.7	5.0
63.3	1	4.0	23.5	25.2	21.8
64.4	2	4.0	34.5	36.5	32.5
65.6	1	2.3	28.0	29.8	26.2
65.6	1	3.7	46.5	49.3	43.6

TABLE G2-6 SUMMARY OF INFORMATION FOR UNL PRODUCT CODE = 2 (PROPOSED LETHALITY = 6.0; WEIGHTED LETHALITY = 7.4). NONLINEAR MODEL.

Temp (°C)	No. Entries	Reported Time (m)	Expected Lethality Reported Time	Upper 99% Confidence Bound	Lower 99% Confidence Bound
60.0	2	3.5	6.7	7.9	5.5
61.1	3	3.5	13.3	15.7	10.9
62.2	1	3.7	27.7	32.5	22.9
62.8	1	3.5	36.6	42.8	30.4
63.3	1	3.5	51.2	59.6	42.8
65.0	1	2.5	98.5	113.7	83.2
65.6	1	3.8	210.8	243.4	178.2
68.9	6	4.9	1682.0	1981.0	1382.0

TABLE G2-7 SUMMARY OF INFORMATION FOR UNL PRODUCT CODE = 4 (PROPOSED LETHALITY = 6.0; WEIGHTED LETHALITY = 17.8). NONLINEAR MODEL.

Temp (°C)	No. Entries	Reported Time (m)	Expected Lethality Reported Time	Upper 99% Confidence Bound	Lower 99% Confidence Bound
62.2	2	3.5	17.3	19.4	15.1
62.2	1	4.0	20.0	22.6	17.4
64.4	1	4.0	51.5	65.9	37.0
64.4	1	3.6	52.7	68.4	36.9
65.0	1	3.7	70.6	94.5	46.7
66.1	1	3.8	122.5	173.4	71.6

TABLE G2-8 SUMMARY OF INFORMATION FOR UNL PRODUCT CODE = 6 (PROPOSED LETHALITY = 6.2; WEIGHTED LETHALITY = 6.4). NONLINEAR MODEL.

Temp (°C)	No. Entries	Reported Time (m)	Expected Lethality Reported Time	Upper 99% Confidence Bound	Lower 99% Confidence Bound
63.3	1	4.0	5.9	6.4	5.5
64.4	2	3.5	9.2	9.8	8.7

TABLE G2-9 SUMMARY OF INFORMATION FOR UNL PRODUCT CODE = 7 (PROPOSED LETHALITY = 5.7; WEIGHTED LETHALITY = 5.8). NONLINEAR MODEL.

Temp (°C)	No. Entries	Reported Time (m)	Expected Lethality Reported Time	Upper 99% Confidence Bound	Lower 99% Confidence Bound
56.7	1	4.0	5.1	5.3	5.0
58.9	1	3.5	14.3	14.6	14.0
58.9	2	8.0	35.1	35.1	33.0
60.0	1	4.0	30.2	30.2	28.2

Plain Egg white

There were 11 entries for this product. However, the pH values were not given for any of them; thus, it is not possible to compute the lethalities that would be obtained for this product since pH is an important variable affecting the lethality.

In the International Egg Pasteurization manual,[4] recommended minimum time and temperature for this product was established using the data with pH = 9.3. For comparison purposes, predicted lethalities assuming a pH = 8.8 are provided below.

TABLE G2-10 SUMMARY OF INFORMATION FOR PLAIN EGG WHITE FOR pH 8.8 (PROPOSED LETHALITY = 5.7; WEIGHTED LETHALITY = 1.7). NONLINEAR MODEL.

Temp (°C)	No. Entries	Reported Time (m)	Expected Lethality Reported Time	Upper 99% Confidence Bound	Lower 99% Confidence Bound
*56.7	6	3.5	1.3	1.7	0.9
56.7	2	4.0	1.6	2.0	1.1
57.2	1	3.5	2.0	2.6	1.5
57.2	1	4.5	2.8	3.5	2.0
57.8	1	3.7	3.3	4.2	2.4
57.8	1	4.2	3.8	4.9	2.8
58.9	1	3.7	7.3	9.9	4.7
59.4	1	3.6	10.4	14.5	6.2
59.4	1	4.1	11.9	16.7	7.2
59.4	1	7.8	23.6	32.7	14.6
60.0	1	3.5	14.6	21.1	8.1

*Present minimum requirement

TABLE G2-11 SUMMARY OF INFORMATION FOR PLAIN EGG WHITE FOR pH 9.3 (PROPOSED LETHALITY = 5.7; WEIGHTED LETHALITY = 4.4). NONLINEAR MODEL.

Temp (°C)	No. Entries	Reported Time (m)	Expected Lethality Reported Time	Upper 99% Confidence Bound	Lower 99% Confidence Bound
*56.7	6	3.5	4.0	4.4	3.7
56.7	2	4.0	4.6	5.0	4.2
57.2	1	4.5	7.0	7.9	6.2
57.2	1	3.5	5.5	6.1	4.8
57.8	1	3.7	7.9	9.1	6.6
57.8	1	4.2	8.9	10.3	7.5
58.9	1	3.7	14.5	17.9	11.2
59.4	1	3.6	19.2	24.4	14.1
59.4	1	4.1	21.9	27.7	16.1
59.4	1	7.8	41.4	52.5	30.4
60.0	1	3.5	25.4	33.1	17.7

*Present minimum requirement

Plain whole egg and yolk products

TABLE G2-12 SUMMARY OF INFORMATION FOR PLAIN WHOLE EGG (PROPOSED LETHALITY = 6.0; WEIGHTED LETHALITY = 9.2 (SHAH ET AL.[2]), 8.1 (MICHALSKI ET AL.[17]), AND 5.1 (NONLINEAR)).

Temp (°C)	No. Entries	Reported Time (m)	Shah et al.[2] Linear Reported Lethality	Michalski et al.[17] Linear Reported Lethality	Nonlinear Reported Lethality
*60.0	10	3.5	8.8	7.8	4.7
60.0	3	4.0	10.1	8.9	4.9
61.1	1	3.5	18.8	15.8	5.6
61.1	1	3.7	19.9	16.7	5.7
61.7	2	3.7	29.0	23.9	6.1
62.8	1	3.5	58.4	46.0	6.9
62.8	1	3.8	62.5	49.3	7.0
62.8	1	3.9	64.2	50.6	7.1
62.8	1	4.0	66.7	52.6	7.1
63.3	1	3.6	87.6	67.5	7.4
63.3	1	4.0	97.4	75.0	7.6
63.9	1	3.7	131.4	99.0	7.9
65.6	1	4.0	441.5	311.1	9.4
65.6	1	4.5	496.7	350.0	9.6
66.1	1	5.0	805.4	555.0	10.2
68.9	1	4.9	4341.4	2704.9	12.1

*Present minimum requirement

TABLE G2-13 SUMMARY OF INFORMATION FOR EGG YOLK PRODUCTS (PROPOSED LETHALITY = 6.2; WEIGHTED LETHALITY = 10.9 (MICHALSKI ET AL.[17]), 6.5 (PALUMBO ET AL.[18;19]), AND 4.7 (NONLINEAR)).

Temp (°C)	No. Entries	Reported Time (m)	Michalski et al.[17] Linear Reported Lethality	Palumbo et al.[18;19] Linear Reported Lethality	Nonlinear Reported Lethality
*61.1	8	3.5	10.5	6.2	4.4
61.1	2	4.0	12.0	7.1	4.6
62.2	1	3.5	19.0	10.5	5.0
62.2	1	3.7	20.1	11.1	5.1
62.8	1	3.7	27.0	14.4	5.4
62.8	1	3.9	28.5	15.2	5.4
63.3	1	3.5	34.3	17.8	5.6
63.3	1	3.6	35.3	18.3	5.6
63.9	1	3.9	50.7	25.5	6.0
64.4	1	8.5	149.5	72.9	7.6
65.6	1	4.5	143.7	65.8	7.1

*Present minimum requirement

Attachment G3: Computation of net lethality for average particle within laminar flow

The proposed regulation calls for specified lethalities to be obtained for the fastest moving particle. The relationship of the velocity of the fastest moving and the average velocity can be deduced for a pure laminar flow, based on a second order differential equation: $\Lambda P = 0 \Lambda^2 \mu$, where P is the pressure, μ is the velocity, and 0 is the dynamic viscosity, a function of temperature. For water, the value of 0 at about 60ºC is near 0.45. The pressure gradient, $-G$, is assumed constant through the pipe. Set $K = G/(40)$. Then, for simple laminar flow, the velocity of the particle flowing through a pipe of radius r, $\mu(s)$, where s is the distance from the center of the pipe is

$$u(s) = u_0 - \frac{G}{4\eta} s^2, \qquad 0 \leq s \leq r \qquad \text{(G3-1)}$$

where $\mu(0) = u_0$ is the largest velocity. The statement made in the Egg Pasteurization Manual[20] is that the average particle velocity is ½ the largest velocity, so that the unknown term, K, can be determined, given that r and μ_0 are known.

It is assumed that all particles get through the pipes and that the amount of product that is at a given velocity and thus at a given distance from the center is proportional to the circumference $2\pi s$. This assumption creates a paradox which can be only be explained by assuming an infinite flowing product and that the pasteurized product is made up of a cross section of the product as it leaves the pipes. Thus, the density of particles moving at velocity $\mu(s)$ at a distance s from the center is proportional to $2Bs$ at any given time. Using this term as a weight, the average particle velocity is

$$\bar{u} = 2r^{-2} \int_0^r s(u_0 - Ks^2)ds = u_0 - \frac{K}{2} r^2 \equiv \frac{u_0}{2} \qquad \text{(G3-2)}$$

Thus, $K = u_0/r^2$. With this substitution, Equation 1 is:

$$u(x) = u_0(1 - x^2), \qquad 0 \leq x = s/r \leq 1 \qquad \text{(G3-3)}$$

Consequently, the assumption that the average particle velocity is ½ the largest velocity is based on an idealized model that posits that at the edges of the pipes the particles are moving very slowly –actually asymptotically with velocity of zero.

One way to relax this assumption is by inserting a value, h, less than 1, as the coefficient of x^2 in Equation G3-3. In addition, the weight, rather than just being proportional to the cross-section circumference, might also include a term reflecting the premise that given, everything else being equal, the relative amount of product moving with a certain velocity would be propositional to that velocity. In other words, density of the product in the final product would be

proportional to the product of the circumference, $2\pi x$, and the velocity, $u_0(1-hx^2)$. This latter assumption was used to perform the calculations.

For computing the average lethality, Equation G3-3 shows that, without loss of generality, the radius r can be assumed equal to 1. For a particle with velocity $\mu(x)$, it will be exposed to the lethality treatment at a constant temperature for time $t(x)$ traveling some distance b, where $t(x) = b/u(x)$. If $t(0) = t_0$ is known (for example 3.5 minutes), then $t(x) = t_0/(1-hx^2)$. Thus the lethality applied by a particle is $L(t(x)) = L(t_0/(1-hx^2))$ and the probability of a *Salmonella* cell surviving is $p(x) = 10^{-L(x)}$. The net lethality, $\overline{L}$, therefore is the integral of $p(x)$, with weights proportional to $2Bx(1-hx^2)$, so that (canceling the term B)

$$\overline{L} = -\log_{10}[\frac{2}{2-h}\int_0^1 x(1-hx^2)10^{-L(t(x))}dx].$$ (G3-4)

This can be simplified by a change of variables: let $v = x^2$, then $L(t(x)) = L(t_0/(1-hv))$, and

$$\overline{L} = -\log_{10}[\frac{2}{2-h}\int_0^1 (1-hv)10^{-L(v)}dv]$$ (G3-5)

where, in an abuse of notation, $L(v) = L(t_0/(1-hv))$.

Linear lethality models

For the linear lethality model, $L(v) = t(x)/D$, where D, the D-value, is constant. Thus $L(v) = t_0/(D(1-hv))$. The quantity t_0/D is the lethality for the fastest moving particle, L_0, so that $L(v) = L_0/(1-hv)$. Thus, the average lethality is a function of the lethality at the fastest particle and the parameter h. For $h = 1$, this entails the assumption that at the edges of the pipe the velocity of the particles is zero. If we relax this assumption and assume that the velocity at the edges is 25% of the velocity of the fastest particle, $h = 0.75$. Table 1 provides some average lethalities given lethalities for the fastest particle for $h = 1$ and $h = 0.75$.

TABLE G3-1 AVERAGE LETHALITIES, GIVEN OBTAINED LETHALITIES FOR THE FASTEST PARTICLE, ASSUMING A LINEAR LETHALITY MODEL. VALUE OF $H = 1$ MEANS PARTICLE VELOCITY AT THE PIPES EDGES IS ZERO; $H = 0.75$ MEANS THAT THE VELOCITY AT THE EDGES IS 25% THAT OF THE LARGEST VELOCITY.

	Lethality (log$_{10}$) for fastest particle								
Value of h	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0
$h = 1$	3.7	4.8	5.9	6.9	8.0	9.0	10.0	11.1	12.1
factor	5.0	6.1	7.3	8.3	9.3	10.2	11.0	11.8	12.5
$h = 0.5$	3.6	4.7	5.7	6.8	7.9	8.9	9.9	11.0	12.0
factor	3.6	4.5	5.4	6.2	7.1	8.0	8.9	9.7	10.6

The results of Table G3-1 show that the difference of the obtained average lethalities and the lethality for the fastest particle increases with lethalities. For $h = 1$, for smaller lethalities shown on the table, the average lethality is less than about 1 $\log_{10}$ larger than that for the fastest particles; when the lethality for the fastest particles is greater than 8 $\log_{10}$, the probability of cells surviving, on the average, is more than 10 times less than the probability of a cell surviving if attached to the fastest particle. However, when the slowest particle velocity is 50% that of the fastest particle, then if the lethality for the fastest particle is greater than 10 $\log_{10}$, the probability of cells surviving, on the average, is more than 10 times less than the probability of a cell surviving if attached to the fastest particle.

Computing net lethalities for product-types used in risk assessment

The University Nebraska data[4] included survival curves for 5 formulated products besides the 10% added salt or sugar products and the plain egg white products at various pH values. A comment to the draft risk assessment presented in October 2004 suggested that the lethalities for plain egg white products are not "representative" of the product because much of the egg white product is formulated (e.g., imitation egg white) and that as a consequence actual lethalities would be higher than those used in the draft risk assessment. FSIS's plant survey confirmed this premise; many of the formulated products that were identified in the survey were matched with the 5 formulated products. Therefore, FSIS incorporated these products into its calculations for net lethalities.

The risk assessment identified 7 product types and computed risk based on assumed lethalities for each product type. The seven product types are: 10% added salt or sugar to liquid whole eggs and egg yolks (4 product types), and plain liquid whole eggs; egg yolks, and egg white. The 5 formulated products (besides the 10% added salt or sugar and plain egg white products) consisted of 3 whole egg formulations; 1 egg yolk formulation; and 1 egg white formulation. Lethalities were computed for each product, as described in the plant–survey report. Briefly, "averaging" lethalities means computing the "average" of the corresponding probabilities of survival, and then taking the negative of the logarithm of the average. Weights are attached to the probabilities when taking the average. Thus the formula for the product-specific net lethality is:

$$L_{prodcut} = -\log_{10}\left[\frac{\sum_{i=1}^{n} n_i 10^{-L_i}}{\sum_{i=1}^{n} n_i}\right] \qquad (G3-2)$$

where L_i is the computed lethality for the ith time – temperature entry for the product (and are functions of h), and n_i (the weight) is the number of times the ith time and temperature combination entry was reported in the survey, and n is the number of entries for the product.

To determine the lethality for the 7 seven product-types, it was assumed a specified weight, w, for the formulated products, representing the assumed proportion of volume, so that the weight for the plain products would be 1-w. For the formulated liquid whole egg product-type:

liquid whole eggs (excluding 10% added salt or sugar) products, the net lethality was computed using equal weights, taking the "average" of the three product-specific net lethalities. Thus, for the product types: whole egg, egg yolk, and egg white, there were two lethalities: one for the plain product (used initially in the draft risk assessment) and one for the formulated product (not used in the risk assessment. These lethalities for each product-type were averaged using the weights w and 1-w.

Thus net lethalities, given times and temperatures are a function of two parameters: (i) h, specifying the relationship between the velocities of the fastest and slowest products; and (ii) w, the proportion of formulated product within the liquid whole egg; egg yolk, and egg white product type category, excluding the 10% added salt and sugar whole and egg yolk products.

The following tables gives computed net lethalities for the seven product–types, based on the above calculation procedures, assuming $w = 1/2$ for Table G3-2, and $w = ¼$, for Table 3. The entries are the net lethalities for different assumption of the velocity of the slowest particle as a percentage of the velocity of the fastest particle.

Table G3-2 Net lethalities for different product types, with different assumptions for velocity of slowest particle, assuming product-type mix of 50% plain and 50% formulated ($w = ½$) for the liquid whole egg, egg yolk, and egg white.

Product Type	All Fastest Particle	Slowest = 50% Fastest Velocity	Slowest = 25% Fastest Velocity	Slowest = 0
10% salt added whole	6.33	6.68	6.77	6.80
10% salt added yolk	6.10	6.53	6.63	6.66
10% sugar added whole	9.10	10.10	10.20	10.23
10% sugar added yolk	9.80	10.42	10.52	10.54
Other whole	5.36	5.72	5.81	5.84
Other yolk	4.95	5.30	5.39	5.42
Other white	4.72	5.39	5.48	5.51

Table G3-3 Net lethalities for different product types, with different assumptions for velocity of slowest particle, assuming product-type mix of 75% plain and 25% formulated ($w = 1/4$) for the liquid whole egg, egg yolk, and egg white.

Product Type	All Fastest Particle	Slowest = 50% Fastest Velocity	Slowest = 25% Fastest Velocity	Slowest = 0
10% salt added whole	6.33	6.68	6.77	6.80
10% salt added yolk	6.10	6.53	6.63	6.66
10% sugar added whole	9.10	10.10	10.20	10.23
10% sugar added yolk	9.80	10.42	10.52	10.54
Other whole	5.19	5.54	5.63	5.66
Other yolk	4.78	5.13	5.22	5.24
Other white	4.56	5.22	5.32	5.34

Attachment G4: Data to derive *Salmonella* lethality curves in eggs and egg products

TABLE G4-1 EGG WHITE RESULTS. ENTRIES ARE MEANS OF THREE MEASUREMENTS.

Replicate Number	pH	Temp (°C)	Time (m)	Log_{10} Level
1	7.8	54.4	0	9.587
1	7.8	54.4	9	9.054
1	7.8	54.4	18	8.254
1	7.8	54.4	27	7.385
1	7.8	54.4	36	6.255
1	7.8	55.5	0	9.587
1	7.8	55.5	5	8.999
1	7.8	55.5	10	8.166
1	7.8	55.5	15	7.052
1	7.8	55.5	20	5.668
1	7.8	56.7	0	9.587
1	7.8	56.7	2	9.014
1	7.8	56.7	4	8.321
1	7.8	56.7	6	7.262
1	7.8	56.7	8	6.408
1	7.8	57.7	0	9.587
1	7.8	57.7	1	9.134
1	7.8	57.7	2	8.500
1	7.8	57.7	3	7.461
1	7.8	57.7	4	6.420
1	8.2	54.4	0	9.605
1	8.2	54.4	8	8.881
1	8.2	54.4	16	8.192
1	8.2	54.4	24	7.598
1	8.2	54.4	32	6.295
1	8.2	55.5	0	9.605
1	8.2	55.5	5	8.901
1	8.2	55.5	10	8.184
1	8.2	55.5	15	7.304
1	8.2	55.5	20	6.025
1	8.2	56.7	0	9.605
1	8.2	56.7	2	9.066
1	8.2	56.7	4	8.472
1	8.2	56.7	6	7.572
1	8.2	56.7	8	6.342
1	8.2	57.7	0	9.605
1	8.2	57.7	1	9.052
1	8.2	57.7	2	8.431
1	8.2	57.7	3	7.684
1	8.2	57.7	4	6.306
1	8.8	54.4	0	9.527
1	8.8	54.4	5	8.804
1	8.8	54.4	10	7.996
1	8.8	54.4	15	7.221
1	8.8	54.4	20	6.374
1	8.8	55.5	0	9.527
1	8.8	55.5	4	8.556
1	8.8	55.5	8	7.594

TABLE G4-2 EGG WHITE RESULTS. ENTRIES ARE MEANS OF THREE MEASUREMENTS.

Replicate Number	pH	Temp (°C)	Time (m)	Log_{10} Level
1	8.8	55.5	12.0	6.729
1	8.8	55.5	16.0	5.500
1	8.8	56.7	0.0	9.527
1	8.8	56.7	3.0	8.347
1	8.8	56.7	6.0	6.587
1	8.8	56.7	9.0	5.253
1	8.8	56.7	12.0	3.026
1	8.8	57.7	0.0	9.527
1	8.8	57.7	1.5	8.518
1	8.8	57.7	3.0	6.857
1	8.8	57.7	4.5	5.564
1	8.8	57.7	6.0	3.440
1	9.3	54.4	0.0	9.247
1	9.3	54.4	4.0	8.194
1	9.3	54.4	8.0	6.535
1	9.3	54.4	12.0	5.171
1	9.3	54.4	16.0	4.113
1	9.3	55.5	0.0	9.247
1	9.3	55.5	3.0	8.040
1	9.3	55.5	6.0	6.121
1	9.3	55.5	9.0	3.834
1	9.3	55.5	12.0	2.360
1	9.3	56.7	0.0	9.247
1	9.3	56.7	2.0	7.496
1	9.3	56.7	4.0	4.307
1	9.3	56.7	6.0	2.201
1	9.3	57.7	0.0	9.247
1	9.3	57.7	1.0	8.185
1	9.3	57.7	2.0	5.924
1	9.3	57.7	3.0	3.586
2	7.8	54.4	0.0	9.584
2	7.8	54.4	10.0	8.799
2	7.8	54.4	20.0	8.313
2	7.8	54.4	30.0	7.639
2	7.8	54.4	40.0	6.732
2	7.8	55.5	0.0	9.584
2	7.8	55.5	6.0	8.567
2	7.8	55.5	12.0	7.659
2	7.8	55.5	18.0	6.576
2	7.8	55.5	24.0	5.563
2	7.8	56.7	0.0	9.584
2	7.8	56.7	3.0	8.538
2	7.8	56.7	6.0	7.374
2	7.8	56.7	9.0	5.745
2	7.8	56.7	12.0	4.839
2	7.8	57.7	0.0	9.584
2	7.8	57.7	2.0	8.481
2	7.8	57.7	4.0	6.669

TABLE G4-3 EGG WHITE RESULTS. ENTRIES ARE MEANS OF THREE MEASUREMENTS

Replicate Number	pH	Temp (°C)	Time (m)	Log_{10} Level
2	7.8	57.7	6.0	4.721
2	7.8	57.7	8.0	2.418
2	8.2	54.4	0.0	9.467
2	8.2	54.4	9.0	8.787
2	8.2	54.4	18.0	7.905
2	8.2	54.4	27.0	6.740
2	8.2	54.4	36.0	5.636
2	8.2	55.5	0.0	9.467
2	8.2	55.5	6.0	8.556
2	8.2	55.5	12.0	7.308
2	8.2	55.5	18.0	6.064
2	8.2	55.5	24.0	4.333
2	8.2	56.7	0.0	9.467
2	8.2	56.7	3.0	8.486
2	8.2	56.7	6.0	6.968
2	8.2	56.7	9.0	5.727
2	8.2	56.7	12.0	3.543
2	8.2	57.7	0.0	9.467
2	8.2	57.7	1.5	8.666
2	8.2	57.7	3.0	7.373
2	8.2	57.7	4.5	5.800
2	8.2	57.7	6.0	4.334
2	8.8	54.4	0.0	9.315
2	8.8	54.4	6.0	8.885
2	8.8	54.4	12.0	7.949
2	8.8	54.4	18.0	7.100
2	8.8	54.4	24.0	6.185
2	8.8	55.5	0.0	9.315
2	8.8	55.5	5.0	8.615
2	8.8	55.5	10.0	7.414
2	8.8	55.5	15.0	6.212
2	8.8	55.5	20.0	4.526
2	8.8	56.7	0.0	9.315
2	8.8	56.7	3.0	8.564
2	8.8	56.7	6.0	7.152
2	8.8	56.7	9.0	5.606
2	8.8	56.7	12.0	3.384
2	8.8	57.7	0.0	9.315
2	8.8	57.7	1.5	8.534
2	8.8	57.7	3.0	7.517
2	8.8	57.7	4.5	5.834
2	8.8	57.7	6.0	4.117
2	9.3	54.4	0.0	9.222
2	9.3	54.4	4.0	7.727
2	9.3	54.4	8.0	6.633
2	9.3	54.4	12.0	5.402
2	9.3	54.4	16.0	3.698
2	9.3	55.5	0.0	9.222

TABLE G4-4 EGG WHITE RESULTS. ENTRIES ARE MEANS OF THREE MEASUREMENTS

Replicate Number	pH	Temp ($^{\circ}$C)	Time (m)	Log_{10} Level
2	9.3	55.5	3	7.499
2	9.3	55.5	6	5.601
2	9.3	55.5	9	3.732
2	9.3	55.5	12	2.301
2	9.3	56.7	0	9.222
2	9.3	56.7	2	6.851
2	9.3	56.7	4	4.396
2	9.3	56.7	6	2.301
2	9.3	57.7	0	9.222
2	9.3	57.7	1	8.011
2	9.3	57.7	2	5.668
2	9.3	57.7	3	2.667
3	7.8	54.4	0	9.472
3	7.8	54.4	10	8.698
3	7.8	54.4	20	7.799
3	7.8	54.4	30	6.899
3	7.8	54.4	40	6.320
3	7.8	55.5	0	9.472
3	7.8	55.5	6	8.671
3	7.8	55.5	12	7.713
3	7.8	55.5	18	6.745
3	7.8	55.5	24	5.712
3	7.8	56.7	0	9.472
3	7.8	56.7	3	8.715
3	7.8	56.7	6	7.548
3	7.8	56.7	9	6.442
3	7.8	56.7	12	4.371
3	7.8	57.7	0	9.472
3	7.8	57.7	2	8.576
3	7.8	57.7	4	6.753
3	7.8	57.7	6	5.531
3	7.8	57.7	8	3.701
3	8.2	54.4	0	9.462
3	8.2	54.4	9	8.587
3	8.2	54.4	18	7.917
3	8.2	54.4	27	6.590
3	8.2	54.4	36	5.780
3	8.2	55.5	0	9.462
3	8.2	55.5	6	8.408
3	8.2	55.5	12	7.238
3	8.2	55.5	18	5.835
3	8.2	55.5	24	3.970
3	8.2	56.7	0	9.462
3	8.2	56.7	3	8.548
3	8.2	56.7	6	7.407
3	8.2	56.7	9	5.710
3	8.2	56.7	12	4.175
3	8.2	57.7	0	9.462

TABLE G4-5 EGG WHITE RESULTS. ENTRIES ARE MEANS OF THREE MEASUREMENTS

Replicate Number	pH	Temp (°C)	Time (m)	Log_{10} Level
3	8.2	57.7	2.0	8.183
3	8.2	57.7	4.0	5.849
3	8.2	57.7	6.0	3.668
3	8.2	57.7	8.0	2.151
3	8.8	54.4	0.0	9.113
3	8.8	54.4	6.0	8.354
3	8.8	54.4	12.0	6.990
3	8.8	54.4	18.0	6.087
3	8.8	54.4	24.0	5.683
3	8.8	55.5	0.0	9.113
3	8.8	55.5	5.0	7.859
3	8.8	55.5	10.0	6.564
3	8.8	55.5	15.0	5.334
3	8.8	55.5	20.0	3.962
3	8.8	56.7	0.0	9.113
3	8.8	56.7	3.0	7.837
3	8.8	56.7	6.0	6.192
3	8.8	56.7	9.0	5.143
3	8.8	56.7	12.0	3.384
3	8.8	57.7	0.0	9.113
3	8.8	57.7	1.5	8.153
3	8.8	57.7	3.0	6.315
3	8.8	57.7	4.5	4.753
3	8.8	57.7	6.0	3.860
3	9.3	54.4	0.0	9.398
3	9.3	54.4	4.0	8.451
3	9.3	54.4	8.0	6.773
3	9.3	54.4	12.0	5.342
3	9.3	54.4	16.0	4.314
3	9.3	55.5	0.0	9.398
3	9.3	55.5	3.0	8.134
3	9.3	55.5	6.0	6.253
3	9.3	55.5	9.0	4.314
3	9.3	55.5	12.0	2.560
3	9.3	56.7	0.0	9.398
3	9.3	56.7	2.0	7.526
3	9.3	56.7	4.0	4.623
3	9.3	56.7	6.0	2.519
3	9.3	57.7	0.0	9.398
3	9.3	57.7	1.0	7.905
3	9.3	57.7	2.0	5.770
3	9.3	57.7	3.0	3.472

TABLE G4-6 OBSERVED *SALMONELLA* LEVELS FOR 10% ADDED SALT PRODUCT.

Product Type	Temp (°C)	Replicate Number	Time (m)	Log_{10} Observed Level
whole	61.1	1	0.0000	9.19478
whole	61.1	1	1.5000	5.94054
whole	61.1	1	3.0000	4.98823
whole	61.1	1	4.5000	4.65692
whole	61.1	1	6.0000	4.21709
whole	61.1	2	0.0000	9.35357
whole	61.1	2	2.0000	5.49944
whole	61.1	2	4.0000	4.72484
whole	61.1	2	6.0000	4.14053
whole	61.1	2	8.0000	3.93067
whole	61.1	3	0.0000	9.26310
whole	61.1	3	2.0000	5.39020
whole	61.1	3	4.0000	4.75530
whole	61.1	3	6.0000	4.20184
whole	61.1	3	8.0000	3.87256
whole	63.3	1	0.0000	9.19478
whole	63.3	1	1.0000	5.55749
whole	63.3	1	2.0000	4.69213
whole	63.3	1	3.0000	3.92587
whole	63.3	1	4.0000	3.53914
whole	63.3	2	0.0000	9.35357
whole	63.3	2	1.0000	5.35680
whole	63.3	2	2.0000	4.44582
whole	63.3	2	3.0000	3.80211
whole	63.3	2	4.0000	3.18337
whole	63.3	3	0.0000	9.26310
whole	63.3	3	1.0000	5.15865
whole	63.3	3	2.0000	4.22900
whole	63.3	3	3.0000	3.68923
whole	63.3	3	4.0000	2.99855
whole	65.5	1	0.0000	9.19478
whole	65.5	1	0.2500	6.14379
whole	65.5	1	0.5000	4.92371
whole	65.5	1	0.7500	4.25347
whole	65.5	1	1.0000	3.15433
whole	65.5	2	0.0000	9.35357
whole	65.5	2	0.2500	6.49567
whole	65.5	2	0.5000	5.02313
whole	65.5	2	0.7500	4.70044
whole	65.5	2	1.0000	3.93427
whole	65.5	3	0.0000	9.26310
whole	65.5	3	0.2500	6.32742
whole	65.5	3	0.5000	5.15192
whole	65.5	3	0.7500	4.34188
whole	65.5	3	1.0000	3.62553
whole	67.8	1	0.0000	9.19478
whole	67.8	1	0.0833	6.54991
whole	67.8	1	0.1667	5.46755

TABLE G4-7 OBSERVED *SALMONELLA* LEVELS FOR 10% ADDED SALT PRODUCT.

Product Type	Temp (°C)	Replicate Number	Time (m)	Log$_{10}$ Observed Level
whole	67.8	1	0.2500	4.81727
whole	67.8	1	0.3333	4.16472
whole	67.8	2	0.0000	9.35357
whole	67.8	2	0.1167	5.79509
whole	67.8	2	0.2333	4.57153
whole	67.8	2	0.3500	3.56328
whole	67.8	3	0.0000	9.26310
whole	67.8	3	0.1167	6.50352
whole	67.8	3	0.2333	5.14314
whole	67.8	3	0.3500	4.25388
whole	67.8	3	0.4667	3.87280
yolk	63.3	1	0.0000	9.21290
yolk	63.3	1	1.0000	5.85873
yolk	63.3	1	2.0000	4.60844
yolk	63.3	1	3.0000	3.76378
yolk	63.3	1	4.0000	3.25398
yolk	63.3	2	0.0000	9.41476
yolk	63.3	2	1.0000	6.57968
yolk	63.3	2	2.0000	5.04019
yolk	63.3	2	3.0000	4.23822
yolk	63.3	2	4.0000	3.29360
yolk	63.3	3	0.0000	9.45224
yolk	63.3	3	1.0000	6.36145
yolk	63.3	3	2.0000	4.74976
yolk	63.3	3	3.0000	3.42728
yolk	63.3	3	4.0000	3.19702
yolk	65.5	1	0.0000	9.21290
yolk	65.5	1	0.5000	6.18479
yolk	65.5	1	1.0000	4.81314
yolk	65.5	1	1.5000	3.79472
yolk	65.5	1	2.0000	3.39992
yolk	65.5	2	0.0000	9.41476
yolk	65.5	2	0.5000	6.06659
yolk	65.5	2	1.0000	4.33856
yolk	65.5	2	1.5000	3.88450
yolk	65.5	2	2.0000	3.42466
yolk	65.5	3	0.0000	9.45224
yolk	65.5	3	0.5000	5.84918
yolk	65.5	3	1.0000	4.32597
yolk	65.5	3	1.5000	3.58568
yolk	65.5	3	2.0000	2.85916
yolk	67.8	1	0.0000	9.21290
yolk	67.8	1	0.1667	6.75529
yolk	67.8	1	0.3333	5.36534
yolk	67.8	1	0.5000	4.24573
yolk	67.8	1	0.6667	3.66814
yolk	67.8	2	0.0000	9.41476
yolk	67.8	2	0.1667	6.77714

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TABLE G4-8 OBSERVED *SALMONELLA* LEVELS FOR 10% ADDED SALT PRODUCT.

Product Type	Temp (°C)	Replicate Number	Time (m)	Log_{10} Observed Level
yolk	67.8	2	0.3333	4.98500
yolk	67.8	2	0.5000	4.27962
yolk	67.8	2	0.6667	3.69174
yolk	67.8	3	0.0000	9.45224
yolk	67.8	3	0.1667	6.83425
yolk	67.8	3	0.3333	5.05178
yolk	67.8	3	0.5000	3.82545
yolk	67.8	3	0.6667	2.69306
yolk	70.0	1	0.0000	9.21290
yolk	70.0	1	0.0833	6.44340
yolk	70.0	1	0.1667	4.70898
yolk	70.0	1	0.2500	3.18495
yolk	70.0	1	0.3333	2.81572
yolk	70.0	2	0.0000	9.41476
yolk	70.0	2	0.0833	6.84075
yolk	70.0	2	0.1667	4.85070
yolk	70.0	2	0.2500	4.32706
yolk	70.0	2	0.3333	3.68281
yolk	70.0	3	0.0000	9.45224
yolk	70.0	3	0.0833	6.86108
yolk	70.0	3	0.1667	5.26543
yolk	70.0	3	0.2500	3.53975
yolk	70.0	3	0.3333	2.84211

TABLE G4-9 OBSERVED *SALMONELLA* LEVELS FOR 10% ADDED SUGAR PRODUCT.

Product Type	Temp (°C)	Replicate Number	Time (m)	Log$_{10}$ Observed Level
whole	60.0	1	0.0000	9.58711
whole	60.0	1	1.5000	8.14119
whole	60.0	1	3.0000	6.11048
whole	60.0	1	4.5000	4.30601
whole	62.2	1	0.0000	9.58711
whole	62.2	1	0.5000	7.93767
whole	62.2	1	1.0000	5.69879
whole	62.2	1	1.5000	3.14905
whole	64.4	1	0.0000	9.58711
whole	64.4	1	0.1667	8.21290
whole	64.4	1	0.3333	6.05399
whole	64.4	1	0.5000	2.96950
whole	66.7	1	0.0000	9.58711
whole	66.7	1	0.0833	8.03727
whole	66.7	1	0.1667	6.16610
whole	66.7	1	0.2500	2.46007
whole	60.0	2	0.0000	9.57592
whole	60.0	2	1.5000	8.25938
whole	60.0	2	3.0000	6.50878
whole	60.0	2	4.5000	4.70856
whole	60.0	2	6.0000	2.56632
whole	62.2	2	0.0000	9.57592
whole	62.2	2	0.5000	8.25022
whole	62.2	2	1.0000	6.12794
whole	62.2	2	1.5000	4.23642
whole	64.4	2	0.0000	9.57592
whole	64.4	2	0.1667	8.55043
whole	64.4	2	0.3333	6.26596
whole	64.4	2	0.5000	4.04986
whole	66.7	2	0.0000	9.57592
whole	66.7	2	0.0833	8.40317
whole	66.7	2	0.1667	6.41794
whole	66.7	2	0.2500	2.83505
whole	60.0	3	0.0000	9.62317
whole	60.0	3	1.5000	8.17125
whole	60.0	3	3.0000	6.10236
whole	60.0	3	4.5000	4.29602
whole	62.2	3	0.0000	9.62317
whole	62.2	3	0.5000	7.92570
whole	62.2	3	1.0000	5.60661
whole	62.2	3	1.5000	2.98475
whole	64.4	3	0.0000	9.62317
whole	64.4	3	0.1667	8.35973
whole	64.4	3	0.3333	6.09889
whole	64.4	3	0.5000	3.04019
whole	66.7	3	0.0000	9.62317
whole	66.7	3	0.0833	8.02201
whole	66.7	3	0.1667	6.32591
whole	66.7	3	0.2500	2.80047

TABLE G4-10 OBSERVED *SALMONELLA* LEVELS FOR 10% ADDED SUGAR PRODUCT

Product Type	Temp (°C)	Replicate Number	Time (m)	Log$_{10}$ Observed Level
yolk	61.1	1	0.0000	9.52703
yolk	61.1	1	2.0000	7.58314
yolk	61.1	1	4.0000	3.20069
yolk	61.1	1	6.0000	2.33333
yolk	63.3	1	0.0000	9.52703
yolk	63.3	1	0.7500	6.16546
yolk	63.3	1	1.5000	4.43654
yolk	63.3	1	2.2500	2.00000
yolk	65.5	1	0.0000	9.52703
yolk	65.5	1	0.2500	6.38230
yolk	65.5	1	0.5000	4.08509
yolk	65.5	1	0.7500	2.51877
yolk	67.8	1	0.0000	9.52703
yolk	67.8	1	0.1167	7.46007
yolk	67.8	1	0.2333	3.19134
yolk	67.8	1	0.3500	2.10034
yolk	61.1	2	0.0000	9.55199
yolk	61.1	2	1.5000	8.18543
yolk	61.1	2	3.0000	5.64710
yolk	61.1	2	4.5000	3.60457
yolk	61.1	2	6.0000	2.25938
yolk	63.3	2	0.0000	9.55199
yolk	63.3	2	0.5000	8.10049
yolk	63.3	2	1.0000	4.95223
yolk	63.3	2	1.5000	3.27995
yolk	63.3	2	2.0000	2.25938
yolk	65.5	2	0.0000	9.55199
yolk	65.5	2	0.2500	6.05870
yolk	65.5	2	0.5000	3.71437
yolk	65.5	2	0.7500	2.98475
yolk	67.8	2	0.0000	9.55199
yolk	67.8	2	0.1167	7.67105
yolk	67.8	2	0.2333	5.51353
yolk	67.8	2	0.3500	3.76065
yolk	67.8	2	0.4667	2.30103
yolk	61.1	3	0.0000	9.48893
yolk	61.1	3	1.5000	8.15313
yolk	61.1	3	3.0000	4.59423
yolk	61.1	3	4.5000	3.62633
yolk	61.1	3	6.0000	2.25938
yolk	63.3	3	0.0000	9.48893
yolk	63.3	3	0.5000	8.07069
yolk	63.3	3	1.0000	5.39478
yolk	63.3	3	1.5000	2.15904
yolk	65.5	3	0.0000	9.48893
yolk	65.5	3	0.2500	6.33449
yolk	65.5	3	0.5000	3.68544

TABLE G4-11 OBSERVED RESULTS FROM MIXED OR FORMULATED EGG PRODUCTS.

Product Type	Temp (°C)	Replicate Number	Time (m)	Log$_{10}$ Observed Level
1	1	60.0	0.00	9.15
1	2	60.0	0.00	9.28
1	3	60.0	0.00	9.28
1	1	60.0	1.50	8.30
1	2	60.0	1.50	8.46
1	3	60.0	1.50	8.26
1	1	60.0	3.00	5.92
1	2	60.0	3.00	6.08
1	3	60.0	3.00	5.82
1	1	60.0	4.50	3.41
1	2	60.0	4.50	3.30
1	3	60.0	4.50	3.52
1	1	62.2	0.00	9.15
1	2	62.2	0.00	9.28
1	3	62.2	0.00	9.28
1	1	62.2	0.50	8.26
1	2	62.2	0.50	8.23
1	3	62.2	0.50	8.15
1	1	62.2	1.00	5.71
1	2	62.2	1.00	5.70
1	3	62.2	1.00	5.68
1	1	62.2	1.50	3.23
1	2	62.2	1.50	3.04
1	3	62.2	1.50	3.00
1	1	64.4	0.00	9.15
1	2	64.4	0.00	9.28
1	3	64.4	0.00	9.28
1	1	64.4	0.25	7.67
1	2	64.4	0.25	7.78
1	3	64.4	0.25	7.76
1	1	64.4	0.50	3.45
1	2	64.4	0.50	3.38
1	3	64.4	0.50	3.15
1	1	64.4	0.75	2.23
1	2	64.4	0.75	2.11
1	3	64.4	0.75	2.11
1	1	66.7	0.00	9.15
1	2	66.7	0.00	9.28
1	3	66.7	0.00	9.28
1	1	66.7	0.17	7.20
1	2	66.7	0.17	7.11
1	3	66.7	0.17	7.23
1	1	66.7	0.33	5.11
1	2	66.7	0.33	5.00
1	3	66.7	0.33	5.00

TABLE G4-11 (CONTINUED)

Product Type	Temp (°C)	Replicate Number	Time (m)	Log$_{10}$ Observed Level
1	1	66.7	0.50	2.30
1	2	66.7	0.50	2.23
1	3	66.7	0.50	2.23
2	2	57.8	0.00	9.34
2	3	57.8	0.00	9.15
2	4	57.8	0.00	9.26
2	2	57.8	2.50	8.53
2	3	57.8	2.50	8.46
2	4	57.8	2.50	8.28
2	2	57.8	5.00	6.80
2	3	57.8	5.00	6.63
2	4	57.8	5.00	6.88
2	2	57.8	7.50	5.76
2	3	57.8	7.50	5.80
2	4	57.8	7.50	5.70
2	2	57.8	10.00	4.49
2	3	57.8	10.00	4.51
2	4	57.8	10.00	4.54
2	2	60.0	0.00	9.34
2	3	60.0	0.00	9.15
2	4	60.0	0.00	9.26
2	2	60.0	1.00	7.82
2	3	60.0	1.00	7.70
2	4	60.0	1.00	7.85
2	2	60.0	2.00	4.99
2	3	60.0	2.00	5.54
2	4	60.0	2.00	5.76
2	2	60.0	3.00	2.43
2	3	60.0	3.00	2.80
2	4	60.0	3.00	3.58
2	2	62.2	0.00	9.34
2	3	62.2	0.00	9.15
2	4	62.2	0.00	9.26
2	2	62.2	0.25	8.77
2	3	62.2	0.25	8.51
2	4	62.2	0.25	8.68
2	2	62.2	0.50	6.85
2	3	62.2	0.50	6.52
2	4	62.2	0.50	6.54
2	2	62.2	0.75	4.41
2	3	62.2	0.75	4.43
2	4	62.2	0.75	4.66
2	2	62.2	1.00	2.00
2	3	62.2	1.00	2.52
2	4	62.2	1.00	2.00

TABLE G4-11 (CONTINUED).

Product Type	Temp (°C)	Replicate Number	Time (m)	Log$_{10}$ Observed Level
2	2	64.4	0.00	9.34
2	3	64.4	0.00	9.15
2	4	64.4	0.00	9.26
2	2	64.4	0.12	8.45
2	3	64.4	0.12	8.36
2	4	64.4	0.12	8.32
2	2	64.4	0.23	5.54
2	3	64.4	0.23	5.63
2	4	64.4	0.23	5.66
2	2	64.4	0.35	2.36
2	3	64.4	0.35	2.48
2	4	64.4	0.35	2.48
4	1	60.0	0.00	8.99
4	2	60.0	0.00	9.20
4	3	60.0	0.00	9.20
4	1	60.0	1.50	7.92
4	2	60.0	1.50	8.11
4	3	60.0	1.50	8.04
4	1	60.0	3.00	5.54
4	2	60.0	3.00	5.28
4	3	60.0	3.00	5.73
4	1	60.0	4.50	2.86
4	2	60.0	4.50	2.63
4	3	60.0	4.50	2.95
4	1	62.2	0.00	8.99
4	2	62.2	0.00	9.20
4	3	62.2	0.00	9.20
4	1	62.2	0.50	8.11
4	2	62.2	0.50	7.88
4	3	62.2	0.50	8.04
4	1	62.2	1.00	5.08
4	2	62.2	1.00	4.63
4	3	62.2	1.00	5.15
4	1	62.2	1.50	2.11
4	2	62.2	1.50	2.48
4	3	62.2	1.50	2.23
4	1	64.4	0.00	8.99
4	2	64.4	0.00	9.20
4	3	64.4	0.00	9.20
4	1	64.4	0.17	8.43
4	2	64.4	0.17	8.59
4	3	64.4	0.17	8.56
4	2	64.4	0.33	5.54
4	3	64.4	0.33	5.63
4	1	64.4	0.33	5.92

TABLE G4-11 (CONTINUED).

Product Type	Temp (°C)	Replicate Number	Time (m)	Log$_{10}$ Observed Level
4	1	64.4	0.50	2.00
4	2	64.4	0.50	3.23
4	3	64.4	0.50	2.60
4	1	66.7	0.00	8.99
4	2	66.7	0.00	9.20
4	3	66.7	0.00	9.20
4	1	66.7	0.08	8.23
4	2	66.7	0.08	8.72
4	3	66.7	0.08	8.45
4	1	66.7	0.17	4.58
4	2	66.7	0.17	5.85
4	3	66.7	0.17	4.20
4	1	66.7	0.25	2.11
4	2	66.7	0.25	3.04
4	3	66.7	0.25	2.23
6	2	61.1	0.00	9.20
6	3	61.1	0.00	9.23
6	4	61.1	0.00	9.26
6	2	61.1	2.50	7.81
6	3	61.1	2.50	8.00
6	4	61.1	2.50	7.63
6	2	61.1	5.00	6.48
6	3	61.1	5.00	6.28
6	4	61.1	5.00	6.90
6	2	61.1	7.50	5.30
6	3	61.1	7.50	5.41
6	4	61.1	7.50	5.78
6	2	61.1	10.00	4.70
6	3	61.1	10.00	4.72
6	4	61.1	10.00	4.77
6	2	63.3	0.00	9.20
6	3	63.3	0.00	9.23
6	4	63.3	0.00	9.26
6	2	63.3	1.00	7.15
6	3	63.3	1.00	7.95
6	4	63.3	1.00	7.04
6	2	63.3	2.00	5.52
6	3	63.3	2.00	5.90
6	4	63.3	2.00	5.83
6	2	63.3	3.00	4.61
6	3	63.3	3.00	4.61
6	4	63.3	3.00	4.63
6	2	63.3	4.00	3.43
6	3	63.3	4.00	3.48
6	4	63.3	4.00	3.49

TABLE G4-11 (CONTINUED).

Product Type	Temp (°C)	Replicate Number	Time (m)	Log$_{10}$ Observed Level
6	2	65.6	0.00	9.20
6	3	65.6	0.00	9.23
6	4	65.6	0.00	9.26
6	2	65.6	0.25	7.90
6	3	65.6	0.25	8.18
6	4	65.6	0.25	7.87
6	2	65.6	0.50	6.53
6	3	65.6	0.50	6.67
6	4	65.6	0.50	6.28
6	2	65.6	0.75	5.11
6	3	65.6	0.75	5.46
6	4	65.6	0.75	5.00
6	2	65.6	1.00	3.81
6	3	65.6	1.00	3.98
6	4	65.6	1.00	3.77
6	2	67.8	0.00	9.20
6	3	67.8	0.00	9.23
6	4	67.8	0.00	9.26
6	2	67.8	0.10	8.04
6	3	67.8	0.10	8.18
6	4	67.8	0.10	7.87
6	2	67.8	0.20	6.43
6	3	67.8	0.20	6.64
6	4	67.8	0.20	6.38
6	2	67.8	0.30	5.63
6	3	67.8	0.30	5.28
6	4	67.8	0.30	5.04
6	2	67.8	0.40	3.61
6	3	67.8	0.40	3.51
6	4	67.8	0.40	3.61
7	2	54.4	0.00	9.08
7	3	54.4	0.00	9.15
7	4	54.4	0.00	9.18
7	2	54.4	3.00	7.91
7	3	54.4	3.00	7.91
7	4	54.4	3.00	7.92
7	2	54.4	6.00	6.57
7	3	54.4	6.00	6.58
7	4	54.4	6.00	6.59
7	2	54.4	9.00	5.18
7	3	54.4	9.00	5.18
7	4	54.4	9.00	5.20
7	2	54.4	12.00	3.40
7	3	54.4	12.00	3.36
7	4	54.4	12.00	3.43

TABLE G4-11 (CONTINUED).

Product Type	Temp (°C)	Replicate Number	Time (m)	Log_{10} Observed Level
7	2	56.7	0.00	9.08
7	3	56.7	0.00	9.15
7	4	56.7	0.00	9.18
7	2	56.7	1.50	7.90
7	3	56.7	1.50	7.92
7	4	56.7	1.50	7.93
7	2	56.7	3.00	6.15
7	3	56.7	3.00	6.11
7	4	56.7	3.00	6.15
7	2	56.7	4.50	4.20
7	3	56.7	4.50	4.18
7	4	56.7	4.50	4.11
7	2	56.7	6.00	2.48
7	3	56.7	6.00	2.60
7	4	56.7	6.00	2.72
7	2	58.9	0.00	9.08
7	3	58.9	0.00	9.15
7	4	58.9	0.00	9.18
7	2	58.9	0.50	8.11
7	3	58.9	0.50	8.15
7	4	58.9	0.50	8.15
7	2	58.9	1.00	5.18
7	3	58.9	1.00	5.30
7	4	58.9	1.00	5.20
7	2	58.9	1.50	2.72
7	3	58.9	1.50	2.72
7	4	58.9	1.50	2.70
7	2	61.1	0.00	9.08
7	3	61.1	0.00	9.15
7	4	61.1	0.00	9.18
7	2	61.1	0.17	8.45
7	3	61.1	0.17	8.41
7	4	61.1	0.17	8.51
7	2	61.1	0.33	6.59
7	3	61.1	0.33	6.62
7	4	61.1	0.33	6.66
7	2	61.1	0.50	4.89
7	3	61.1	0.50	4.90
7	4	61.1	0.50	4.92
7	2	61.1	0.67	2.00
7	3	61.1	0.67	2.00
7	4	61.1	0.67	2.30

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