ANNEX G

Pasteurization of Liquid Egg Products and Shell Eggs

A common method used to eliminate or reduce the number of viable bacterial cells is pasteurization, a process in which liquid egg products are heated at below boiling to kill vegetative cells. Consequently, 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 survive 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. 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 actual data analysis used for selecting the distribution functions for these lethality models.

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 products. 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 $p(t)$.

ANALYSIS

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, then $p(t)$ 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 then 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 (e.g., temperature) within the range of those studied. It is assumed that 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, $h(t) = p'(t) / p(t)$, 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 $\ln(p(t)) = -\int h(t)\, dt$. 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 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

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

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 previous to t. For liquid egg products, since the product is heated relatively fast, it is not necessary to rely on Equation G2; 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. But for pasteurization of shell eggs, because the temperature of the eggs changes slowly there may be a significant amount of inactivation before the temperatures reach the maximum (equilibrium) temperature. If Equation G2 were valid, then the survival curve would be:

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

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 G3 becomes,

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

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 $\log_{10}$).

An issue addressed in these risk assessments is strain variability of survival kinetic parameters. Comparisons of D-values have been given 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).[2]

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 = 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 samples 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 study,[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.

Definition of Lethality
The term 'lethality (at time *t*)' is defined to be '$-\log_{10}(p(t))$' (or, negative value of $\log_{10}(p(t))$), following normal usage of the word.

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.[4-10] The reason was because *S.* Blockley exhibits higher heat resistance than other serotypes. On the other hand, 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 that were 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, in so far as the asymptotic behavior of the survival curves reflects that of the most heat-resistant strain or serotype of the cocktail. Thus, is the assessment includes parameters in the lethalities models that account for bias and 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: 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, $n = 5$; 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®, 1999)). The value of σ is assumed to be 0.13, corresponding to the estimate derived above. A standardized random normal variate, ζ, is generated and the quantity $0.13(\zeta - 1.163)$ is added to derived value of $\ln(p(t))$.

Statistical Methods

Pasteurization of shell eggs

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

Liquid egg white and 10% added salt or sugar for yolk and whole product

For the above listed liquid products, experimental data consisted of observed survival curves for fixed temperatures.[5]. 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.

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, there are many studies that report nonlinear survival curves for *Salmonella*. For example, Blackburn et al.[11] described non-linear inactivation of SE in culture broth. Based one the data from the United Egg Producers[5] and the results from Blackburn et al.,[11]

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.[11] are not available, the reported results are credible for use in these risk assessments.

Many functions have been used to fit nonlinear survival curves.[11-15] 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[16] and is often characterized by the shape of the curves for a short period of time and for times approaching infinity. The more common types are: 1) initially concave (negative second derivative) and approaching a straight line – often this type of curve is referred to as having a "shoulder"; 2) a straight line; 3) initially convex and approaching a straight line with non-zero slope; 4) convex such that the probability of survival approaches zero, so that the lethality has no upper bound; and 5) 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,[11] were not used in the risk assessments.

It often difficult to determine for sigmoidal or convex survival curves whether or not they are tailing.[3] For data from the United Egg Producers,[5] this is particularly true because the experiments often included only four or five data points. Consequently, convex shaped survival curves are fit to functions for which tailing is observed in plots of the data. Such functions are derived by either assuming that 1) the hazard function (or the instantaneous inactivation rate) is constant for a given cell and that over the population of cells the instantaneous inactivation rates are distributed as some designated distribution such as the gamma or normal distributions,[17] or 2) a survival distribution for $p(t)$.[14] 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

$$\ln(p(t))=\ln\left[\phi_F(t)\right] \tag{G5}$$

where $\phi_F(t)$ is the Laplace transform of F. If there is an initial shoulder, then there would be additional terms,[15] 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 further considered here.

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 6 4, the derivative of $\log_{10}[p(t)]$ 6 -0, and as t 6 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 α <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,[3,12,12] is

$$\log_{10}\left[p(t)\right] = -\log_{10}\left[1+\exp(a\ln(t)+b)\right] \quad \text{(G6)}$$

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 $-e^{b}at^{a-1}/\ln(10)$, 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 5 for the gamma distribution and by Equation 6 fit the observed lethalities equally well, then, provided $a < \alpha$, the asymptotic lethality (as t 6 4) for the gamma function is larger. This is seen by noting that the ratio of the limit as t 6 *4* of the function of Equation 6 to that of the function of Equation 5 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 α > 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. If the logistic function were selected, then the implied greater degree of heterogeneity as applied to the general population might be inappropriate, increasing, artificially, by at least some amount, the cell heterogeneity of the population. The use of a cocktail with five bacterial strains imposes an increased heterogeneity; however, at issue is the asymptotic behavior of the survival curves, which reflects the most heat resistance of the studied *Salmonella*. Consequently using the logistic may represent properly the asymptotic behavior of the most heat resistance serotype. Thus, both functions were considered and "function" was treated as a state of knowledge variable.

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.

A third function considered, based on assuming a Weibull distribution for the survival function[14] is where a and b are parameters. The shape of this curve depends on the value of b: 1)

$$\ln\left[p(t)\right] = -at^{b} \quad \text{(G7)}$$

if $b > 1$, then the curve is convex; 2) if $b = 1$, then the curve is linear; and 3) 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 when assuming F is the gamma distribution.

For the 10% added sugar 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 lethality is considered large enough in order to validate the asymptotic behavior of the model. 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. Therefore, for egg white products, a different concave function was considered, motivated by assuming a two-stage process[15] 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: 1) initial stage; 2) second stage, on the verge of inactivation; or 3) 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:

$$P_t(I) = \int_0^t \left[1-e^{-k_2(t-\tau)}\right] k_1 e^{-k_1 \tau} d\tau \qquad \text{(G8)}$$

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

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

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

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

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

Once a function is selected it is necessary to estimate the values of the parameters that characterize the function. Because environmental conditions such as temperature and pH vary, in

order to derive equations for survival curves for any given set of environmental conditions regression analyses are performed by fitting the estimated parameter values 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 to be random error variables, reflecting the experimental error structure that would cause correlations between observations. Procedures used to determine models follow those as given in Pinheiro and Bates[18] 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.[18]

The lethality models used in the risk assessments 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 in Pinheiro and Bates,[18] 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. We thus used graphical data from a lethality study for SE PT4 (P167807) in tryptone soy broth (TSB) at various pH and salt levels using submerged-coil heating apparatus.[11] Non-linearities for the SE survival curves were reported. To fit survival curves, the authors used a function referred to as the log-logistic,

$$\log_{10}[p(t)] = -\frac{a}{1+e^{-b(\ln(t)-c)}} \qquad \text{(G11)}$$

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.

Blackburn et al.[11] did not state why the log-logistic function was selected. It is reasonable to assume they examined the data and selected this function based on their observations of SE

survival curves 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.[11]) showing the predictions of times needed to obtain 3- and 5-$\log_{10}$ 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 6. 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:

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

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

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.[11]). A portion of this table, corresponding to the whole and yolk egg products, is presented below in the subsection entitled '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.

Survival curves for liquid egg white products

The protocol for the United Egg Producer's study,[5] approved by FSIS, is briefly described below. Three trials were conducted at each of four tested temperatures (54.4, 55.5, 56.7, and 57.7°C). 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 $\log_{10}$ 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, only 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 data used in the analysis is presented in an attachment, Table W. The entries are the means of three measurements (data not shown).

The observed data indicated survival curves with an initial shoulder, followed by an asymptotic linear line. This was readily noticed by examining the residuals of a linear regression for each experiment (48 in total) and noting that only 5 residuals were positive (3 of which were at a pH of 9.3). If in general the survival curves were linear, the expected number of positive residuals would be 24. Consequently, it appears that the curves are not linear.

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, $\log_{10}(\text{asym}D)$, 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, at a pH of 9.3, these values were substantially lower than the projected values that would be obtained at this pH using the line determined from the other pH values. The pH of 9.3 appears to be higher than those that are typically obtained when the product is pasteurized, and thus data at this pH were excluded from the following analyses.

Using the remaining results, analyses of variances on the $\log_{10}(\text{asym}D)$ were performed, assuming pH and temperature and their interaction as fixed factors and replicate as a random factor. The analyses indicated a significant interaction between temperature and pH, the replicate effect (within pH) was statistically significant ($p = 0.04$), and the intra-replicate correlation was approximately 31%. The reason for this relatively high intra-replicate correlation can be seen from the following table (G2), which presents the estimated $\log_{10}$ (asym*D*).

TABLE G2 ESTIMATED ASYMPTOTIC LOG_{10} D-VALUES, BY PH, TEMPERATURE AND REPLICATE

		Temperature (°C)				
pH	**Replicate**	**54.4**	**55.5**	**56.7**	**57.7**	**All**
	1	0.987	0.653	0.353	0.037	0.508
7.8	2	1.163	0.774	0.372	-0.003	0.577
	3	1.095	0.785	0.327	0.101	0.577
	4	0.981	0.721	0.343	0.047	0.523
8.2	5	0.928	0.635	0.271	0.013	0.462
	6	0.965	0.610	0.306	-0.006	0.469
	7	0.792	0.601	0.239	-0.042	0.398
8.8	8	0.826	0.570	0.244	0.002	0.411
	9	0.828	0.588	0.319	0.017	0.438

An examination of the relationship of the $\log_{10}$ *D*-values among the three replicates for the pH values reveals that the replicate number 4 for pH 8.2 has consistently higher *D*-values than the

$$\ln\left[p(t)\right] = -kt + \ln\left[1+\frac{k}{w}(1-e^{-wt})\right] + \varepsilon \qquad \text{(G14)}$$

values for the other two replicates at the same pH. When the results for this replicate are deleted, then intra-replicate correlation is reduced to 18% and the replicate effect may be considered marginally significant, with a *p*-value of 0.15. A further examination of Table G2 reveals that the first replicate for the pH of 7.8 has, with one exception, lower *D*- values. Thus, it cannot be dismissed that there is a replicate effect.

The function defined in Equation 10,

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 to be 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,

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

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 more manageable coefficients. For $\ln(w)$, a similar function,

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

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 16, 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 = 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 to be 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. The observed and fitted survival curves are given below (Table G3; Figure G1; Figure G2).

TABLE G3 ESTIMATED VALUES OF PARAMETERS USED FOR MODELING LETHALITY IN EGG WHITE PRODUCTS.

Parameter	Value	Std. Error	DF	*t*-value	*p*-value
a	-4.76610	0.22143	102	-21.52417	<.0001
b	0.71335	0.03135	102	22.75718	<.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

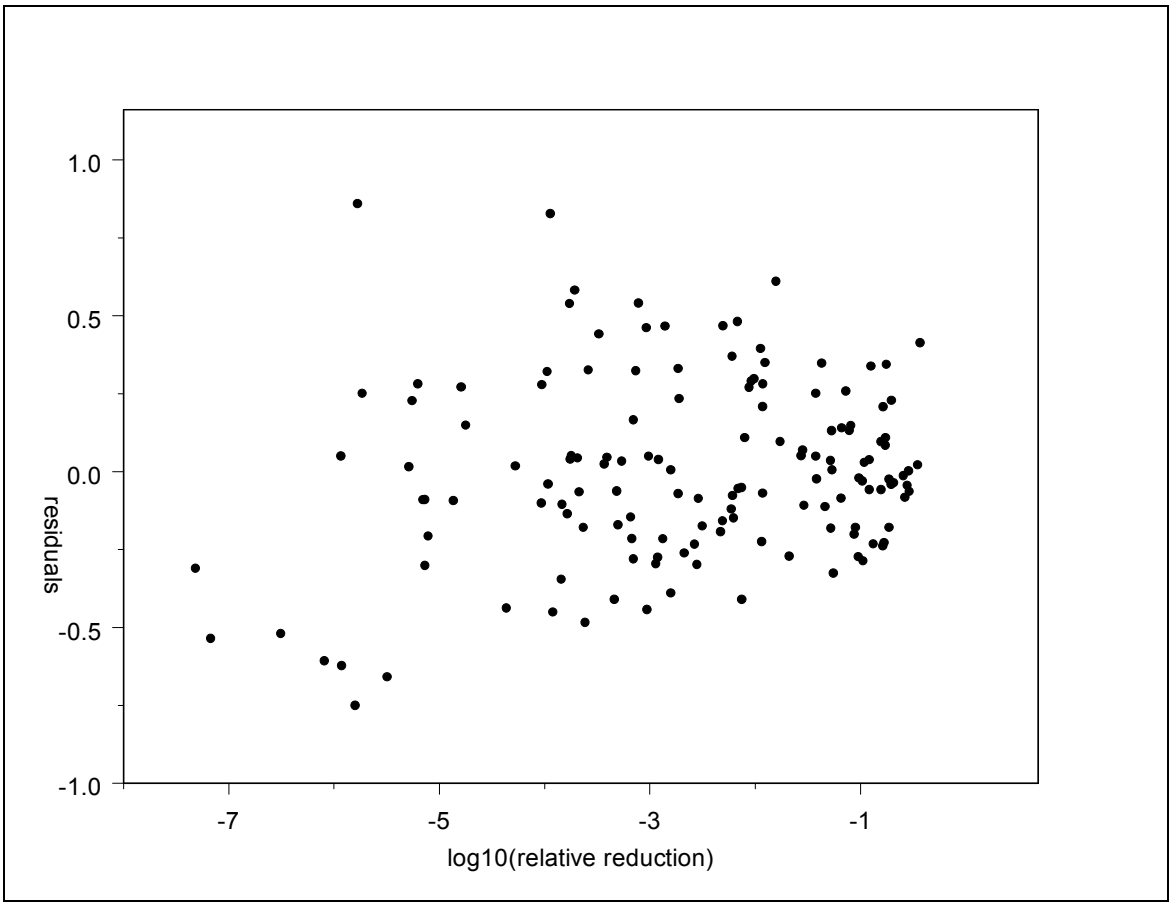

FIGURE G1 PLOT OF RESIDUALS VERSUS OBSERVED LOG_{10} RELATIVE REDUCTIONS FOR SELECTED MODEL USED FOR MODELING LETHALITY *SALMONELLA* SPP. FOR EGG WHITE PRODUCTS.

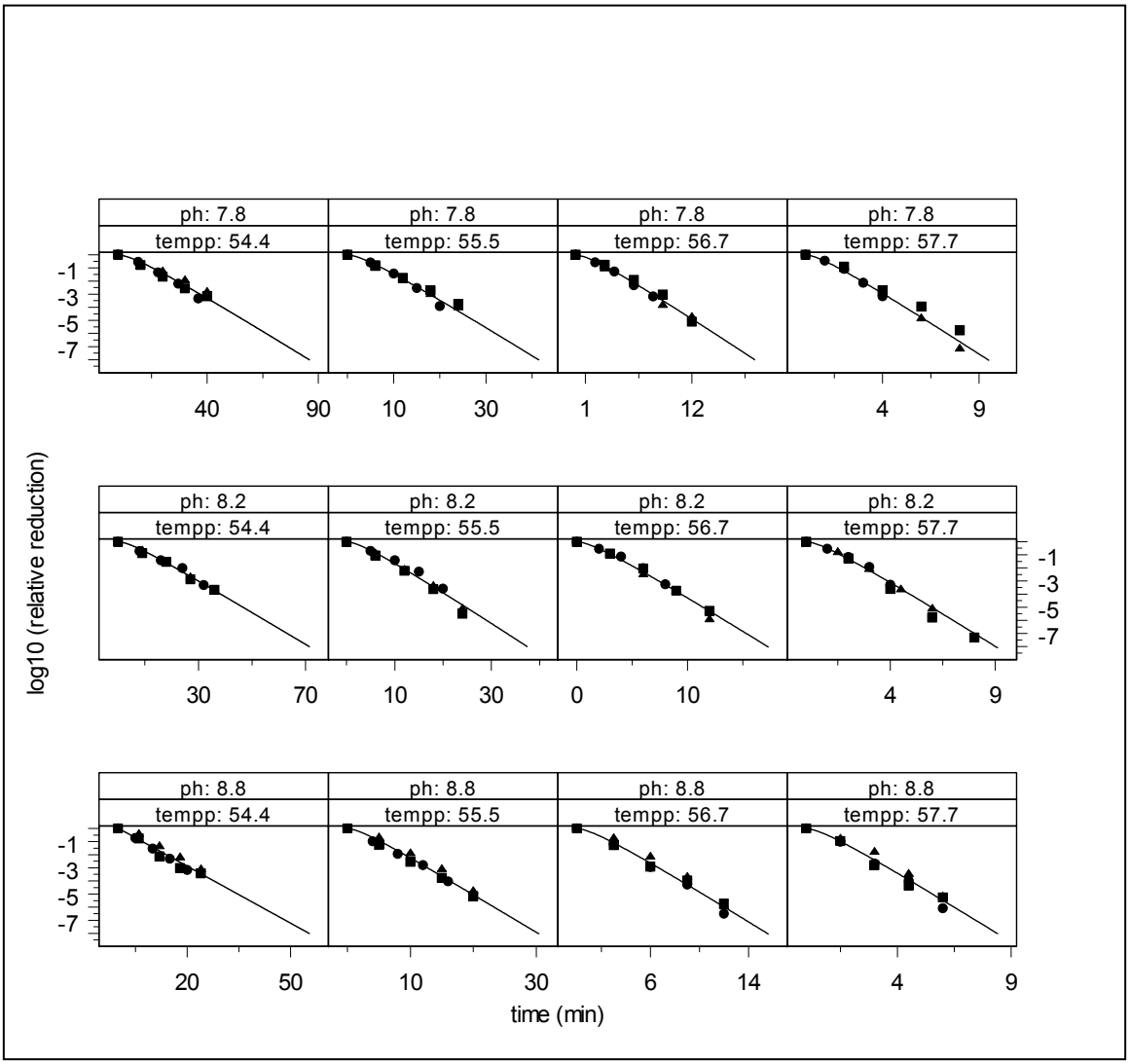

FIGURE G2 PLOTS OF OBSERVED DATA POINTS AND FITTED SURVIVAL CURVES (LOG_{10}(RELATIVE REDUCTION) VERSUS TIME (MIN) FOR EGG WHITE PRODUCT FOR GIVEN TEMPERATURES AND PH'S.

Survival curves for 10% added salt to whole and yolk egg products

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 was added on a 10% weight basis for testing at day 0 and after 4 days of storage at 6°C. However, data from product stored for 4 days were not used in this analysis in so far as it is not thought typical that product would be held this long after breaking.

The data indicated survival curves that were convex. Each replicate consisted of a set of 4 experiments at different temperatures for a given type of product. The temperatures for the yolk egg product were 63.3, 65.5, 67.8, and 70.0°C; and for the whole product they were 61.1, 63.3, 65.5, 67.8°C. For each type of product there were 3 replicates, so in total, for the 2 types of

products studied, there were 6 replicates for a total of 24 experiments. The observed levels ($\log_{10}$) used in the analysis is presented in the attachment, Table S. The entries are the means of three measurements (not shown).

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) with $\log_{10}$ of the observed relative reduction as the dependent variable and the values at $t = 0$ were of course deleted. Table G4 provides pooled root mean square errors for the two types of products.

TABLE G4 POOLED ROOT MEAN SQUARE ERRORS (RMSE) FROM NONLINEAR REGRESSIONS FOR YOLK AND WHOLE EGG PRODUCTS, BASED ON 12 EXPERIMENTS PER PRODUCT TYPE.

Type of product	RMSE logistic	RMSE gamma	RMSE Weibll
Yolk	0.1885	0.1981	0.2616
Whole	0.1383	0.1274	0.1224

The pooled root mean square errors of the regressions (based on 48 degrees of freedom) were as follows: 0.165 for logistic (Equation 6); 0.167 for the gamma (Equation 5); and 0.204 for the Weibull (Equation 7). For the yolk product, the Weibull assumption yields the highest RMSE, and among the 12 experiments, almost always had the highest RMSE among the three. Because of the relatively poor fits for the Weibull assumption for the yolk product, this function was not considered further. Consequently, the gamma and the logistic functions were considered.

The following function, based on Equation G5 assuming F is the gamma distribution with two parameters: α and $\beta > 0$,

$$\log_{10}[p(t)] = -\alpha \log_{10}(1+\beta t) + \varepsilon \qquad \text{(G17)}$$

where ε is an error term that is considered. For each experiment, nonlinear regressions were performed. Table G5 contains the estimates of α, β, the mean, and standard deviation of the estimated gamma distribution. For modeling let $\delta = -1$ for the yolk product and = 1 for the whole product. Linear mixed effects regressions, where the replicate was considered a random factor, were performed with, α and β, the mean and standard deviation as dependent variables. These analyses indicated that the replicate effect was not statistically significant, based on likelihood ratio tests. For example, with the variable a as the dependent variable, the model with the highest loglikelihood included only terms for temperature and type of product. For this model, the likelihood ratio test (- 2 log-likelihood ratio) was about 2, which is not statistically significant. When the mean was the dependent variable, the intra-replicate correlation was estimated to be zero (or negative). Consequently, it can be assumed that the replicate effect is negligible and need not be accounted in the model.

TABLE G5 RESULTS OF NONLINEAR REGRESSION: $LOG_{10}(P(T)) = -ALOG_{10}(1+BT)$

Product Type	Temp °C	Replicate Number	a	b	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	3	5.452	19.679	107.30	45.95	42.8

Nonlinear mixed effects regressions were performed on Equation G16, where it was assumed that α and β are at most quadratic with respect to temperature. The value of 60 was subtracted from temperature. Simple functions were tried and terms were added until there was not significant improvement in the model, based on the likelihood ratio test. The following relations for α and β were found to provide adequate fits to the data:

$$\begin{aligned} \alpha &= a + b\delta + c(T\text{-}60) \\ \beta &= f + h(T\text{-}60) + i\delta(T\text{-}60) + j(T\text{-}60)^2 \end{aligned} \qquad \text{(G18)}$$

where T is temperature and a, b, c, f ,h, i, and j parameters. Different error structures were assumed as follows: 1) a and f are random (resulting in a high correlation between them and thus this assumption was not considered further); 2) assuming just a is random; and 3) assuming a is fixed but ε - the error term - has nested structure of replicate and experiment within replicate. The last two assumptions provided nearly the same predicted results, but the latter did have a higher likelihood value. Thus the last assumption was used in estimating the values of the parameters. The coefficient of T - 60 for β (what would be g) if included did not improve the model by a significant amount (P- value = 0.19). Using the above model, the replicate effect was not significant (P- value = 0.46), confirming its exclusion from the model, as discussed above. Figure G3 is a plot of the residuals versus the observed $\log_{10}$ relative reductions. The RMSE of

the residuals is 0.2877; the mean of the standard error of the predicted lethalities for the observed conditions of the experiments is 0.1047 and the CVs are all less than 5%.

In a similar fashion as above, a model using Equation G6, the logistic function, was also developed where *a*, *b*, *c*, *d*, *e*, *f*, and *g* are parameters. For this model, the replicate effect had a *P*-

$$\log_{10}\left[p(t)\right] = -\log_{10}\left[1+\exp(b+c\delta+d(T-60))\ln(t)+a+e\delta+f(T-60)+g(T-60)^2\right] \quad \text{(G19)}$$

value of 0.43 by the likelihood ratio test. The loglikelihood was slightly larger, by about 1 (using the REML method) confirming, as above, that the logistic provides a slightly better fit, but that the difference between models using the gamma and logistic assumptions is not significant. (Note that a negative residual implies a predicted lethality less than the observed lethality.) The mean of the residuals for the gamma model is 0.010, while that for the logistic model is 0.0028; the standard deviations are both approximately 0.28, the skewness for both are about -0.26, the kurtosis for the gamma assumption is -0.23, while that for the logistic is 0.36; the range of the residuals are 1.45 for the gamma and 1.48 for the logistic; the means of the standard errors of the predicted lethalities are 0.1047 for the gamma assumption and 0.1063 for the logistic. For observed lethalities greater than 4.5 $\log_{10}$, the average of the residuals for the gamma model are more negative than those for the logistic model, though the difference is small (-0.0170 for the gamma model versus -0.0108 for the logistic model).

While there are not statistically significant differences between the results derived from two sets of assumptions, there can be profound differences when the predicted times are beyond the range of the data. To achieve an 8-$\log_{10}$ lethality, the predicted times when using the logistic model are up to 33% larger than the corresponding predicted times when using the gamma model. The adjoining figure shows an example (whole egg product at 63.3ºC) of the difference of the predicted survival curves for the two models.

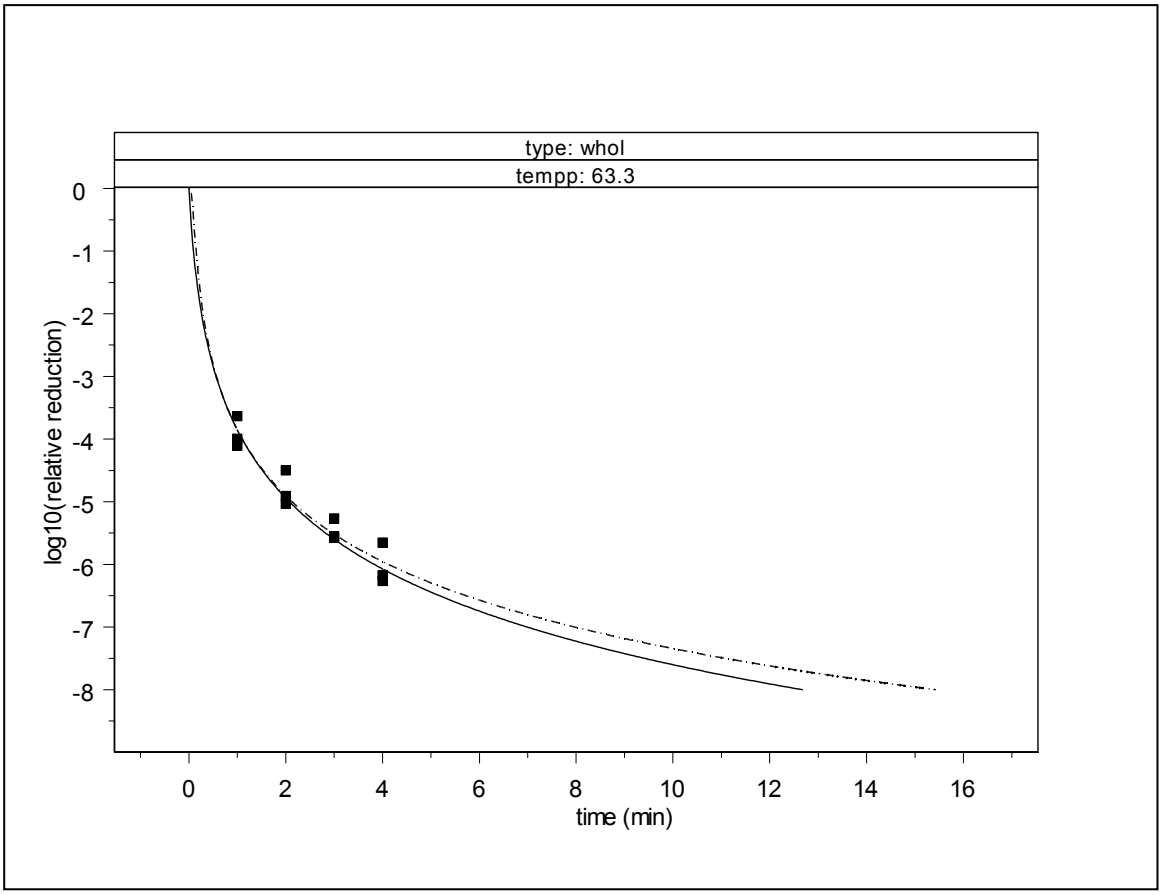

FIGURE G3 PLOT OF OBSERVED LOG_{10}(RELATIVE REDUCTION) AND PREDICTED SURVIVAL CURVES FOR THE TWO MODELS FOR PREDICTING LETHALITY FOR EGG PRODUCT WITH 10% ADDED SALT. THE DOTTED LINE IS THE PREDICTED SURVIVAL CURVE USING THE LOGISTIC ASSUMPTION, AND THE SOLID LINE IS THE PREDICTED CURVE USING THE GAMMA ASSUMPTION.

As a result of not being able to distinguish between the goodness-of-fit measures of the two functions, both functions are used in the risk assessment where "function" is considered a state of knowledge variable. The estimated values, standard errors, and the correlation matrix of the estimated parameters, copied from the S-plus output, for the two models are given below in Table G6.

TABLE G6 ESTIMATED VALUES, STANDARD ERRORS, AND THE CORRELATION MATRIX OF THE ESTIMATED MODEL PARAMETERS.

For the Gamma model (Equations G17 and G18):

	Value	Std. Error	DF	*t*-value	*p*-value
a	3.635166	0.277987	65	13.07677	<.0001
b	-0.911574	0.198646	65	-4.58893	<.0001
c	0.360656	0.052292	65	6.89693	<.0001
f	6.298565	1.181291	65	5.33194	<.0001
h	-1.160951	0.409893	65	-2.83233	0.0061
i	0.894829	0.200157	65	4.47063	<.0001
j	0.297161	0.039165	65	7.58732	<.0001

Correlation	*a*	*b*	*c*	*f*	*h*	*i*
b	-0.835					
c	-0.759	0.658				
f	-0.590	0.307	0.300			
h	0.528	-0.359	-0.394	-0.926		
i	0.528	-0.757	-0.775	-0.031	0.261	
j	-0.175	-0.019	-0.228	0.801	-0.754	0.349

For the logistic model (Equation G.19)

	Value	Std. Error	DF	*t*-value	*p*-value
a	4.810836	0.5946470	65	8.09024	<.0001
b	3.263478	0.2114460	65	15.43409	<.0001
c	-0.539650	0.0825574	65	-6.53666	<.0001
d	0.231221	0.0346593	65	6.67125	<.0001
e	0.655073	0.1285531	65	5.09574	<.0001
f	0.701920	0.2211998	65	3.17324	0.0023
g	0.101009	0.0193673	65	5.21545	<.0001

Correlation	*a*	*b*	*c*	*d*	*e*	*f*
b	-0.522					
c	-0.198	-0.308				
d	0.423	-0.905	0.329			
e	-0.230	-0.034	0.176	0.112		
f	-0.933	0.444	0.248	-0.404	0.130	
g	0.824	-0.419	-0.234	0.455	-0.016	-0.963

Plots of the residuals versus the observed $\log_{10}$ relative reductions and the fitted curves for the gamma model are given below.

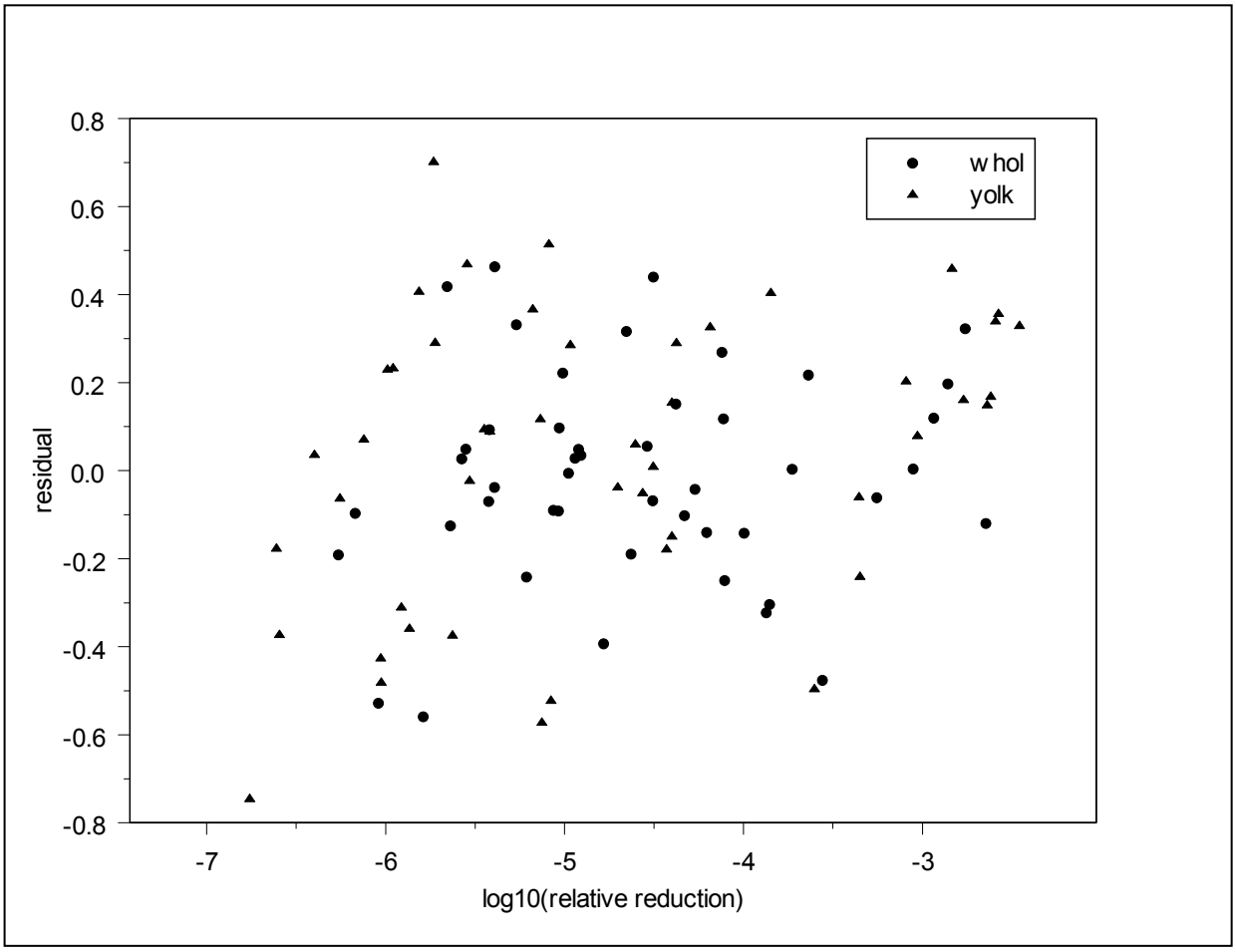

FIGURE G4 PLOTS OF RESIDUALS VERSUS OBSERVED LOG_{10} RELATIVE REDUCTION FOR THE GAMMA FUNCTION (EQUATIONS 17 AND 18) FOR MODELING THE LETHALITY OF 10% SALTED EGG PRODUCTS

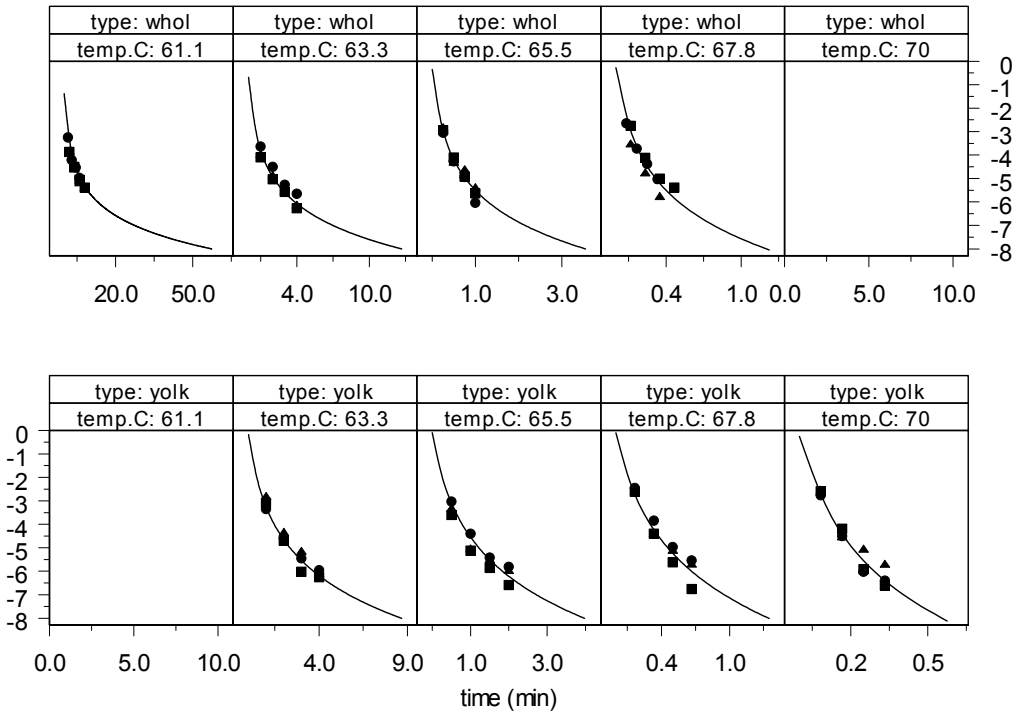

FIGURE G5 PLOTS OF OBSERVED DATA POINTS AND FITTED SURVIVAL CURVES, LOG_{10}(RELATIVE REDUCTION THE Y-AXIS) VERSUS TIME (MIN)- THE X-AXIS, ASSUMING THE GAMMA ASSUMPTION (EQUATIONS 16 AND 17) FOR 10% ADDED SALT IN WHOLE AND YOLK EGG PRODUCT AND GIVEN TEMPERATURES.

Survival curves for 10% added sugar in whole and yolk egg products

10% added sugar to whole egg products

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, so in total, there are 12 survival curves. Data recorded as 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 less than 100 cfu/ml and the other one at 100 cfu/mL), were also excluded. The data are presented in the attachment.

The function defined by Equation 7 with parameters $k = b$ and $w = \log_{10}(e)a > 0$, was considered for modeling $\log_{10}(p(t))$. Equation 7 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 eggwhite product, though that function provided a good fit with the possible exception for data at 66.7°C. Table G7 provides the estimates of w and k from nonlinear regression of Equation 7 for each temperature and replicate.

TABLE G7 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

The above results indicate increasing values of w and b with increasing temperature.

It is assumed that $\ln(k)$ and $\ln(w)$ were at most quadratic polynomial functions of temperature. Nonlinear mixed effects regressions were performed using the functions

$$\begin{aligned} \ln(k) &= a + b(T\text{-}55) + c(T\text{-}55)^2 \\ \ln(w) &= d + e(T\text{-}55) \end{aligned} \qquad \text{(G20)}$$

where T is temperature and the variable d is assumed to be a random variable with a nested error structure of replicate and experiment within replicate. For the above model, the replicate effect was significant at the 0.007 level. Adding a quadratic temperature term for $\ln(w)$ did not improve the fit of the model (P- value = 0.59). The root mean square of the residuals is 0.3072 and the average standard error of the predictions is 0.2166; however, for lethalities of approximately 8, for higher temperatures near 67ºC the CV is about 8%. The estimated values, standard errors, and the correlation matrix of the estimated parameters, from the S-plus output, are given below in Table G8.

TABLE G8 ESTIMATED VALUES, STANDARD ERRORS, AND THE CORRELATION MATRIX OF THE ESTIMATED PARAMETERS.

	Value	Std. Error	DF	*t*-value	*p*-value
a	0.331788	0.1464089	21	2.26617	0.0341
b	-0.070704	0.0360617	21	-1.96065	0.0633
c	0.007454	0.0020786	21	3.58588	0.0017
d	-3.394085	0.1252402	21	-27.10059	<.0001
e	0.655432	0.0151426	21	43.28385	<.0001

Correlati	*a*	*b*		*d*
b	-0.984			
c	0.971	-0.996		
d	-0.471	0.361	-0.363	
e	0.423	-0.312	0.324	-0.944

Plots of the observed $\log_{10}$ relative reductions and the fitted curves are given below in Figure G6 and Figure G7.

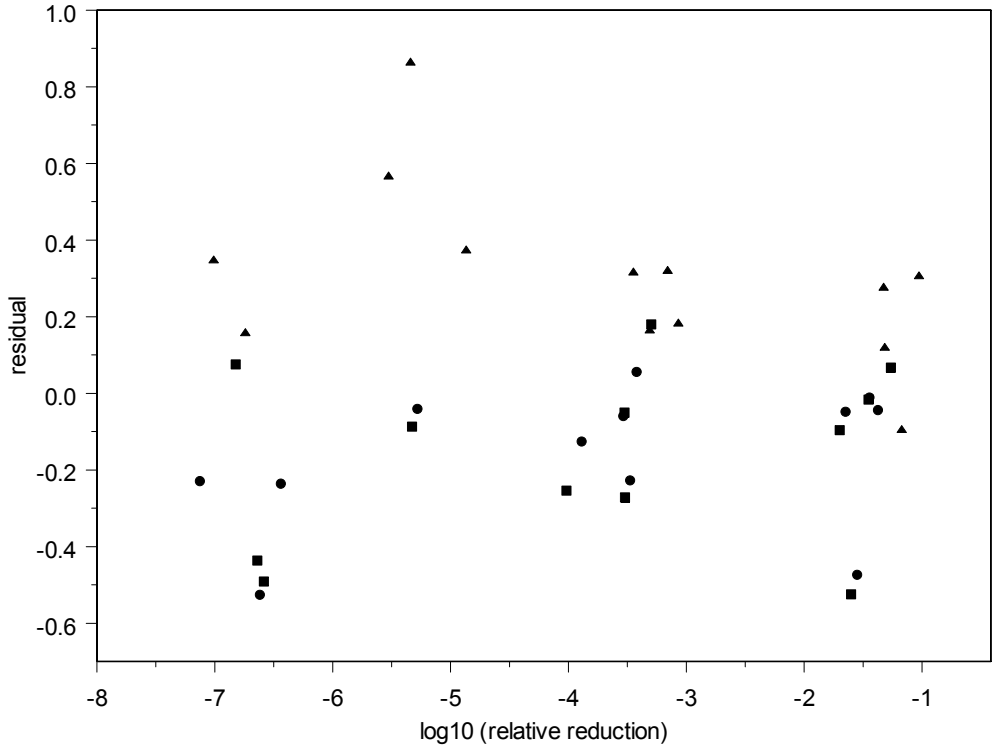

FIGURE G6 PLOT OF RESIDUALS VERSUS OBSERVED $\log_{10}$ RELATIVE REDUCTIONS FOR 10% ADDED SUGAR IN WHOLE EGG PRODUCTS. DATA POINTS OF THE SAME SYMBOL ARE FROM THE SAME REPLICATES.

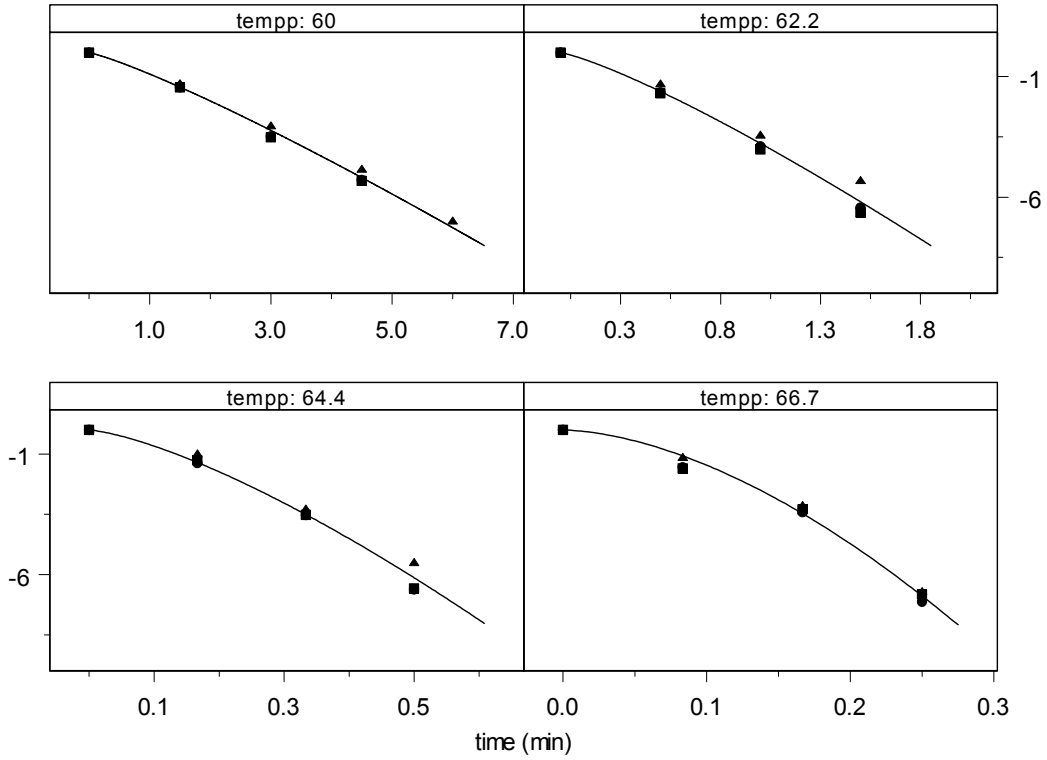

FIGURE G7 PLOTS OF OBSERVED AND FITTED SURVIVAL CURVES (LOG_{10}(RELATIVE REDUCTION) VERSUS TIME (M) FOR 10% ADDED SUGAR IN WHOLE EGG PRODUCT FOR GIVEN TEMPERATURES.

10% added sugar to yolk egg products

In this section of the study there were four replicate sets of experiments, where each replicate set consisted of experiments at the four 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 data are presented in the attachment, Table YS.

The observed data indicated asymptotic convexity of the survival curves and in some cases an initial shoulder. Regarding convexity, linear regressions, fitting quadratic polynomials, were performed and the coefficients of the quadratic terms were positive in 14 of the 16 regressions. In addition, for many of the observed survival curves, the slope of the line connecting data for the two smallest times was larger than the slope connecting the data points of the points with the second and third smallest times, suggesting a possible shoulder effect. There did not appear to be any easily recognizable pattern of curves other than the asymptotic convexity. Consequently, the function given in Equation G.6 was used for modeling survival curves.

Nonlinear regression for experiment using Equation 6 was performed and the estimates of *a* and *b* are presented in Table G8.

TABLE G8 ESTIMATED VALUES OF A AND B FOR EQUATION G6 FOR EACH EXPERIMENT OF 10% ADDED SUGAR TO YOLK EGG PRODUCT.

Temp (°C)	Number of Replicates	Obs	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 G8 reveals that the values of *a* appear not to be related strongly with temperature whereas the values of *b* appear are. An analysis of variance with the estimated values of *a* and *b* as dependent variables did not indicate significant replicates effects. The correlations (Pearson, Spearman and Kendall) of *a* and temperature had significances of approximately 0.13; the regression of *b* on a quadratic polynomial of temperature provided a

significance of approximately 0.05 for the quadratic term. Nonlinear regressions were performed and the functions which provided adequate fits, based on maximum likelihood ratio tests, were

$$a = e + f(T\text{-}55)$$
$$b = g + h(T\text{-}55) + k(T\text{-}55)^2 \qquad \text{(G.21)}$$

where T is temperature, e, f, g, h, and k are parameters. By the likelihood ratio test, f is significantly not zero at a 0.024 significance level. Assuming e and g to be random variables did not increase the log-likelihood by significant amounts. Further, assuming a to be random provided a higher log-likelihood value than when assuming g to be random. Thus, a was assumed to be random over experiments. Imposing a nested error structure of replicate and experiment within replicate was not statistically significant. The estimated values, standard errors, and the correlation matrix of the estimated parameters, copied from the S-plus output, are given below in Table G9.

TABLE G9 ESTIMATED VALUES, STANDARD ERRORS, AND THE CORRELATION MATRIX OF THE ESTIMATED PARAMETERS.

	Value	Std. Error	DF	t-value	p-value
e	11.65200	1.261182	15	9.238947	<.0001
f	-0.28275	0.128761	15	-2.195889	0.0346
g	-46.69955	5.076583	15	-9.199012	<.0001
h	9.28490	1.087982	15	8.534055	<.0001
k	-0.29105	0.057338	15	-5.076042	<.0001

Correl	***e***	***f***	***g***	***h***
f	-0.964			
g	-0.695	0.644		
h	0.685	-0.654	-0.993	
k	-0.680	0.674	0.971	-0.992

Plots of the residuals versus the observed $\log_{10}$ relative reductions and the estimated survival curves are given below.

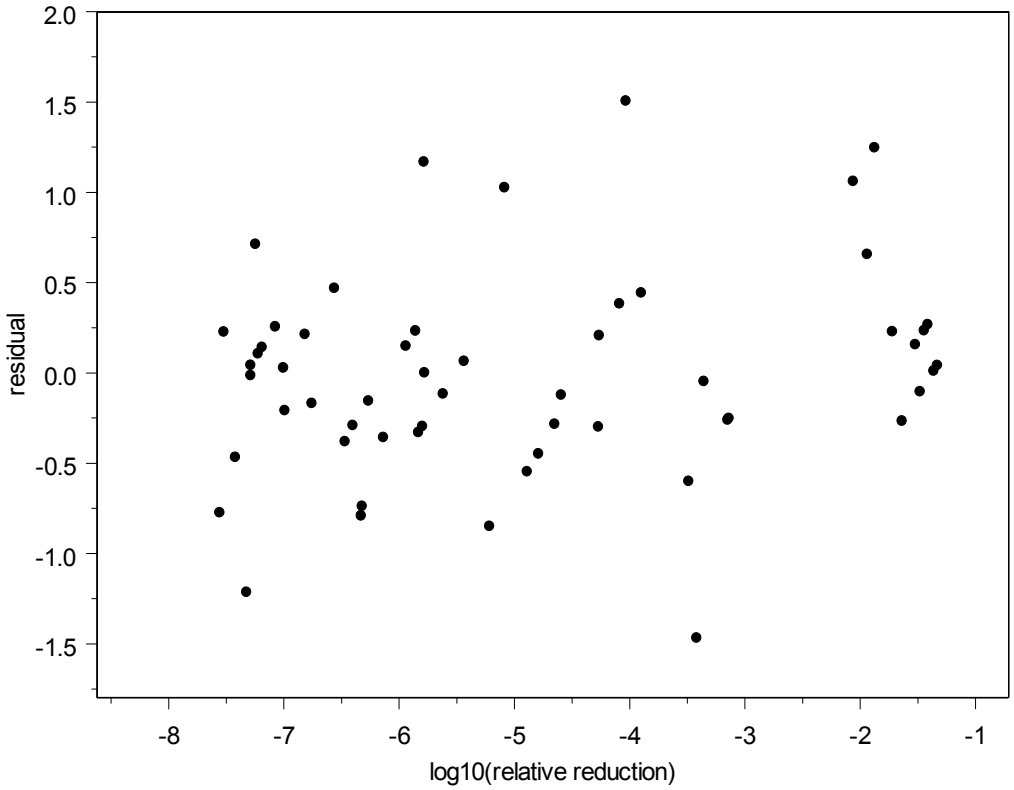

FIGURE G8 PLOT OF RESIDUALS VERSUS OBSERVED LOG_{10} RELATIVE REDUCTIONS FOR 10% ADDED SUGAR TO YOLK EGG PRODUCT.

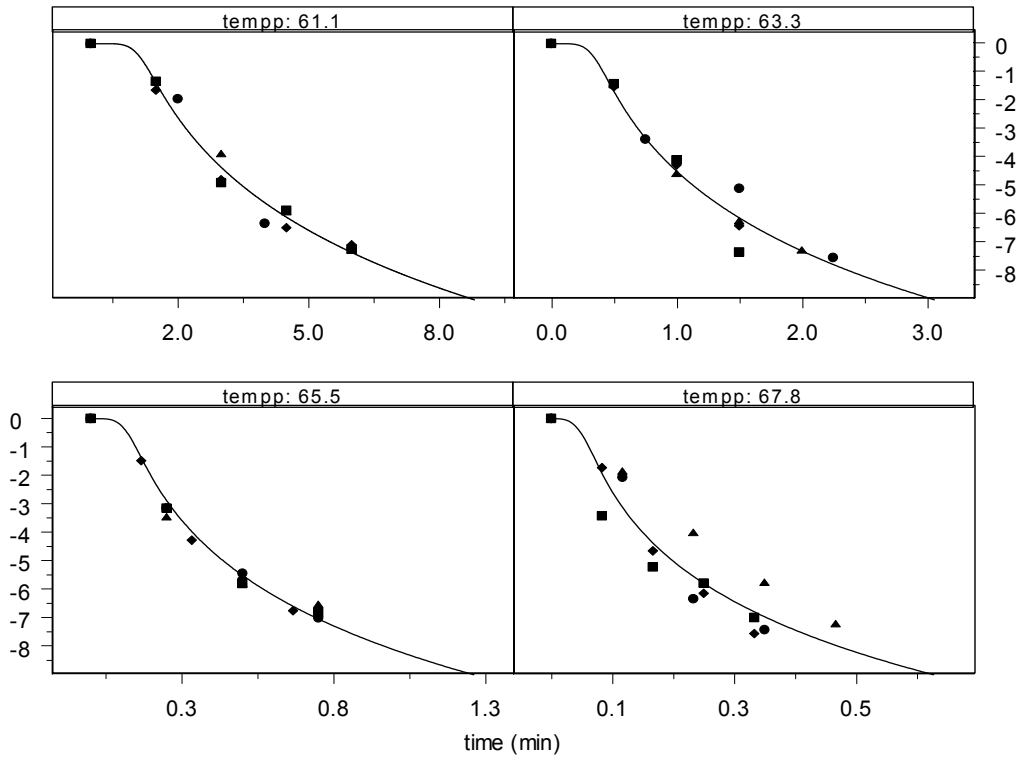

FIGURE G9 PLOTS OF OBSERVED AND FITTED SURVIVAL CURVES (LOG_{10}(RELATIVE REDUCTION) VERSUS TIME (M) FOR 10% ADDED SUGAR IN YOLK EGG PRODUCT FOR GIVEN TEMPERATURES.

Whole and yolk egg products

As explained in the Introduction, data were not available for these products. However, the lethality model of Blackburn et. al.[11] with SE phage type 4 (P167807) in tryptone soy broth (TSB) was assumed to represent these products in the draft risk assessment model. The 88 survival curves generated in this 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 6), where the parameters are determined from Equations 12 and 13, based on predicted *D*-values that are given in Blackburn et al.[11] (Table 6). 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.[11] 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.

Type of Product	Strain	Temperature (°C)	Predicted D- values (min)
Whole	SE	55	4.46
Whole	various	58	1.12
Whole	various	60	0.41
Whole	SE	57.2	1.64
Whole	SE	60	0.43
Whole	SE	60	0.41
Whole	S. Typhimurium	60	0.41
Whole (pH = 5.5)	S. Typhimurium	57.8	1.86
Whole	various	60	0.41
Yolk	various	62.2	0.36
Yolk	S. Typhimurium	59.5	0.85

Whole Egg products

Figure G10 shows the thermal death curve ($\log_{10}$(*D*-value) versus temperature) for the 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:

$$\log_{10}(D\text{-}value) = 12.1199 - 0.20834\ T \qquad \text{(G22)}$$

To determine the survival curve, values of *a* and *b* are determined from Equations G.12 and G.13, which are used in Equation G.6. The uncertainty associated with the above procedure is accounted for by generating random variables *f* and *g* such that the standardized variables

$$(f - 12.1199) / 0.13879 \text{ and } (g\text{-}0.20834)/0.00236$$

are distributed as bivariate t-distribution with 3 degrees of freedom and correlation -0.999551.

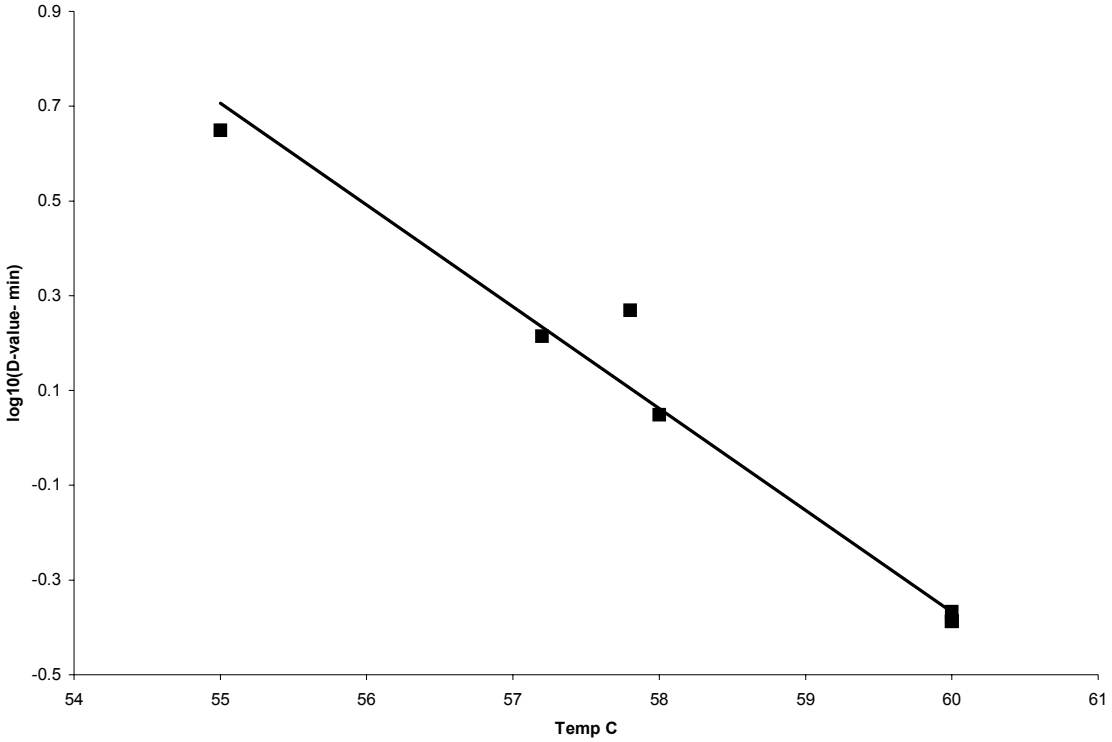

FIGURE G10 THERMAL DEATH CURVE DERIVED FROM D- VALUES REPORTED IN BLACKBURN ET AL[11] IN BROTH USED FOR PREDICTING LETHALITIES IN WHOLE EGG PRODUCTS.

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

$$\log_{10}(D\text{-}value) = 8.1518 - 0.1382\,T \qquad \text{(G23)}$$

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

$(f - 8.1518) / 0.3750$ and $(g - 0.1382) / 0.00616$

are distributed as bivariate t-distribution with 3 degrees of freedom and correlation -0.999754.

Pasteurization within eggs

Schuman et al.[19] have previously 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 $\log_{10}$ 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 G11 and G12 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:

$$T(t) = T_w + (T_i - T_w)\, e^{-kt} \qquad \text{(G24)}$$

where k is the exponential heating rate, T_i is the initial temperature, and T_w is the water-bath temperature. Figures G11 and Figure G12 provide the temperature profiles for the two temperatures. Estimates of k are given in Table LS3. To estimate T(t) in lag period in the temperature profiles (Figures G11 and G12), linear interpolation was used. The actual equation used to fit the data, derived from Equation G4 is,

$$\log_{10}\left[S(t)\right] = \log_{10}\left[S(0)\right] - \log_{10}(\mathrm{e})\int_0^t e^{a+bT(\tau)}d\tau \qquad \text{(G25)}$$

where $\log_{10}[S(0)]$ 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 25 were estimated by minimizing the mean square errors of the predicted values using Microsoft Excel® 2000_Solver. Figures G13 and G14 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 G11 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_{10} cfu/g)		Samples *Salmonella*-positive by enrichment testing	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
0	21.2	20.6	6.67	6.73	3/3	3/3
24	55.9	55.9	4.88	5.35	3/3	3/3
35	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	57.1	57.5	< 1.0	< 1.0	1/3	0/3
57.5	57.1	57.5	< 1.0	< 1.0	0/3	0/3
65	57.6	57.6	< 1.0	< 1.0	0/3	0/3

TABLE G12 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_{10} cfu/g)		Samples Salmonella-positive by enrichment testing	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
0	21.1	19.6	6.77	6.80	3/3	3/3
35	55.3	56.2	4.3	4.83	3/3	3/3
45	55.5	56.3	2.83	3.62	3/3	3/3
55	56.8	56.6	1.00	1.41	2/3	3/3
65	56.2	56.4	< 1.0	< 1.0	1/3	0/3
75	56.2	56.9	< 1.0	< 1.0	0/3	0/3
85	56.2	57.0	< 1.0	< 1.0	0/3	0/3

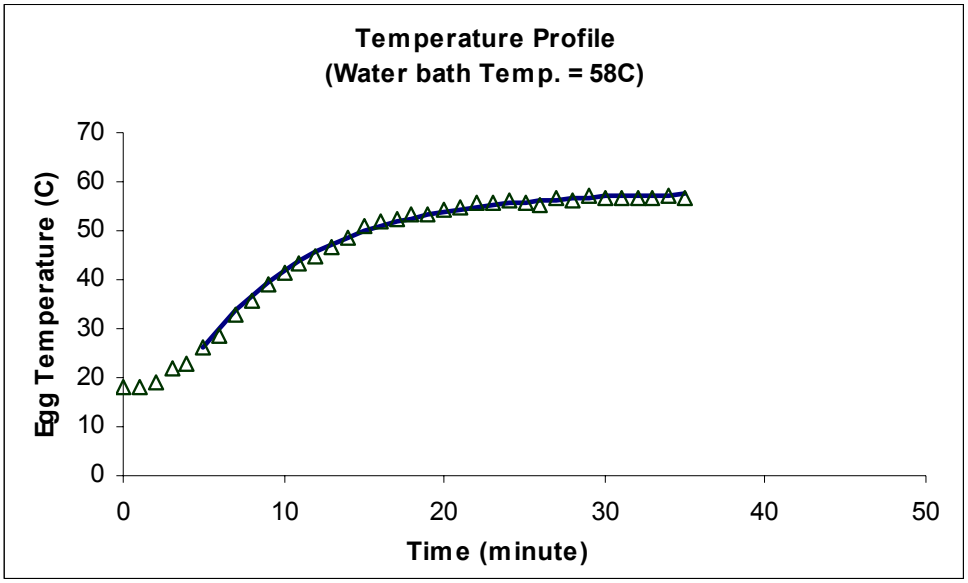

FIGURE G11 TEMPERATURE PROFILE FOR SHELL EGG PASTEURIZATION AT 58°C

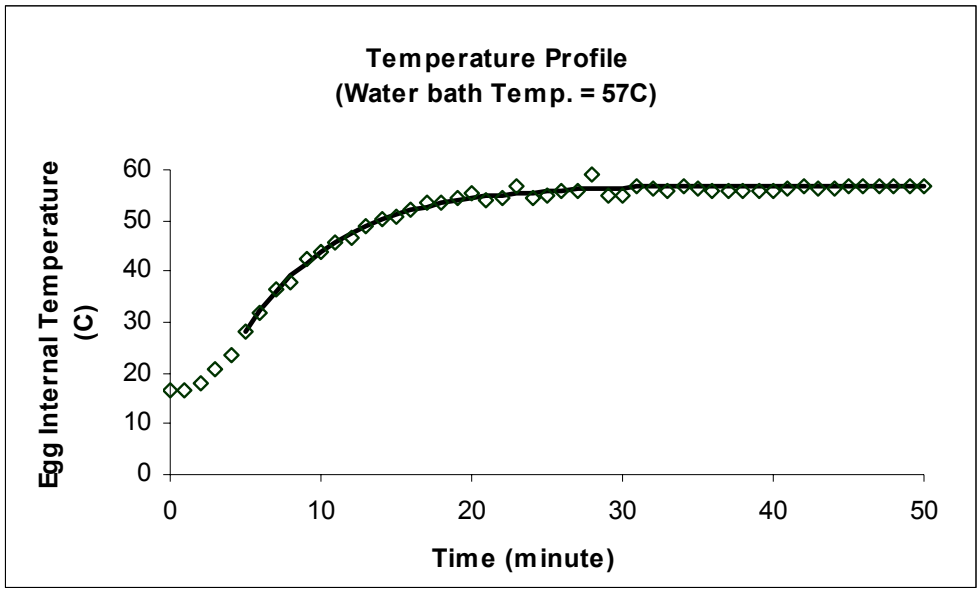

FIGURE G12 TEMPERATURE PROFILE FOR SHELL EGG PASTEURIZATION AT 57°C.

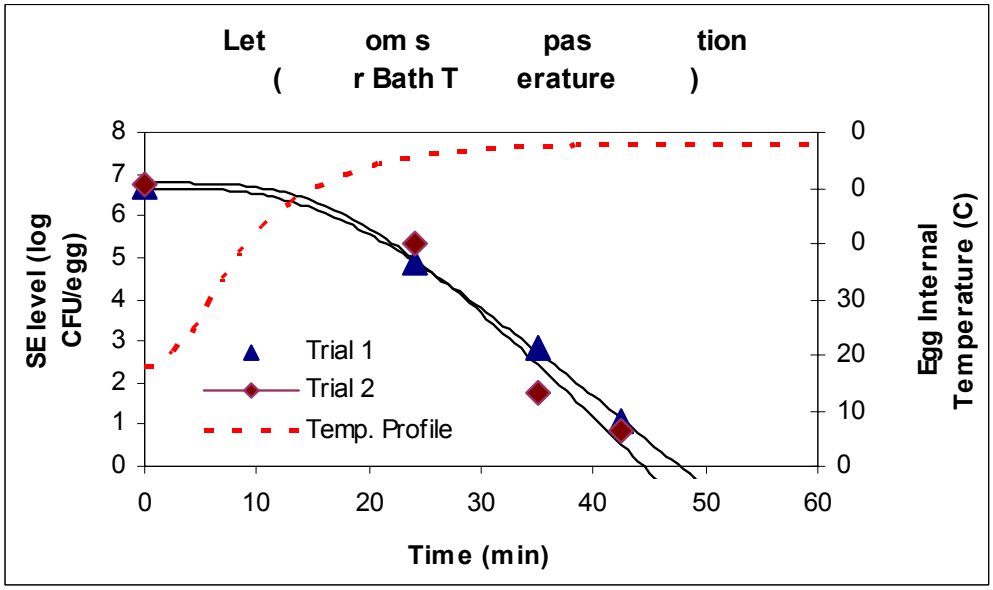

FIGURE G13 THE LOG_{10}-REDUCTION OF SE DURING THE SHELL EGG PASTEURIZATION AT 58°C.

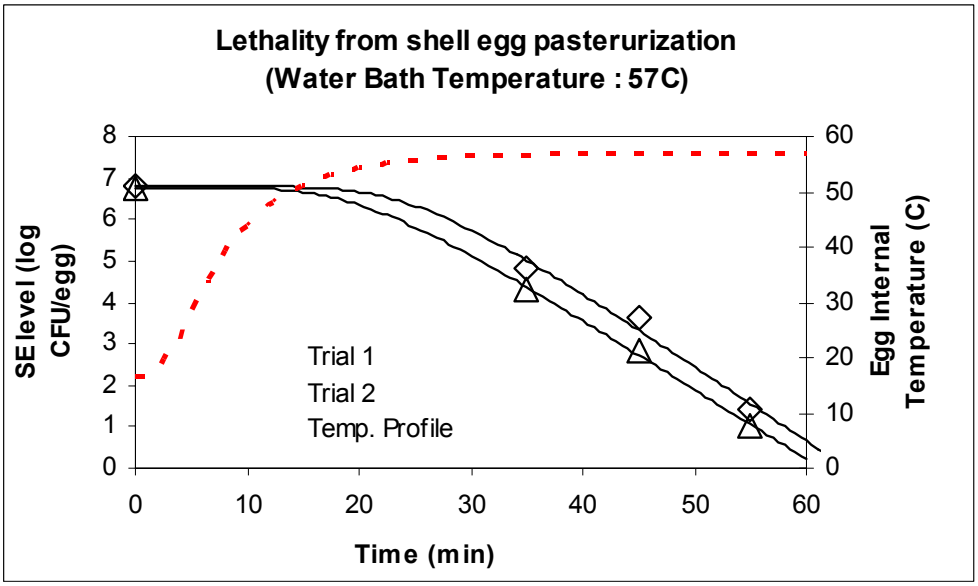

FIGURE G14 THE LOG_{10}-REDUCTION OF SE DURING THE SHELL EGG PASTEURIZATION AT 57°C.

Table G13 gives the estimated values of *a* and *b*.

TABLE G13 THE INITIAL SE LEVEL BEFORE PASTEURIZATION AND THE ESTIMATE OF PARAMETERS OF EQUATION G.25.

Experiment		Initial SE level (log_{10} 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 to be 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. Nevertheless, these results will be used. 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, α = -1.1969, with standard error of s_α = 0.3965; the intercept is, β = 67.225, with standard error of, s_β = 22.798; and the correlation is, ρ = -0.9056, based on two degrees of freedom.

Assumptions for modeling risk assessment

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,

$$\ln\left[p(t \mid T_w)\right] = -\int_0^t e^{a(T_w)+b(T_w)T(\tau)}d\tau \qquad \text{(G26)}$$

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 R, α, β, such that, the quantity $r = (R - R)/0.03827$ is distributed as a t-distribution with 2 degrees of freedom, independently, and the vector, $v = (v_\alpha, v_\beta) = ((\alpha - \alpha)/s_\alpha , (\beta - \beta)/s_\beta)$, 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 α and β. Using the parameter values estimated above, the values of $b(T_w)$ and $a(T_w)$ are determined from the relationships, $b(T_w) = \exp(\gamma + \delta T_w)$ and $\ln(a(T_w)) = R + \ln(b(T_w))$.

Features of the protocol used to generate data

For the analysis of pasteurization of shell eggs, the data studied by Schuman et al.[19] was used to model the lethality of SE from shell egg pasteurization. Features of the protocol are:

1. 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).

2. 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.[20,21]

3. 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.[20,21]

Methodology of Schuman et al[19]

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 $\log_{10}$ cfu/ml were obtained (transferring 10ml 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 sec. By 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 *l* 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 min.
  2. Submersion in a pre-heated 57°C water-bath for up to 85 min.

- 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 *l* of water (22°C sterile deionized water) → cool for 5 min.
- 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 cystine 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 as previously described.

ATTACHMENT G1

Data used in deriving lethality curves for *Salmonella* in eggs and egg products

TABLE G14 EGG WHITE RESULTS USED FOR DETERMINING MODEL ENTRIES ARE MEANS OF THREE MEASUREMENTS.

Replicate number	pH	Temp °C	time (min)	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

Annex G

TABLE G15 EGG WHITE RESULTS USED FOR DETERMINING MODEL. ENTRIES ARE MEANS OF THREE MEASUREMENTS.

replicate number	pH	Temp C	time (min)	$\log_{10}$ 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 G16 EGG WHITE RESULTS USED FOR DETERMINING MODEL. ENTRIES ARE MEANS OF THREE MEASUREMENTS.

replicate number	pH	Temp C	time (min)	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 G17 EGG WHITE RESULTS USED FOR DETERMINING MODEL. ENTRIES ARE MEANS OF THREE MEASUREMENTS.

replicate number	pH	Temp C	time (min)	$\log_{10}$ 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 G18 EGGWHITE RESULTS USED FOR DETERMINING MODEL. ENTRIES ARE MEANS OF THREE MEASUREMENTS.

replicate number	pH	Temp C	time (min)	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 G19 OBSERVED LEVELS FOR 10% SALTED ADDED PRODUCT.

product type	temp C	replicate number	time (min)	$\log_{10}$ 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 G20 OBSERVED LEVELS FOR 10% SALTED ADDED PRODUCT.

product type	temp C	replicate number	time (min)	$\log_{10}$ 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

Annex G

TABLE G21 OBSERVED LEVELS FOR 10% SALTED ADDED PRODUCT.

product type	Temp C	replicate number	time (min)	$\log_{10}$ 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 G22 OBSERVED LEVELS FOR 10% ADDED SUGAR PRODUCT.

Product type	Temp C	replicate number	time (min)	observed log_{10} 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 G23 OBSERVED LEVELS FOR 10% ADDED SUGAR PRODUCT.

Product type	Temp C	replicate number	time (min)	observed $\log_{10}$ 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

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