3 Exposure Assessment

EXPOSURE ASSESSMENT OF *SALMONELLA* ENTERITIDIS IN SHELL EGGS

Introduction

Salmonella Enteritidis (SE) colonize the reproductive tissues of hens and, consequently, the eggs they lay. Once inside an egg, SE survives cleaning and disinfecting of the shell surface. Furthermore, SE can multiply within the egg depending on how the egg is handled between the times it is laid and consumed. The first part of this exposure assessment estimates the frequency with which people are exposed to different doses of SE in servings prepared from shell eggs. The second part estimates exposures to all *Salmonella* spp. in servings of pasteurized eggs products.

The amount of SE present when an egg is consumed depends on whether SE were present when the egg was laid and, if so, whether they grew (or died) during handling. This exposure assessment follows eggs from the farm to the pasteurizer and from the pasteurizer to consumption. Figure 3-1 shows the most important components of this process. Pasteurization has special prominence in this assessment because it is the principal risk management measure under evaluation.

The occurrence of SE within an egg depends on whether the hen that laid it was infected with SE. Although SE-contaminated eggs only come from infected hens, not all eggs produced by infected hens are SE contaminated. Furthermore, infected hens are only found on farms in which SE is present, and on such farms, not all hens are infected. Thus, for an egg to be contaminated with SE, three conditions must exist: SE must be present on the farm, SE must infect one or more hens, and SE-infected hens must be susceptible to producing SE-contaminated eggs.

If an egg is laid with SE inside, the SE may die, remain dormant, or multiply. Multiplication depends primarily on time and temperature of storage. Higher temperatures (up to 37°C) favor SE growth, and longer storage times at temperatures permitting growth favor greater amounts of SE growth. Thus, the interaction of time and temperature determines how much SE growth occurs inside an egg.

On farms, eggs are typically stored for a short time in the laying house. The laying house holds all the hens of the flock; eggs are stored there from the time they are laid until they can be gathered, either mechanically or by hand. After the egg is gathered, it is stored in a warehouse on the farm for a variable period whereupon it may be either processed at the farm or trucked to a processing facility and stored in another warehouse.

Processing involves candling of eggs to detect defects and washing the shell; it may or may not include pasteurization and packaging of eggs into cartons. If the eggs are pasteurized, they are done so just before packaging. Pasteurization of shell eggs involves submersing the eggs in hot water for sufficient time to destroy SE, but not so long to cause changes to the liquid inside the egg. Consequently, a properly pasteurized shell egg appears grossly similar to an unpasteurized egg.

After processing, further growth of SE within an egg is possible, even in pasteurized eggs. Either some SE may survive pasteurization and grow or the egg may not be pasteurized and the SE inside continue to grow.

An egg is shipped to retailers or wholesalers to be purchased for food. The egg may be stored for varying times and temperatures before shipment, during shipment, and after shipment. For example, an egg may stay on a grocery shelf for several days before it is purchased. Furthermore, the egg will likely be stored for some time (days to weeks) in a consumer's refrigerator at home before it is consumed. All of these steps could present additional opportunities for SE growth.

Eggs are served in a wide array of foods, and a single egg may contribute to a meal that serves many people. During preparation of a meal, SE within an egg dish seems likely to be distributed homogeneously within the meal; therefore, when multiple servings from a single egg are simulated, there are multiple exposures per egg, albeit with fewer SE per serving than what were in the original egg.

Most meals prepared with eggs are typically cooked prior to consumption. Cooking can kill some, most, or all of the SE in a serving. Nevertheless, cooking of meals containing eggs is highly variable, and some meals, such as eggnog, are not heated before consumption.

This exposure assessment, and the risk characterization that follows in chapter 5, will help decision makers determine the extent to which different factors influence human exposures to SE and subsequent illnesses, based on data and assumptions that are inputs to the risk assessment model. Specifically, the risk characterization evaluates the log reduction from pasteurization in reducing exposures of consumers to SE from shell eggs. Pasteurization of shell eggs is not currently a common practice in the egg industry. FSIS wants to establish standards for pasteurizing shell eggs to ensure a consistent and safe product for consumers purchasing pasteurized eggs. Greater consumer demand for pasteurized shell eggs may consequently reduce the occurrence of human illness associated with SE in eggs.

The exposure assessment will help identify combinations of time and temperature of storage before pasteurization that result in no or very limited growth of SE within contaminated eggs. A decision could be made to require eggs to be stored according to specific guidelines before egg pasteurization. Alternatively, if storage conditions allow for substantial growth of SE within eggs, the log reduction from the pasteurization procedure itself should be adjusted to kill more bacteria.

A quantitative model has been developed to represent the most important elements of the process described above. The model estimates the number of SE at various points in time as they grow in an individual egg, from the times it is laid until it is consumed. The basic mathematical structure of that model is presented initially in the next section. Additional details will be found

in the remainder of this chapter, and a complete development of the concepts presented here can be found in the various supporting annexes. Figure 3-1 shows the farm-to-table progression of eggs as modeled in this risk assessment.

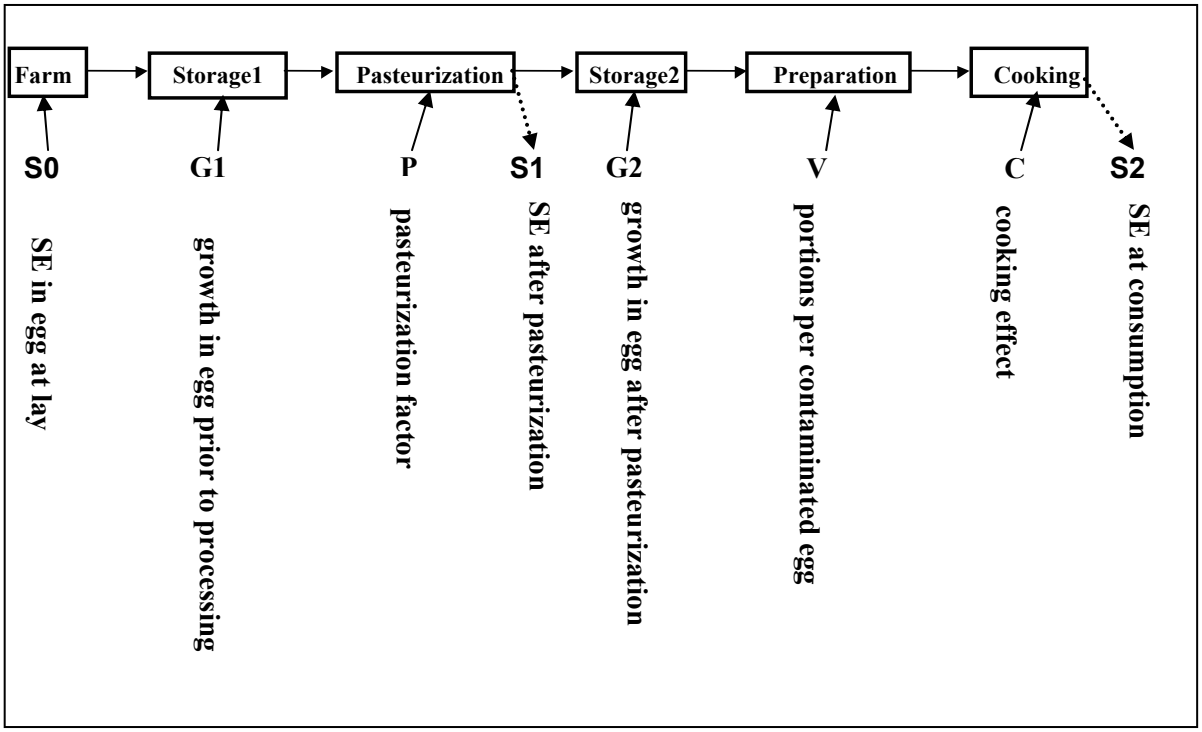

FIGURE 3-1 FARM-TO-TABLE PROGRESSION OF EGGS IN EXPOSURE ASSESSMENT.

Overview of the Shell Egg Exposure Assessment Model

Four equations summarize the SE in shell eggs model. Although this section does not follow the same chronological progression shown in Figure 3-1, it serves to introduce all the key variables and inputs addressed in this risk assessment. Subsequent sections in this part of the risk assessment will describe these inputs and provide the chronological development of the process. This model overview is presented at the outset to provide a better understanding of how the farm and first storage steps, etc. fit into the overall exposure assessment. The model presented in this chapter begins by estimating the number of SE that remains after pasteurization (Equation 3.1). Equation 3.2 estimates the dose of SE consumed by an individual. Illness is not necessarily the outcome from consuming SE. Therefore, the probability that illness occurs for a given dose in a serving is estimated using the dose-response relationship developed in chapter 4 (Equation 3.3). Finally, this probability of illness per serving is converted to a probability of illness per egg to account for some eggs that contribute to multiple servings (Equation 3.4). Each of these relationships is developed below.

Bacteria after pasteurization

The number of SE in an egg after it is pasteurized depends on the number of SE in the egg at lay, growth of these bacteria before processing, and the log reduction from pasteurization in reducing SE numbers within contaminated eggs (Equation 3.1).

$$S_1 = S_0 \text{ x } G_1 \text{ x } P \qquad (3.1)$$

where S_1 = the number of SE cells per egg after pasteurization; S_0 = the number of SE cells per egg at the time of lay; G_1 = the relative growth of SE from the time of lay to the time of pasteurization. This value generally ranges over the [1, 10^{10}] interval where 1 means that no growth occurred and 10^{10} means that one organism in an egg at the time of lay grew to 10 billion organisms at the time of pasteurization; P = the fraction of SE cells that survive pasteurization. This fraction can range over the [0,1] interval where 0 is complete elimination of the bacteria and 1 is complete survival.

Example
S_0 = 134 SE
G_1 = 2.6 $\log_{10}$ of growth (a multiplier of $10^{2.6}$ = 398)
P = 5 $\log_{10}$ reduction due to pasteurization (a multiplier of 10^{-5} = 0.00001)
S_1 = 134 x 398 x 0.00001 = 0.53, which is the expected number of SE.
Note that there are no units for any of the values except S_0 and S_1. G_1 and P are simply multipliers.

Equation 3.1 shows that the number of SE present at the time of lay are allowed to increase until the time of pasteurization. At pasteurization, the total number of bacteria is reduced to the S_1 level of contamination by the pasteurization process. Clearly, the determination of these variable values is a critical task of this risk assessment. The values for S0 are estimated using probability distributions to represent the variability in bacteria per egg. The value for G_1 is based on the predicted behavior of SE within eggs, which depends on time and temperature probability distributions. The value for P is constant for all eggs and is a selected input for the model. Given that thousands of contaminated eggs were modeled, the output of Equation 3.1 is a distribution of values that capture the variability attending the estimate of this post-pasteurization value.

Bacteria after cooking

The number of SE consumed in a given serving depends on the number of SE in the product after pasteurization (S_1 above), the growth of these bacteria after pasteurization, the attenuating effect of cooking, and the number of servings per egg.

$$S_2 = (S_1 \text{ x } G_2 \text{ x } C)/V \qquad (3.2)$$

Example
S_1 = 0.53 SE / egg
G_2 = 4 $\log_{10}$ of growth (a multiplier of 10^4 = 10,000)
C = 0.9 log reduction due to cooking (a multiplier of $10^{-0.9}$ = 0.126)
V = 3 servings / egg
S_2 = 0.53 x 10,000 x 0.126 / 3 = 222, which is the estimated number of SE per serving.
Note that there are units only for S_1 and V. G_2 and C are multipliers.

where S_1 is as defined above and: S_2 = the number of *Salmonella* cells per serving of an egg meal at the time of consumption. An egg meal can be any meal prepared from shell eggs; G_2 = the relative growth of SE from the time of pasteurization to the time of preparation and cooking. Its values can range as described for G_1; C = the fraction of cells that survive cooking. As described for pasteurization, this fraction can range over the [0,1]

interval where 0 is complete elimination of the bacteria and 1 is complete survival; V = the number of portions or servings created from a meal containing an egg.

Equation 3.2 starts with the SE that survive pasteurization and allows them to grow until the egg meal is cooked. This number is then reduced by the effect of cooking, and the resultant surviving number of cells is divided by the number of servings to produce the number of bacteria per serving.

Probability of illness per serving

The likelihood of illness per serving is calculated using a dose-response function with the number of SE per serving as its argument.

$$I_S = DR(S_2) \tag{3.3}$$

where I_S = the probability of illness resulting from consuming a serving of an egg meal. This probability can range over the [0,1] interval; S_2 = as defined above.

Example
S_2 = 222 SE / serving
DR(222) = 0.25 likelihood of illness given a dose of 222 SE per serving
Thus, out of 100 individuals experiencing this dose, 25 individuals would become ill.

The function relating the dose to the probability of illness is discussed at length in the Hazard Characterization chapter. Given a particular dose resulting from a contaminated egg, Equation calculates the probability that the dose would cause illness.

Illnesses per egg

The number of illnesses per egg is simply the probability of illness per serving times the number of servings per egg.

$$I_E = I_S \text{ x } V \tag{3.4}$$

Although the probability of illness per serving is between 0 and 1, if multiple servings were generated from a contaminated egg, it is possible to have many illnesses that result from the consumption of that egg. For example, if an egg was used to prepare a meal that served four people, and the egg contained sufficient SE to result in the probability of illness per serving being 1.0, then we would expect four illnesses from that single egg. Nevertheless, if only one person consumed an egg, and the serving contained just a few SE, then less than one illness could result from consuming that egg.

Example
I_S = 0.25 likelihood of illness per serving
V = 3 servings / egg
IE = 0.25 x 3 = 0.75 per egg
Thus, this egg has a 75% chance of causing an illness.

Modeling Plan

The four relationships described above are combined in a probabilistic mathematical model. The model begins with an estimate of the variation in the number of SE per egg, which is obtained

from analyzing the prevalence of SE in flocks, hens, and eggs found in Annex B and summarized below. The resulting probability distribution of SE per egg is sampled repeatedly to estimate the number of SE in each particular egg. Specific parameters, also the result of sampling probability distributions, for time, temperature, cooking, and other inputs are applied to the egg. These parameters are themselves the results of equations whose inputs are uncertain and/or variable. The values of these equation inputs are likewise sampled from other probability distributions. Thus, the variables in the four-equation model above are themselves the outputs of complex analytical processes. For example, although the relative growth might enter an equation as a rather simple numerical value the process of deriving that simple value is quite complex. The details of the derivation of these variables' values can be found in the annexes to this main report. A summary of those derivations follows. The model is programmed in Visual Basic for Applications. Inputs and outputs are stored in Excel spreadsheets. The model is available at the FSIS website (http://www.fsis.usda.gov).

The shell egg exposure assessment is complex. A large number of variables and parameters are needed to estimate the inputs described in the four-equation model above. To model growth, for example, equations that predict growth behavior of SE in eggs are needed. These equations depend on the storage times and temperatures an egg experiences during the various stages it traverses between the time it is laid and the time it is consumed. These equations depend on mathematical parameters that have been estimated from available data. Furthermore, probability distributions that describe how time and temperature during storage vary for eggs in these stages are needed. These distributions are estimated from data as well.

Estimation of parameters and distributions results in uncertainty about the true values or distributions of these parameters and variables. Estimates produced by the model are conditional on the values of the model's inputs. One set of model inputs will result in an estimate of a single value in the resulting distribution of illnesses per egg. Because input values are variable, the model must be run repeatedly using different input values to estimate the full range of possible outcomes. This enables decision makers to examine and consider the effect of this variation in possible outcomes on the answers to their risk management questions. An example of variability in model inputs is that some eggs are stored for two days on the farm while other eggs are stored for four days.

That this variability exists is only part of the estimation challenge. There is also uncertainty about the variability. In the example above, the number of days of storage varies. That variability can be modeled as a continuous or a discrete variable. There is uncertainty about the frequency with which the varying numbers of days occur. For purposes of presentation in this chapter, the input values or distributions presented are the best estimates from the annexes to this report. The discussion in this chapter does not include explicit references to the estimates' uncertainty. Chapter 5 on risk characterization will examine the effect of uncertainty on the expected number of human illnesses by making changes in different model assumptions. This sensitivity analysis will assess which inputs most influence the output of this exposure assessment.

One use of the uncertainty analysis is to identify critical research needs. Model important and highly uncertain inputs can be identified and researched to improve knowledge of human health risks resulting from SE in eggs. The current modeling approach satisfies this purpose.

A description of the scientific evidence and procedures used to estimate model inputs can be found in the annexes. This chapter makes extensive use of the science presented in those annexes. It is assumed that the interested reader will pursue the details of any elements of further

interest in the appropriate annex. In those few instances where inputs are not developed in the annexes, the relevant data and estimation procedures are presented in this chapter.

SE per egg at lay, S_0

The number of SE per egg varies from egg to egg. The distribution of all these values is described by a probability distribution. Most eggs do not contain SE at the time of lay. Eggs that are contaminated may contain 1, 10, 100, or more bacteria. The purpose of this section is to describe how the variability in SE per egg is distributed. Figure 3-2 is a schematic illustration of this estimation.

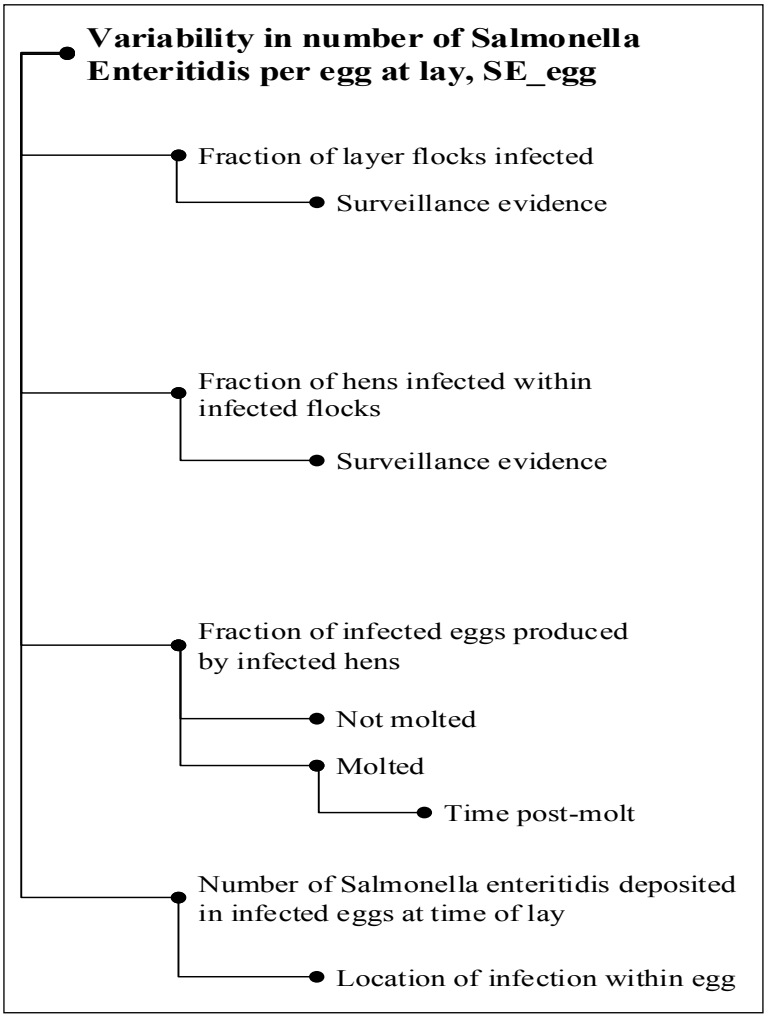

FIGURE 3-2 KEY INPUTS TO DISTRIBUTION OF SE PER EGG.

The output is shown at the top of the model in Figure 3-2. The four branches stemming from this output are the primary inputs. Each of these in turn has one or more inputs and so on. For example, the fraction of infected eggs laid by infected hens depends on whether the flock is molted. If the flock is molted, this fraction further depends on the time in weeks since molting

was completed. The number of SE deposited within a contaminated egg depends on the site of contamination. Sites of contamination include the internal surface of the shell, the albumen, the vitelline membrane that separates the albumen from the yolk, and the yolk.

Fractions of eggs contaminated with SE

The model is based on the assumption that only an infected hen can lay an egg that is internally contaminated with SE. Some whole flocks of hens are believed to be free of SE. Therefore, if the flock is not infected or the flock is infected but the hen is not infected, then the egg is not infected and the number of SE per egg is zero. To estimate the fraction of all eggs produced with no SE, the algorithm summarized in Table 3-1 is used. The algorithm shows the nature of the calculation directly, and it suggests the extent of the scientific evidence that was required to arrive at those calculations. The fraction of all U.S. flocks that are infected is estimated to be 20%. No further distinction is made about the extent of infection within a flock; it is simply a yes/no estimation. Given that a flock is infected, the fraction of hens within that flock that is infected varies from flock to flock. The variation in the number of infected laying hens is represented by a Weibull distribution. The best parameter estimates for this distribution are α = 0.43 and β = 0.0054.

TABLE 3-1 DEFINITION AND DESCRIPTION OF INPUTS USED TO CALCULATE THE FRACTION OF EGGS CONTAMINATED WITH SE.

Variable	Description	Estimation
f	Fraction of flocks detected as infected via surveillance	9.6% from data
g	Surveillance adjustment multiplier	2.065 from data
h	Fraction of flocks infected	$f \times g$ = 20%
K	Fraction of infected hens within a flock given that the flock is infected	Weibull(0.43, 0.0054) distribution estimated from data
j	Fraction of flocks molted	22% from data
e_{nm}	Fraction of infected eggs produced given that hen is infected and flock is not molted	8.6% from data
W	Time (weeks) post-molt	Uniform(0,20)
$M(w)$	Multiplier, as function of time post-molt, to adjust infected egg fraction for molted flocks	$\frac{e^{-6.1-0.23W}}{0.00023\times\left(1+e^{-6.1-0.23W}\right)}+1$ where the coefficients are estimated from data
e_m	Fraction of infected eggs produced given that hen is infected and flock is molted	M(w) x e_{nm}
E	Fraction of infected eggs among all eggs produced	EV [K x h x (e_{nm} x {1 – j} + e_m x j)]

Effect of molting

Molting is the shedding and regrowth of feathers by hens. Flocks are molted because the process rejuvenates hens' production of eggs. If a flock is not molted, it begins egg production at about 20 weeks of age and continues producing eggs for 1 year. Rates of egg production decline as the flock approaches its anniversary and the flock ceases to be economical. Therefore, the flock is

destroyed and replaced by a new flock of hens. Molting is an alternative management strategy that maintains the same flock in production for an extended period. A flock is typically molted several weeks before its anniversary. Molting is forced by restricting feed and light. The molting period can last 10 weeks and no eggs are produced during this time. Once molting is complete, the hens regain their earlier productivity and will lay eggs for nearly another year.

The stress of molting is thought to result in an increased susceptibility of hens to SE infection. Evidence from field studies suggests that molted flocks, in the first 20 weeks of post-molt production, will produce SE-contaminated eggs more frequently than non-molted flocks.

At any given time of year, the fraction of all flocks that are molted is estimated to be about 22%; only those flocks that are molted and in their first 20 weeks of production post-molt are of interest for this part of the exposure assessment. A non-molted flock will produce eggs for 52 weeks. Therefore, over 2 years there are 104 weeks of production. If the flock molts, the period in molt is about 10 weeks, and there are 94 weeks of production available. As such, the pre-molt and post-molt production periods constitute about 47 weeks each. The first 20 weeks of one of these production periods is about 42% of the production year. Consequently, 9.4% (22% x 42%) of flocks are molted and in their first 20 weeks of post-molt production. This fraction of infected flocks represents the flocks producing contaminated eggs at higher frequencies than the remainder of infected flocks.

Estimating the fraction of contaminated eggs per hen

The fraction of eggs produced by an infected hen is provided in Annex B. The best estimate of the fraction of eggs that is contaminated given that the hen is infected and the flock is not molted is 8.6%. For molted flocks, the fraction of eggs that is contaminated depends on the number of weeks post-molt. Early in the post-molt period, the fraction of eggs contaminated is much greater than that estimated for a non-molted flock. As the flock approaches 20 weeks post-molt, the fraction of eggs contaminated reduces to a level equivalent to that of a non-molted flock. This value varies as a function of the time post-molt and does not lend itself to a simple numerical expression.

Initial contamination by location in egg

Given that an egg is contaminated with SE, the number of organisms initially deposited inside the egg depends on the location of the bacteria. Table 3-2 lists nine types of contaminated eggs considered in this analysis and the proportions of each of these egg types. SE may initially be deposited in the albumen, in the yolk, in the vitelline membrane (VM), or on the inner shell membranes (shell).

TABLE 3-2 BASELINE ESTIMATES OF FRACTIONS OF VARIOUS TYPES OF SE-CONTAMINATED EGGS.

Type	Frctn.[a]	Type	Frctn.	Type	Frctn.	Type	Frctn.	Type	Frctn.
Shell	0.19							Shell	0.19
						Growth	0.79	Alb C G	0.07
Internal	0.81	Albumen	0.75	Close	0.15	No growth	0.21	Alb C N	0.02
				Far	0.85	Growth	0.39	Alb F G	0.20
						No growth	0.61	Alb F N	0.31
		VM or Yolk	0.25	VM	0.90	Low value	0.93	VM low	0.17
						High value	0.07	VM high	0.01
						Low value	0.93	Yolk low	0.02
				Yolk	0.10	High value	0.07	Yolk high	0.00

[a]Fraction

For albumen-contaminated eggs (Alb), the site of contamination is further distinguished as being close to or far from the yolk. These types of eggs must be modeled separately. A higher fraction of albumen-contaminated eggs will support growth if the SE is deposited close to, as opposed to far from, the yolk. Yolk- and VM-contaminated eggs are further separated into those that have a low number of SE and those with high numbers of SE initially inside them (low value and high value, respectively). Figure 3-3 shows the relative frequency of different contamination locations.

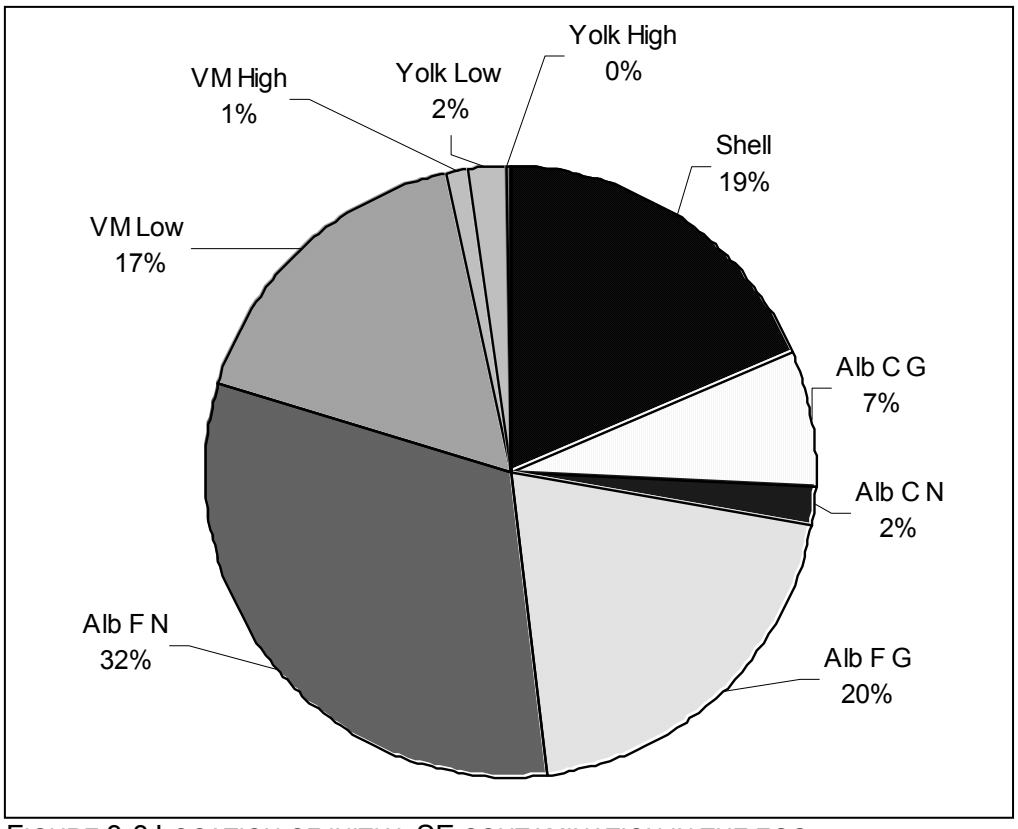

FIGURE 3-3 LOCATION OF INITIAL SE CONTAMINATION IN THE EGG.

The location of the initial contamination determines the number of bacteria present at the time of lay. The number of bacteria per egg varies and is represented by a probability distribution, which is a composite of the variable types of eggs and the variability in initial bacteria deposited. Roughly 80% of all contaminated eggs are contaminated in the albumen or shell. For these eggs, the initial number of SE deposited is lognormally distributed (Table 3-3). Equivalently, ln(bacteria per egg) is a normal distribution. The best-fitting parameters for this normal distribution are a mean of 2.6 and a standard deviation of 1.3. Therefore, we expect that S_0 is a random value from this distribution for about 80% of contaminated eggs.

TABLE 3-3 INITIAL NUMBERS OF SE DEPOSITED IN CONTAMINATED EGGS BY EGG TYPE.

Egg Type	Initial Bacteria Estimate
Shell	
Alb C G	
Alb C N	
Alb F G	
Alb FN	$e^{Normal\ (2.6,\ 1.3)}$
VM low	
Yolk low	*Poisson*(1.39) without zeros
VM high	
Yolk high	Assume one organism begins exponential growth immediately at lay

Roughly 19% of all contaminated eggs (Table 3-2) are low-value VM or yolk-contaminated eggs. For these types of contaminated eggs, the initial number of bacteria is estimated using a Poisson distribution with zero values censored. The best-fitting parameter for this Poisson distribution is 1.39. Therefore, we expect S_0 is a random value from this distribution for about 19% of contaminated eggs.

Roughly 1% of all contaminated eggs are high-value VM or yolk-contaminated eggs. For these types of contaminated eggs, the initial number of bacteria is assumed a single organism that can grow immediately. This organism does not experience any lag period and multiplies exponentially soon after lay. Such growth can be substantial but is variable from egg to egg, depending on how the egg is stored. Predicting this growth requires modeling the exponential growth occurring within contaminated eggs. Although these eggs seemingly start with the minimum amount of contamination possible, the warm temperature of the egg at the time it is laid guarantees substantial multiplication of bacteria within just a few hours. Therefore, we expect S_0 to be one organism for 1% of contaminated eggs, but this one organism becomes several very quickly.

The distribution for S_0 also includes those eggs that are not contaminated. The fraction of all eggs that are not contaminated, and for which S_0 is equal to zero, is 1 minus the fraction of contaminated eggs among all eggs produced (E from Table 3-1). For the remaining fraction of eggs that are contaminated, the probability (or fraction) of eggs with differing amounts of S_0 must be estimated using Monte Carlo simulation. This simulation will sample distributions for S_0 according to the fractions shown in Table 3-4. In this manner, the variability in S_0 across all eggs produced in the U.S. can be estimated.

TABLE 3-4 THE FRACTION OF ALL EGGS CONTAINING VARIOUS LEVELS OF INITIAL BACTERIA AS PREDICTED BY VARIOUS DISTRIBUTIONS. THESE DISTRIBUTIONS ARE MIXED TO ESTIMATE THE NUMBER OF SE INITIALLY DEPOSITED INSIDE EGGS, S_0.

Fraction	S_0
$1 - E$	0 (no contamination)
E x 80%	$e^{Normal(\mu,\sigma)}$
E x 19%	*Poisson(λ)* without zeros
E x 1%	Assume one organism begins exponential growth immediately at lay

Growth effect before processing, G_1

The risk associated with eggs laid with *Salmonella* depends on the number of *Salmonella* present at the time of consumption. Because *Salmonella* have the ability to reproduce and grow inside the egg, the nature of this growth is of special importance to this exposure assessment. *Salmonella* have specific requirements for growth. The most important of these is temperature, but factors such as pH and the availability of iron also affect growth of *Salmonella*. This section presents background material, mathematical concepts, derivation of inputs, functional relationships, and computer programming topics that concern growth of SE in contaminated eggs before processing (G_1).

A contaminated egg may bear a nominal amount of SE. In the conceptual model presented in Equations 3.1 through 3.4 above, the amount of SE growth per egg before processing, G_1, is presented as a growth factor that functions as a multiplier. In the computer model G_1 is the result obtained by dividing the number of SE in an egg just before processing by the number of SE in that egg at the time of lay. Thus, G_1 can be thought of as a summary representation of a complex set of interactions.

G_1 is treated separately from G_2 (below) to better model the log reduction from pasteurization during the processing of eggs. The amount of bacteria surviving pasteurization depends on the initial number of bacteria and the treatment efficacy. The growth behavior of SE in eggs after pasteurization (G_2) is also influenced directly by growth before processing and the log reduction from pasteurization. The model simulates individual eggs from the point of lay through consumption. To aid transparency, the individual stages of the model are presented as if these stages were independent. As shown later, the storage conditions that influence growth vary for individual eggs. Thus, G_1 is estimated for each individual egg. Ultimately, G_1 is represented by a distribution representing the variation in growth possible in all eggs. Thus, the value of G_1 varies from egg to egg. The values of G_1 developed here are expressed by a probability distribution. This distribution reflects the different amounts of growth that could occur in the population of SE-contaminated eggs from the laying house to the processor.

Growth of SE within eggs is a complex phenomenon about which the scientific evidence is somewhat vague. Conventionally, it has been argued that most eggs are initially contaminated in the albumen of the egg. The albumen is an environment that is suboptimal for SE growth. The scientific explanation for slow or poor growth of SE in albumen is based on mineral-nutrient limitation in albumen. For instance, presence of iron-binding molecules (siderophores) within albumen limit the availability of this critical element to SE (for further discussion, see Annex E).

Growth of any prokaryotic organism involves the process of binary fission, or cell division. The bacteria require nutrients in the environment to divide. Albumen does not provide the same nutritive environment as the yolk. The yolk in an egg is separated from the albumen by a thin membrane, the VM (or yolk membrane). It is hypothesized that, as the egg ages, the yolk membrane deteriorates so it ceases to completely separate nutrients in the yolk from the albumen. This deterioration depends on the internal temperature of the egg: high temperatures hasten the rate of deterioration, while low temperatures lessen it.

The hypothesis of yolk membrane deterioration, or breakdown, appears equivocal based on conflicting data sets. At this time, experimental and observational studies suggest there is some time in the life of a contaminated egg when the rate of growth of SE increases dramatically. This time is considered to be when yolk membrane breakdown (YMB) occurs. Hypothetically, the rapid growth of bacteria after this time is thought to be a result of either the bacteria penetrating the deteriorating yolk membrane or some yolk nutrients passing through the yolk membrane into the albumen where the bacteria reside (Figure 3-4).

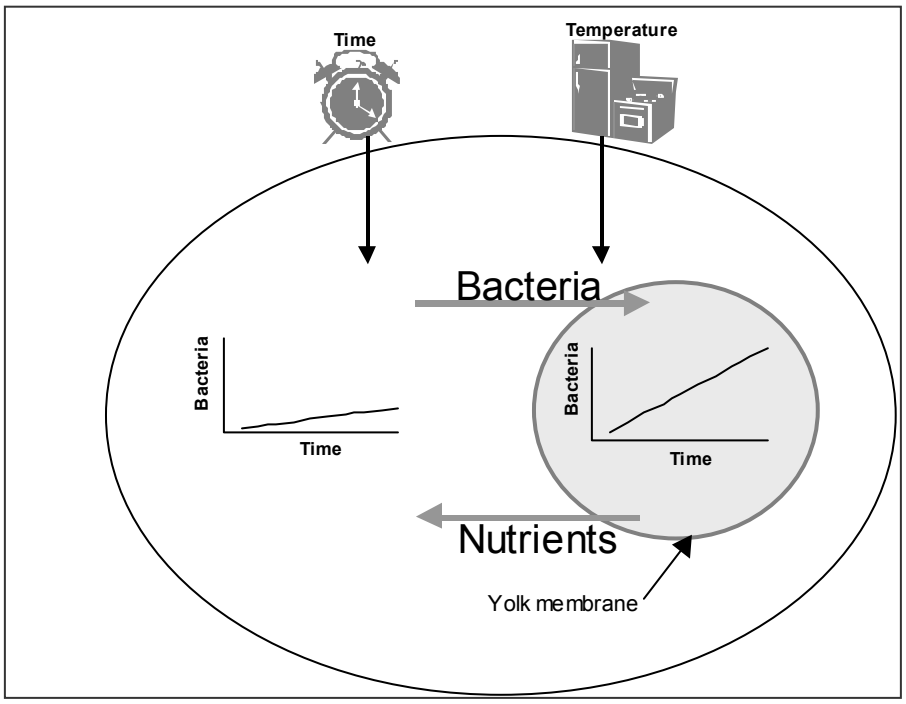

FIGURE 3-4 SCHEMATIC REPRESENTATION OF FACTORS AFFECTING THE GROWTH OF BACTERIA IN SHELL EGGS.

Both mechanisms may play a role in the sudden change in SE growth behavior in eggs. There is still much to learn about

this phenomenon. Nevertheless, at the least, assessing the risk from SE inside eggs hinges on predicting when this rapid growth can occur. Once YMB occurs, growth behavior of SE is assumed consistent with experimental studies where SE is inoculated directly into yolk material. The rate of growth inside albumen is a function of the internal egg temperature but is generally much slower than growth inside the yolk.

Mathematical concepts

The probability distribution of G_1 must be estimated for the population of all contaminated eggs. The number of bacteria in an individual egg can be modeled by estimating the growth between the time it is laid and the time just before it is processed. Dividing the ending number of bacteria in the egg by the starting number of bacteria in the egg produces the growth factor, G_1, for that egg, as shown below.

$$G_1 = \frac{\text{bacteria in egg just before processing}}{\text{bacteria in egg when laid}} \quad (3.5)$$

Let S_t be the number of bacteria in the egg at time t, where $t = 0$ when the egg is laid. The number of bacteria in an egg depends on several things, including: the number of bacteria were in the egg at the time of lay (S_0); the age of the egg (A); the type of contaminated egg (e.g., contamination initially in the albumen, on the vitelline membrane, or in the yolk) (E_i); the growth rate in the applicable compartment (G); and the time at which YMB occurs (M). S_t can then be defined as:

$$S_t = S(S_0, A, E_i, G, M) \quad (3.6)$$

Growth of bacteria in an egg depends on the factors just introduced (Figure 3-4). Along the right side of Figure 3-4 is the portion of the farm-to-table path eggs travel before pasteurization. The model determines the number of bacteria inside a particular egg at the end of storage in the layer house, after storage on farm, after transportation, and after storage at the processor. The left side of this figure shows that YMB (M) depends on storage time and temperature, the rate of cooling, and the initial bacteria in the egg (S_0). The exponential growth rate depends on time and temperature, the type of egg, and the serologic status of the egg. Because storage temperatures change as the egg moves from the layer house to on-farm storage to transport to the processor, the calculations of YMB and exponential growth rate change with time in the model. This graphic depiction of the dependencies of critical model calculations introduces the mathematical relationships described further in this section covering G_1. Furthermore, the principles of estimating growth inside eggs discussed for G_1 apply to estimating growth after pasteurization until the egg is consumed. This portion of the farm-to-table path is defined as G_2 above.

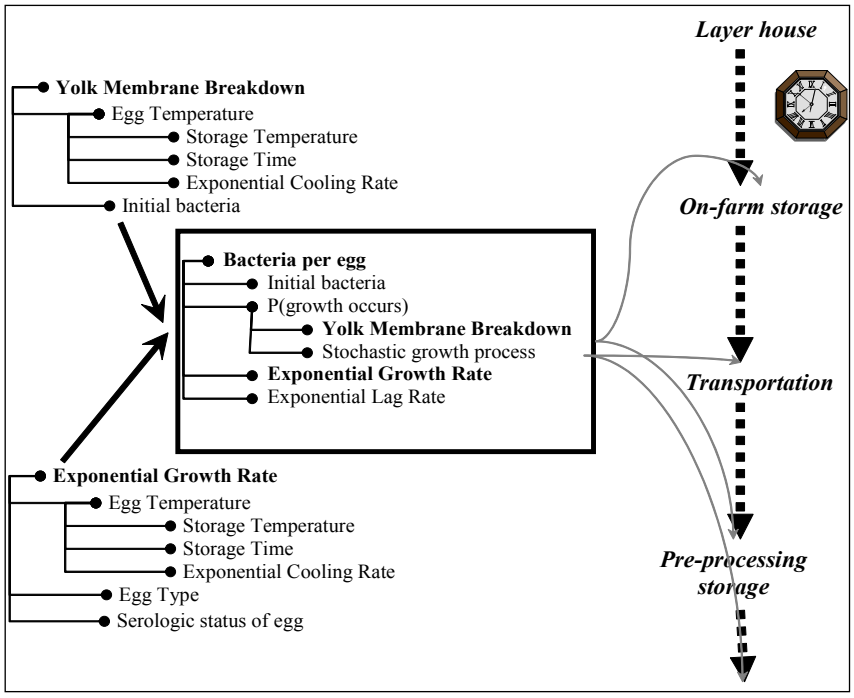

FIGURE 3-5 SCHEMATIC OF CRITICAL DEPENDENCIES AND STEPS WITHIN THE *G1* MODEL.

We know that growth rate and YMB depend on the internal egg temperature (T_t):

$$G_t = G(T_t) \tag{3.7}$$

where G_t is the exponential growth rate per day at time t and T_t is the egg temperature at time t.

$$M_t = M(T_t) \tag{3.8}$$

where M_t is the time to YMB at time t.

Later in this chapter, an additional argument is added to Equation introduce the presence of detectable anti-SE antibodies in a particular egg. The initial bacteria in the egg also influences the time of YMB.

The internal egg temperature (T_t) depends on the initial egg temperature (T_0), the ambient temperature (T_a) of storage, the time of storage (t), and the rate at which the internal egg temperature changes. For now, this cooling rate is assumed constant and equal to k. The functional dependencies of T_t are the following:

$$T_t = T(T_0, T_a, t, k). \tag{3.9}$$

By substitution, Equation 3.5 can be rewritten:

$$G_1 = \frac{S(S_0, E_i, T_0, \{t\}, \{T_a\}, \{k\})}{S_0} \tag{3.10}$$

For an individual egg, the initial number of bacteria at lay and the initial temperature at lay are fixed at the values returned by sampling from their parent distributions. An individual contaminated egg is also of a specific type. For an individual egg, however, the ambient temperature of storage is likely to vary between the times of lay and processing. The ambient temperatures also apply to particular times of storage. Similarly, the cooling rate, which depends on how the egg is packaged, is likely to change. These changes are addressed by using vectors of ambient temperatures, times, and k values (vectors are signified by the {} brackets). The time and ambient temperature profile for this egg can also be referenced.

The calculation of Equation 3.10 is not simple, in part because growth rate and YMB (Equation 3.7 and Equation 3.8) are functions of the internal egg temperature (Equation 3.9), which changes across time for a given ambient temperature and k value. The ambient temperature and k values also change across time. In the sections to follow, a solution method that

Egg Age and Time

Egg age refers to time that has elapsed since it was laid. In this model, egg age and time are the same. For example, consider an egg that is laid and remains in the layer house for 6 hours, then is stored an additional 24 hours on the farm before transportation to a processor. Transport takes 3 hours, after which the egg is stored another 24 hours before processing. When laid, the egg's age is zero and time elapsed is zero. When the egg begins to be processed, its age is the time that elapsed since lay. Therefore, Age = t = 6 + 24 + 3 + 24 = 57 hours for this particular egg.

calculates the bacterial growth in an individual egg across time by recalculating growth in small time increments is described.

Although the inputs S_0 and E_i are described in Annex B, the other inputs to Equation 10 are introduced below. Furthermore, the specific functional relationships, which are only alluded to above, must be explained. Inputs are described below under "Derivation of Storage Times, Temperatures, and Exponential Cooling Rates." Functions are described below under "Functional Relationships." Next, however, a brief description of the modeling protocol for G_1 is given. This protocol provides perspective on how the inputs and functions are used in the model. Descriptions of inputs and functions follow this section.

Modeling protocol for G_1

This section explains the computer model calculations for the G_1 phase of this risk assessment. Beginning when an egg is laid the model steps through time increments to determine the amount of growth inside a contaminated egg. The G_1 phase of growth ends just as the egg begins to be processed. The following explanation describes how the model determines the number of bacteria in a contaminated egg just before it is processed.

- Step 0: The model iteratively simulates the fate of a single egg.
- Step 1: Select the type of egg production facility where the egg was laid: The first step in modeling is selection of an in-line or off-line egg production facility from a probability distribution. The type of egg production facility determines the number of steps modeled within G_1. Distinctions between in-line and off-line facilities are explained below in "Derivation of Storage Times, Storage Temperatures, and Exponential Cooling Constants."
- Step 2: Select ambient temperature, time, and k values for steps: The time and temperature profile for the egg is determined next. Storage temperatures, times, and exponential cooling rates for the egg are selected probabilistically from frequency distributions described below. For an egg produced by an off-line facility, this profile amounts to determining several factors. These factors include: the time spent in the layer house and the ambient temperature in the layer house; the time spent in storage on the farm and the ambient temperature in the storage facility; the time spent being transported to the processing facility and the ambient temperature of the transport vehicle; and the time spent in storage at the processing facility before processing and the ambient temperature at this facility. Therefore, before the model calculates growth within the egg, it determines the total time and ambient temperature history for that egg. Similarly, exponential cooling rates applicable to each storage period are determined for the egg.

  Time and temperature of egg storage are not correlated in the model. In other words, eggs stored for 18 days are just as likely to be held at 67.5°F as those stored for 2 days. It may seem reasonable to assume that someone storing eggs for a longer period would be more likely to refrigerate the eggs. On the other hand, an argument can be made against this possibility because someone storing eggs for a long period may be less able to manage storage times and temperatures and these eggs could be stored at higher temperatures. Lacking direct evidence of a correlation of time and temperature, it is not reflected in the model.
- Step 3: Select egg contamination location: The type of egg is selected probabilistically based on the frequencies described in Table 3-2. The type of contaminated egg

determines the initial level of SE within the egg and the growth characteristics for that egg.

Nine types of shell eggs are modeled.

1. Shell: Inner shell membrane contaminated, no growth until after YMB
2. Alb C G: Albumen contaminated close to yolk, growth can occur before and after YMB
3. Alb C N: Albumen contaminated close to yolk, no growth until after YMB
4. Alb F G: Albumen contaminated far from yolk, growth occurs before and after YMB
5. Alb F N: Albumen contaminated far from yolk, no growth until after YMB
6. VM Low: Vitelline membrane contaminated, low initial contamination egg
7. VM High: Vitelline membrane contaminated, high initial contamination egg
8. Yolk Low: Yolk contaminated, low initial contamination egg
9. Yolk High: Yolk contaminated, high initial contamination egg

The initial level of contamination for types 1, 2, 3, 4, and 5 eggs are randomly selected according to the lognormal distributions described in Table 3-3. Initial levels of contamination for type 6 and 8 eggs are randomly selected according to a Poisson distribution. Contamination types 7 and 9 start with one organism but begin immediate exponential growth.

- Step 4. Aging of the egg: Time is incremented for each egg in fraction of day units that can be specified and varied by the user. The model allows the user to set the increment at any amount desired. The stability of the outcome distribution for G_1 depends somewhat on size of the time increment. A smaller increment allows more precision in bacterial growth calculations but takes additional run time.
- Step 5. Calculate internal temperature at each time increment. The internal temperature of the egg for each time increment is calculated. This internal temperature, in turn, determines how much growth will occur in that egg during that time.
- Step 6. Calculate time of YMB: For each time increment, YMB occurrence is modeled for all egg types but 8 and 9 above. Once YMB occurs in an egg, this step is skipped for future time increments.
- Step 7. Exponential growth rates: Depending on where the contamination resides within the egg, an exponential growth rate multiplier is calculated for each time increment. Because egg types 3 and 5 do not experience growth within the albumen, this step is skipped for these eggs until YMB occurs.
- Step 8. Calculating growth in eggs: An algorithm is used to select the number of bacteria in the egg at each time increment in a deterministic fashion. Alternatively, if stochastic growth is assumed, as explained under the "Functional Relationships" section, then the number of bacteria is only determined at the end of each step in the model. In this case, the number of bacteria is calculated after layer house storage, after on-farm storage, after transportation, and after pre-processing storage.

Derivation of storage times and temperatures and exponential cooling constants

An egg experiences different environments as it moves from the layer house to on-farm storage to a truck for transport and to a processor. In the model, these environments are characterized by their ambient temperatures and the packaging material used to store the eggs. For a particular

egg, the ambient temperature in the layer house is probably not the same as the ambient temperature when it is stored at the processor. Furthermore, eggs may be stored in a variety of manners. In the layer house, they simply sit on conveyor belts awaiting collection. Elsewhere, they may be stored in trays on racks, in boxes, or in cardboard or Styrofoam cartons. The manner of storage affects the rate at which the internal egg temperature equilibrates to the ambient temperature. The cooling rate, therefore, depends on the packing method and material.

Non-data-based Assumptions
In some instances data is not available to estimate parameters for the model. In such cases values are assumed, as explained below for times and temperatures of egg storage.

As mentioned before, an individual egg's temperature can be characterized by vectors of ambient temperature, storage time, and exponential cooling rate. For example, it is assumed that the layer house an egg was laid in has a particular ambient temperature during the time the egg remains in the house. The first element of the ambient temperature vector for this egg is the layer house temperature. The first element of the storage time vector is the time that the egg spends in the layer house. The first element of the exponential cooling rate vector is the applicable cooling rate for an egg sitting on a conveyor belt. Subsequent elements for these vectors will refer to on-farm storage, transportation, and preprocessing storage. Therefore, these vectors each contain four different values reflecting the environmental characteristics of the different places an egg travels between lay and processing. For example, the ambient temperature will include a single air temperature for each of the on-farm, storage, transportation, and pre-processing storage steps. These will each have been sampled from a distribution of possible air temperatures. The same is true for storage time and exponential cooling rate.

In the model, each egg is modeled independently of every other egg. If two eggs are handled in exactly the same manner, then these eggs are probably produced in the same layer house and are packaged, transported, and processed together. Such associations are likely to occur through processing. However, because the model determines the likelihood from a single egg during each iteration, it seems reasonable to treat each egg independently.

The probability distributions for storage time and temperature and the exponential cooling rates are estimated from available data to represent the natural variability in these values. The data and the estimation procedures are described in the remainder of this section.

Eggs are produced in either an in-line or an off-line facility. In-line facilities have egg-processing equipment on the same premises as the layer houses. Eggs produced in such facilities generally take less time to process than off-line facilities. Off-line facilities must transport their eggs to an off-site processor. Eggs produced in these facilities are usually stored somewhere on the farm to await transport to a processing facility some distance from the farm. A national survey of the layer industry in 1999[1] found 13.5% of egg-producing farms were in-line facilities. Off-line processing was used by the remaining 86.5% of farms. Egg handling between the time of lay and the time they are processed for retail sale varies based on whether the eggs are produced in an in-line or off-line facility. The model reflects these differences.

To account for changing ambient temperatures and cooling rates, k values and time and temperature effects are explicitly considered in the model for the following steps in the handling process: laying house; on-farm storage (off line only); transportation to the processor (off line only); and pre-processing storage.

Although the times, ambient temperatures, and k values for each of these steps vary among the population of all eggs produced in the U.S., these values are constant for individual eggs in the model. To illustrate this assumption, consider the ambient temperature inside laying houses.

It varies from laying house to laying house because it depends on management practices such as the thermostat setting a particular manager chooses, the number of fans in the house, climate, and weather. Nevertheless, the ambient temperature an individual egg produced in a specific laying house experiences may be reasonably constant during the time that egg awaits collection. This model treats it as constant.

Storage times

Table 3-5 shows available data for time inputs.

TABLE 3-5 AVAILABLE INFORMATION ON TIME INPUTS FOR G1.

On Farm[a]		Pre-processing[b]		
Average Number of Days between Egg Pickups	Percent Farm Sites	Average Number of Days	Percent Producers (in line)	Percent Packers (off line)
1 to 2	48.5	<1	23%	51%
3 to 5	45.1	1 to 3	46%	29%
6 to 9	6.2	4 to 6	22%	11%
10 or more	0.2	7 to 10	7%	6%
Total	100	11 to 15	1%	1%
		16 to 20	1%	1%
		> = 20	0%	0%
		Total	100%	99%

[a]Source: National Animal Health Monitoring System.[1]

[b]Source: Research Triangle Institute.[2]

Information was available only for the time of on-farm storage and the time eggs were stored at the processor for both in-line and off-line processors. Furthermore, the information was reported in ranges. For on-farm storage, the reported value is the "average number of days between egg pickups." Thus, it is reasonable to assume that the average egg being picked up would have been stored for about half of the time reported for the range.

Lognormal distributions were fit to the average number of days between egg pickups. These distributions were assumed to describe the variability in average storage times among farms and among processors. Distributions were fit by minimizing the squared differences between the cumulative empirical distribution and the theoretical cumulative lognormal distribution. A lognormal distribution was chosen because it "is useful for modeling naturally occurring variables that are the *product* of other naturally occurring variables."[3] The times of storage are considered the products of many other factors (e.g., management, weather, market). Reasonable visual fits to relatively limited information, as seen in Figure 3-6, are consistent with the choice of a lognormal distribution. Figure 3-7 compares a lognormal distribution with the storage time at the processor for off-line eggs, and Figure 3-8 shows a similar comparison for in-line eggs. Note that because these inputs are modeled with lognormal distributions, values for storage time more extreme than those observed can be returned. These extreme values are limited in the model by truncating the lognormal distribution at the 99.9th percentile.

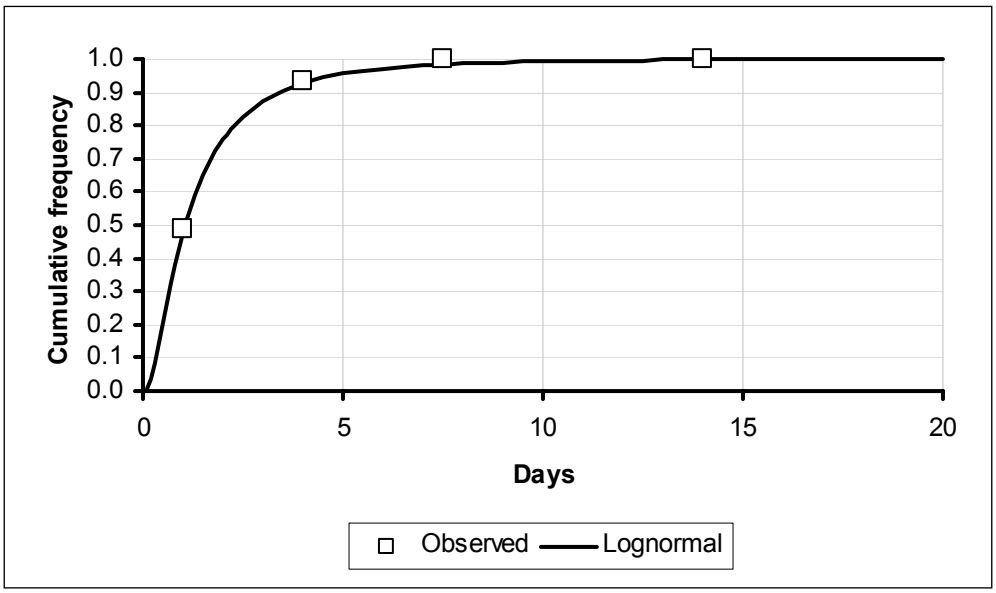

FIGURE 3-6 COMPARISON OF OBSERVED AND PREDICTED RESULTS FROM A LOGNORMAL DISTRIBUTION FOR ON-FARM STORAGE TIME.

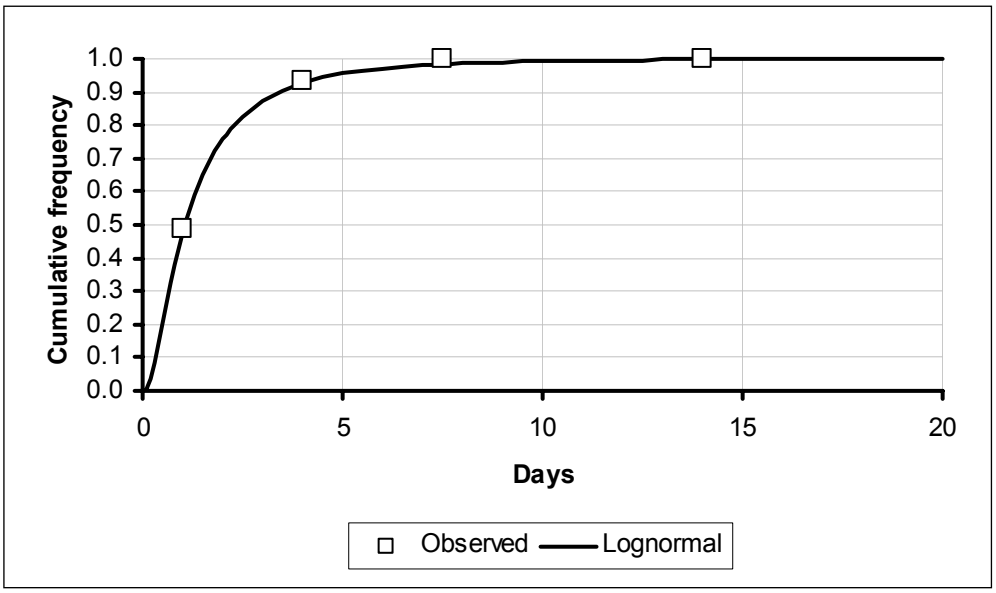

FIGURE 3-7 COMPARISON OF OBSERVED AND PREDICTED RESULTS FROM A LOGNORMAL DISTRIBUTION FOR STORAGE TIME OF OFF-LINE EGGS BEFORE PROCESSING.

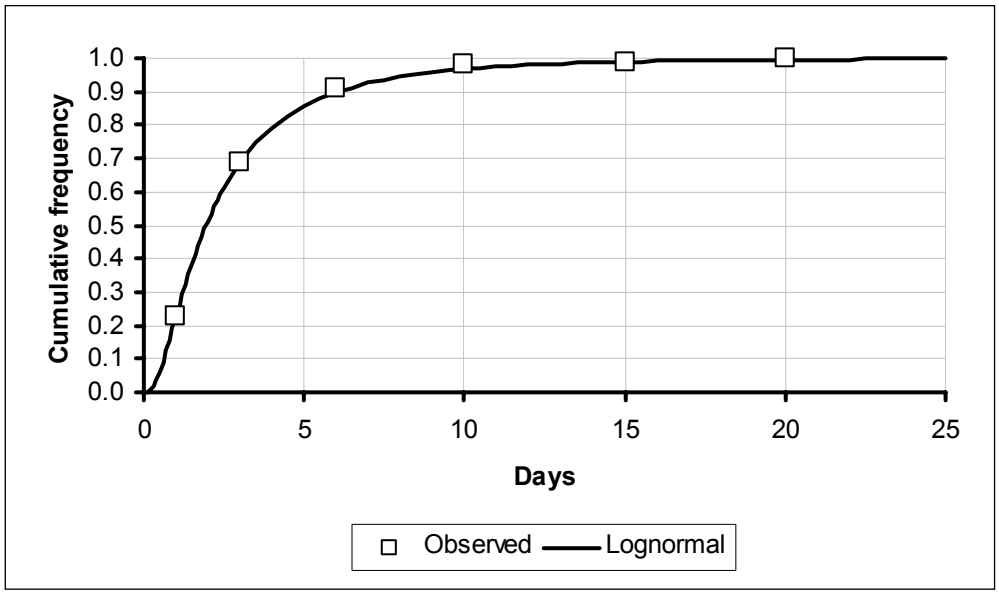

FIGURE 3-8 COMPARISON OF OBSERVED AND PREDICTED RESULTS FROM A LOGNORMAL DISTRIBUTION FOR STORAGE TIME OF IN-LINE EGGS BEFORE PROCESSING.

Data to estimate the time eggs remain in the layer house, distinct from the time eggs are stored on the farm, were unavailable. A lognormal distribution was used to represent the variability in this time. Eggs are normally collected from the layer house twice a day; thus, the average egg remains in the layer house 6 hours or 0.25 days until it is collected. A value of ln(0.25 days) = –1.39 was used for the mean of the lognormal distribution. The standard deviation was set equal to the 0.59 standard deviation for on-farm storage time. There are also no data for the time it takes to transport eggs to the processor. An arbitrary value of 6 hours was selected to represent the time it takes to transport eggs from the farm to the processor. Assuming a lognormal distribution, the standard deviation was set by default to the same value used for the layer house and on-farm storage. Table 3-6 shows the modeled parameters for the lognormal distributions of storage time for the four steps before processing.

TABLE 3-6 PARAMETERS FOR LOGNORMAL DISTRIBUTIONS FOR TIME OF EGG STORAGE AT DIFFERENT MODEL POINTS.

		Time	
Input	**Supported by Data?**	**Mean**	**Std Dev**
Layer house	No	-1.39	0.59
On-farm	Yes	0.72	0.59
Transportation from farm	No	-1.39	0.59
Pre-processing off line	Yes	-0.04	1.33
Pre-processing in line	Yes	0.67	0.89

Storage temperatures

The temperature of the egg is critically important to the growth of any SE present in the egg. To estimate this growth, ambient air temperatures are needed to estimate changes in the temperature of the egg. This section presents information on ambient air temperatures in the layer house, on the farm, during transport, and in storage before processing. Table 3-7 shows available information regarding ambient temperature during on-farm storage, during transport to processing, and during pre-processing storage. This information does not directly pertain, however, to the layer house environment.

TABLE 3-7 AVAILABLE TEMPERATURE INPUTS FOR G_1.

On-Farm[a]		Transportation to Processor[b]		Storage before Processing[b]		
Temperature for Egg Storage	**% Farm Sites**	**Temperature of Refrigerated Trailer**	**% Trailers**	**Temperature of Refrigerated Storage Space**	**% Producers (in line)**	**% Packers (off line)**
< 50°F (10°C)	21%	Unrefrigerated	6%	Unrefrigerated	0%	0%
50-59°F (10-15°C)	51%	<45°F (7.2°C)	18%	<45°F (7.2°C)	12%	37%
≥ 60°F (15.6°C)	28%	45-59°F (7.2-15°C)	66%	45-59°F (7.2-15°C)	66%	56%
Total	100%	60-75°F (15.6-23.9°C)	10%	60-75°F (15.6-23.9°C)	21%	7%
		≥75°F (23.9°C)	0%	≥ 75°F (23.9°C)	1%	0%
		Total	100%	Total	100%	100%

[a]Source: National Animal Health Monitoring System.[1]
[b]Source: Research Triangle Institute.[2]

Information in Table 3-7 is available in ranges only. Lognormal distributions were fitted using the mid point of temperature class as the most likely empirical value. The following figures compare cumulative empirical frequency distributions with lognormal distributions for temperature of on-farm storage (Figure 3-9), transportation to processor (Figure 3-10), pre-processing storage of off-line eggs (Figure 3-11), and pre-processing storage of in-line eggs (Figure 3-12).

The distribution for ambient temperature in the layer houses is derived as follows. Although commercial egg-laying facilities generally monitor and control the house environment closely, there was no survey evidence available describing the variability of temperatures across layer houses. There is, however, evidence that suggests likely temperature ranges. First, the Agricultural Research Service states "Laying houses maintained between 57 and 79°F (14 and 26°C) are desirable."[4] Table 3-8 provides evidence of recommended temperature variability in layer houses. It gives recommended ambient temperatures for layer houses by week of flock production. Recall that flocks begin production when the hens are about 20 weeks of age. Together, this evidence suggests that ambient temperatures might vary from layer house to layer house by age of hen and might vary within houses by time of day. Furthermore, we can assume that ambient temperature is influenced by time of year—the temperature would be hotter in summer and cooler in winter.

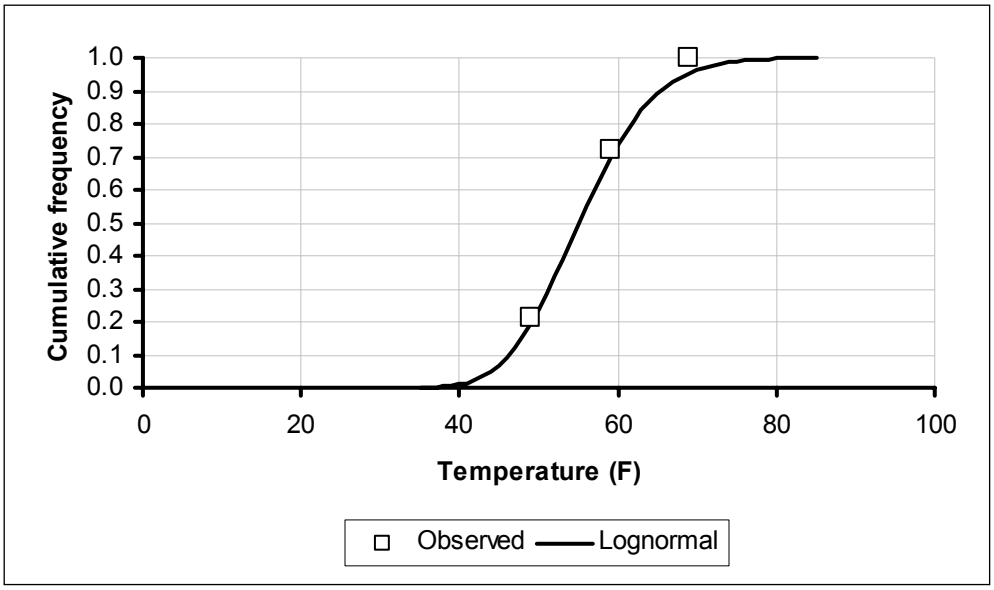

FIGURE 3-9 COMPARISON OF OBSERVED AND PREDICTED RESULTS FROM A LOGNORMAL DISTRIBUTION FOR STORAGE TEMPERATURE OF EGGS STORED ON THE FARM BEFORE TRANSPORTATION TO THE PROCESSOR.

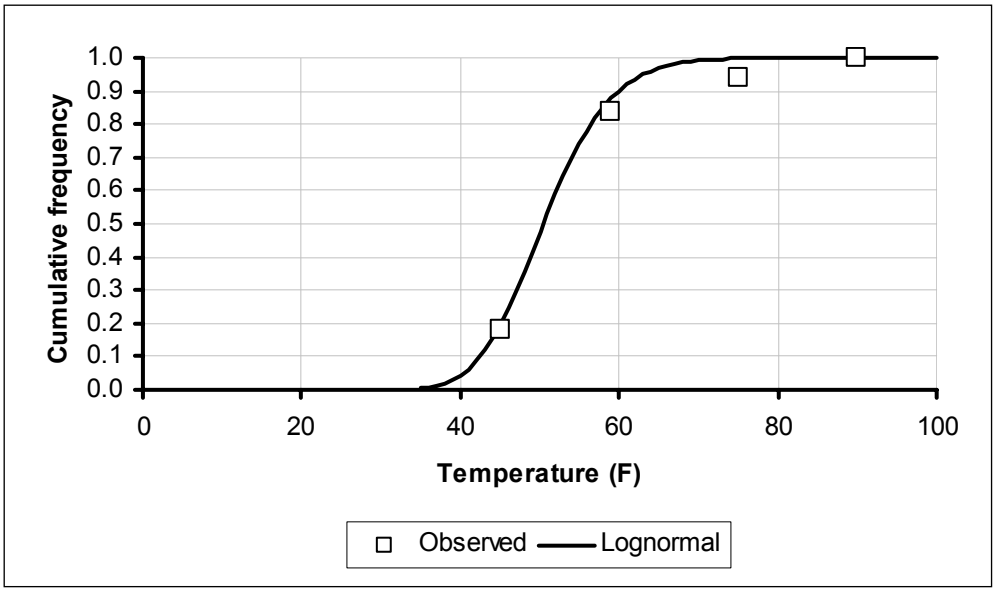

FIGURE 3-10 COMPARISON OF OBSERVED AND PREDICTED RESULTS FROM A LOGNORMAL DISTRIBUTION FOR AMBIENT TEMPERATURE DURING TRANSPORTATION OF EGGS TO THE PROCESSOR.

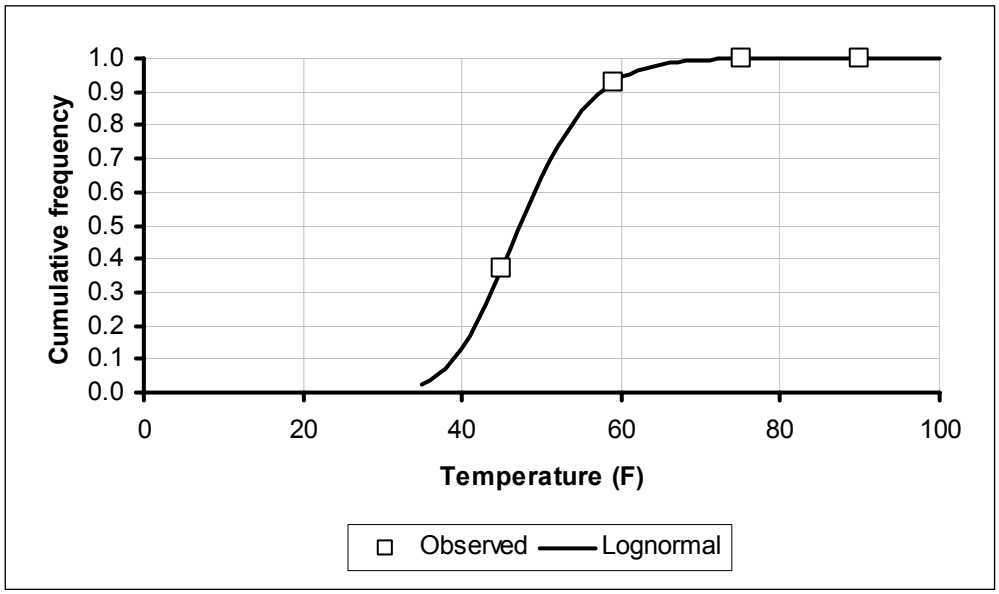

FIGURE 3-11 COMPARISON OF OBSERVED AND PREDICTED RESULTS FROM A LOGNORMAL DISTRIBUTION FOR STORAGE TEMPERATURE OF OFF-LINE EGGS BEFORE PROCESSING.

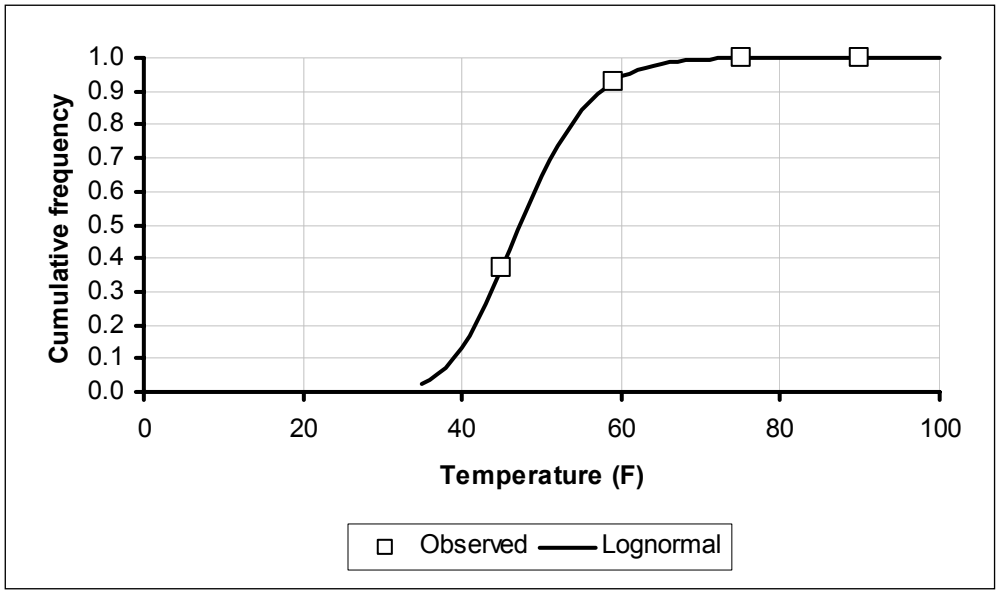

FIGURE 3-12 COMPARISON OF OBSERVED AND PREDICTED RESULTS FROM A LOGNORMAL DISTRIBUTION FOR STORAGE TEMPERATURE OF IN-LINE EGGS BEFORE PROCESSING.

TABLE 3-8 RECOMMENDED AMBIENT TEMPERATURES BY WEEK OF FLOCK PRODUCTION.[5]

Week of Production for Flock	Ambient Temperature (F)
1	90
2	85
3	80
4	75
5	70
6 until end of production	70

Given the complexity of factors influencing ambient temperature in layer houses and the absence of survey data from which to infer a probability distribution that captures the natural variability in temperatures, it is assumed that the variability in temperatures among layer houses follows a lognormal distribution. Furthermore, the mean temperature within layer houses is assumed to be 75°F (i.e., room temperature). Because the standard deviation varies little among the steps for which there are data, the standard deviation is assumed approximately the same within the layer house as for all the other steps, in this case 0.15. Table 3-9 shows the modeled parameters for the lognormal distributions of storage temperature for the four steps before processing.

TABLE 3-9 PARAMETERS FOR LOGNORMAL DISTRIBUTIONS FOR TEMPERATURE OF EGG STORAGE AT DIFFERENT MODEL POINTS.

		Temperature	
Input	Supported by Data?	Mean	Std Dev
Layer house	No	4.32	0.15
On-farm	Yes	4.01	0.14
Transportation from farm	Yes	3.92	0.14
Preprocessing off-line	Yes	3.86	0.15
Preprocessing in-line	Yes	3.97	0.14

Determination of exponential cooling rates

As eggs are stored, temperatures may change; when stored in refrigerated environments, eggs cool. Cooling slows or stops the growth of *Salmonella* and, as such, warrants separate consideration.

The cooling rate, k, describes the reduction in degrees of temperature per hour of storage at an ambient temperature and its units are in $\frac{1}{hrs}$. The smaller the value of k, the less change in egg temperature occurs in an hour. The more insulated an egg is from its environment, the lower the k value is likely to be. For example, an egg stored in a large cardboard box with hundreds of other eggs surrounding it is insulated from the ambient air temperature. In contrast, an egg sitting on a conveyor belt is not insulated and quickly adapts to the ambient air temperature. Very rapid changes in egg temperature are associated with large k values. The function that

An Example of Using *k* Values to Determine Internal Egg Temperature

Using a k value of 0.10, an ambient temperature (T_a) of 12°C and a starting internal egg temperature (T_{i0}) of 20°C, the equation for determining the internal temperature of an egg after 3 hours is

$$T_{i3} = e^{\left(\frac{-k}{hours} x\, t\, hours\right)} \text{ x } (T_{i0} - T_a) + T_a$$
$$= e^{(-0.10 \text{ x } 3)} \text{ x } (20 - 12) + 12 = 17.9$$

predicts how egg temperature changes with time, using this cooling rate, is described later in this chapter. A detailed discussion of the derivation of cooling rates (i.e., *k* values) is provided in Annex D. Some key findings of that analysis are presented in Table 3-10. This analysis suggests that *k* values range from 0.0063 to 0.615 depending on how the eggs are packaged. Note that the *k* values in Table 3-10 are averages estimated from the experimental evidence. Furthermore, these *k* values were estimated from measurements of eggs in the center of flats, cases, or pallets.

TABLE 3-10 ESTIMATED COOLING RATES OF EGGS WITH VARIOUS PACKING METHODS.

Packing Method	Exponential Cooling Rate per Hour, *k*
Pallet of cardboard (off line) (constant ambient temperature)	0.0063
Pallet, cardboard (off line) (fluctuated ambient temperature)	0.0064
Pallet of cardboard cases	0.0075
Pallet of cardboard (in line)	0.0094
Individual case/basket temperature	0.0131
Pallet, cardboard cases (traditional cooling)	0.0215
Pallet of cardboard cases (flats)	0.0472
Pallet of plastic basket cases	0.0524
– Plastic and fiber filler flats, fiber case, closed – Formed and folded cartons, fiber case, closed – Formed and folded cartons, open stack – Formed and folded cartons, wood case – Plastic and fiber filler flats, wood case – Plastic and fiber filler flats, fiber case, open	0.0628 0.1000
(1) Filler flats (2) Fiberboard case (30 dozen)—foam cartons (closed top) (3) Fiberboard case (30 dozen)—foam cartons (slotted top)	 0.2280
Plastic and fiber filler flats, open stack	0.2750
Fiber filler flats or fiber cases with forced air cooling through opening in cases	0.6150

The above table shows that the cooling rate differs by packing methods. It also varies somewhat for the same basic packing method. To simplify the analysis, three basic packing methods are selected and the cooling rate for the center egg in each is assigned as shown in Table 3-11. These selected cooling rates for each packing method are supported with three separate arguments: simplicity, consistency with the data, and model predictions.

TABLE 3-11 EXPONENTIAL COOLING RATES FOR USE IN BASELINE MODEL (CENTRAL EGG).

Packing Method	Exponential Cooling Rate per Hour, *k*
Cases within a pallet	0.01
Stacks of cartons or flats within or without a case	0.10
Egg exposed to ambient air or carton in home refrigerator	1.00

Simplicity

Packing and packaging materials for eggs vary, as do cooling methods and airflow in layer houses, farms, processing plants, vehicles, retail facilities, and homes. Attempts to disaggregate cooling constants by packing material or cooling methods are likely to be frustrated lack of data. Furthermore, the effect of differences in cooling rates on internal egg temperature diminishes as

the cooling constant increases. Figure 3-13 reveals the effect of cooling rate on the change in egg temperature. If we calculate the change in temperature on an hourly basis, we can show that at an ambient temperature of 10°C and an internal egg temperature of 41.1°C, it would take approximately 15 days to cool the internal temperature below 11°C given a cooling rate of 0.01, and approximately 1 day if the cooling rate is 0.1. Nevertheless, it would take 5 hours given a cooling rate of 0.75 and approximately 4 hours if the cooling rate were 1.0. Therefore, little difference is apparent between cooling rates of 0.75 and 1.0.

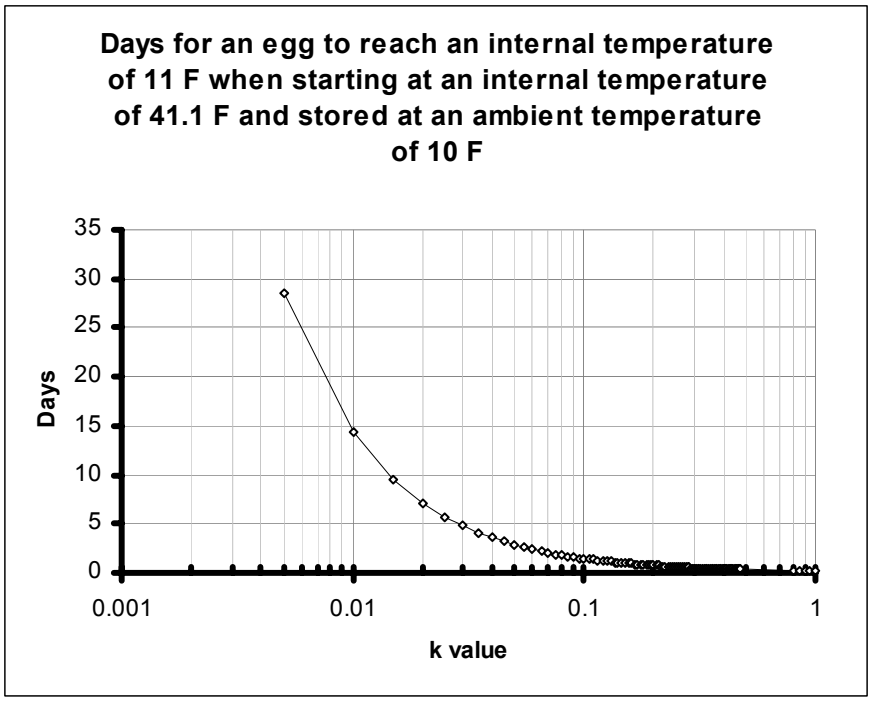

FIGURE 3-13 EFFECT OF *K* VALUE ON DAYS FOR AN EGG TO REACH A GIVEN INTERNAL TEMPERATURE.

Exponential cooling rates for all eggs are represented by three values: 0.01 for pallets, 0.1 for cases, and 1.0 for ambient air and individual cases. Nevertheless, the exponential cooling rate for an individual egg can vary from 0.01 to 1.0 depending on where that egg is stored within a case or pallet. These cooling rates are used to predict the internal temperature of eggs at different times along the farm-to-table continuum. For example, an applicable cooling rate for an egg held in the layer house is used to predict the internal temperature of that egg just before the egg moves into storage elsewhere on the farm. Because internal egg temperature directly influences the rate of *Salmonella* growth inside an egg, this value must be selected from a distribution before estimating growth.

Consistency with data

Table 3-10 shows six *k* values for eggs that have been palletized: 0.0063, 0.0064, 0.0075, 0.0094, 0.0215, and 0.0472. These values are applicable to eggs in the center of pallets. The average of these six values is 0.016. For simplicity, eggs in the center of pallets are assumed to have a *k*

value of 0.01. Table 3-10 shows 11 values for eggs in cases or flats: 0.0131, two instances of 0.0628, four instances of 0.10, three instances of 0.228, and 0.275. The *k* value for "fiber filler flats or fiber cases with forced air cooling through opening in cases" is not included because it is believed to be more representative of eggs that are exposed to ambient air than eggs in the center of a case or stack of flats. The average of these 11 values is 0.136. For simplicity, eggs in the center of cases or flats are assumed to have a *k* value of 0.1.

No *k* values are shown in Table 3-10 for eggs exposed to ambient air or in a single carton in a refrigerator. In a layer house, eggs are generally exposed to ambient air. These eggs usually sit on an egg belt until collected. In many home refrigerators, eggs are in a single dozen container in which all eggs are outside of the container. Although these situations are not shown among the packing methods in Table 3-10, these eggs are assumed to have a *k* value at least as large as or larger than that reported for "fiber filler flat or fiber cases with forced air cooling through the openings in the cases." This is because forced air cooling provides mechanical ventilation that should move air into the container and nearly surround eggs with the ambient air. The average value of *k* for this packing method is 0.615; Annex D shows it ranged from 0.39 to 0.97. Consequently, a *k* value of 1.0 is used for eggs exposed to ambient air or in a carton in a refrigerator.

Model predictions

The discussion above shows that the cooling rates used are consistent with the data. This section presents the results of modeling the rate of cooling. As noted earlier, *k* values were estimated from measurements of the temperature of the eggs in the center of flats, cases, or pallets. These eggs do not represent all eggs within a pallet. They are the extreme instance. To adjust for the nonrepresentative nature of the center egg cooling rate, the rate is adjusted by the following formula for eggs not in the center of a pallet (found in Annex D).

$$\begin{pmatrix}\text{Adjusted}\\ \text{cooling}\\ \text{constant}\end{pmatrix} = \begin{pmatrix}\text{Cooling}\\ \text{constant}\\ \text{in center}\\ \text{of pallet}\end{pmatrix} \times \left(\frac{\text{Distance from perimeter to center of pallet}}{\text{Distance from perimeter to specified egg}}\right)^2 \tag{3.11}$$

A pallet measures approximately 3 ft wide x 4 ft long x 6 ft high. Given these dimensions, approximately 40% of eggs would be within 4 inches of the perimeter of the pallet and would thus have an adjusted cooling constant of at least 20 times that of an egg in the center:

$$(0.01)\text{ x }\left(\frac{18\,\text{inches}\,(\text{distance from nearest edge to center of pallet})}{4\,\text{inches}}\right)^2 \approx 0.20 \tag{3.12}$$

The calculation is the same for a case except a case measures approximately 18 inches by 12 inches by 14 inches. If we assume a cooling constant of 0.01 for pallets and a cooling constant of 0.1 for cartons, then the predicted cooling constants at varying distances from the perimeter can be calculated and are shown in Figure 3-14.

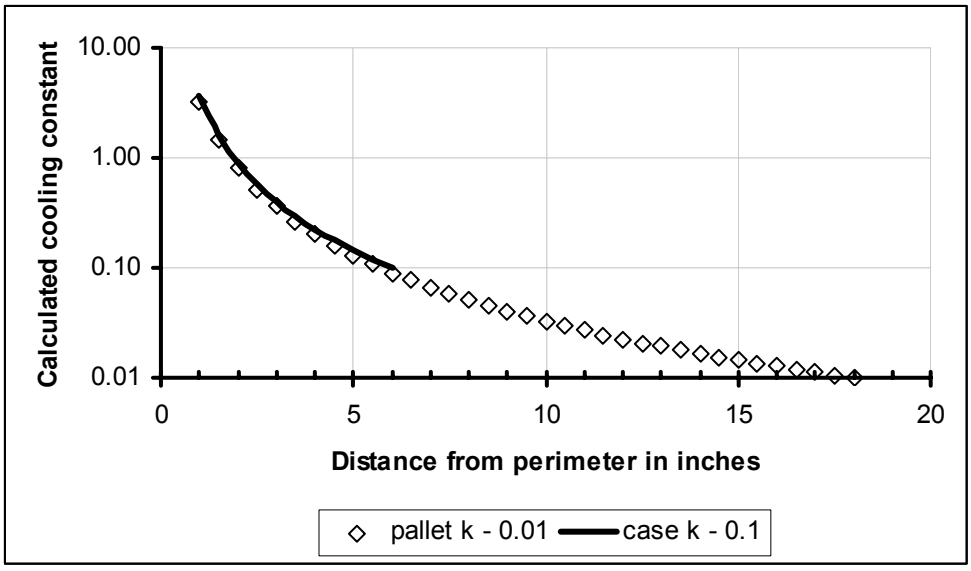

FIGURE 3-14 PREDICTED COOLING CONSTANTS FOR VARYING DISTANCES FROM PERIMETERS FOR PALLETS ASSUMING A CENTRAL EGG COOLING CONSTANT OF 0.01 AND CASES ASSUMING A CENTRAL EGG COOLING CONSTANT OF 0.1.

Figure 3-14 shows that the cooling rates for cases are very close to those for pallets over a limited range of distances. For instance, at a distance of 6 inches from the perimeter (the central egg in a case), the predicted cooling rate for the pallet is 0.09 per hour. At a distance of 2 inches from the perimeter, the predicted cooling rate for the pallet is 0.81 per hour and for a case, it is 0.9 per hour. Thus, the model predictions give consistent results across cases and pallets. Furthermore, the predictions of cooling constants around 1.0 for eggs within 2 inches of the perimeter in pallets or cases lends support to the assumption that eggs exposed to ambient air or in cartons in a refrigerator have a cooling constant of 1.0.

Distribution of exponential cooling rates in production and processing

Among egg producers and processors, egg storage practices vary. For example, some producers may use pallets to store their eggs, while others prefer to use cartons or flats. The following describes the estimated fraction of production or processing facilities that use the three basic storage practices of cases within a pallet, stacks of cartons or flats within or without a case, and eggs exposed to ambient air in a carton in a refrigerator. These fractions are used to determine the applicable cooling rate for each modeled egg during its travels from the layer house to the processor.

Layer House

In a layer house, eggs are provided reasonably unfettered access to the ambient air. These eggs usually sit on an egg belt until collected. An exponential cooling constant of 1.0 is assumed for all eggs in a layer house.

On-Farm Storage

Within the model, on-farm storage is assumed to apply to off-line facilities only. What might be thought of as on-farm storage for in-line facilities is modeled as pre-processing storage. The

NAHMS survey[1] of the U.S. layer industry found 81.5% of farms that were off-line facilities used reusable plastic flats to store and transport eggs off the farm. The remaining 18.5% of such farms used disposable fiber flats. These findings actually highlight the fact that most commercial egg producers are likely to store and transport eggs, in line or off line, in flats that are placed on wheeled racks for ease of movement. It seems unlikely that eggs would be stored in boxes on pallets before the eggs are processed. This possibility is accounted for in eggs stored on the farm by assuming that 1% of all eggs might be transported in cases on pallets from the farm to the processor. These eggs would have a *k* value of 0.01. The other 99% of eggs would be transported in flats on racks and would have a *k* value of 0.1.

Cooling constants for each egg are adjusted to account for the egg's distance from the perimeter. Random draws are taken from three uniform distributions to represent the egg's three-dimensional location in a case or pallet. The value representing the closest outside surface is selected as representing the egg's distance to the perimeter. In this manner, a different cooling rate is chosen for each egg that passes through this processing step.

Transportation

Within the model, transportation applies to off-line facilities only. The same packaging used for storing eggs on the farm is assumed to be used for transportation. Thus, the same *k* values and frequencies are used for transportation that were used to model on-farm storage. For an individual egg, the *k* value is equal to the *k* value the egg had on the farm.

Pre-processing storage

Storage before processing is common to both in-line and off-line facilities. The same packaging used for storing eggs on the farm and for transportation is used for pre-processing storage. Thus, the same *k* values and frequencies used for pre-processing storage for off-line eggs are used to model on-farm storage. Cooling constants for storing eggs at in-line facilities are the same as for off-line facilities with the exception that no eggs would be stored in cases and pallets.

Table 3-12 summarizes the exponential cooling constants used in the model. Note that a cooling constant of 0.01 represents storage in pallets, and a cooling constant of 0.1 represents storage in individual cases or racks. These cooling constants are for the central egg; the cooling constant for a specific egg is adjusted with Equation 3.11.

TABLE 3-12 FRACTION OF THE CENTRAL EGGS AT DIFFERENT COOLING CONSTANTS IN THE STEPS BEFORE PROCESSING.

Location		Fraction of Central Eggs at Given *k* Value		
		0.01	**0.1**	**1**
Off line	Layer house			1.00
	On-farm storage	0.01	0.99	
	Transportation	0.01	0.99	
	Pre-processing storage	0.01	0.99	
In line	Layer house			1.00
	Pre-processing storage		1.00	

Functional relationships

In this section, the relationships presented earlier are revisited to provide the detailed calculations for G_1. The complexities alluded to earlier are added to the model in this section. Internal egg temperature is an important input calculated from the ambient temperature and cooling rate. YMB depends on internal egg temperature and time of storage. The rate of growth of *Salmonella* inside the egg depends on YMB and internal egg temperature. Finally, the rate of growth, in conjunction with the initial number of SE and amount of time available, determines the number of bacteria in an egg serving. The algorithms for predicting internal egg temperature and for estimating YMB, growth rate, and the total bacteria inside the egg are presented in this section.

Internal egg temperature, T_t

Internal egg temperature changes with time as a function of its initial temperature, the ambient temperature, and the rate of cooling (see Table 3-13). Note that the units for time (hours) must match up with the units for the k value (hours^{-1}). This equation's derivation can be found in Annex D.

TABLE 3-13 DETERMINATION OF INTERNAL EGG TEMPERATURE (T_T).

Variable Name	Description	Estimation
T_a	Storage temperature for applicable time	Lognormal distribution from data
T_0	Internal egg temperature at time of lay	40°C (104°F)
k	Exponential cooling rate (hours^{-1})	
T	Storage time in hours	Lognormal distribution from data
T_t	Internal egg temperature at time = t	$e^{(-kt)}(T_0 - T_a) + T_a$

Yolk membrane breakdown, M_t

YMB is a concept that applies to eggs that are not initially contaminated in the yolk. For the SE inside such eggs, growth is assumed to occur slowly or not at all until the bacteria have access to the rich nutrients of the yolk. The yolk membrane provides a physical barrier to rapid bacteria growth, but the membrane's permeability increases across time as a function of the internal temperature of the egg. The likelihood that YMB occurs for a specific egg at a specific time depends on the current and past ambient temperatures that the egg has experienced. See Annex E for more detail.

Estimation of the cumulative probability of YMB, $P(M_t)$, is based on the calculations shown in Table 3-14. Although the cumulative likelihood of YMB increases monotonically with time, the actual time YMB occurs is a random occurrence for a particular egg. Therefore, two eggs handled in exactly the same conditions will have identical cumulative probability distributions across time for YMB. However, one egg's yolk membrane may break down at the 5th percentile of this distribution, while the other may not break down until the 95th percentile of this distribution.

TABLE 3-14 ESTIMATION OF THE CUMULATIVE PROBABILITY OF YMB.

Variable name	Description	Estimation
T_t	Internal egg temperature at time = *t*	See Table 3-13
Ω	Multiplier to account for data discrepancies	Either 1 or 2.53
S_0	Initial bacteria in egg at time of lay	Random value depending on egg type, E_i
d, f, g, k	Coefficients estimated from statistical fitting to data	Constants
YMB_b	Intermediate calculation for estimating $P(M_t)$	$\Omega e^{(e^{(F+G \times T_t)}-K)}$ + [0.0032(S_0 – 500) ÷ (8Ω)
t	Storage time in hours	Lognormal distribution from data
$P(M_t)$	cumulative probability of YMB	$1 - e^{(-e^{(-e^{D}+YM\beta b \times t)})}$

Annex E provides more detail about the input Ω in Table 3-14. It is a multiplier included to account for discrepancies in predictions from two sets of data concerning YMB. If Ω equals one, then the estimated $P(M_t)$ is consistent with one dataset. If Ω equals 2.53, then $P(M_t)$ is consistent with the other dataset. For the baseline model, Ω is assumed to equal one. For more detail about this parameter, see Annex E (section 2).

Imagine an egg that is 25 hours old. Suppose the incremental change in the probability of YMB ($P(M_t)$) during the past hour is desired. The change in cumulative probability for that egg is calculated as

$$\Delta P(M_t) = P(M_t = 1.04) - P(M_t = 1.00) \quad (3.13)$$

where we calculate $P(M)$ at time = 1.04 days and subtract from it $P(M)$ at time = 1.00 day when the time increment is 0.04 day or 1 hour. If internal egg temperature varies, but we know $P(M)$ at time = 1.00 day, then the value for $P(M)$ at time = 1.04 days is approximated as

$$P(M_{1.04}) = P(M_{1.00}) + \Delta P(M_{0.04}) \quad (3.14)$$

where $\Delta P(M_{0.04})$ is solved for using and assuming a constant internal egg temperature during the past hour. This does not require assuming the internal egg temperature was constant before time = 1.00 day. If the internal egg temperature declined between time = 1.00 day and time = 1.04 days, then $\Delta P(M_{0.04})$ will be smaller than that predicted assuming the temperature remained constant. Alternatively, $\Delta P(M_{0.04})$ will be larger if the temperature increased during that time interval.

This example can be generalized for any value of time and sufficiently small values for the time increment. This is how $P(M_t)$ is recalculated as the age of an egg increases and internal egg temperature varies. In the model, a random value (p) from 0 to 1 is drawn at the beginning of the iteration. As subsequent increments are modeled the value for $P(M_t)$ is updated and compared to p. When $P(M_t)$ exceeds p then YMB has occurred and $P(M_t)$ is no longer estimated.

Growth rate

The exponential growth rate for *Salmonella* in eggs depends on the initial contamination site, level of *Salmonella*, and internal egg temperature. Annex E presents the detailed data and estimation of growth rate functions. The algorithms for predicting the exponential growth rate in albumen, *VM*, and yolk are shown in Table 3-15.

TABLE 3-15 EXPONENTIAL GROWTH RATES FOR YOLK-CONTAMINATED, VITELLINE MEMBRANE-CONTAMINATED, AND ALBUMEN-CONTAMINATED EGGS

Variable name	Description	Estimation
T_t	Internal egg temperature at time = *t*	See Table 3-14
Tmax	Maximum temperature at which growth occurs	114°F (45.6°C)
B, E, FY, W	Coefficients estimated from statistical fitting to data	Constants, see Annex E
Ws	Seropositivity indicator	Is 1 if seropositive egg, is zero if seronegative egg
μ^{Yolk}	Predicted exponential growth rate in yolk	$(1 - W \times W_s) \times \left((E + FY\,x\,T_t) \times (1 - e^{(B\,x\,(T_t - T_{max}))})\right)^2$
V	Coefficient estimated from data	Constant, see Annex E
$\mu^{Vitelline}$	Predicted exponential growth rate on the vitelline membrane	$V \times \mu_{Yolk}$
K	Constant of proportionality between vitelline and albumen growth rates	0.07, see Annex E
$\mu^{Albumen}$	Predicted exponential growth rate in albumen	$K \times \mu_{Vitelline}$

Number of bacteria inside egg, S_t

Calculating growth inside an egg requires consideration of the initial number of bacteria inside the egg, how long the bacteria have been growing, where the bacteria reside in the egg, the exponential growth rate, and the time when YMB occurs. If an egg is albumen contaminated, then SE growth is unlikely to occur until YMB commences. The same general pattern applies to VM-contaminated eggs.

Figure 3-15 provides an illustrative example of our conception of the growth phases for an albumen-contaminated egg with a constant internal temperature of 12.5°C and initially contaminated with one SE bacterium. This bacterium in the albumen may begin to adapt to the relatively difficult environment of the albumen. This initial adaptation period, the lag phase, lasts up to 8 days after which the bacterium is able to grow exponentially at a slow rate. This particular egg's yolk membrane is assumed to break down at 42 days, the 10th percentile of the cumulative distribution at a constant temperature of 12.5°C. Following this breakdown, the organisms adapt again to a new environment and experience an abbreviated lag phase, before growing exponentially at a fast rate in the yolk. As the bacteria population approaches the maximum population density achievable inside an egg, theoretically about 10 $\log_{10}$, growth

slows and eventually ceases, or equilibrates to the death rate, inside that egg about 50 days after lay.

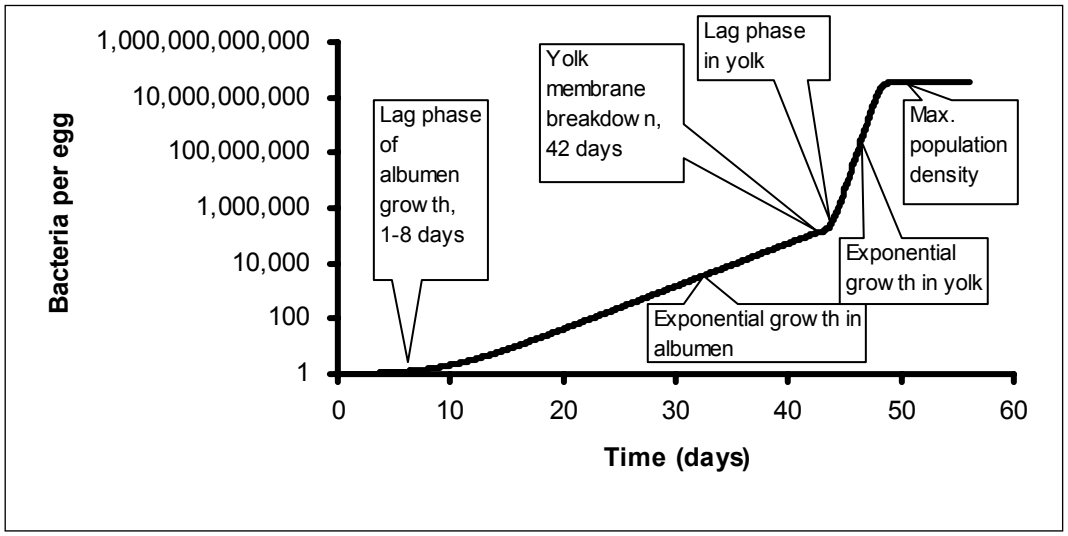

FIGURE 3-15 ILLUSTRATIVE EXAMPLE OF PHASES OF GROWTH MODELED FOR SE IN CONTAMINATED EGGS. IN THIS EXAMPLE, INTERNAL TEMPERATURE IS CONSTANT AT 12.5°C AND YMB OCCURS AT THE 10TH PERCENTILE OF THE POPULATION (I.E., APPLICABLE $P(M) = 0.10$).

For modeling purposes, the exponential growth rate in albumen is estimated according to Table 3-15. The number of days of albumen growth depends on when YMB occurs. The growth in the yolk is also estimated according Table 3-15. The number of days of yolk growth depends on the remaining time the egg is stored before it is consumed and the maximum density of organisms allowed in the egg. Growth rates and YMB depend on internal egg temperature. Internal egg temperature further depends on ambient storage temperatures, length of time in storage, and the cooling rate. The ambient temperatures, storage times, and cooling rates are described by probability distributions. Therefore, calculating the number of bacteria in an egg at any point between the times it was laid and the time it is processed requires all of the inputs and calculations previously described in this section. Given all these previous calculations, the final calculations for estimating the number of bacteria in the egg are presented here.

Deterministic calculations

The amount of bacteria in an egg can be estimated using deterministic or non-random calculations. Alternatively, these estimates can be completed using stochastic or random techniques. The deterministic calculations are described first.

In the absence of any constraints, bacteria within an egg would grow according to the following differential equation:

$$\frac{dS(t)}{dt} = S(t)\mu \tag{3.15}$$

where $S(t)$ = number of bacteria at time t and μ = the daily exponential growth rate.

The exponential growth rate per day, μ, is assumed independent of time for a sufficiently small time interval. In reality, the exponential growth rate is changing across time because internal egg temperature is a function of time. Nevertheless, for the purposes of modeling, approximates growth for minor increments when μ is fixed.

The lag phase occurs because the bacteria are adjusting to changing environmental conditions. The consequence of the lag phase is that the bacterial growth rate is less than μ for some initial period. Baranyi et al.[6] proposed a variable, α , as the adjustment function. It is a function of time and describes the modulating effect of environmental influences on μ. Therefore, μ is considered the maximum growth rate, and α essentially reduces the maximal rate for some period. The variable $\alpha(t)$ ranges from zero to one and exerts its influence early in the growth period of bacteria. The adjustment factor ($\alpha(t)$) was defined as a function of some critical substance that serves to limit or constrain growth. Much complexity surrounds the notion of rate-limiting nutrients or processes. Table 3-16 describes the elements needed to calculate $\alpha(t)$and more detail is provided in Annex E.

The lag adjustment is not the only consideration in modeling the growth of bacteria. The maximum population density that can be achieved by the bacteria is another consideration. While the lag phase reflects the bacteria adjusting to their new environment, the maximum population density reflects the limitations of the environment or genetic factors to support an ever-increasing population size. As the maximum population density is approached in an egg, the growth rate slows down and eventually becomes zero. Therefore, a second adjuster of μ that ranges from one to zero and exerts its effect late in the growth period is introduced, $\beta(t)$. Its estimation is also shown in Table 3-16.

Including the $\alpha(t)$and $\beta(t)$ terms, the differential equation becomes

$$\frac{dS(t)}{dt} = S(t)a(t)\mu\beta(t) \tag{3.16}$$

which is a complicated expression to solve. Baranyi and Roberts[6] provide a solution for the case where μ is constant, but such a solution is not easily applied to a computer model where μ is changing with time. Instead, for each sufficiently small time increment in the model the terms α, μ and β are assumed constant. Therefore, the solution is approximated as

$$S_{t+1} = S_t \text{ x } e^{\alpha \text{ x } \mu \text{ x } \beta \text{ x } \Delta t} \tag{3.17}$$

where S_{t+1} is the number of bacteria at the end of one time increment, S_t is the number of bacteria at the beginning of the time increment, and Δt is the size of the time increment (i.e., fraction of days for this model). This calculation is completed for each egg individually throughout the time it is modeled.

Using Equation 3.17, the model steps through cumulative time increments and recalculates the bacterial levels in a contaminated egg. For example, if the egg is albumen contaminated, the model determines the growth rate in albumen for the applicable temperature and time increment, then calculates the corresponding α and β terms, and finally calculates the number of SE in the egg for each point in time. This step is repeated for each successive time increment until the time when YMB occurs. Once this occurs, subsequent time increments calculate the growth rate in yolk for the temperature applicable to the time increment. Because $\alpha(t)$ for yolk growth is a

different function of time relative to that for albumen growth, the calculation for this input is based on the cumulative time since YMB. Time begins again at zero when YMB occurs for the purposes of calculating $\mu(t)$. For each time increment after YMB, the number of bacteria is recalculated using with the appropriate substitutions for $\mu(t)$ and $\alpha(t)$.

TABLE 3-16 ESTIMATING THE NUMBER OF BACTERIA IN AN EGG.

Variable	Description	Estimation
μ	Exponential growth rate	See Table 3-15
LPD	Lag period duration	$\frac{\ln\left(1+\frac{1}{q_0}\right)}{\mu}$ from Baranyi and Roberts[6]
GT	Time for cells to double in number (generation time)	$\frac{\ln(2)}{\mu}$
R	Ratio of lag period duration to generation time	$LPD \div GT$ (Assumed to be 5)
		$R = \frac{\ln\left(1+\frac{1}{q_0}\right)}{\ln(2)}$, so $q_0 = 0.03$, (Annex E)
Q_0	Ratio of exponential lag rate (λ) to μ	
t	Storage time in hours	Lognormal distribution from data
$\alpha(t)$	Lag adjustment to μ	$\frac{q_0}{q_0 + e^{-\mu t}}$ from Baranyi and Roberts[6]
MPD	Maximum population density for SE in eggs	$10^{10.59}$
S_0	Initial number of bacteria in egg	Random value depending on egg type, E_i
$\beta(t)$	Maximum density adjustment to μ	$1 - \frac{S_t}{MPD}$
$\Delta(t)$	Time increment	Model setting in days^{-1}
S_t	Number of bacteria within egg at time t	$S_0 \times e^{\alpha \times \mu \times \beta \times \Delta}$

Variability in growth

The process described above estimates growth of SE in a deterministic fashion when in fact there could be a great deal of variability in the growth behavior of the SE cells in shell eggs. To examine these effects, an alternative algorithm for calculating the number of bacteria in an egg using stochastic theory is presented. This theory and the derivation of these equations are presented in detail in Annex E. The algorithm for estimating the number of bacteria in an egg at time t is shown in Table 3-17.

Modeling stochastic growth processes is computationally intensive. To examine the value of including the stochastic calculations, the results of the model using deterministic and stochastic predictions are compared as part of the risk characterization.