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10.1 An Introduction to Modeling *Salmonella* in Low Water Activity Foods

The presence and survival of *Salmonella* in low water activity (a_w) foods continues to pose a challenge for the food industry. Peer-reviewed literature data on prevalence and levels of contamination of *Salmonella* in low water activity foods in the United States are limited. Available published data include those on: *Salmonella* contamination on nuts and peanuts (Calhoun *et al*., 2013), almonds (Danyluk *et al*., 2007; Bansal *et al*., 2010), pecans (Brar, Strawn, and Danyluk, 2016), and walnuts (Davidson *et al*., 2015); prevalence and levels of *Salmonella* on spices (Van Doren *et al*., 2013); as well as data on prevalence of *Salmonella* in animal feed (Li *et al*., 2012). On the other hand, data on survival and inactivation of *Salmonella* in low water activity foods have been collected extensively. Examples include *Salmonella* in a wide variety of nuts (Uesugi, Danyluk, and Harris, 2006; Uesugi and Harris, 2006; Danyluk *et al*., 2008; Beuchat and Mann, 2010, 2011; Abd, McCarthy, and Harris, 2012; Blessington, Mitcham, and Harris, 2012, 2014; Kimber *et al*., 2012; Beuchat *et al*., 2013b; Blessington *et al*., 2013a; Blessington *et al*., 2013b; Brar *et al*., 2015), whey protein (Santillana Farakos, Frank, and Schaffner, 2013), peanut butter (Ma *et al*., 2009; Lathrop, Taylor, and Schnepf, 2014; Li, Huang, and Chen, 2014), dry confectionary raw materials (Komitopoulou and Penaloza, 2009), spices (Keller *et al*., 2013), and several others as detailed in the reviews by FAO/WHO (2014), Beuchat *et al*. (2013a) and Podolak *et al*. (2010). In these studies, *Salmonella* is shown to be very resistant to desiccation and, once the cells are dry, have an increased resistance to heat. A high degree of variability is seen among studies, substrates, and the environmental conditions under which the experiments take place. Water activity is one critical factor in *Salmonella* inactivation and survival. Other influencing factors

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include the interaction between water and *Salmonella* cells and the effect of temperature, as well as the interaction between water and other components of the food matrix (e.g., sugars and fats). How these factors and interactions influence survival is still not well understood.

The Weibull model has been determined to be the best applicable model to describe survival of *Salmonella* in low water activity foods (Santillana Farakos, Frank, and Schaffner, 2013; Santillana Farakos *et al*., 2016). Predictive models developed for *Salmonella* survival in these foods include models for survival on almonds at −20, 4, and 24 °C assuming log-linear declines developed by Danyluk, Harris, and Schaffner (2006) and Lambertini *et al*. (2012). Santillana Farakos, Frank, and Schaffner (2013) developed secondary models using a Weibull primary model to predict survival in a low-fat food model system at temperatures ranging from 21 to 80°C and a_w <0.6. More recently, a Weibull-type model able to predict survival of *Salmonella* in tree nuts at typical storage temperatures, incorporating variability and uncertainty separately was developed (Santillana Farakos *et al*., 2016).

The first step in developing a predictive model is to collect data on survival and/or inactivation. Once developed, predictive models can be used for different purposes, including quantitative microbial risk assessment (QMRA) and to provide information useful for setting performance standards and food safety objectives. Quantitative risk assessment is a tool able to estimate the risk of adverse health effects from exposure to a hazard in the food supply and the associated burden of illness for a specific population. It consists of four steps: (i) hazard identification, (ii) hazard characterization, (iii) exposure assessment, and (iv) risk characterization (CAC, 1999; CFSAN, 2002). In addition to data and models on *Salmonella* survival, the exposure assessment component of QMRA requires prevalence and contamination levels. Available QMRA of *Salmonella* in low water activity foods include those by Danyluk, Harris, and Schaffner (2006), Lambertini *et al*. (2012) and the US Food and Drug Administration (FDA, 2013, 2016). Lambertini *et al*. (2016) have also conducted a quantitative assessment of the risk of salmonellosis to both humans and also to pets associated with the consumption of dry pet foods.

The aim of this chapter is to provide an introduction to modeling *Salmonella* in low water activity foods focusing on the statistical issues related with model development and the development of a QMRA. *Salmonella* on peanuts are used in an example case study.

10.2 Developing a Predictive Model for Salmonella in Low Water Activity Foods

Predictive models in food microbiology are used to estimate microbial concentration levels given certain conditions. The first step in developing a predictive model is to develop a primary model. Primary models determine the magnitude of a response of interest (e.g., growth rate, survival rate, inactivation rate, lag phase, etc.) given certain conditions (e.g., temperature (T), a_w) that are considered fixed. For example, it may be necessary to know the survival rate of

Salmonella in peanuts during storage at ambient temperature. The specific response of interest could be the time it takes to reduce the population of *Salmonella* by 2-$\log_{10}$ CFU in peanuts at 25°C and a_w 0.45. Once the data are collected on *Salmonella* survival under the temperature and water activity conditions of interest, primary models would be selected to fit the data. A commonly used primary model is the classic linear model (Bigelow model, Equation 10.1), where survival numbers can be estimated using the traditional *D*/*z* concept:

$$N_t = N_0 * exp^{(-k_{max} * t)} \tag{10.1}$$

where N_0 is the population at time 0, N_t is the population at time *t*, *t* is the time (min), k_{max} is the maximum specific inactivation rate (min^{-1}), and $D_{value} = \frac{ln(10)}{k_{max}}$.

The Weibull primary model (Equation 10.2) can also be used to describe survival curves that do not follow log-linear kinetics and show asymptotic tails. This is most commonly the case for *Salmonella* survival in low water activity foods.

$$log_{10}(N_t) = log_{10}(N_0) - (t/\delta)^{\rho} \tag{10.2}$$

where N_0, N_t, and *t* are as defined above, δ is the time required for first decimal reduction (e.g., min) and ρ is a parameter that defines the shape of the curve.

By fitting these models to the data, an estimate of the model parameters is obtained: the maximum specific inactivation rate when fitting the Bigelow model (k_{max} in Equation 10.1); or the time it takes for the first $\log_{10}$ reduction (δ in Equation 10.2) and a parameter that defines the shape of the survival curve (ρ in Equation 10.2) when fitting the Weibull model. The models can then be used with these fitted parameter values and, given an initial population of *Salmonella* (N_o), the time it takes to reduce the population by a certain $\log_{10}$ CFU can be calculated or the $\log_{10}$ reduction given a certain amount of time (e.g., 10 min). Using this approach, the predictive model obtained would be applicable to the specific strain, temperature, water activity, and other environmental conditions present when collecting the data used to derive the model. The Bigelow equation is valid at any time *t* (and independent of *t*), that is, $N_{t+dt} = N_t * exp^{(-k_{max} * dt)}$, while this is not the case for the Weibull model, where $log_{10}(N_{t+d}) = log_{10}(N_t) - (d/\delta)^{\rho}$ should not be used. The correct equation to use with the Weibull model would be $log_{10}(N_{t+d}) = log_{10}(N_0) - ((t+d)/\delta)^{\rho}$ or $log_{10}(N_{t+d}) = log_{10}(N_t) - ((t+d)^{\rho} - t^{\rho})/\delta^{\rho}$.

If data are collected over a range of temperatures (e.g., 25–50°C) and a_w (e.g., 0.25–0.55), secondary models can be developed to cover survival prediction at this temperature and a_w range. Once primary models are derived directly from experimental data, secondary models are derived to predict changes in primary model parameters as a function of independent variables. In the *Salmonella*-peanut example, secondary models could be used to predict primary model parameters of the Bigelow and Weibull models (k_{max} if using the Bigelow model or δ and ρ if using the Weibull model) and determine their relationship with the

experimental conditions (T and a_w). Survival of *Salmonella* in raw peanuts at any temperature and water activity within the range of those used to develop the model can then be determined.

In the following subsections, using the data collected by Brar *et al*. (2015) on *Salmonella* survival on raw peanuts at three different temperatures (−24, 4, and 22°C), a case study on how to develop a primary and secondary predictive model for survival of *Salmonella* is shown This approach could be used for survival data on any pathogen in any food, and is explored here for survival of *Salmonella* in low water activity foods.

10.2.1 Primary Models

Survival of *Salmonella* in low water activity foods may be characterized by curves with a relatively rapid initial decline followed by long-term persistence, with little decline over time (Podolak *et al*., 2010; Santillana Farakos, Schaffner, and Frank, 2014). The shapes of the survival curves have been observed to vary depending on the study, the substrate, and the environmental conditions under which the experiments take place. Various primary models are available and can be used to fit microbial survival data: the log-linear model (Bigelow and Esty, 1920), the Geeraerd-tail model (Geeraerd, Herremans, and Van Impe, 2000), the Weibull model (Mafart *et al*., 2002), the Coroller model (Coroller *et al*., 2006), and the biphasic linear model (Cerf, 1977). Of the aforementioned models, the Weibull model has been shown to provide the best description of *Salmonella* survival kinetics in low water activity foods (Mattick *et al*., 2001; Ma *et al*., 2009; Abd, McCarthy, and Harris, 2012; Santillana Farakos, Frank, and Schaffner, 2013). All these models are available in GInaFiT (Geeraerd, Valdramidis, and Van Impe, 2005), a free software fitting tool (http://cit.kuleuven.be/biotec/software/GinaFit [last accessed 10 February 2017]).

The first step in the process of choosing a primary model is to visually analyze the data. In Figure 10.1, *Salmonella* survival on raw peanuts at three different

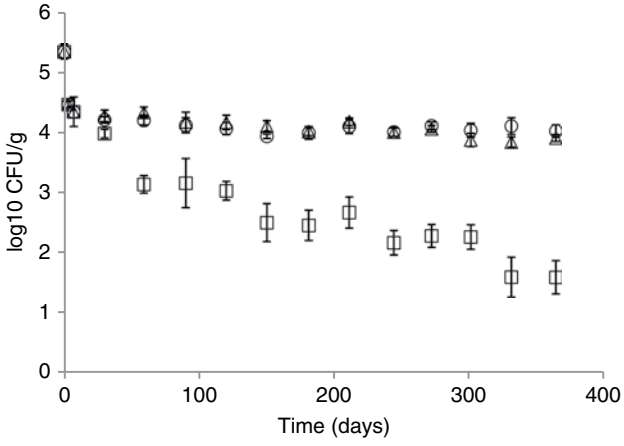

Figure 10.1 Survival of *Salmonella* on raw peanuts at (○) −24, (Δ) 4, and (□) 22°C for 365 days (Brar *et al*. (2015), reprinted with permission of the authors).

temperatures (–24 , 4, and 22°C), as collected by Brar *et al.* (2015), is plotted against 365 days.

There was no significant decline of *Salmonella* on raw peanuts at freezing or refrigeration temperatures (Brar *et al.*, 2015). The average decline over 365 days was 0.3-$\log_{10}$ CFU at –24°C and 0.4-$\log_{10}$ CFU at 4°C. *Salmonella* populations had higher declines on raw peanuts at 22°C, with an average log reduction of 2.0-$\log_{10}$ CFU over 365 days (Brar *et al.*, 2015). *Salmonella* survival is characterized by an initial decline during the first seven days of storage, which does not seem to be influenced by temperature (Figure 10.1). At all temperatures, the *Salmonella* population dropped 1-log in the first seven days. In the Brar *et al.* (2015) study, day 0 was the day of the (wet) inoculation, day 3 was three days after storage at room temperature, and day 7 was after an additional four days of equilibration at room temperature. Day 7 was thus the first time point of storage at the specific temperature. The influence of temperature began to show around day 30, after which a significant decline was observed for the data at 22°C, while no significant decline was observed at either –24 or 4°C (Figure 10.1). These observed survival kinetics for *Salmonella* on raw peanuts are in line with other published literature, where survival curves for *Salmonella* in low water activity foods do not follow log-linear kinetics and show significant asymptotic tails (Uesugi, Danyluk, and Harris, 2006; Beuchat and Mann; 2010, Blessington, Mitcham, and Harris, 2012; Kimber *et al.*, 2012; Blessington *et al.*, 2013a; Blessington *et al.*, 2013b; Keller *et al.*, 2013; Santillana Farakos, Frank, and Schaffner, 2013). The data presented in Figure 10.1 indicate that the best description of *Salmonella* survival under these conditions requires a model that includes a nonlinear inactivation rate and the ability to incorporate tailing. In the extensive data collection and analysis of *Salmonella* survival in low water activity foods by Santillana Farakos, Frank, and Schaffner (2013), the Weibull model was determined to be the best model to describe the survival kinetics at temperatures ranging from 21 to 80°C and water activity levels below 0.6. An extension of the Weibull survival model to the double Weibull model was proposed by Coroller *et al.* (2006) (Equation 10.3) to describe a mixture of two subpopulations, one subpopulation being more sensitive to the environmental stress than the other:

$$N_t = \log_{10}\left(\frac{10^{N_0}}{1+10^{\alpha}}\left[10^{-\left(\frac{t}{\delta_1}\right)^{\rho}+\alpha} + 10^{-\left(\frac{t}{\delta_2}\right)^{\rho}}\right]\right) \tag{10.3}$$

where N_0 , N_t , ρ, and t are described as above, $\alpha = \log_{10}\left(\frac{f}{1-f}\right)$ and f is the fraction of bacteria in population 1; δ_1 and δ_2 are the time to the first $\log_{10}$ reduction for population 1 and 2, respectively.

The Weibull (Equation 10.2 with $\rho \neq 1$) and double Weibull (Equation 10. 3) models were thus selected as candidates for primary modeling of the peanut data set, and their fits were compared with those found when using the more traditional log-linear Bigelow model (Equation 10.1). Given the experimental design of the survival study, the data were modeled using day 7 (the first day of storage at the specified temperature) as time 0.

10.2.2 Criteria for Choosing the Best Applicable Model

The models were fit using GInaFiT. An f_{test} was used to determine the capacity of the model to describe the data well (95% confidence, $f_{test} < F_{table}$). If more than one model fit the data well for all conditions, the model with best statistical parameter fits was chosen (lowest AIC, lowest RMSE, and highest R_{adj}^2).

10.2.3 Primary Model Fits

The statistical analysis results of the fits with the log linear, Weibull and double Weibull models are presented in Table 10.1. All three models describe the data well ($f_{test} < F_{table}$; $F_{table} = 1.45$) for all conditions, except one where the double Weibull model did not provide an appropriate fit to the 4°C survival data. Survival of *Salmonella* on raw peanuts at 4°C showed log-linear persistence of the pathogen over time and the double Weibull model was, thus, unable to fit the data. Statistical analysis results show the Weibull model (with $\rho \neq 1$ in Equation 10.2) provided a better fit to the data at freezing and ambient temperature storage (Table 10.1) (lower AIC and RMSE, highest R_{adj}^2), while the log-linear survival model provided the lowest AIC and RMSE and highest R_{adj}^2 for survival at refrigeration temperature. In all, the Weibull model provided better fits for most of the conditions under study, and statistical parameters indicated the best fit. The Weibull model can also produce linear fits (with $\rho = 1$ in Equation 10.2), and thus describe linear inactivation kinetics as obtained at 4°C. As such, it was selected as the best applicable model for the data set. In Table 10.2, the δ and ρ values of the Weibull model fits for all conditions under study are presented. Because δ values for data at distinct temperatures differed by several orders of magnitude, these values were transformed to the log scale and are presented in Table 10.2.

10.2.4 Secondary Models

Linear models relating the time required for first decimal reduction (log δ) and shape factor values (ρ) to temperature were fit using multiple linear regression and are shown in Equations 10.4 and 10.5, respectively. These models are secondary models of a Weibull primary model and can be used to predict primary model parameters (δ and ρ) given certain T values. These δ and ρ values as obtained with the secondary models could be used to predict survival of *Salmonella* on raw peanuts at the range of temperatures at which the data were collected (−24, 4, and 22°C) and water activity levels specifically associated with the storage temperature (detailed in Table 10.2).

$$\log \delta = -0.065 * T + 3.4 \quad R^2 = 0.96 \tag{10.4}$$

$$\rho = 0.0069 * T + 0.38 \quad R^2 = 0.99 \tag{10.5}$$

In Equation 10.4 the standard error (s.e.) of log δ was 0.18, that of the temperature parameter (T) was 0.013, and that of the constant 0.25. In Equation 10.5, the s.e. of ρ was 0.00025, that of the T parameter was 0.0005, and that of the constant was 0.01.

Table 10.1 Statistical parameter fit results of the log-linear, Weibull and double Weibull models for *Salmonella* survival on raw peanuts, where day 7 was considered time 0, at −24, 4, and 22°C by adjusted R^2 (R^2adj), Root Mean Square Error (RMSE) and Akaike Information Criterion (AIC). Best statistical parameter values are shown in bold.

		Log-linear[a]				Weibull[b]				Double Weibull[c]			
Water activity	**T (°C)**	**AIC**	**R^2adj**	**RMSE**	**f_{test}**	**AIC**	**R^2adj**	**RMSE**	**f_{test}**	**AIC**	**R^2adj**	**RMSE**	**f_{test}**
0.69 ± 0.05	−24 ± 1	−310.0	0.17	0.14	0.01	**−323.3**	**0.31**	**0.12**	0.00	−319.6	0.26	0.13	0.00
0.94 ± 0.09	4 ± 2	**−327.6**	**0.60**	**0.12**	**0.00**	−310.4	0.51	0.13	0.01	—	—	—	—
0.56 ± 0.08	22 ± 1	−158.7	0.81	0.36	0.06	**−182.3**	**0.86**	**0.30**	**0.04**	−182.3	0.86	0.31	0.04

a) Bigelow and Esty (1920);
b) Mafart *et al.* (2002);
c) Coroller *et al.* (2006); GInaFiT was unable to fit the data because of a systematic error with the data.

Table 10.2 δ and ρ values of the Weibull model fits for *Salmonella* survival on raw peanuts at −24, 4, and 22°C.

		Weibull[a]			
Water activity	**T (°C)**	**log δ[b] (log days)**	**log se δ[c]**	**ρ[d]**	**se ρ[e]**
0.69 ± 0.05	−24 ± 1	4.83	5.18	0.21	0.09
0.94 ± 0.09	4 ± 2	3.49	3.27	0.39	0.11
0.56 ± 0.08	22 ± 1	1.76	1.15	0.54	0.06

a) Mafart *et al.* (2002);
b) time required for first decimal reduction (measured in log days);
c) standard error of δ parameter value;
d) fitting parameter that defines the shape of the curve;
e) standard error of ρ parameter value.

If more data are available at different temperatures and different water activity levels at the same temperature, the linear predictive models presented above can be extended in their prediction potential using the same approach. Even more sophisticated modeling techniques can be used to develop a predictive model that includes an estimate of uncertainty and variability in the parameter estimates. Further reading on a proposed approach to incorporating variability and uncertainty in a predictive model for survival of *Salmonella* in tree nuts is available elsewhere (Santillana Farakos *et al.*, 2016).

10.3 Model Validation

Methods for selecting a model or to determine whether a given model is valid for a given set of conditions has always been an important part of predictive microbiology commonly referred to as validation. Models can be validated using an independent set of data from that used to develop a model. One of the earliest efforts to provide a standard method or criteria to validate a model was by Ross (1996), who proposed indices called the bias and accuracy factors. These factors were soon updated by Baranyi, Pin, and Ross (1999), who modified and generalized the bias and accuracy to enable comparisons between growth models, as well as with observations. Baranyi, Pin, and Ross (1999) describe the revised accuracy and bias factors as essentially methods of determining "averages," where the averages are computed in slightly different ways. Accuracy is computed using mean square differences, while bias is computed using the arithmetical mean of the differences. The revised factors enable direct comparison of models to one another, rather than to a specific data set, which might not be representative of "true" behavior. Baranyi, Pin, and Ross (1999) note that a direct comparison of models in this manner can indicate whether models differ significantly from one another, or describe the same information equally well within the limits of the data. Finally the authors reiterate that it is still important to visually examine a

Table 10.3 Different criteria for definitions of fail-safe, accurate, and fail-dangerous model predictions as proposed by Mohr *et al*. (2015) based on the values of the residual (observed minus predicted value).

	Fail-safe	Accurate	Fail-dangerous
Criterion 1	Residual <−1.0 log	−1.0 < Residual <0.5-log	Residual >0.5-log
Criterion 2	Residual <−1.0 log	−1.0 < Residual < 1.0-log	Residual >1.0-log
Criterion 3	Residual <−0.5 log	−0.5 < Residual < 0.5-log	Residual >0.5-log

plot of predicted versus observed values to guard against any systematic deviations that might not be revealed by the bias and accuracy factors.

Mohr *et al*. (2015) published an extensive validation and assessment of six *Clostridium perfringens* cooling models, with some relevant insights on model validation in general. These authors define a model as "validated" when its predictions have been "extensively compared to laboratory data, provided the model's performance is acceptable." Mohr *et al*. (2015) used three sets of criteria, each based on different definitions for "accurate," "fail-safe," and "fail-dangerous" to evaluate model performance. These criteria are summarized in Table 10.3 and are all modifications of the acceptable prediction zone (APZ) method (Oscar, 2005a), although Mohr *et al*. (2015) redefine "A" in "APZ" to be "accurate" and not "acceptable" as in the original definition by Oscar (2005a).

The boundaries of the APZ are based on an evaluation of the standard deviation of observed log counts among replicate experiments. Mohr *et al*. (2015) explain that Criterion 1 has a fail-safe boundary set at twice the level of the fail-dangerous boundary because greater error can be tolerated in the fail-safe direction, as originally noted by Oscar (2005b, 2007). The boundaries for Criterion 2 are based on the levels of microbial growth that an expert food microbiologist would consider significant. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF) used growth of <1-log as the criterion for determining the absence of measurable growth of pathogens of concern in its publication on microbial challenge studies (NACMCF, 2010). The boundaries for Criteria 3 are based on the observation that 0.5-log is generally accepted as the resolution limit of microbial testing, resolution being the capability of distinguishing two sets of results. NACMCF (2010) noted that a difference of greater than 0.5-log CFU/g may be an appropriate criterion for determining microbial growth but that this may depend on the food, inoculum level, and method of enumeration.

NACMCF (2010) also offers useful advice on when models alone are used to make a food safety decision, noting that "proper use of models requires judgment and experience, both in food microbiology and modeling." Models must be shown to be valid for the food in question when models alone are used to make a decision (NACMCF, 2010). Any decisions should also take into consideration any lot-to-lot variation in the formulation and composition of the food (NACMCF, 2010). While a detailed discussion of the proper design of microbiological experiments, including sampling intervals, inoculation methods, and

testing procedures, is beyond the scope of this chapter, interested readers are directed to NACMCF (2010).

10.4 Models in Risk Assessment

Risk assessment is a scientific process that addresses the magnitude of public health risk and identifies factors that control it. Risk managers can then use a risk assessment to make decisions regarding the management of the safety of a food product. A quantitative risk assessment provides enhanced information to risk managers particularly when a multistep food process is being evaluated (Brocklehurst, 2004). Risk assessments can also identify data gaps that when filled will refine the risk assessment model, in some cases reducing uncertainty in the risk estimates.

10.4.1 The Risk Assessment Model

In developing a QMRA, the first step is to identify the hazard and to determine what question(s) the risk model will be designed to answer. Using *Salmonella* and peanuts as an example, a risk model using Monte Carlo simulations could evaluate the risks of salmonellosis associated with the consumption of roasted peanuts and the impact of different log reductions in reducing the risk of roasted peanut–associated illness. To facilitate this process, a flow diagram of the process being evaluated can be established. Figure 10.2 shows a simplified flow diagram for roasted peanuts.

Following hazard identification and defining the risk management question(s) to be addressed, the next step is to collect data to establish an exposure analysis,

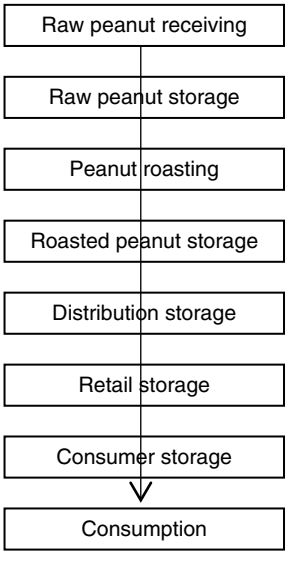

Figure 10.2 Flow diagram for roasted peanuts process.

(i.e., chance of consumer exposure). This step requires an understanding of both the prevalence and concentration of the microbe on the product throughout the process (ideally starting at the initial step in the model), and subsequent information on how that population changes during the process. Because of changes in microbial populations, predictive food microbiology, or the development and use of mathematical models that describe the growth, survival or inactivation of microorganisms (NACMCF, 2010), has a natural application in the exposure assessment component of risk models (van Gerwen and Zwietering; 1998). In addition to information on microbial populations, information related to the consumption of the product also needs to be collected.

Using *Salmonella* and peanuts as an example, data on prevalence and concentration of *Salmonella* on raw, shelled peanuts are available from Calhoun *et al*. (2013), where a screening of three crop years resulted in 2.33% positive samples (out of 944 samples, testing done between several days and several weeks after sample collection), and that of Miksch *et al*. (2013), where *Salmonella* was found in 0.67% of shelled raw runner peanut samples (out of 10 162 samples, testing done between 1 and 18 months after sample collection) over a three-year period. *Salmonella* concentrations obtained from these studies were <0.03–2.4 MPN/g (<3–240 MPN/100 g) (Calhoun *et al*., 2013) and 0.74–5.25 MPN/350 g (0.21–1.5 MPN/100 g) (Miksch *et al*., 2013). As previously discussed, *Salmonella* populations decrease on raw peanuts during storage (Brar *et al*., 2015). Models such as those described earlier based on the storage times and temperatures typical of peanuts at different points in the model will need to be considered to calculate the reduction level. The "kill step" of peanut roasting is also included in the model; the log-reductions associated with peanut roasting are variable, depending on the type of roasting (oil versus dry). In the absence of published information, assumptions are made on peanut storage times and temperatures (which can be made following discussions with industry experts). Consumer storage data from published studies of consumer behavior can be used (Lee *et al*., 2011). Production data may be obtained from USDA reports like the ERS (2014) report on tree nut production in the United States. Consumption data can be estimated using data originating from the National Health and Nutrition Examination Survey (NHANES) (CDC, 2013). If data are not available, expert elicitations can take place with industry members and other subject matter experts.

The next step is to establish a dose–response analysis, or a way of translating the data from the exposure analysis into an output measure of human health. To translate the exposure analysis, statistical models are used to analyze or quantify dose–response relationships. The term "infective dose" implies that at a certain pathogen load illness or infection will occur for an entire population or subpopulation of hosts. Infective dose has also been used to describe pathogenicity or likelihood of an illness. However, this is not the case in many foodborne outbreak situations and has limited use in QMRA. In the complex situation of foodborne disease, the actual ingested dose required to cause illness varies based on a number of factors, including the host, pathogen, food matrix, and other environmental interactions (Marks *et al*., 1998). For risk assessment purposes, there is a need to account for the variability and, if possible, uncertainty that results from incompletely characterized ingested doses and population responses. The result is a

dose–response distribution that models a distribution reflecting the uncertainty of many factors rather than a point estimate (Marks *et al.*, 1998). These distributions, often beta-Poisson in nature (Haas, 1983), indicate the relative possibility of illness (usually plotted on the y-axis) at any given ingested dose (plotted on the x-axis), and use two parameters (α and β) to describe the distribution of susceptibility to the pathogen to characterize the variability among members of the population (Cassin *et al.*, 1998). Because published human feeding studies of foodborne pathogens are rare, development of dose–response models often depends on epidemiological data, using samples of remaining contaminated lots of the suspect food to infer an actual ingested dose based on the contamination level of the pathogen found. For the example of *Salmonella* and peanuts, a dose–response curve published by FAO/WHO (2002) can be used to determine the probability of salmonellosis based on the ingested dose. This model is based on 20 documented outbreaks of salmonellosis in eggs and broiler chickens, and remains the most commonly used dose–response model for salmonellosis for all food commodities. More complete and complex alternative dose–response models are available elsewhere (Bollaerts *et al.*, 2008; Teunis *et al.*, 2010).

The final step of a risk assessment involves developing a risk characterization, or the complete picture of the assessed risk, by combining the hazard identification, exposure analysis, and dose–response analysis. Any data from the hazard identification, exposure analysis, and dose–response analysis where there is inherent variability should be transformed into a probability distribution to be fed into the Monte Carlo analysis. The use of probability distributions for all variables allows for the description and an inclusion in the risk model of the variability in the inputs. Common types of probability distributions used in risk models include normal, log-normal, uniform, triangle, beta-PERT, and beta-Poisson; should the data not fall into one of these distributions, it is also possible to utilize a histogram of the data as is (empirical distribution).

10.4.2 The Monte Carlo Analysis

Monte Carlo simulations are the most commonly used technique in the field of food safety microbial risk assessment. This technique utilizes simulations to obtain statistics of output variables for a set scenario based on the statistics of input variables, allowing the user to account for variability in the risk in quantitative analysis and decision making. In each scenario, the values of the input random variables are sampled based on their distributions, and the output variables are calculated using a computational model (Mahadevan, 1996). Statistics for output variables are calculated following a number of iterations of the Monte Carlo simulation, allowing the user to determine how likely the resulting outcomes are. This output of the range of possible outcomes and probabilities allows the user to evaluate the likelihood of possible outcomes based on changes to the input variables.

During a Monte Carlo simulation, a different value is selected randomly from each of the distributions for each of the inputs to calculate an output; each set of random samples collected from the distributions inputted into the model is called an iteration. Each of these outputs is recorded and the process is repeated

for multiple iterations; typical Monte Carlo simulations are run for thousands, if not tens or hundreds of thousands, of iterations. When the simulation is complete, the multiple outputs from the model provide information on probability of reaching the model result; the result indicates not only what may happen, but how likely it is to happen.

Continuing the example of *Salmonella* and peanuts, based on the percentage of peanuts that get roasted in the United States, a distribution of the serving sizes of roasted peanuts in the United States could be obtained using the NHANES survey data. The consumption data can then be linked to a distribution of *Salmonella* prevalence on raw peanuts (as determined by available data from Calhoun *et al*. (2013) and Miksch *et al*. (2013)) to determine the likelihood of that serving of roasted peanuts containing *Salmonella*. Should that serving contain *Salmonella*, the appropriate distribution for *Salmonella* concentration determined from the data presented in Calhoun *et al*. (2013) and Miksch *et al*. (2013) would determine the amount of *Salmonella* on that serving. Uniform distributions for storage time can be assumed when no additional data are available (with a minimum and maximum estimated value). If a most likely value for storage time is known together with a minimum and maximum value, a triangular distribution can be used instead. The temperature of storage can be estimated under all conditions. To calculate *Salmonella* reductions during different storage conditions, data from previous studies (Brar *et al*., 2015) can be used to calculate estimated log reductions at each storage step. The predictive models developed for *Salmonella* survival in peanuts as described earlier can also be used. These calculated log reductions can then be subtracted from the concentration of *Salmonella* on a peanut serving. The likelihood of that peanut serving leading to illness can then be calculated from the dose–response curve. Utilizing multiple iterations from the Monte Carlo model, ultimately the output from the model is the probability of a number of cases of human illnesses per year. To evaluate the independent variable, reductions during roasting, a reduction step can be added where a fixed reduction with certain amount of variability is assumed. For example, 4 ± 1, 4 ± 0.5, and 4 ± 0.0, or 5 ± 1, 5 ± 0.5, and 5 ± 0.0 log reductions could be selected, and Monte Carlo simulations for each scenario conducted to determine likelihood of illnesses and how those illness levels differ from the baseline.

The numerical distribution outputs of a Monte Carlo simulation and their precision depend on the convergence properties of the sampling algorithm (Smid *et al*., 2010). Convergence can be defined as the number of iterations the Monte Carlo simulation requires for it to obtain samples that are truly representative of the underlying distribution of interest (Cowles and Carlin, 1996). The number of iterations required for convergence can be calculated according to Brooks (1998).

10.4.3 Sensitivity Analysis

One of the advantages of utilizing a Monte Carlo simulation is the ability to determine which inputs or assumptions entered into the model have the greatest impact on the risk outcome. A sensitivity analysis can easily be conducted on all

the different scenarios tested. Spearman's rank correlation coefficients (R^2) of parameters can be used as a simple measure of the impact of any input on the output. If the R^2 of a parameter is positive, there is an increase in risk with an increase of the parameter value; if negative, there is a decrease in risk with the increasing value. Other sensitivity analysis methods are available (Frey, Mokhtari, and Danish, 2003), notably when strong interactions between parameters are expected in the model. If the input with the greatest impact on the risk outcome has a high amount of uncertainty associated with it, the user then has the ability to collect additional data and update the input.

10.4.4 Modeling Variability and Uncertainty

In the risk assessment model previously described for *Salmonella* in peanuts, all distributions represent variability of the parameter in the considered process. Variability is usually described as the distribution of the variable inherent to the system that cannot be reduced by further measurements. Serving size is a typical example: on a typical day, some individuals will consume a large serving size of peanuts (or peanut-containing products) while others will consume a small serving size, however precisely this serving size is known. Similarly, the time (and temperature) at which peanuts will be stored by the consumer is variable from one consumer to the other, and will be variable even if all times are precisely recorded. This variability distribution of the storage time could be modeled, as an example, using a triangular distribution with minimum value of 0 (some consumers eat peanuts without further storage), with a mode of two weeks (most frequently, the consumers eat the product after 15 days of storage) and a maximum of one year (the maximum time a consumer keeps peanuts is assumed to be one year). As an output of the Monte Carlo simulation, including the variability distributions, the final distribution will then reflect a distribution of the risk, variable from serving to serving, as a function of the serving size, the consumer storage, and all the possible combinations of all parameters implemented in the model. The simple multiplication of the mean risk per serving (output of the Monte Carlo simulation) by the number of servings provides a mean estimate of the expected mean number of cases in the population. Parameters in a "*Salmonella* in peanuts a_w" simulation could include the prevalence variability (from lot to lot, from year to year and/or from region to region), the variability of the time, water activity and temperature of storage at the different steps, the variability of log reduction during inactivation, and the variability of the serving size.

Additionally, there are some parameters that may be known with uncertainty (lack of knowledge of the parameter). As an example, the mode of the storage time was set to two weeks in the previous peanut example, but could actually be one week or even three weeks. Uncertainty can be reduced by further studies, in contrast to variability. The uncertainty can be characterized by a set of discrete values (e.g., the mode of the storage time distribution could be one week, two weeks, or three weeks), or may be characterized by a distribution (e.g., the uncertainty around the mode of the storage time may be characterized by a uniform distribution ranging from one to three weeks). Uncertainty and

variability dimensions should be separated in QMRA. Nauta (2000) illustrated how the mixing of uncertainty and variability distributions could lead to biases in a risk assessment outcome. The simplest way to evaluate the impact of uncertainty is to run the model multiple times, changing the uncertain parameter from one simulation to the next one. In the previous peanut example, the model would be run using a mode of one week, then a second time using a mode of two weeks, and a third time using a mode of three weeks. The results from these three simulations would then be compared. As a minimum, the different sets of results associated with the various assumptions made for the parameter should be presented and discussed. Depending on the results, risk managers may decide that additional research must be undertaken to reduce the uncertainty in a parameter.

When more than one parameter in the model is known with uncertainty, the assessment can get complicated, notably in the presence of strong interactions between parameters. As such, simulation processes considering variability and uncertainty separately can be developed. This is known as a second order or two-dimensional Monte Carlo simulation. In this second order process, the Monte Carlo simulation of variability is embedded in the Monte Carlo simulation of uncertainty (Frey, 1992). By considering variability and uncertainty of the parameters separately, a measure of uncertainty of the outputs can be obtained, aiding in the interpretation of the resulting probability distributions of a risk assessment. Major sources of uncertainty (most are not only uncertain but also variable) in survival of *Salmonella* in peanuts and other low water activity foods can include the prevalence of *Salmonella* in the product, the survival parameters, and certain characteristics of the processes.

10.4.5 Available Tools in Risk Assessment

Due to the nature of Monte Carlo simulations, where multiple probability distributions are sampled in each iteration and numerous iterations are conducted in each simulation, software programs must be used to run the analysis. An example of such a program is FDA-iRISK®, a free software tool that can be used to estimate the public health outcome and economic burden of hazards (microbial and chemical) in foods (http://foodrisk.org/exclusives/fda-irisk-a-comparative-risk-assessment-tool/). It is a web-based system that enables users to relatively rapidly conduct a fully quantitative full probabilistic risk assessment of food safety hazards. The tool requires the user to input data but provides (behind the scenes) the challenging computational infrastructure that supports a risk assessment. For more information on FDA-iRISK® and example case studies, the reader is referred to Chen *et al*. (2013). Other software tools available that require prior knowledge on the mathematics and computational challenges behind a quantitative risk assessment are @RISK by Palisade Corporation, Crystal Ball by Oracle, and Model Risk by Vose Software. These tools are add-ins to Microsoft Excel that allow the flexibility of Monte Carlo simulations in a spreadsheet model format. Others, like Analytica by Lumina Decision Systems, require independent software to run Monte Carlo simulations, often integrating probabilistic analysis from the start, which may increase ease of use and speed of computation. Risk

assessments have also been published using general statistical software such as SAS, R or MatLab; these require a risk assessor fully experienced in quantitative risk assessment to perform the analyses.

10.5 Summary

This chapter has provided an introduction to modeling the survival of *Salmonella* in low water activity foods, serving as a framework on key aspects to consider when developing predictive models and their use in quantitative microbial risk assessment. It has included a step-by-step approach on how to develop predictive models for *Salmonella* using raw peanuts as the example food product. It has also included an overview on the factors to take into account when choosing available nonlinear versus linear inactivation models, a framework on how to incorporate variability and uncertainty as well as the importance of model validation. The scientific process of a quantitative microbial risk assessment for *Salmonella* has been presented using peanuts as the example low water activity food, including a reference to the available tools to conduct a risk assessment. Data on *Salmonella* contamination levels (prevalence and concentration) are scarce or lacking for many low water activity food commodities; and survival and inactivation studies have collected data using inocula at high concentrations (which are not those typically found in these types of products). Data on actual frequency and contamination levels, as well as studies collecting survival and inactivation data at lower concentrations, would be useful and aid in improving the prediction potential of current available models for *Salmonella* in these types of foods, as well as models to assess the risk of human salmonellosis arising from the consumption of low water activity food products.

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