

Risk Assessment and Predictive Microbiology

ROBERT L. BUCHANAN* and RICHARD C. WHITING

ABSTRACT

The need and desirability of being able to perform quantitative microbial risk assessments for food processing and preparation operations has been discussed extensively for the past several years. However, there has been little application of this approach in large part due to the need to account for the changes in bacterial populations as a result of food environments and processing. The use of predictive food microbiology models has the potential for overcoming these limitations. Through integration of predictive modeling with risk assessment, it is possible to estimate how changes in unit operations are likely to effect the overall safety of a food. Hypothetical examples of how these techniques could be applied to both single-step and multiple-step food-processing and preparation operations are provided.

Key words: Modeling, risk analysis

During the past ten years the development of concepts and data has allowed predictive food microbiology to become a distinct specialization within food microbiology. These scientific advances have resulted a variety of mathematical models, particularly for foodborne pathogens, and the transfer of this technology via the development of user-friendly application systems. Simultaneously, there has been a call for the application of quantitative risk assessment techniques to microbiological food safety issues. The increased interest in these interrelated areas is not coincidental, but instead reflects a need on the part of the food industry and its associated regulatory agencies for enhanced analytical tools to assist in the evaluation of risk management options. The food industry's movement to "second-" and "third-generation" foods that rely on multiple barriers to control microorganisms requires an ability to deal quantitatively with a range of factors that influence safety. The general acceptance and implementation of HACCP programs throughout the food industry are having a similar impact. This process control approach to food safety is emphasizing the need for quantitative assessment techniques. However, quantitative microbial risk assessment is an approach that is

in its infancy, with many of the concepts and techniques awaiting elucidation. The purpose of the current manuscript is to (i) provide a brief introduction to some of the requirements and concepts that underlie microbial risk assessments, (ii) establish the critical role that predictive microbiology must play in applying microbial risk assessment techniques to foods, and (iii) give examples of how these two approaches can be coupled to predict how changes in food processing and preparation protocols are likely to impact public health.

MICROBIAL RISK ASSESSMENT

Risk analysis is traditionally subdivided into three inter-related, but distinct phases: risk assessment, risk management, and risk communication. The focus of the current presentation will be on risk assessment, which attempts to establish the risk, either relative or absolute, that an event or factor will negatively affect a population. In the case of microbial food safety issues, the focus is the ability of a foodborne pathogen to produce an infection or cause a disease. For the purposes of the current discussion, consideration will be primarily limited to enteric bacterial pathogens. When discussing enteric pathogens, infection and disease are differentiated using the following definitions: *Infection*, the ability to colonize and reproduce in the intestinal tract; and *morbidity*, the production of a measurable disease response.

A risk assessment is actually composed of three separate components. The first is a measure of the dose-response relationship between the pathogen and the host. This can either be qualitative or quantitative. However, to fully realize a quantitative microbial risk assessment, an estimate is needed of the relationship between pathogen numbers and the percentage of the target human population evidencing a response. In the case of enteric pathogens, most data currently available relates to the ability of these bacteria to establish infections. The second factor is the relative severity of the disease. This requires an attempt to measure the relative public health impact of the various diseases. For example, while *Clostridium perfringens* and *Shigella dysenteriae* both cause enteric infections, the latter has the potential for being much more dangerous. The final factor is

estimation of exposure faced by the population. This is an estimate of the levels of a pathogen ingested by consumers on a population basis and must take into account both the incidence and prevalence of the microorganism. In microbial risk assessment, each exposure is considered a distinct event. We are not generally concerned with cumulative exposure effects except possibly in relation to the development of immunity or chronic autoimmune diseases.

When considering the potential uses of quantitative microbial risk assessment techniques, it is important to emphasize that they are tools to be used for reaching risk management decisions and not a substitute for sound judgement. They provide estimates of probabilities of infection or disease. It is up to the users of that information to determine if that probability is significant or should lead to a specific course of action. Such questions are typically complex and require the integration of science, politics, economics, and human behavior. For example, the use of risk assessment in developing public policy implies that there is a level of risk that the public is willing to tolerate. The specific uses of microbial risk assessment for establishing public policy have been very limited, with the most important application being the Environmental Protection Agency (EPA) use of risk assessment techniques to establish standards for enteric pathogens in drinking water. However, there are a number of food regulations and guidelines that have a strong basis in risk assessment. For example, the low-acid canned food regulations are based on subjecting foods to a thermal process capable of achieving a 12-D reduction in *Clostridium sporogenes* spores. Another way of phrasing this is that the can receives a heat treatment that reduces the probability of an initial single spore surviving by a factor of 10^{12} .

It is important to note that when dealing with microorganisms, the infectious unit is a single cell. While the probability of acquiring an infection by ingesting a single pathogen cell may be exceedingly small, it is not zero. The concept of minimum infectious doses is not accepted by many microbial risk analysis investigators, who instead assume that there is no threshold when dealing with human populations and infectious bacteria. However, all agree that the risk (probability) of infection and morbidity increases with increasing dose.

It should be noted that the above assumption is pertinent only to infectious foodborne pathogens such as *Salmonella* spp., *Shigella* spp., *Escherichia coli* O157:H7, and *Listeria monocytogenes*. In the case of toxigenic foodborne microorganisms such as *Staphylococcus aureus* and *Clostridium botulinum*, a minimum dose may be pertinent because sufficient amounts of growth may be required to produce enough toxin to elicit a response. In this instance, microbial risk assessments require a somewhat different approach that couples chemical risk assessment techniques with predictive microbiology. While this class of foodborne pathogens will not be discussed further, the concepts for performing reasonable risk assessments are similar to those for infectious bacteria.

Currently, a key complication in the application of risk assessment techniques to microbial food safety issues is that, unlike most chemical toxins, the levels of bacteria are not

constant. They can change drastically as the result of growth or inactivation. The ability to run risk assessment scenarios to explore the potential impact of changing food-processing or food-preparation protocols is dependent on being able to make reasonable estimates of the levels of a pathogen that consumers are ingesting. The ability to estimate exposure is, in turn, dependent on being able to estimate (i) the probability that the pathogen is present in the food ingredients, (ii) the initial levels of the pathogen that can be expected when present, and (iii) how these levels change as a result of operations associated with the processing, preparation, and storage of the food.

PREDICTIVE FOOD MICROBIOLOGY AS THE KEY FOR PERFORMING QUANTITATIVE MICROBIAL RISK ASSESSMENTS OF FOODS

While estimating exposure appears a monumental task, a goal of this manuscript is to propose that it is within current capabilities to begin using microbial risk assessment techniques. Information on the presence and levels of foodborne pathogens can be acquired by a food manufacturer from historical data on the microbiological characteristics of their products and raw ingredients. Alternatively, data from large-scale surveys such as the recent U.S. Department of Agriculture (USDA) Food Safety and Inspection Service baseline study for steers and heifers can provide estimates. However, knowing the initial levels of foodborne pathogens is only the start for estimating risk actually faced by the consumer. We feel that a key tool for estimating the impact of food environment and processes, and thus the exposure faced by the consuming public, is predictive food microbiology. The remainder of this manuscript will focus on introducing a potential approach for coupling and using risk assessment and predictive microbiology models. To underly this basic assertion, we make two working hypotheses.

The first is that predictive food microbiology models provide an effective means for rapidly estimating the impact of various unit operations on the growth, inactivation, and survival of foodborne pathogens. It is further assumed that models available for different unit operations can be linked to get an estimate of the overall impact of food processing, preparation, and storage on the levels of the pathogen being evaluated. Of course, the accuracy and variability of the predictions will be dependent on how well the models fit the specific food being considered, and ideally each of the models used will have been validated for the product being evaluated. The characteristics and use of predictive microbiology models have been reviewed extensively (7). However, even moderately accurate models can provide a means for exploring relative risks through the use of risk scenarios. This can help identify critical steps in a process in relation to control of microbiological concerns (1).

The second hypothesis is that once a dose-response relationship has been established, unless one affects a pathogen's resistance or a host's susceptibility, the key data for a microbial risk assessment in foods are estimates of exposure (i.e., the numbers of pathogens ingested by consumers). Again, since there are no cumulative effects associated

with most foodborne pathogens, exposures are unique events, and the dose that is used to predict a response is the number of cells of the pathogen that are eaten by a population of consumers.

We make this assumption understanding that there are instances where there may be exceptions. For example, if one were considering the susceptibility of a specific subpopulation, an altered dose-response relationship might have to be assumed. One could argue that this is a different population with its own distinct characteristics, instead of a subset of the general population. It is always important to remember that risk assessment is a statistical tool that deals with populations, and is not for predicting the risk for any one individual.

On the pathogen side, one could postulate instances where a food process alters the virulence or resistance of a pathogen. For instance, preexposure to mild acids can increase the ability of enteric pathogens to survive passage through the stomach and thus increase their virulence. Likewise, specific food ingredients and entrapment in lipid droplets have been hypothesized to protect enteric pathogens from gastric acids, and thus increase their relative infectivity (2, 4). However, in most instances, food-processing operations are more likely to physiologically stress bacterial cells, and thus decrease their virulence. In this case, assuming no change in virulence provides a safety margin in the final calculations.

In developing examples of how predictive food microbiology and risk assessment models can be linked, we have opted to use the beta Poisson distribution model for describing dose-response relationships (5):

$$P_i = 1 - (1 + N/\beta)^{-\alpha}, \quad (1)$$

where P_i is the probability of infection, N is exposure (pathogen level in colony-forming units [CFU]), α and β are coefficients specific to the pathogen.

This empirical model has been used extensively and is considered particularly effective for describing dose-response relations when assessing low levels of bacterial enteric pathogens (5). It describes a sigmoidal dose-response relationship that assumes no threshold (Fig. 1). Instead, this assumes that there is a small but finite risk that an individual can become infected after exposure to a single cell. Examples of such probabilities for enteric pathogens are provided in Table 1.

As an initial example of combining predictive microbiology and dose-response models to explore questions of food safety interest, let us examine the risk of being colonized with *Shigella flexneri* (one of the species responsible for bacillary dysentery) if someone temperature abused a ready-to-eat food that was initially contaminated with a single cell of the pathogen. An aerobic storage temperature of 15°C in combination with a pH of 6.5 and a NaCl concentration of 0.5% will be assumed and used to solve growth kinetics models of Zaika et al. (8) for *S. flexneri*. These polynomial models simultaneously consider temperature, pH, NaCl, and NaNO₂, modeling the Gompertz parameters. The models were developed using a large set of

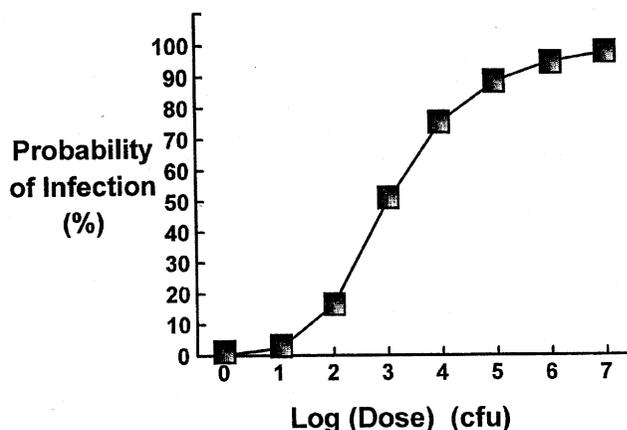


FIGURE 1. Example of the type of dose-response curve generated by the beta Poisson distribution model. The specific example is for a *Salmonella* population using the α and β values reported by Rose and Gerba (6).

experimental data. The Gompertz equation is a sigmoidal relationship that has been used extensively as a primary model for describing changes in bacterial populations (i.e., growth) as a function of time (3):

$$\log(N_t) = A + Ce^{-e^{-B(t-M)}}, \quad (2)$$

where N_t is population density (CFU/ml), A is initial population density (log [CFU/ml]), B is relative maximum growth rate ([log (CFU/ml)]/h), C is difference in initial and maximum population densities (log [CFU/ml]), and M is time of maximum growth rate (h). This equation can be subsequently used to calculate more commonly used growth kinetics such as lag-phase durations, generation times, and maximum population densities.

The N_t value generated as the output of the predictive microbiology model was then used as the input for the beta Poisson distribution model, using published α and β values (6). The results for this example are presented graphically in Figure 2. It is apparent that the risk of infection remains relatively low over the course of the temperature abuse period until the pathogen enters exponential growth, whereupon calculated risks increase dramatically. Using the same approach we could run a series of temperatures or time periods to estimate the relative impact of different abuse situations.

TABLE 1. Examples of reported probability of infection values (P_i) for exposure to a single microorganism of various enteric pathogens (6)

Enteric Pathogen	Probability of infection (P_i)
<i>Salmonella</i> spp.	2×10^{-3}
<i>Shigella</i> spp.	1×10^{-3}
<i>Vibrio cholerae</i>	7×10^{-6}
Rotavirus	3×10^{-1}
<i>Entamoeba histolytica</i>	3×10^{-1}
<i>Giardia</i> spp.	2×10^{-2}

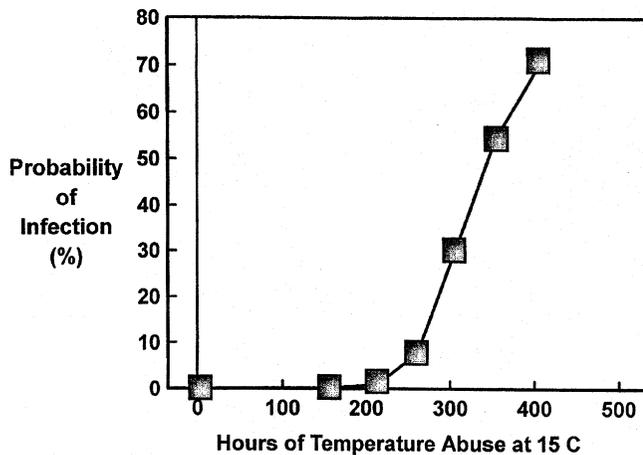


FIGURE 2. The effect of duration of temperature abuse at 15°C on the probability of *Shigella flexneri* infection. The probability of infection values were calculated using α and β values reported by Rose and Gerba (6) and the growth kinetics models of Zaika et al. (8). An initial level of 1 cell was assumed, and the points represent the times when cell number increases to 10, 100, 1000, 10,000, 100,000, and 1,000,000. The following formulation variables were assumed for solving the growth kinetics models: pH, 6.5; NaCl, 0.5%; NaNO_2 , 0 $\mu\text{g/g}$.

APPLICATION TO MULTISTEP FOOD PROCESSES

The same approach can be used to solve more complex problems by sequentially solving models that represent the different phases of a food process. Again using a hypothetical example, we will consider two what-if scenarios for a hypothetical three-step process where a raw food that may have *Salmonella* cells present is initially stored at chill temperatures, cooked at 60°C, stored for a second period at chill temperatures, and then consumed without further processing. To further demonstrate the potential of this approach to consider "real" food systems, we will also introduce the concept of population distributions. The initial level of a pathogen is likely to vary among the individual samples examined, both in regard to its presence and its initial levels. That reflects the fact that pathogens, particularly in raw foods, are not homogeneously distributed. The hypothetical distribution of *Salmonella* cells in this set of examples is presented in Figure 3. The growth model employed was that of Gibson et al. (3). It was assumed that the pH of the hypothetical food was 7.0 and the sodium chloride content was 0.5%. The beta Poisson distribution model was used in conjunction with the α and β values reported by Rose and Gerba (6). A hypothetical D-value of 0.4 min was assumed, and the effect of the thermal processing step was calculated using the equation

$$\log(N) = \log(N_0) - (t/D), \quad (4)$$

where N is the number of microorganisms after the cook step (CFU/g), N_0 is the initial number of bacteria (CFU/g), D is the D-value ($[\log(\text{CFU/g})]/\text{min}$), and t is the duration of the cook step (min). For the sake of simplicity, neither come-up nor cool-down times are considered.

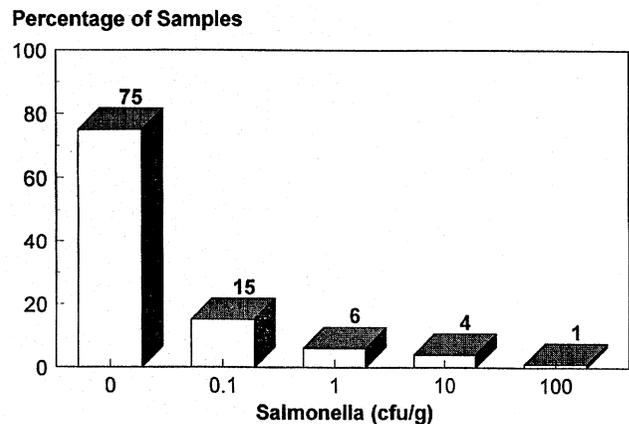


FIGURE 3. Distribution of initial *Salmonella* levels in raw food used in hypothetical multistep microbial risk assessment scenarios.

In the first scenario (Table 2), an initial storage at 10°C for 48 h was assumed, followed by a cook period of 3 min and a second storage period of 72 h at 10°C. The 10°C storage, which would be considered a marginal abuse temperature, was selected because it is the lowest temperature within the valid range covered by the model of Gibson et al. (3). This model includes an estimate at the lag phase duration which substantially delays growth at this temperature. However, shorter lag times could be estimated based on the prior history of the inoculum. The cook time was selected on the basis of it being approximately a 7-D process; this is often the target level for many heat processes for elimination of *Salmonella* spp. Sequentially solving the growth and thermal inactivation models for each of the initial population levels allows one to estimate the levels of *Salmonella* cells that a population of consumers is likely to be exposed to when consuming the product. In this scenario, even in the 1% of the product that had the highest initial *Salmonella* counts, the levels of the pathogen are reduced to less than one surviving cell for every 10,000 g of food. Once these final bacterial population levels have been calculated, they can then be converted to probabilities of infection values using the dose-response relationship. It is apparent on

TABLE 2. Example of a hypothetical risk assessment calculation for a multistep process of a ready-to-eat food using predictive microbiology models: Scenario #1

Process step ^a	<i>Salmonella</i> population (log CFU/g)				
	Distribution of initial population levels (%)				
	75	15	6	4	1
IN	—	-1.0	0.0	1.0	2.0
IS	—	-0.8	0.2	1.1	2.1
TP	—	-8.3	-7.4	-6.4	-5.4
FS	—	-7.5	-6.6	-5.7	-4.8
P_i^b	0	8.8×10^{-11}	6.5×10^{-10}	5.1×10^{-9}	4.1×10^{-8}

^a IN, before initial storage; IS, after initial storage at 10°C for 48 h; TP, after thermal process at 60°C for 3 min; FS, after final storage at 10°C for 72 h.

^b P_i , probability of infection per gram of food consumed.

TABLE 3. Example of a hypothetical risk assessment calculation for a multistep process of a ready-to-eat food using predictive microbiology models: Scenario #2

Process step ^a	Salmonella population (log CFU/g)				
	Distribution of initial population levels (%)				
	75	15	6	4	1
IN	—	-1.0	0.0	1.0	2.0
IS	—	1.6	2.6	3.6	4.6
TP	—	-3.4	-2.4	-1.4	-0.4
FS	—	1.8	2.7	3.7	4.7
P_i^b	0	1.1×10^{-1}	4.1×10^{-1}	7.0×10^{-1}	8.6×10^{-1}

^a IN, before initial storage; IS, after initial storage at 15°C for 48 h; TP, after thermal process at 15°C for 72 h.

^b Probability of infection per gram of food consumed.

the basis of the assumptions made in this example that the risks associated with the consumption of a food generated with this process would be minimal.

Once a template for solving the risks associated with a set of unit operations has been established, it is then a simple matter to rapidly examine the effect of altering one or more of the steps. An additional scenario of the above process is provided as an example. In this second scenario (Table 3), the temperature during the initial storage period is raised from 10°C to 15°C, the cooking time is reduced from 3 to 2 min (7-D to 5-D), and the final storage temperature is raised to 15°C.

These multistep scenarios demonstrate quantitatively three concepts that food microbiologists deal with daily in a qualitative manner. The first is that regardless of the abuse encountered, the probability of disease from a specific microbial agent is nonexistent if the pathogen is never present. It is apparent that for the 75% of the product that initially did not have *Salmonella* cells in it, the probability of infection remains zero despite the extent of the abuse. The second and third concepts are interrelated. The second is that each of the steps has an impact, and it is the integration of the all of the unit operations that determines the overall microbiological safety of the product. However, this leads to the third concept, which is that some steps in food processing and preparation have a greater impact than others. In the case of the current example, it is apparent that the duration of the cook step and the final storage conditions have a large impact on the relative safety of the process. In the current example, this reflects the fact that the initial storage time was selected such that at 10°C, the *Salmonella* population would be expected to be just completing the lag phase. The ability to evaluate the steps of a process in this manner should be particularly useful in the hazard analysis and critical control point identification phases of developing and implementing a HACCP plan.

NEW TOOLS

The ability to perform quantitative risk assessments and to include within these analyses predictive microbiology

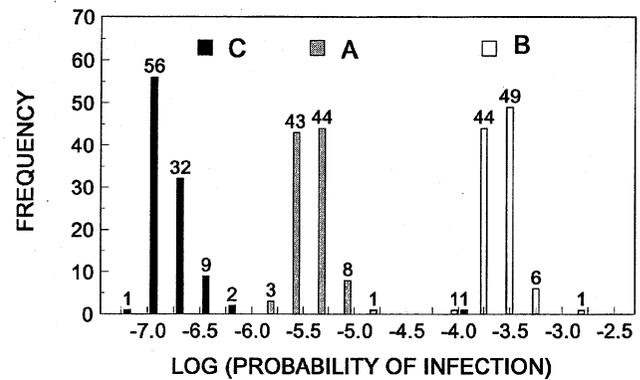


FIGURE 4. Hypothetical example of the use of “@Risk” software for performing multistep microbial risk assessment. The frequency (%) of occurrence in the simulations of the log probability of infection is presented for three different process sequences. IS, after initial storage (at); TP, after thermal process (at); FS, after final storage (at). A: IS, 10°C for 72 h; TP, 2-D reduction; FS, 20°C for 8 h. B: IS, 10°C for 72 h; TP, 2-D reduction; FS, 20°C for 12 h. C: IS, 10°C for 72 h; TP, 5-D reduction; FS, 20°C for 12 h.

models is being enhanced by introduction of new risk analysis software. One such program that our laboratory is currently exploring is “@Risk” (Palisade Corp., Newfield, NY). Instead of dealing with a single-point analysis, this software allows the incorporation of a variance term for each step where there is a distribution of potential values. Then using statistical simulation techniques, the models are repeatedly solved to generate a predicted distribution of the risks associated with the overall process. As an “add-on” for either Lotus 1-2-3 or Excel, the software is in a familiar format and is quite easy to use. Three scenarios based on a simple “@Risk” analysis using the same multistep food process as above are presented in Figure 4. This example assumes an initial distribution of *Salmonella* of 74% with 0.03 log CFU/ml of food, 25% with 0.3, and 1% with 3.0. The food (pH 6.5 and 1% NaCl) is given a 2-D cook followed by a mild 8-h abuse (A). Most of the simulations result in a probability of infection in the range of 10^{-5} to 10^{-6} , although one simulation was $10^{-2.8}$. Phrased differently, a probability of 10^{-5} to 10^{-6} is equivalent to feeding a million people each 1 g of this food and expecting between 1 and 10 to acquire a *Salmonella* infection. When the abuse period was increased to 12 h (B), the probability of infection increased to 10^{-3} to 10^{-4} , a situation that would probably be considered unsafe. If the food were given a 5-D cook (C) the same 12-h abuse would have a probability of only 10^{-6} to 10^{-7} . However, in this last situation there was one simulation with a probability of 10^{-4} . This illustrates, along with situation A, the consequence of a few food samples with high initial pathogen populations.

CONCLUDING REMARKS

Risk analyses have been conducted for chemical toxicants with increasing sophistication over the last two decades. During the past several years there has been increasing interest internationally in conducting risk analyses on microbial food safety issues. However, this call has

been largely unanswered because of the lack of risk assessment techniques that could account for the growth or inactivation of pathogenic bacteria. The integration of risk assessment and predictive food microbiology models has the potential for overcoming this problem. Using the "unitoperations" risk assessment approach introduced in this manuscript, it should be possible to perform rudimentary assessments of multistep food-processing and preparation systems, in a manner similar to the admittedly simple examples provided. With further refinement of these and other techniques, it should be possible to develop sophisticated risk assessment models that could help rapidly estimate the impact of changing a food process or formulation on public health and safety. These techniques should significantly aid in the establishment and implementation of HACCP programs within the food industry. However, in doing so, it must always be remembered that while these techniques will provide a potentially powerful tool that will assist in the generation of risk assessments, they can only augment but never substitute for sound scientific judgment.

REFERENCES

1. Buchanan, R. L. 1994. Enhancing food safety through the use of predictive microbiology, p. 169–204. Proc. 3rd ASEPT Int. Conf. Food Safety. Laval, France, June, 1994.
2. D'Aoust, J.-Y. 1985. Infective dose of *Salmonella typhimurium* in cheddar cheese. Am. J. Epidemiol. 122:717–720.
3. Gibson, A. M., N. Bratchell, and T. A. Roberts. 1988. Predicting microbial growth: growth response of salmonellae in a laboratory medium as affected by pH, sodium chloride, and storage temperature. Int. J. Food Microbiol. 6:155–178.
4. Greenwood, M. H., and W. L. Hooper. 1983. Chocolate bars contaminated with *Salmonella napoli*: an infectivity study. Brit. Med. J. 286:1394.
5. Haas, C. N. 1983. Estimation of risk due to low doses of microorganisms: A comparison of alternative methodologies. Am. J. Epidemiol. 118:573–582.
6. Rose, J. B., and C. P. Gerba. 1991. Use of risk assessment for development of microbial standards. Water Sci. Technol. 24:29–34.
7. Whiting, R. C., and R. L. Buchanan. 1994. IFT scientific status summary: microbial modeling. Food Technol. 48(65):113–120.
8. Zaika, L. L., J. G. Phillips, and R. L. Buchanan. 1992. Model for aerobic growth of *Shigella flexneri* under various conditions of temperature, pH, sodium chloride and sodium nitrite concentrations. J. Food Prot. 55:509–513.