Part 3.

Exposure Assessment

3.1 INTRODUCTION

In a quantitative microbiological risk assessment, the exposure assessment describes the pathways through which a pathogen population is introduced, distributed and altered in the production, distribution and consumption of food. The result desired from the exposure assessment is the prevalence, concentration and, if possible, virulence of the pathogen in foods at the point that they are eaten and the level of consumption of the food by the population of interest.

In many cases, data necessary to complete the exposure assessment are usually not known, in particular the frequency of contamination of foods and the total pathogen numbers ingested by consumers. An estimate can be derived, however, based on knowledge of contamination levels and prevalence at some earlier point in the farm-to-fork chain, and on models of the effect of physical processes and conditions that the food undergoes from then until the point of consumption, i.e. final pathogen numbers ingested by consumers.

This section aims to identify the data needed to assess human exposure to *L. monocytogenes* in ready-to-eat (RTE) foods; potential sources of that data; tools and techniques to overcome gaps in the data; and approaches for synthesizing data using models to enable estimation of exposure.

Conceptual and mathematical approaches that can be used in exposure assessment are also described, such as "predictive microbiology" models that can help provide necessary information and fill some of the data gaps. Such models need to be validated in products of similar microbial ecology to the product of interest. Existing data concerning current understanding of the microbial ecology of *L. monocytogenes* in foods is presented to assist in assessment of predictive microbiology models for use in exposure assessment.

Thus, an assessment of foodborne exposure to *L. monocytogenes* typically requires acquisition of data that:

- describe the prevalence of *L. monocytogenes* in ingredients, or specific finished products of interest, or both;
- describe the concentration of *L. monocytogenes* in ingredients, or specific finished products of interest, or both;
- describe the amount of the product eaten at each meal or serving and the frequency of eating, and, if possible, the consumption characteristics of sub-groups of the population that are particularly susceptible to listeriosis;

- enable the prevalence and concentration at one point in the food chain to be determined from an earlier point in the chain, e.g. storage times and temperatures, and from the microbial ecology, e.g. growth potential in the food; and
- determine the simplifying assumptions and process model that the exposure assessment will include. It is impossible to include in a model all of the situations that a food may experience.

Many of these data are typically derived from studies intended for other purposes and are not ideally suited for the objectives of exposure assessment. Often, they are published in the scientific literature, or appear in reports from regulatory authorities performing routine monitoring. Other sources for these data are import and export control services for quarantine purposes; outbreak investigation reports; and industry files. Unpublished reports from government or industry are not always accessible because of confidentiality concerns. Ideally, the studies used for exposures assessment should be comprehensive national surveys of the specific foods in question, with information on the extent of contamination (prevalence) and level of *L. monocytogenes* contamination in the product (concentration). These are rarely available, and smaller surveys within several countries often have to be used to estimate the contamination of RTE foods by *L. monocytogenes*.

In such studies, information about concentration is often lacking. Under the zero-tolerance regulatory approach adopted by many authorities towards *L. monocytogenes* in RTE foods, concentration is not of particular interest to the requestor and supervisor of the surveys, particularly when faced with the fact that concentration data are more time consuming and costly to acquire. Zero tolerance implies regulations that require that the hazard not be detectable in a test sample of specified size. Many countries specify the absence of *L. monocytogenes* in a 25-g test sample in RTE foods as the tolerable limit.

Data about consumption of RTE foods are also limited. These are usually available only from government sources, usually through national or regional nutrition surveys. The surveys often capture covariate information about those consumers and non-consumers. Those data help, for example, to estimate consumption patterns separately for age and gender classes, enabling inferences to be drawn about consumption by at-risk groups. Some surveys, though, do not have the level of detail to identify a specific RTE food, the "foods eaten" tending to be grouped into broader categories based on nutritional composition, but which may not be related to the risk of listeriosis. More specific consumption data can be derived from the individual records of each consumer surveyed. These data are kept by some survey authorities, and are available under some circumstances, but are not publicly released for reasons of confidentiality. If available, those data can also be used to better determine the consumption patterns of at-risk groups. For example, the Australian National Nutrition Survey (ABS, 1995) included a health status survey, but few of the health-related questions addressed known susceptibility factors for listeriosis.

Another source of data, complementary to that of the consumption surveys, is the inventory databases of food retailers, which provides complete and specific data on the number of units of every product type sold. Most stores and chains can provide estimates of their market share and "wastage" (i.e. product not sold but discarded because of spoilage, damage or other loss), and, from this, estimates of specific consumption levels from national to local levels can be derived. Commercial confidentiality and consumer privacy are a potential issues in collecting and accessing these data. Information is also available

commercially from market research companies that specialize in determining consumer preferences and volume of products purchased. These reports are used by industry for marketing, but risk assessors may purchase some data from them.

These data help to get close to consumption characteristics like the ones listed above. The statistical agencies of many countries publish aggregate disappearance data – production, import and export – for some raw and processed foodstuffs. Some of the data are detailed enough for purposes of exposure assessments.

3.2 EXPOSURE DATA

3.2.1 Introduction

In a quantitative risk assessment, the key desired output of the exposure assessment is prevalence, concentration and, if possible, physiological state of *L. monocytogenes* in foods at the point of consumption. In the case of *L monocytogenes*, although the final numbers ingested by consumers are usually not known, an estimate can be derived based on models of the effect of physical processes and conditions that the food undergoes through the farm-to-fork chain. Such estimates are based on predictive microbiology models, and these are discussed in Appendix 3, including their limitations and methods for assessing their reliability. A strength of the risk assessment approach is that it can assess contributions to risk from all points along a food's journey from the point of harvest or slaughter to when it is consumed, enabling prioritization of risk management actions. While much attention has been paid to modelling risk from farm to fork, it is not always necessary to include the entire food chain to answer the risk management question, as in the case of the questions addressed in the current risk assessment (see Part 5 of this report).

The models are parameterized by data from studies carried out on products or their ingredients at different stages in the production-to-consumption chain. Information on what is in a serving requires information on the extent (prevalence) and level (concentration) of *L. monocytogenes* in a single package of the food, i.e. individual consumer units. Even if this is known at the point of manufacture, an estimate of the extent of growth or die-off during retail and consumer storage and handling has to be made. The only practical means of doing this is through modelling different components in the production-to-consumption chain. Mathematical models have been developed for growth, survival and inactivation of *L. monocytogenes* in laboratory broth media and some foods. The most reliable of these models are developed from systematic studies under carefully controlled conditions known to exert a major influence on *L. monocytogenes* growth, namely temperature, water activity (a_w) or NaCl concentration, pH and levels of preservatives, including organic acids and nitrite, etc. These models may have to be modified for specific foods and their full complement of ingredients. A last step estimates the meal sizes for the RTE foods and frequency of eating.

Process models are sometimes developed to examine how prevalence and concentration changes at points along the food chain. Models for microbial growth, survival or inactivation are developed for each step (unit operation – production, processing and handling, transportation, storage, and consumer preparation) in the progression from production up to preparation prior to consumption. The concentration at the conclusion of one step is the initial concentration for the next.

3.2.2 Prevalence

Recorded prevalence of *L. monocytogenes* in RTE foods varies with the product type and the stage in the production-to-consumption chain at which it is measured. The degree of *L. monocytogenes* contamination in ingredients differs substantially, depending on whether they are derived from farm animals, fish or shellfish, or produce. *L. monocytogenes* occurs in both uncultivated and cultivated soils and in silage and manure piles. It is less frequent in water or fish. Some geographical differences in prevalence may occur. For example, the prevalence of *L. monocytogenes* is considered to be much lower in fish products harvested from tropical waters than those derived from temperate waters (FAO, 1999). Prevalence on raw ingredients can be affected by various factors such as climate or health status of workers. Although *L. monocytogenes* in RTE foods is primarily reported in industrialized countries, it has been detected in foods produced in developing countries (Kovacs-Domjan, 1991; Salamah, 1993; Arumugaswamy, Ali and Hamid, 1994; Gohil et al., 1995; Luisjuanmorales et al., 1995; Warke et al., 2000; Xiumei Liu, pers. comm., 2000; A.S. Anandavally, pers. comm., 2000; Carlos, Oscar and Irma, 2001; Eleftheriadou et al., 2002; Dhanshree et al., 2003) and its occurrence in these countries may be more frequent than the literature suggests.

Contamination of foods by *L. monocytogenes* appears to occur most often at the processing level. *L. monocytogenes* may be present on processing equipment and facilities (walls, floors, drains, etc.), and contaminate food via water droplets, splashing, dust particles from the ceiling, and contact surfaces, including transfer by workers hands (Grau, 1993). Some RTE products may not undergo thermal or other processing sufficient to inactivate *L. monocytogenes*. In those products receiving a listericidal treatment, the presence of the pathogen is generally associated with recontamination from environmental sources prior to final packaging. Other RTE foods may be contaminated at the point of sale, for example, due to slicing of processed meats. Within the home, opened packages may be contaminated from *L. monocytogenes* present within the refrigerator or in other refrigerated foods, from the kitchen environment or from family members. Surveys of *L. monocytogenes* prevalence, conducted for purposes other than risk assessments are usually available for at least some of the RTE foods.

Section A2.8.2 in Appendix 2 describes the beta-binomial model for combining prevalence estimates from disparate sources.

3.3 MODELLING EXPOSURE: APPROACHES

3.3.1 Introduction

Microbial food safety risk assessment is a relatively new development. For developing and structuring a risk assessment, there is no one standard accepted at international or even national levels. Primarily, the exposure assessments in risk assessments conducted to date have been conducted beginning from either production stages or retail stages. Some have modelled prevalence and concentration at the time of consumption by allowing for the effects of time and temperature on growth and survival of *L. monocytogenes* from an earlier point in the chain. If necessary to meet the purpose of the risk assessment, a few have started the exposure assessments as far back in the food chain as the farm, or the water for fisheries or aquaculture products. However, lack of data about the impact of various environmental sources of contamination means that knowledge of the significance of early production stages is limited, at best. To date, they have not been used to any great extent in published exposure

assessments. In risk assessments, and therefore in exposure assessments, there is a gradation of approaches – from descriptive, through qualitative to fully quantitative – for characterizing the variable of interest, whether risk or exposure. These include:

- qualitative expressions, e.g. high, average, low, more than, less than;
- an estimate relative to some known or existing level of exposure;
- a single numerical estimate for the end result based upon a series of point estimates, e.g. the average, or the worst case;
- a set of estimates that describes the range of possible outcomes as well as the one considered most likely, e.g. an average, worst-case and conservative estimate based on series of average, worst-case and conservative estimates for each variable in the assessment affecting exposure; and
- an estimate derived by combining the frequency distribution of variables in the assessment, characterized by a frequency distribution of possible outcomes. This approach gives as complete a representation as possible of the range of possible outcomes and the probability of each, providing all the information that the other methods do, and considerably more. This approach requires the greatest amount of information and the use of mathematical modelling techniques.

Van Gerwen et al. (1997) presented a three-step plan for hazard identification in the context of risk assessment, aimed at discerning those perceived hazards that represented the greatest risk, and which warranted more detailed study. Their plan involved "rough", "detailed" and "comprehensive" hazard identification. "Rough" hazard identification selects pathogens that have been implicated in foodborne outbreaks in the food of interest. The "detailed" hazard identification selects pathogens that have been reported as being *present* in the ingredients of the food of interest. The "comprehensive" procedure considers all pathogens, and even those less likely to arise in a specific food are included in the assessment. By including those hazards currently considered to be unlikely to be present, it should be possible to create an estimate of potential problems and to deal with them proactively. That philosophy can be extended to the performance of exposure assessments. The effort expended to undertake an exposure assessment must be commensurate with the magnitude of the risk. Pre-screening of the magnitude of exposure, using simple methods, can aid decisions about the value of investing in fully quantitative assessment methods. The approach can also show where greater detail should be built into the risk assessment model and where higher quality data will be required. If a risk assessment, for example, is intended to evaluate various options in a food process, details about on-farm contamination are unnecessary and modelling the consumer handling of the food can be simplified.

Microbial hazards in foods can arise at any stage in the food chain, and be affected by subsequent processing and handling steps. Thus, the system under analysis is a continuum, often from the point of production (farm, sea) to the point of consumption, and the risks presented by hazards at one point in the chain cannot be considered in isolation from the system as a whole.

To assess exposure it is necessary to understand both:

- the amount of food consumed and by whom, and
- where in that system the hazards arise, and all factors that affect the prevalence and concentration of the hazard in the food at the time of consumption.

This section provides an overview of methods used to estimate exposure. The ideas introduced here will be discussed further when reviewing existing exposure assessments.

3.3.2 Prevalence and concentration

Prevalence and concentration of *L. monocytogenes* in foods can change as a result of:

- initial and subsequent contamination;
- physical processes, e.g. dilution by mixing with uncontaminated ingredients, or division of batches into smaller units for distribution and sale; and
- growth or inactivation in the product.

To date, despite some assessors (Bemrah, et al., 1998; FDA/FSIS, 2001) noting that *L. monocytogenes* is probably heterogeneously distributed in some foods, all published exposure assessments have assumed that pathogens are distributed homogenously within a food. Multiple sampling of a food would presumably show a normal distribution of the $\log_{10}$ CFU/g of the microorganisms. This is a clearly a simplification. A consequence of the assumption of homogeneity is that in exposure assessments prevalence and concentration of *L. monocytogenes* in foods are often considered to be related properties, particularly at very low concentrations. The observed prevalence will depend on the sample size and the extent of contamination of the batch. If the batch is contaminated at a level of >1 CFU/g, there is high probability that each 25-g sample would test positive for *L. monocytogenes.* If, however, the sample size were only 1 g, some samples would test negative. If the contamination level were 1/100 g, we would expect only 1 in 4 samples of 25 g would test positive and it would be more typical to describe this concentration as "25% prevalence".

The distribution of bacteria in a homogeneous sample is likely to follow a Poisson distribution. In that case, if the mean concentration is *X* per gram, and there are *Y* grams per sample the count of *L. monocytogenes* per *sample* is Poisson distributed, with mean *X*Y*. More importantly, the probability of a positive result for a sample of Y grams then becomes 1 -exp(-*X*Y*). Therefore, for large amounts of product, the prevalence and concentration are related and the estimate of the prevalence depends on the level of contamination and sample size. This is explicitly considered in a recent risk assessment (FDA/FSIS, 2001), although sample data for RTE foods were in some cases aggregated without regard to sample size. Thus, when incorporating data from many sources into an exposure assessment, it is important to consider the sampling methodology and test protocols, because sample sizes may differ and test methodology may differ in sensitivity Furthermore, some methods offer better sensitivity for specific types of foods than do other methods.

Similarly, products that permit the growth of *L. monocytogenes* may exhibit a low prevalence of contamination at the point of production and a higher prevalence at the point of consumption. This is not necessarily due to re-contamination, but may arise because the product was initially contaminated at a very low level. Subsequent growth in the product increases the probability of detection of that contamination. It is important, then, to recognize prevalence as "detected" prevalence. Also, the use of a more sensitive analytical method will find a higher prevalence of contaminated samples than will a less sensitive method. The estimated prevalences of the studies carry introduced uncertainty from the test methods and protocols used.

Qualitative risk assessments may be undertaken, for example, using the process of "expert elicitation". Synthesizing the knowledge of experts and describing some uncertainties permits at least a ranking of relative risks, or separation into risk categories. No true qualitative risk assessment has been conducted, however, in the area of microbial food safety. As assessors understand how qualitative risk assessments are done, they may become effective tools for risk managers because they can be conducted quickly and used to address specific questions or to demonstrate that extensive, fully quantitative exposure, and risk, assessment is not required. While there is no universally agreed methodology for qualitative exposure assessment, a useful discussion is presented in FAO/WHO [2004], which also includes a detailed example.

Many assessments of exposure of human populations to foodborne *L. monocytogenes* have been undertaken (Peeler and Bunning, 1994; Farber, Ross and Harwig, 1996; Hitchins, 1996; Lindqvist and Westöö, 2000; Buchanan et al., 1997; Bemrah et al., 1998; FAO, 1999). Most have included numerical epidemiological and prevalence data and some included concentration data for *L. monocytogenes* in specific RTE foods or classes of RTE foods. Nonetheless, in some cases the resulting assessments are descriptive or have simply ranked exposure or risk relative to some other, unquantified, level of risk (FAO, 1999; Ross and Sanderson, 2000; FDA/FSIS, 2001). Few have quantified exposure in terms of probability and magnitude of exposure, and fewer still (FDA/FSIS, 2001) have reported rigorously on the sources and magnitude of uncertainty in the estimates.

Methods for modelling growth and inactivation are discussed in detail in Appendix 2.

3.3.3 Conceptual model

The food production and distribution system being assessed can be described in a number of ways, but it is often easiest to start the process using diagrams, such as flow charts, to show the origin of hazards and the relationships and operations that can change the level and prevalence of the hazard in the food. An example of a flow chart, describing a very generic model for microbial food safety exposure assessment, is shown in Figure 3.1. That qualitative description of the factors that affect exposure (or more generally the risk), and the relationships among them, is described as a "conceptual model".

Semi-quantitative assessments can be developed using descriptors for each variable such as {high, low, normal}, or {better, worse, same}, or {+, -, 0}, or by applying a weighting system, or a combination. These methods rely implicitly on some known reference value, and have not been widely used in food safety risk assessments. Such approaches are often found in decision trees, such as that shown in Figure 3.2.

3.3.4 Mathematical models

A refinement of the conceptual model is to construct a mathematical model of the relationships. In principle, the entire system and the relationships between all variables could be explicitly defined by expressing the relationships mathematically, i.e. using algebraic notations and equations. By substituting data or values based on expert opinion for the variables in the model, the equations describing the origin and amount of *L. monocytogenes* in the food and the factors that impinge upon it can, in principle, be solved to yield a numerical estimate of exposure. Mathematical expertise is required to accurately describe the system, but it is now possible to model very complex systems relatively easily using the so-

called Monte Carlo techniques and "spreadsheet models" written using computer spreadsheet software. Frequently the conceptual model can be very complex, and the solution of the corresponding mathematical model is also made easier using spreadsheet models. While it is easy to develop spreadsheet models, it is also easy to make mathematical and logical errors in the construction of the model. It is therefore very important to verify both the accuracy of the mathematical model as a description of the system being assessed and its mathematical reliability (Starfield, Smith and Bleloch, 1990; Morgan, 1993; Vose, 1996). Texts that teach modelling skills are available (e.g. Starfield, Smith and Bleloch, 1990).

3.3.5 Point estimates

When solving exposure assessment models, a decision has to be made regarding the value of the variables to be used in the model. Typically, the factors in a system that affect exposure do not have single, fixed values but are characterized by a range of possible values. The most obvious method is to characterize the variable quantity by its central tendency value (e.g. mean, median). Thus, the mathematical model would produce an estimate of the risk characterized by the most commonly occurring scenario.

Point in Food Continuum	**Variables Affecting Dose**		
	Consumption	Concentration in contaminated units	Prevalence of contaminated units
Raw Ingredients		environmental sources affecting concentration in ingredients	season, harvest area, fodder and feeding regimes, irrigation water, etc.
⇓			
Processing		*volumetric changes:* mixing with other ingredients, changes due to dilution or concentration steps (e.g. evaporation, removal of whey) *growth or inactivation changes* brining, heating steps, holding times and temperatures,	cross-contamination, mixing with other bulk ingredients, splitting into smaller units for retail or food service
⇓			
Transport and Storage		time, temperature, product composition	
⇓			
Retail Sale		time, temperature, product composition, breakdown to smaller units	packaging and cross-contamination, portioning, breakdown to smaller units
⇓			
Home/food service		time, temperature, product composition	cross-contamination, combination with other foods
⇓			
Consumption	frequency and amount consumed affected by: season, wealth, age, sex, culture/region, etc.	heating; mixing with other components (e.g. vinegar in salads); breakdown to smaller units	breakdown to smaller units/serving portions

Figure 3.1 A generic exposure assessment model for pathogens in foods.

However, this ignores important risk characteristics, as will be discussed in more detail in the risk characterization, as the highest risk is associated with the small percentage with the highest levels of *L. monocytogenes*. An alternative approach is to use worse-case scenarios, based, for example, on the 90th or 95th percentiles. One problem with this approach, particularly when dealing with a multiple step conceptual model, is the "compounding conservatism" (Cassin et al., 1996). If conservative or worst-case values are taken for each variable, the resulting risk estimate is characterized by an extremely improbable event. It should also be noted that point estimates based on measures of central tendencies, e.g. average, or modes will not necessarily lead to an answer that represents the most likely outcome and can lead to large errors (Cassin et al., 1996).

The use of point estimates of parameters determining the probability of an adverse event has severe limitations in relation to providing an "accurate" assessment of risk (Buchanan and Whiting, 1997), and, increasingly, stochastic modelling techniques are being employed for hazard characterizations, exposure assessments and risk characterizations.

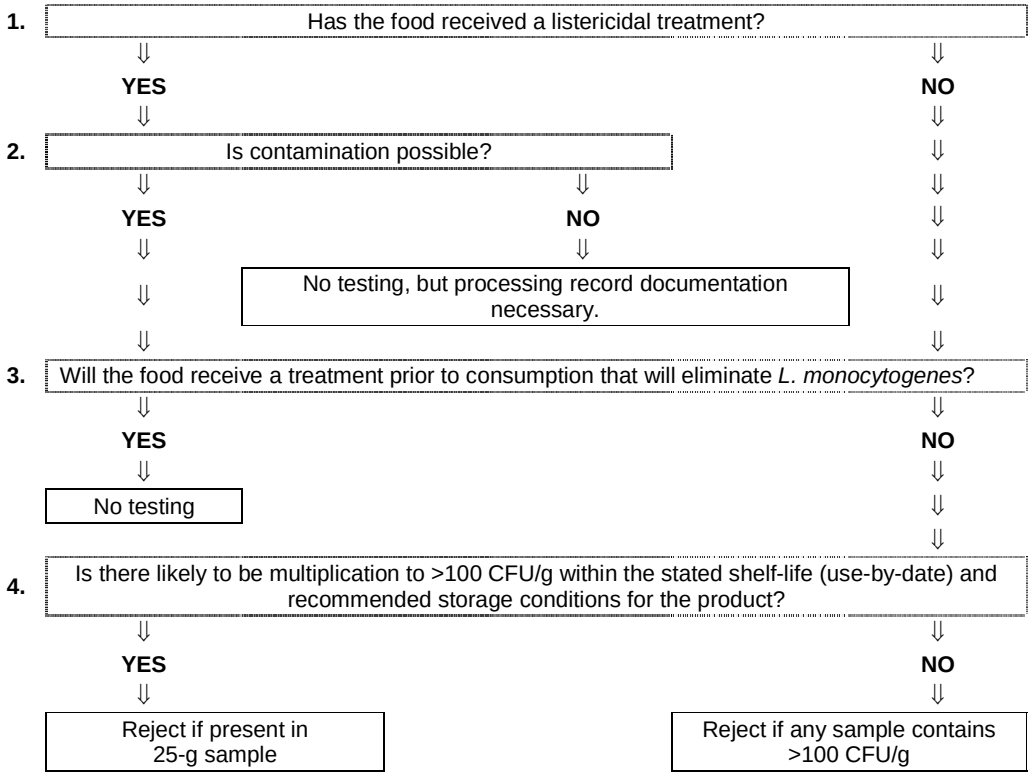

Figure 3.2 A decision tree to aid the management of the hazard of listeriosis from foods showing qualitative risk assessment decisions within a risk management scheme.
SOURCE: Reproduced from CCFH, 1999.

While increasing the potential accuracy of microbial risk assessments, two potential disadvantages are associated with stochastic modelling methods. The first is that the time it takes to develop such models may delay risk-management decisions. The second is that the complexity of the model increases the bounds of uncertainty and variability, which may become so wide as to lead to questions on the part of the risk manager regarding the reliability of the information. However, this must be put in context, namely that, in most instances, food safety decisions will be reached with or without the availability of a risk assessment.

3.3.6 Distributions and stochastic approaches

Point estimates are useful to provide a quick estimate of the magnitude of risk. To support critical decisions, however, a more accurate estimate conveys an understanding of the complete range and probability of all possible outcomes, and their consequences.

The *range* of possible values can be characterized by a minimum and maximum. More information is conveyed if some central, or *most-likely*, value is also used. In general, the possible values form a continuous spectrum of values, some of which are more likely to occur than others, i.e. they form a *distribution*. These distributions can be described mathematically.

The normal distribution is well known, but many data sets are better described by other distributions. For example, the uniform distribution describes a variable in which a value is known to vary between two limits. It is frequently used for variables for which there is no knowledge of the probability of any of those values within the limits occurring. The triangular distribution is the simplest description of minimum, maximum and most-likely values and is used to represent a possible range when extensive data are not available. The Beta-Pert distribution is similar, but gives greater emphasis to the most-likely value and less to the upper and lower limits (the "tails" of the distribution) than does the triangular distribution (see Figure 3.3).

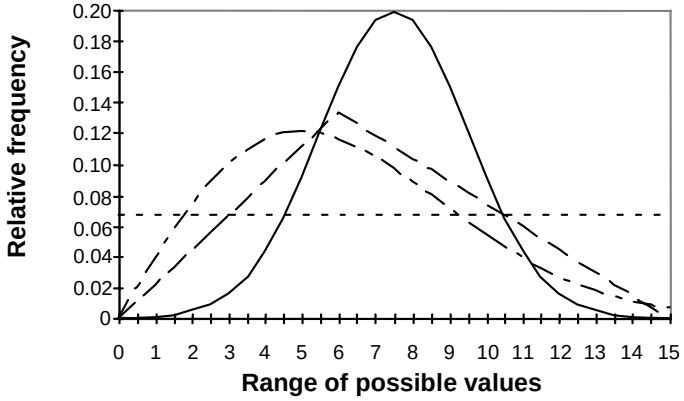

Figure 3.3 Some types of distributions used to describe ranges of values for observations. Normal (————); triangular (—— —— ——); uniform (- - - -); Beta-Pert (— - — - — -).

In certain instances, naturally occurring phenomena can be described by a mathematical equation (e.g. decay of a radionuclide) instead of the fixed variable values in the conceptual model. Lognormal, beta, gamma and Weibull distributions, for example, are used frequently to describe data. This results in the solution of the model being a distribution of possible values, based on all the possible combinations of scenario sets. The results are the range of possible outcomes. The answer obtained by this method is called an *explicit* solution. The explicit solution offers a complete representation of the range and probability of possible outcomes of a process, and provides much more insight than does a calculation based on average values. In most cases, however, the calculations and resulting equations for an explicit solution become so complicated so quickly that they cannot be solved for anything but the simplest models.

A third approach to describing risks is through simulation modelling. This is based on the Theory of Large Numbers, which effectively states that an accurate answer to a complex model can be deduced if the model is solved repeatedly using the various distributional inputs in accordance with the likelihood of occurrence.

3.3.7 Simulation modelling

Computer simulation modelling software (e.g. @Risk, Crystal Ball, Analytica,) offers a means to calculate the results for complex systems or processes for which explicit mathematical models do not exist or are difficult, if not impossible, to solve analytically. After the model is constructed, the software calculates all of the possible combinations of factors by calculating the answer many times, each cycle of which is called an iteration. At each iteration a value is selected from each variable range, at random according to the probability distribution describing that variable. The outcome is then calculated for that specific set of circumstances, i.e. that iteration. All of those values are collated to generate a distribution of possible outcomes. Because some or all of the independent variables in the model are characterized by a range of possible values, there is a range of outcomes, some of which will occur more often than others.

The distributions used to describe both the inputs and outputs of a model are composed of two components: variability and uncertainty. It is important to be able to differentiate between uncertainty and variability. Variability describes diversity that is inherent in any population. Uncertainty refers to the situation where assumptions have to be made about the ranges of values and their probabilities of occurrence. The degree of uncertainty will be reduced by the acquisition of new data or knowledge, whereas additional data will not decrease variability.

The results of an exposure assessment that employs simulation modelling techniques depend on the model, the data ranges and distributions that are used, and on the assumptions made in setting up the model. Detailed consideration of the potential pitfalls in simulation modelling are available in general references and guidelines for the use of simulation modelling in risk assessment (e.g. Vose, 1996; Morgan, 1993; Burmaster and Anderson, 1994; EPA, 1997).

3.3.8 Uncertainty and variability

Acceptance of a degree of uncertainty and variability is fundamental to an estimation of exposure in any model. Uncertainty refers to information that is required for completion of

the assessment but that is not available and has to be assumed or inferred. The basis of uncertainty is twofold: information uncertainty and model uncertainty. The information on which the exposure estimates are made is often limited. Population characteristics must be inferred from observations made on a sample drawn from the population at a specific point in time, and observed phenomena must be extrapolated to the situation under study. The assumptions on which the exposure estimates are based introduce uncertainty: simplification of complex processes into mathematical models for physical processes, inactivation and growth introduce uncertainty; small sets of scenarios are generalized to all scenarios of importance; and assumptions are made about how recognizable components of processes operate. In addition, the limitations in testing methods for *L. monocytogenes* also introduce uncertainty in the levels of the pathogen in the food supply. Many surveys test only for presence per 25 g of product.

Variability is an inherent property of all physical, chemical and biological systems. There is natural variability (heterogeneity) among the constituents of a population. In the case of the current risk assessment there are multiple factors influencing risk that each have inherent variability. The prevalence and concentration of *L. monocytogenes* in RTE foods vary, and the composition of the foods, serving sizes and frequencies, the virulence of *L. monocytogenes* isolates and the susceptibility of infected individuals were among the long list of variable parameters encountered in the risk assessment.

3.4 MODELLING THE PRODUCTION-TO-CONSUMPTION CHAIN

3.4.1 Environmental niche

Sources of *L. monocytogenes* in the environment were described in Section 1.2.

3.4.2 Preharvest

A complete exposure assessment starts at the earliest stages in the production of a food so that it can include the effect of the environment. Green vegetables or berry crops might be affected by contamination from soil, manure, irrigation, silage and the pathogens in them, for example. Insects may also play a role in the spread of organisms to crops. Pathogens may survive in manure or soil for long periods (Dowe et al., 1997); inside protozoa (Barker and Brown, 1994); and some may also penetrate the vasculature of leafy plants like lettuce, and alfalfa or mung bean seeds. *L. monocytogenes* does not occur naturally in oceans. Some aquatic environments may become contaminated with *L. monocytogenes* from human or animal sewage or from soil from cultivated and uncultivated fields carried in rainwater runoff. In such cases *L. monocytogenes* might contaminate fish and shellfish.

3.4.3 Production

After harvest, preliminary washing or cleaning of the product may remove some of the initial contamination. Transport may introduce additional or new pathogens. At each of the succeeding stages of production, changes in prevalence and concentration are likely to occur. However, unless actual measurements are taken at each these stages, they must be modelled based on the knowledge that already exists.

3.4.4 Processing and packaging

Subsequent production steps include holding, mixing and aggregation, fermentation, heating, pasteurization, brining, smoking and pickling. Some of these steps increase, but most decrease, the prevalence and concentration of pathogens. Much of *L. monocytogenes* contamination arises from environmental contamination in the processing plant. For example, aerosols from cleaning water and dirty equipment may be sources. Cooked products, e.g. processed RTE meats, should be free of *L. monocytogenes,* but may become recontaminated during subsequent handling and contact with equipment before final packaging. Slicing operations appear to be common sources of re-contamination of cooked products. Sources and routes of contamination of food with *L. monocytogenes* in food processing facilities are extensively reviewed in Ryser and Marth (1999). More recent studies include those by Norton et al. (2001) and Chasseignaux et al. (2002).

3.4.5 Transportation

Changes in the frequency of *L. monocytogenes* contamination can occur after final packaging for products that remained sealed until consumption. The number of *L. monocytogenes* can increase if the food and the storage conditions support the growth of the microorganism. This can lead to an apparent increase in the frequency of contamination if the product was initially contaminated at a level below the limit of detection of the method used to enumerate *L. monocytogenes* (see Table 3.1).

3.4.6 Retail

Changes to populations of the microorganisms can take place during storage and display. The prevalence and levels of a pathogen may change through recontamination from portioning of the opened packaged products through slicing, chopping and then repackaging. Other packages or other RTE foods then may be cross-contaminated by the same process. Ambient temperatures can permit the growth of the pathogen on contaminated slicing equipment, cutting boards, etc., and could increase the level of hazard.

Table 3.1 Ranges of environmental factors that permit growth of *Listeria monocytogenes* when all other factors are optimal.

Environmental Factor	Limits	
	Lower Limit	Upper Limit
Temperature (°C)	-2 to +4	~ 45
Salt (% water phase NaCl)	<0.5	13 – 16
(and corresponding a_w)	(0.91–0.93)	(> 0.997)
pH (HCl as acidulant)	4.2–4.3	9.4 – 9.5
Lactic acid (water phase)	0	3.8–4.6 mM, MIC[1] of undissociated acid[2] (800–1000 mM, MIC of sodium lactate[3])
Acetic acid	0	~20 mM (MIC of undissociated acid)
Citric acid	0	~3 mM (MIC of undissociated acid)
Sodium nitrite	0	8.4 – 14.4 µM (undissociated)

NOTES: (1) MIC = minimum inhibitory concentration, i.e. the minimum concentration that prevents growth. (2) From Tienungoon, 1998. (3) From Houtsma, de Wit and Rombouts, 1993.

SOURCES: The overall ranges are summarized from Ryser and Marth, 1991; ICMSF, 1996; and Augustin and Carlier, 2000a.

3.4.7 Home and foodservice

For foods that support growth of *L. monocytogenes*, time and temperature of storage are the most critical parts of this stage since RTE products may be kept refrigerated for long periods. In addition, cross-contamination to opened RTE food packages may occur in the refrigerator from other foods with *L. monocytogenes*. For some RTE foods that do not support its growth, such as dry fermented sausages, levels of *L. monocytogenes* are expected to diminish during storage, and probably at a faster rate if held at ambient temperature than if refrigerated. If there is no final heating step prior to eating, as is the usual case for RTE foods, the concentration of *L. monocytogenes* at the end of the storage period in the home or foodservice establishment will be the concentration when the food is eaten.

3.5 MICROBIAL ECOLOGY OF *LISTERIA MONOCYTOGENES* IN FOODS

3.5.1 Introduction

The dose ingested, and hence the risk of listeriosis, is dependent on the mass of food consumed and the level and frequency of contamination. However, surveys of the level of *L. monocytogenes* in foods are not conducted; instead, the dose must be inferred from exposure data acquired earlier in the food chain. In the case of the current risk assessment, retail data were employed in conjunction with predictive microbiology models and data on storage times and temperatures to predict the levels ingested. The need for this modelling reflects that when *L. monocytogenes* is present in food its numbers may increase, decrease or remain constant as a result of growth, death (or inactivation) or stasis, respectively. The degree to which growth and inactivation occur is governed by the composition of the food, the conditions under which the food is stored or subject, and the time during which those different conditions apply.

While the distributions of serving sizes of RTE foods generally only differ by a 5–10-fold range (e.g. 10–100 g), the concentration of *L. monocytogenes* within the serving can range over many orders of magnitude. Given sufficient time, *L. monocytogenes* can reach concentrations of 10^6 to 10^9 CFU/g in many RTE foods that support microbial growth. Conversely, heat treatments can effectively eliminate the microorganism in a matter of minutes. Typically, microbial populations increase or decrease exponentially over time. Consequently, if growth is possible in the product, the predicted risk resulting from that growth generally changes exponentially with time. The same is true of pathogen inactivation.

Since predictive microbiology plays such an important role in the current microbiological risk assessment, it is important that the application of predictive microbiology methods and its limitations are well understood by risk assessors, stakeholders and risk managers. A review of predictive microbiology concepts and limitations, methods of assessing predictive model performance, and techniques for the application of predictive models in risk assessment is given is Appendix 3, including a compendium of published predictive models for *L. monocytogenes* relevant to foods.

The current section presents patterns of microbial behaviour in foods and food processing, and identifies unifying principles to aid understanding of the factors that affect the ecology of *L. monocytogenes* in foods. Reviews of the ecology and physiology of *L. monocytogenes* in food products in general (Lou and Yousef, 1999) and in specific food products (Ryser, 1999a,b; Farber and Peterkin, 1999; Cox, Bailey and Ryser, 1999; Jinneman, Wekell and

Eklund, 1999; Brackett, 1999) have recently been presented. Many relevant data are collated and tabulated in ICMSF (1996) and Augustin and Carlier (2000a). The following material is based on Ross, Baranyi and McMeekin (1999) and Ross, Dalgaard and Tienungoon (2000) who reviewed the microbial ecology of *L. monocytogenes* in relation to the risk assessment of RTE seafood.

3.5.2 Growth limits

The ranges of environmental factors that permit growth of *L. monocytogenes* are discussed in detail in a number of reviews (Lou and Yousef, 1999; ICMSF, 1996; Augustin and Carlier, 2000a), as summarized in Table 3.1. These limits are not absolute, however, as discussed below, but represent the widest range of that factor when all other factors are optimal for growth. When several factors are suboptimal for growth, the ranges of each that will permit growth of *L. monocytogenes* are restricted. This is the basis of the Hurdle Concept, or "multiple barrier methods" in food preservation. There are exceptions to this behaviour. While slightly elevated salt concentration may inhibit growth rate, it has also been reported to increase the high-temperature tolerance of many bacterial species, though the effect is not universal (Gould, 1989).

For several foodborne pathogens, including *L. monocytogenes*, greatest tolerance to sub-optimal conditions is exhibited at conditions optimal for growth yield[1] (George, Richardson and Peck, 1996; Presser, Ross and Ratkowsky, 1998; Tienungoon, 1998). Conditions that maximize the growth *rate* of *L. monocytogenes* are not necessarily the same as those that maximize growth *yield*. For *L. monocytogenes*, yield is maximal when temperature is in the range of 20° to 25°C, while the growth rate is fastest at ~37°C. It is often important in growth modelling of *L. monocytogenes* to calculate the growth yields at temperatures in the 0° to 7°C range. At temperatures above or below 20–25°C, the water activity or pH growth limits of *L. monocytogenes* will not be as wide as the extreme values listed in Table 3.1. Similarly, recovery of *L. monocytogenes* from injury is most rapid at 20–25°C (Mackey et al., 1994; see also Figure 3.4).

3.5.3 Growth: rate, lag and maximum population density

Where the interaction of factors permits growth, the amount of growth that occurs in a specified time will be governed by:

- the growth rate;
- whether there is a lag time before growth is initiated; and
- the total concentration of bacteria that the food will support.

These three topics are considered individually below.

3.5.3.1 Growth rate

Growth rate is affected by factors that include:

- temperature;
- storage atmosphere;

1. In this context, yield is taken to represent the maximum cell biomass produced in a given (batch) environment. An analogous measure is maximum population density.

- salt or sugar content (often expressed as water activity);
- pH and presence of organic acids;
- preservatives such as nitrite, sorbate, etc.; and
- the presence of high levels of other microorganisms of other strains or species.

Many of these factors act independently and can be understood in terms of the relative inhibition of growth rate due to each factor. Under completely optimal conditions, each microbial strain has a unique maximum growth rate. For *L. monocytogenes,* the fastest doubling time is in the range of 35 to 40 minutes, and occurs at temperature of ~37°C, when pH is neutral, and in a rich medium that contains sufficient nutrients and has a water activity in the range 0.990 to 0.995 (1±0.5% NaCl). As any environmental factor becomes less optimal, the growth rate declines in a predictable manner. The cumulative effect of many factors at suboptimal levels can be estimated by multiplying the relative inhibitory effect of each factor. The relative inhibitory effect can be determined from the "distance" between the optimal level of the factor and the minimum (or maximum) level that completely inhibits growth. This concept is embodied in the structure of a number of the square-root type models (Ratkowsksy et al., 1982, 1983; Presser, Ross and Ratkowsky, 1998), "gamma" models (Zwietering, De Wit and Notermans, 1996) and "cardinal parameter" models (Rosso et al., 1995) derived from them.

Interactions can occur between some factors used to preserve foods. The activity of many preservatives is pH dependent. The effect is best described for organic acids. The inhibitory effect of organic acid is almost completely determined by the concentration of the undissociated form of the acid. The concentration of undissociated form can be readily calculated from the total concentration of the organic acid and the pH. If the inhibitory activity of organic acids is described in terms of the undissociated form of the acid the simple multiplicative rule (as described above) works well, as illustrated by Presser, Ross and Ratkowsky (1998) and by Tienungoon (1998) for *L. monocytogenes*. Nitrite activity is also reported to be pH dependent (Woods, Wood and Gibbs, 1989) and the results of studies by the USDA Agricultural Research Service Eastern Regional Research Centre in Philadelphia (embodied in the Pathogen Modelling Program[2]) also show a pH dependence of nitrite on the growth rate of *L. monocytogenes,* particularly at levels >125 ppm in broth. The relative inhibition of a specific concentration of nitrite is equivalent at all experimental conditions of pH, temperature and water activity. That inhibition is approximately linearly related to the total nitrite concentration.

In general, the growth of *L. monocytogenes* is reported to be little affected by anaerobic, or oxygen reduced, atmospheres (Buchanan and Phillips, 1990; Pelroy et al., 1994; Buchanan and Golden, 1995; ICMSF, 1996). However, growth is reduced by CO_2 when it used in modified atmosphere packaging (Davies, 1997; Bell, Penney and Moorhead, 1995; Ingham, Escude and McCown, 1990; Szabo and Cahill, 1998; Nilsson, Huss and Gram, 1997).

Growth rate may also be affected by the presence of high levels of other microorganisms, in a phenomenon described as the "Jameson effect" by Stephens et al. (1997). Jameson (1962), in studies concerning the growth of *Salmonella*, reported the suppression of growth of

2. Pathogen Modelling Program. Available free of charge from USDA. Download from: http://www.arserrc.gov/mfs/pathogen.htm

all microorganisms on the food when the total microbial population achieved the maximum population density (MPD) characteristic of the food. The same effect has been reported for *Staphylococcus aureus* in seafood (Ross and McMeekin, 1991), *L. monocytogenes* in meat products (Grau and Vanderlinde, 1992), in fresh-cut spinach (Babic, Watada and Buta, 1997), co-cultures of *L. monocytogenes* and *Carnobacterium* spp. in laboratory broth, fish juice and seafood (Buchanan and Bagi, 1997; Duffes et al., 1999; Nilsson, Gram and Huss, 1999), and was discussed by Peeler and Bunning (1994) in relation to their predictions of the growth of *L. monocytogenes* in raw milk.

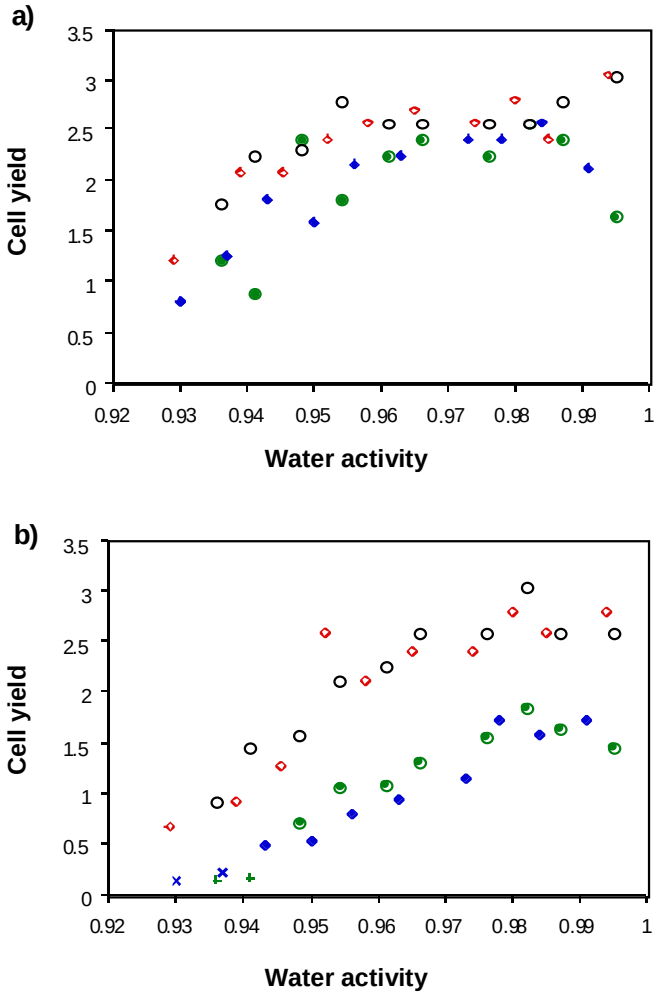

Figure 3.4 The observed cell yield of *Listeria monocytogenes* "corrected" for the non-linearity of the Optical Density (OD)-concentration relationship using the function of Dalgaard et al. (1994) and plotted against water activity (NaCl as humectant), demonstrating the influence of lactic acid, and pH; a) pH ≈5.7, and b) pH ≈5.4. Strain Scott A; growth in the absence of lactic acid (◊), and growth (◆) and no growth (×) in the presence of 50 mM lactic acid. Strain L5; growth in the absence of lactic acid (⌗), and growth (●) and no growth (+) in the presence of 50 mM lactic acid.
SOURCE: Reproduced from Tienungoon, 1998.

3.5.3.2 Maximum concentration

A corollary of the Jameson effect is that there is an upper concentration limit to the growth of *L. monocytogenes* and other bacteria in foods. Under optimal conditions, this level is of the order of 10^9 CFU/g or CFU/ml. However, the conditions of growth may limit the maximum concentration of *L. monocytogenes* that can occur. This phenomenon was reviewed by FDA/FSIS (2001) and incorporated in that exposure assessment. Specifically, at lower temperatures, the maximum growth predicted to occur was limited to levels up to 1000-fold lower than at temperatures above 8°C. Similar behaviour as a function of water activity, pH and lactic acid in broths was described by Tienungoon (1998). At pH 6.1, decline in final population numbers did not occur unless water activity (NaCl) was less than 0.935. As pH decreased, or lactic acid concentration increased, or both, the final cell density began to be reduced at progressively higher water activities, suggesting that multiple hurdles to growth reduce the maximum population density. Figure 3.4 shows this phenomenon at pH 5.4 and 5.7 and with or without 50 mM lactic acid for two strains of *L. monocytogenes*.

3.5.3.3 Lag phases or recovery from injury

Upon transfer to a new environment, microorganisms may experience a lag phase before growth begins or recommences. The effect is to reduce the amount of growth predicted. Lag time duration has often been considered erratic and evaluations of predictive models have shown that lag times are less reliably predicted than generation times (Walls and Scott, 1997; Dalgaard and Jørgensen, 1998; Augustin and Carlier, 2000a,b). This variability has often been attributed to the prior history of cells (e.g. Hudson, 1993), which is usually ill-defined or unknown, affecting the duration of the lag time.

Robinson et al. (1998) formalized a concept of the lag time as being dictated by two elements: (i) the amount of work required of the cell to adjust to a new environment or to repair injury due to the shift to the new environment, or both; and (ii) the rate at which those repairs and adjustments can be made. The latter rate is presumed to respond to the environment in the same way, relatively, as generation time, i.e. if the environment causes the generation time to double, the lag time will also double, and so forth. In recognition of this, the ratio of the lag time : generation time has been introduced to enable comparison of lag times measured in different environments (Mellefont, McMeekin and Ross, 2003) This ratio can be considered as the relative lag time (RLT). The RLT can be considered as the amount of work (whether adjustment or repair) that the cell must perform in a new environment or after injury before growth can recommence.

Systematic studies have considered the effect of the prior history of the cell, including prior temperature and osmotic stresses, on the duration of lag time and RLT of *L. monocytogenes* (Bréand et al., 1997, 1999; Delignette-Muller, 1998; Robinson et al., 1998; Ross, 1999; Whiting and Bagi, 2002, Mellefont, McMeekin and Ross, 2003; Mellefont and Ross, 2003). These studies have supported the concept that the RLT is greater, i.e. more work is required, when there is a larger shift in environmental conditions. Generally, the effect is more pronounced when cells are shifted away from optimal conditions rather than towards conditions more optimal for growth.

Ross (1999) undertook a review of published lag time data for *L. monocytogenes*, expressing the results as RLTs. The distribution of reported RLTs has a sharp peak in the range 3 to 6. Augustin and Carlier (2000a) presented similar information expressed as

ln(RLT). Both analyses are highly consistent. These distributions of RLT can be exploited for "exposure assessment", either as point values taken from the cumulative distribution, or by providing a distribution of lag times from which to sample in Monte Carlo simulations (Ross and McMeekin, 2003).

It has also been proposed that lag times may be a function of the concentration of cells present, with fewer cells leading to longer lag times (Zhao, Montville and Schaffner, 2000; Robinson et al., 2001). This may reflect the probability of a cell being ready to grow; with more cells present, it is more likely that at least one cell will have a short lag.

The integration into a conceptual model of factors that may affect the rate and amount of growth of *L. monocytogenes* is shown in Figure 3.5.

3.5.4 Death or inactivation

3.5.4.1 Death rates

When conditions are outside the ranges that permit growth, microorganisms will either survive or be inactivated. Inactivation has traditionally been considered to follow log-linear kinetics, characterized by D and *z*-values (see next section), although the actual kinetics may be complex and involve several distinct phases, each with its own log-linear rate (Cerf, 1977; Augustin, Carlier and Rozier, 1998; Humpheson et al., 1998; Peleg and Cole, 1998). Until recently, D and *z* values were the primary methods of modelling thermal inactivation of microorganisms.

Recent reports indicate that log-linear models are inadequate to describe the death kinetics of *L. monocytogenes*, and that more complex (e.g. sigmoidal) functions are needed. Augustin, Carlier and Rozier (1998) used the concept of heat resistance *distributions* to develop models. The issue of variability in responses between strains, or due to uncontrolled variables, is currently a major theme in predictive microbiology.

The use of temperatures above the biokinetic range to inactivate microorganisms may be termed "thermal" processes, while the use of other growth preventing conditions, e.g. high salt or low pH, that result in inactivation have been called "non-thermal inactivation".

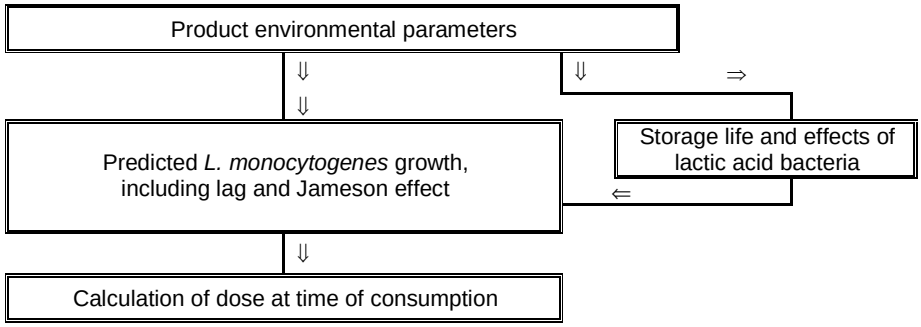

Figure 3.5 Overall model structure for the conceptual model and influence diagram for the interaction of factors governing the extent of growth of *Listeria monocytogenes* in ready-to-eat foods. Each of the boxes represents a "module" of calculations. Details of the predictive growth module are shown in Figure 3.6.

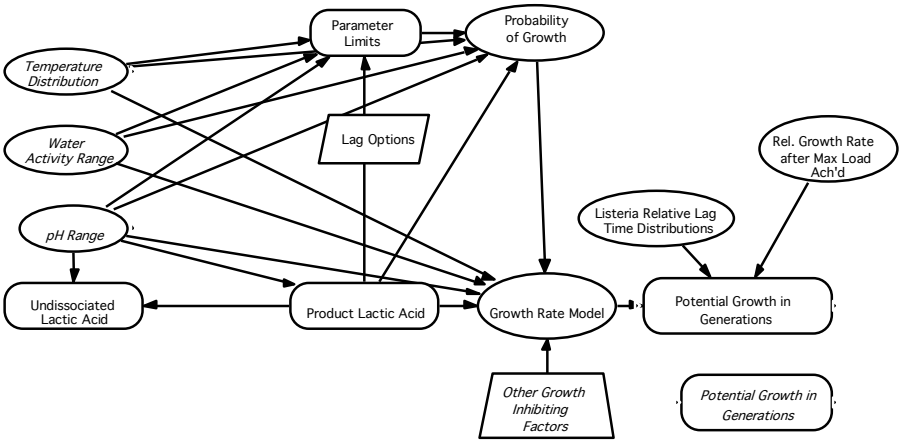

Figure 3.6 Detail of "predicted growth …" module in the conceptual model (Figure 3.5) and influence diagram of the interaction of factors governing the extent of growth of *Listeria monocytogenes* in ready-to-eat foods. Each of the boxes represents a "module" of calculations.

3.5.4.2 Thermal inactivation

A number of measures are used to describe the effect of heat, expressed as temperature, on the rate of death of microorganisms. The first of these is the D value, which is the time required for a ten-fold reduction, i.e. 90% of the population killed, at some specified condition of temperature and other factors. Implicit in the use of the D value to describe death rate is the assumption that death follows log-linear kinetics. Temperature is very effective in killing microorganisms and the rate of killing by temperatures even slightly above the upper limit for growth of vegetative bacteria is many times faster than death due to other factors (Shadbolt, Ross and McMeekin, 1999). Once temperature exceeds the physiological range for the organisms, small increases in temperature cause large increases in mortality. The increase in lethality due to heat is described by the z value, which is the temperature increase required to increase the lethality by a factor of ten. z values are typically of the order of 5–15°C.

Lou and Yousef (1999) reviewed in detail the large expanse of literature on the thermal inactivation of *L. monocytogenes*. ICMSF (1996) provides extensive lists of thermal inactivation times under different conditions and food types. Those data do not support the opinion sometimes still expressed that *L. monocytogenes* has unusually high thermal tolerance.

Heat tolerance of *L. monocytogenes* can be maximized by prior sub-lethal shocks, stress or applying to cells having reached stationary phase. These effects, and effects on subsequent lag and growth, have been studied and modelled (Stephens, Cole and Jones, 1994; Bréand et al., 1997, 1999; Augustin, Carlier and Rozier, 1998).

3.5.4.3 Freezing

Freezing damages and kills some microorganisms, mainly due to the increasing osmotic potential, i.e. as the water in the food freezes it increases the effective concentration of

solutes in the remaining liquid water, causing osmotic stress to those organisms suspended in that water. As water freezes, ice crystals may also cause physical disruption of cell membranes, further reducing the viability of organisms that have frozen. During thawing, further damage to cells can occur. Freezing and thawing, however, cannot be relied upon to eliminate contaminating microorganisms. Typical reductions in viable cell numbers on freezing and thawing for foodborne microorganisms of public health significance are of the order of a 10 to 100-fold reduction in the most susceptible types of organisms. Multiple freeze-thaw cycles are more lethal than a single freeze-thaw cycle.

The most important factor influencing the effect of freezing on microbial cells is the suspending medium. Certain compounds enhance, while others diminish, the effects of freezing. Glycerin, saccharose, gelatin and proteins in general act as cryoprotectants. Common salt (NaCl) increases the effect of freezing, due to depression of the freezing point of the water in the system, which has the effect of prolonging the cell's exposure to damaging high osmotic stress. The rate of freezing and thawing will also affect the lethality of these processes, with more rapid rates of both being less lethal. During frozen storage there will be a gradual loss of viability, the rate being slower at colder temperatures below freezing. Fluctuations in temperature during frozen storage will increase the rate of loss of viability. Cells are also re-exposed to damage through osmotic stress during thawing. A review of the studies concerning freezing and thawing effects on foodborne microorganisms is given in Lou and Yousef (1999) and Singhal and Kalkarni (2000).

3.5.4.4 Non-thermal inactivation

Conditions that prevent growth of microorganisms ultimately lead to their inactivation. Low temperature seems to be an exception to the general rule that more extreme conditions accelerate rates of microbial inactivation. Lower temperatures reduce the rate of death when other factors prohibit growth: very low temperature is routinely used as a method of culture preservation.

Non-thermal inactivation may be very slow. Seeliger (1961) reported that *L. monocytogenes* can survive for up to a year in 16% NaCl (a_w = 0.883). The mechanisms of non-thermal inactivation are currently poorly understood but have recently been reviewed (Mackey, 1999).

Buchanan and his colleagues have provided much of the published non-thermal inactivation data for *L. monocytogenes* (Buchanan and Golden, 1994, 1995; Golden, Buchanan and Whiting, 1995; Buchanan, Golden and Phillips, 1997). In most of those studies, organic acid was considered the main factor causing inactivation. A single predictive model encompassing much of the USDA data was presented in Buchanan, Golden and Phillips (1997). The inactivation kinetics were not log-linear. The model predicts the time required to reduce the original population by 99.99%, a time termed t_{4D}. It should be pointed out that because inactivation rates are not log-linear, the model cannot be used reliably to predict inactivation times beyond a 4 D kill, i.e. an 8 D kill will not necessarily occur after two t_{4D}s.

Data for rates of radiation inactivation are summarized in ICSMF (1996). The lethality of irradiation depends on the medium in which the cell is suspended, including factors such as temperature, water activity and pH.

3.6 SUMMARY

This section has attempted to identify the data needed to assess human exposure to *L. monocytogenes* in RTE foods, as well as tools and techniques to overcome missing data, and approaches for synthesizing data through models to enable estimation of exposure. Those data include the incidence of contamination; level of contamination; point of contamination; time and temperature history between contamination and consumption; volume of food per meal; and total consumption of the food in the community of interest.

Incidence and prevalence data at the point of consumption will rarely be available and quantitative or semi-quantitative assessment of exposure will probably have to rely on predictive microbiology models. Those models will have to have been successfully validated in products of similar microbial ecology to the product of interest. Some models have been shown to be too "fail-safe" and to produce unrealistically high estimates of exposure. Any exposure assessment should explicitly recognize the limitations of existing data, our understanding of the microbial ecology of *L. monocytogenes* and of the current generation of predictive microbiology models, so that the risk assessment process remains transparent.

Subsequent sections will provide specific examples of exposure assessments of *L. monocytogenes* in RTE foods, demonstrating the above principles.

Part 4.

Example Risk Assessments

4.1 OVERVIEW

4.1.1 Introduction

This section presents four risk assessments of *L. monocytogenes* in specific RTE foods: pasteurized milk; ice cream; fermented meats; and cold-smoked, vacuum-packed fish. All were modelled from production or retail to the point of consumption.

The four commodities were selected to exemplify estimation of the difference in risk associated with foods that do support and those that do not support the growth of *L. monocytogenes*, and foods with different contamination rates, shelf-life and levels of consumption. They also serve to answer one of the CCFH questions on the risk from *L. monocytogenes* in foods that support growth and foods that do not support growth under specific storage and shelf-life conditions. Various microbiological and mathematical and statistical considerations are also discussed and illustrated using different modelling approaches. Two examples attempt to estimate risk to a consumer in a specific nation, and two examples attempt to assess an average risk for any consumer in the world. The former approach is limited in its applicability to other nations, while the latter "ignores" the effect on risk of differences between nations, e.g. in consumption.

The dose-response relationship elaborated in Part 2 was linked with an exposure assessment developed for each commodity in order to generate an estimate of the risk of acquiring listeriosis. The risk estimates were expressed per 100 000 population and per 1 million servings, to illustrate the importance of the risk metric in understanding comparative risk. "Cases per 1 million servings" illustrates the risk to an individual consumer of that food, whereas "cases per 100 000 population" includes the effect of number of servings per year in a country, and reflects the comparative risk to a population originating in different foods.

Risk estimates ranged from 1 case per 20 million servings for smoked fish to 4 cases per 100 000 million servings for fermented meats, or from 9 cases per 10 million consumers per year for pasteurized milk to 7 cases per 100 000 million consumers per year for fermented meats.

4.1.2 Approaches taken

The risk characterization begins with the prevalence and concentration of *L. monocytogenes*, nominally at the point of completion of production or retail, in packages or containers of the selected RTE foods. Changes are followed in the pathogen population in contaminated product through to the point where the consumer eats a portion but the risk assessment does

not consider cross-contamination. The aim is to simulate the prevalence and levels of *L. monocytogenes* in those portions. Ancillary information is simulated for the frequency of consumption and the annual number of meals in a large population of susceptible and non-susceptible people.

To characterize the risk to consumers, the variables that have to be considered in these exposure assessment examples include: *L. monocytogenes* prevalence and concentration in finished products; product formulation; growth and inactivation rates; period and temperature of storage; and national and regional consumption patterns. The aim of these examples is to illustrate the effect of (i) potential for *L. monocytogenes* growth; (ii) low contamination levels in products that do not permit growth of *L. monocytogenes*; (iii) long-term storage on *L. monocytogenes* concentration; (iv) consumption patterns and volumes on dose eaten; and (v) low prevalence, or low concentration, of contamination of product on the risk of listeriosis from RTE foods.

The foods were selected based on various criteria, to exemplify various issues and effects of factors such as: different food commodities; potential for growth or not during long-term storage; cold-chain integrity; inactivation processes (e.g. pasteurization); post-process contamination; expected high contamination load of final RTE foods; high consumption rates; and products in international trade.

In addition to being used to estimate the risk of listeriosis from various RTE foods, and to contribute to providing answers to the questions posed by CCFH, the examples chosen are used to illustrate approaches to estimation of the risk of foodborne microbial illness. Examples 1 and 2 illustrate, in detail, appropriate statistical approaches to modelling the risk of microbial foodborne illness, including a description of prevalence and concentration of contaminants, while examples 3 and 4 emphasize modelling of the microbial ecology of *L. monocytogenes* in foods. Both of these topics – the statistical aspects of modelling and the microbial ecology of *L. monocytogenes* in foods – have been discussed in detail earlier in this report.

4.1.3 Choice of example risk assessments

4.1.3.1 Example 1: Fluid milk

The criteria for choosing milk were that it is widely consumed and the source is from many local suppliers. The variables were the prevalence and concentration of contamination with *L. monocytogenes*, post-processing contamination and growth during consumer refrigeration, and consumption patterns. The aim is to illustrate the interactive effects on risk deriving from consumption levels, contamination levels, shelf-life, contamination rates per package, and effects of times between exposures from a single contaminated unit.

4.1.3.2 Example 2: Ice cream

The criteria for selecting ice cream were the fact that no growth should occur during storage life and that the product is eaten worldwide, with a high consumption rate, particularly for some immunocompromised persons. The variables were contamination levels and national and regional consumption rates. The aim is to illustrate the relative risk of low contamination in a non-growth-permissive product, i.e. to estimate whether ice cream represents an important potential source of risk of listeriosis.

4.1.3.3 Example 3: Semi–dry fermented meats

The criteria for selection of fermented meats were that they are frequently contaminated but do not support growth. These products are widely consumed around the world, with many different varieties. The purpose in this risk assessment is to illustrate the effects of product formulation on potential for growth and the subsequent risk, and to attempt to contrast this with the risk from RTE foods that do allow the growth of *L. monocytogenes*.

4.1.3.4 Example 4: Cold-smoked fish

The criteria for selecting cold-smoked, vacuum-packed fish were that it is frequently contaminated; its formulation, storage conditions and long shelf-life suggest potential for extensive *L. monocytogenes* growth; and there is extensive international trade in the product. Variables modelled include formulation of the product, contamination levels, time and temperature of storage, national and regional consumption data and, in particular, the complex microbial ecology of the product, including the effect of lactic acid bacteria on product shelf-life and potential for growth of *L. monocytogenes.* The aim of the assessment is to illustrate the effects of the interaction of patterns and volumes of consumption with contamination frequency and potential for growth in a long-shelf-life product.

4.1.4 Common elements used in risk assessments

4.1.4.1 Definition of risks that were calculated

Key elements of the exposure assessment are the probability of consuming the food and the levels of pathogen consumed on each eating occasion. The latter reflects the hazard identification, namely the risk arises from the acute hazard attributable to exposure to individual meals, rather than a chronic hazard from repeated exposure. Two measures are used to characterize the risk: the number of illnesses per 100 000 population per year, and the number of illnesses per 1 000 000 servings of the food.

In examples 1 and 2, the risk to "susceptible" populations and normal consumers was estimated separately, as described below (see Section 4.1.4.5 – Dose-response modelling). In examples 3 and 4, data to enable differentiation of consumption by these groups was not available. Development of the dose-response relationships for these two groups relied on epidemiological data that indicate that the susceptible population ranges from 15 to 20% of the total population, and that individuals within the susceptible population account for 80–98% of all cases of listeriosis. As such, calculation of the risk to each sub-population would only reflect the assumptions concerning their relative susceptibility (defined by the r-value used) and the proportion of the population that each group represents (also defined in the modelling as between 15 and 20% of the total population) and would not provide additional insight. Thus, in examples 3 and 4, the risk to the total population alone was estimated.

It is probable, though not certain, however, that a more precise estimate of risk is generated by calculating separately the risk outcome for the susceptible and non-susceptible populations, and then combining the estimates to obtain the final total population outcome.

4.1.4.2 Simulation modelling

Simulated results are, themselves, subject to uncertainty introduced by the modelling algorithms used to perform the computations. The extremely low probabilities associated

with acquiring listeriosis as a result of consuming any single serving of food means the estimates from risk characterization are very sensitive to extreme values from input distributions (the right-hand tails of the distributions). Those values are infrequently sampled but, when sampled, greatly increase the risk estimate. To overcome this problem, models were simplified to reduce processing time so that more iterations could be performed, and more replicates of each simulation model run. Summary statistics of replicated runs of the models using different random seeds to initialize the software were used to describe some notion of that variability (A. Fazil, pers. comm., 2001; G. Paoli, pers. comm., 2001).

Simulations were done using Analytica™ 1.1.1, Analytica™ 2.0.1 or Analytica™ 2.0.5, software using Median Latin Hypercube sampling, generating random numbers using the Minimal Standard method (multiplicative congruential), with various random seeds. The seeds 203132, 6821, 113307, 651757, 201246, 421952, 323512, 71796, 311868, 300896, 197545, 496893, 692118, 726146, 242899 and 959784 were selected at random from a Uniform(0, 1 000 000). For examples 1 and 2, the simulations were run on a personal computer with a Pentium®III processor. For examples 3 and 4, the simulations were run using Analytica™ 1.1.1 on a Macintosh Powerbook G4 computer. Unless otherwise noted, each simulation involved 32 000 iterations.

4.1.4.3 Estimation of consumption

Two approaches were taken to estimate consumption. In examples 1 and 2, Canadian consumption data were used and enabled the differentiation of consumption patterns by age and gender for adults in that population. In examples 3 and 4, the approach taken was to attempt to estimate the risk to a consumer from any nation. Estimates of annual per capita consumption were derived from national consumption and national population estimates for five nations. This approach resulted in very coarse estimates of consumption, and did not allow differentiation of consumption by age or gender.

Relatively few countries collect information on consumption that is useful for risk assessment purposes, i.e. on a daily or per-serving basis; most databases are cumulative over a year for nutritional purposes. The Canadian Nutrition Surveys (CFPNS, 1992–1995) for pasteurized milk and ice cream were used because the exposure assessment working team members were more familiar with this set of data than others, and there was enough information to have distributions based on daily meal portions by gender and age. For both these products, however, the consumption by young children and teenagers, as well as those >74 years old, were not considered in the survey, despite those in these age ranges possibly being high consumers. The exposure assessment, therefore, is most meaningful for a Canadian adult situation, although many other countries probably have similar consumption patterns. This differs from the consumption data generated for cold-smoked fish and semi-dry fermented meats, where survey data from several countries were combined. These scenarios show two approaches to generating information on eating practices, one at a national level and one with a more global focus.

4.1.4.4 Temperature data

Several studies reporting temperatures of distribution, retail display and commercial or home storage are available (Willocx, Hendrickx and Tobback, 1993; Notermans et al., 1997; Sergeledis et al., 1997; O'Brien, 1997; Johnson et al., 1998; MLA, 1999). For simplicity, in

the example studies reported here, all product temperature data were derived from Audits International (2000) survey of home refrigerators in the United States of America.

4.1.4.5 Dose-response modelling

The functional form for the dose-response relationship is $Pr\{illness|dose\} = 1-e^{-r.dose}$. Two distributions for the r-value of the exponential model were used, for consumers of increased susceptibility and for healthy consumers, respectively. Uncertainty about the appropriate parameterization and variability across the population of interest in the response to the same *L. monocytogenes* dose (e.g. due to variability in individual consumers health status at any given time, the type of meal and factors that could affect the survival of *L. monocytogenes* during passage through the stomach, variability in virulence of strains of *L. monocytogenes*, etc.), a distribution of r-values for each subpopulation was generated from 5000 iterations of the dose-response model, following the procedure described in Sections 2.3 and 2.4. The dose-response distribution for individuals from the susceptible population is stochastically smaller than the dose-response distribution for individuals from the non-susceptible population.

In each iteration of the model, the calculated dose is combined with an estimate of the r-value from the distribution outlined above for either a susceptible or normal consumer. For examples 3 and 4, the models were constructed so that in 15–20% of iterations, an r-value was drawn from the distribution of r-values for a susceptible consumer, but in all other cases a value was selected from the r-value distribution for "normal" consumers. The dose-response model is then combined with serving size data, and the modelled contamination level data, to predict probability of illness from the serving in that iteration.

4.2 EXAMPLE 1. PASTEURIZED MILK

4.2.1 Statement of purpose

This pasteurized milk assessment begins with the prevalence and concentration of *L. monocytogenes*, nominally at retail, in packages or containers of this RTE product and traces growth of the pathogen population in contaminated product through to the point where the consumer drinks a portion. The aim is to simulate the prevalence and levels of *L. monocytogenes* in those portions that, along with serving sizes, determine the size of the dose of *L. monocytogenes* that a consumer might ingest. Ancillary information is simulated for the frequency of consumption and the annual number of servings in a large population of susceptible adults and non-susceptible adults. Among those annual servings are some contaminated milk portions, which might lead to illness, according to the hazard characterization. The situation modelled is based upon Canadian data and practices.

4.2.2 Hazard identification

L. monocytogenes is found throughout the farm environment and can be transmitted to cows through consumption of silage and hay (Farber and Peterkin, 2000; Ryser, 1999a). The pathogen can also cause mastitis that allows the organism to be continually excreted into milk. It has frequently been isolated from milking barns and parlours and from dairy processing equipment. It is therefore not surprising that it has been found in raw milk around

the world. It has been implicated in one outbreak of listeriosis attributed to pasteurized milk and another attributed to chocolate milk. In 1983, in Massachusetts, 49 people suffered from listeriosis after consuming one brand of 2% fat pasteurized milk (Fleming et al., 1985). The milk came from several farms, one of which had animals with bovine listeriosis at the time of the outbreak. The milk was apparently properly pasteurized, which indicates there was such a high level of contamination in the milk that some organisms survived the pasteurization or, more likely, post-process contamination occurred in the plant. In 1994, in the midwest United States of America, 54 people at a summer picnic developed gastroenteritis following consumption of chocolate milk in cartons that were later found to contain up to 10^9 *L. monocytogenes* CFU/ml (Dalton et al., 1997; Ryser, 1999a). Again, post-process contamination and storage for at least 2 hours at ambient temperatures was the most likely scenario.

4.2.3 Exposure assessment results

4.2.3.1 Prevalence of L. monocytogenes *at retail in pasteurized milk*

Prevalence at retail is based on 10 separate prevalence estimates for *L. monocytogenes* in pasteurized bovine milk produced in various countries, from retail or distribution, in packaged amounts. *L. monocytogenes* prevalence ranged from 0 to 1.1% of samples, in studies reporting from 14 to 1039 samples, 2157 samples in total (Table 4.1). Considered, but not included in the results, is the information from the study that reports only prevalence without noting also a sample size (in Baek et al., 2000). Also considered, but not included in the results, is the information from the studies that reported prevalence in samples drawn from bulk tanks of pasteurized milk. Samples drawn from bulk amounts were considered to represent prevalence of *L. monocytogenes* in pasteurized milk, but at a stage in the production-to-consumption chain earlier than the starting point used here. Among samples drawn from bulk tanks, prevalence of contamination was also generally very low, except for one study that found contamination in 21.4% of samples (Fleming et al., 1985; Fernandez-Garayzabal et al., 1986; Venables, 1989; Destro, Serrano and Kabuki, 1991; Harvey and Gilmour, 1992; Moura, Destro and Franco, 1993; Pitt, Harden and Hull, 1999). The stochastic structure of the collection of studies presented in Table 4.1 is represented by attributing binomial variability to the within-study estimates to account for their individual precision, and attributing a Beta distribution to the between-study variability of the true study prevalences, π_i, from data y_i of n_i samples positive for *L. monocytogenes* in the i^{th} study, a two-stage hierarchical model $\boldsymbol{Y}_i|n_i,\pi_i \sim$ Binomial(n_i,π_i), i=1, …, 9 and $\pi_i \sim$Beta(α,β). This leads to the inference that average prevalence is 3.50×10^{-3} [4.39×10^{-4}, 3.87×10^{-3}] at the 95% confidence interval when maximum likelihood estimates are $\hat{\alpha}$=0.55 and $\hat{\beta}$=155.47 (Figure 4.1).

4.2.3.2 Concentration of L. monocytogenes *in contaminated milk at retail*

No studies that described *L. monocytogenes* concentrations in pasteurized milk samples were found. This assessment relies on information summarized in FDA/FSIS (2001) (Table 4.2a), assumes that these are concentrations as if measured at retail, and constructs a distribution with estimated minimum and maximum concentrations (Table 4.2b). Minimum concentration in positive samples was assumed to be 0.04 CFU/ml and maximum concentration was assumed to be 250 CFU/ml, based on the authors' judgment. Variability in *L. monocytogenes* concentrations in contaminated pasteurized milk, at retail, is constructed by simulating

concentrations in [0.04, 250] CFU/ml, assuming that concentrations are block Uniform between the $\log_{10}$ quantiles in Table 4.2b.

Table 4.1 Data sets used to estimate prevalence of *Listeria monocytogenes* in pasteurized milk, at retail.

Food	Stage	Country of study	Positive	Samples	Proportion +ve	Source
Pasteurized milk	Retail	Brazil	0	20	0	[1]
Pasteurized milk	Retail	Canada	0	14	0	[2]
Pasteurized milk	NA	Germany	0	651	0	[3]
Pasteurized milk	Retail	Korea	0	26	0	[4]
Pasteurized milk	Retail	Poland	0	73	0	[5]
Cow cream pasteurized	Retail	UK	0	40	0	[6]
Pasteurized milk	Retail	UK	11	1039	0.011	[6]
2% low-fat milk	Retail or distribution	USA	0	125	0	[7]
Whole milk	Retail or distribution	USA	1	169	0.006	[7]
Pasteurized milk	Retail	Japan	NA	NA	0.009	[6] [8]

NOTES: NA = not available

SOURCES: [1] Casarotti, Gallo and Camargo, 1994. [2] Farber, Sanders and Johnston, 1989. [3] Hartung, 2000. [4] Baek et al., 2000. [5] Rola et al., 1994. [6] Greenwood, Roberts and Burden, 1991. [7] US FDA, 1987, (cited in Hitchins, 1996). [8] MacGowan et al., 1994 (cited in Baek et al., 2000).

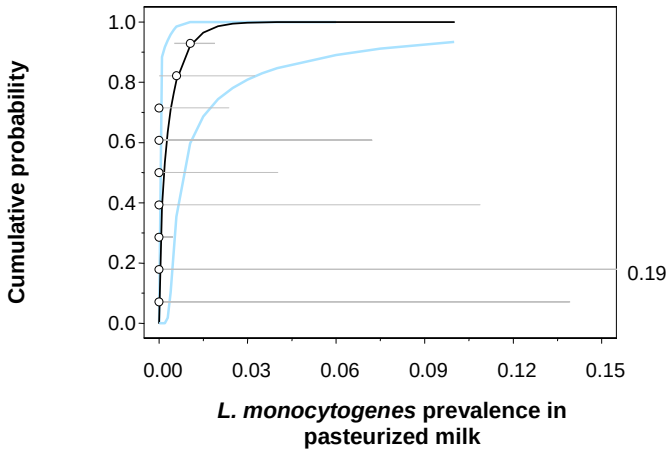

Figure 4.1 Empirical cumulative distribution function (open circles with 95% confidence intervals) for Table 4.1's individual studies' prevalence estimates, and fitted Beta distribution (solid line with shaded lines for 95% confidence limits) show the plot of the 2-stage hierarchical model that combined individual studies' estimates into a single estimate for the distribution of the prevalence of *Listeria monocytogenes* in pasteurized milk.

Table 4.2 *Listeria monocytogenes* concentration in contaminated pasteurized milk.

4.2a Data set used to estimate *L. monocytogenes* concentration in contaminated pasteurized milk.

Data set	1 CFU/ml	≤10^2 CFU/ml	Samples
FDA/FSIS, 2001	39	2	41

4.2b Assumed distribution function for *L. monocytogenes* concentration in contaminated pasteurized milk, with estimated minimum (0.04 CFU/ml) and maximum (250 CFU/ml) concentration.

CFU/ml	$\log_{10}$ CFU/ml	Cumulative probability [95% confidence interval]
0.04	-1.4	0
1	0	0.935 [0.835, 0.994]
100	2	0.984 [0.914, 1]
250	2.4	1

4.2.3.3 Growth of L. monocytogenes *in milk*

L. monocytogenes in pasteurized milk can grow at refrigerator temperatures, increasing the concentration in milk at the point of consumption from the concentration that is observed at retail. Although other conditions also explicitly define boundaries between growth and no growth and parameterize the growth rate, this exposure assessment has accounted for only the effect of storage temperature and the length of time that the product is stored before consumption.

4.2.3.4 Growth rate of L. monocytogenes *in milk*

Simulations of the exposure assessment incorporate variability in growth rates at 5°C as Uniform(0.092, 0.434) $\log_{10}$/day (FDA/FSIS, 2001) and scale them to represent growth rates at the storage temperatures in Table 4.3 using the relationship $\sqrt{\mu_T} = \sqrt{\mu_5}(T - T_{\min})(5 - T_{\min})^{-1}$ (McMeekin et al., 1993) to incorporate variability in *L. monocytogenes* growth associated with storage temperature. The amount of *L. monocytogenes* growth until consumption of a pasteurized milk portion is the product of the daily growth rate and the length of the storage time. It is assumed also that the *L. monocytogenes* detected in the milk were in the milk sufficiently long for the lag phase to have been passed.

4.2.3.5 Pasteurized milk storage temperature

Storage temperature was simulated from the data reported (Audits International, 2000) from a survey of home refrigerator temperatures in the United States of America (Table 4.3).

Table 4.3 Selected quantiles (1%, 5%, 50%, 95% and 99% points) from simulated distributions of characteristics controlling *Listeria monocytogenes* growth.

Storage temperature (Audits International, 2000)		**Storage time** (FDA/FSIS, 2001) truncated to respect pasteurized milk storage life (Neumeyer, Ross and McMeekin, 1997; Neumeyer et al., 1997)	
Cumulative probability	Storage temperature quantile (°C)	Cumulative probability	Storage time quantile (days)
0.01	0.06	0.01	1.58 (0.0002 s.e.)
0.05	0.53	0.05	2.31 (0.0002 s.e.)
0.50	3.41	0.50	5.29 (0.0003 s.e.)
0.95	6.88	0.95	10.47 (0.0016 s.e.)
0.99	8.61	0.99	12.68 (0.0026 s.e.)

4.2.3.6 Pasteurized milk storage time

The number of days that the consumer stores pasteurized milk before consumption is described by a Triangular(1, 5, 12) distribution, nominally, allowing the most likely value to vary as Uniform(4, 6) and the maximum value to vary as Uniform(6, 18) (FDA/FSIS, 2001), but restricting storage time to be shorter than storage life. Storage life for pasteurized milk depends on the growth of spoilage bacteria, which is assumed to be 12 days at 4°C, with storage life at other temperatures determined by the relationship $Life(T) = 12 \times [\frac{4+7.7}{T+7.7}]$ (Neumeyer, Ross and McMeekin, 1997; Neumeyer et al., 1997). Quantities vary among 16 simulations, each involving 32 000 iterations from the input distributions (Table 4.3).

4.2.3.7 Concentration of L. monocytogenes *in contaminated milk at consumption*

Concentrations in contaminated milk at retail (Table 4.2) increase due to growth of *L. monocytogenes* during the storage time and under the temperature conditions modelled (Table 4.3), leading to a simulated distribution of *L. monocytogenes* concentrations at the point of consumption (Table 4.4). Growth was assumed to occur when simulated storage temperatures exceeded a minimum temperature that varied from iteration to iteration (Uniform(-2ºC, -1ºC)). Maximum population densities are modelled to depend on temperature, after FDA/FSIS (2001): <5°C, 10^7 CFU/g; 5°–7°C, $10^{7.5}$ CFU/g; >7°C, 10^8 CFU/g. Initial concentrations were low enough and growth rates were low enough that limits imposed by maximum population densities were seldom invoked in the simulations that gave the results in Table 4.4. Quantities in Table 4.4 vary among 16 simulations, each involving 32 000 iterations.

Table 4.4 Selected quantiles from simulated distributions of *L. monocytogenes* concentration in contaminated milk at point of consumption.

Quantile ($\log_{10}$ CFU/ml)	**Cumulative probability**
-1	0.011 (3.39×10^{-5} s.e.)
0	0.374 (8.38×10^{-5} s.e.)
1	0.771 (1.13×10^{-4} s.e.)
2	0.914 (5.19×10^{-5} s.e.)
3	0.970 (5.52×10^{-5} s.e.)
4	0.991 (2.46×10^{-5} s.e.)
5	0.9977 (2.05×10^{-5} s.e.)
6	0.9996 (6.67×10^{-6} s.e.)
7	0.99998 (1.82×10^{-6} s.e.)
8	1

Only servings from contaminated milk will contain any *L. monocytogenes* organisms. Furthermore, only some

servings from a multiple-serving container of contaminated milk that contains very low levels of contamination will contain any of the pathogen. Assumptions about homogeneity or heterogeneity of the organisms in a contaminated foodstuff can have a great effect on the simulated results. Clustering of colonies of pathogens would introduce extra variability into the results (Haas, Rose and Gerber, 1999). Here, it is assumed that the organism is distributed homogeneously through the product in a way that counts of organisms in samples from the product would follow a Poisson distribution, but will ignore the small variations in the number of *L. monocytogenes* organisms present in servings drawn from a packaged product with the same average concentration. It is assumed also that all organisms present would be in a part of the milk that would be consumed.

4.2.3.8 Consumption characteristics for milk

Defining milk consumption

Selection of foods from Canadian Federal-Provincial Nutrition Surveys' (CFPNS, 1991–1995) databases reflects both consumption frequency and amount of milk consumed on eating occasions. Results are based on the reported consumption practices of the 12 089 consumers who were respondents to the Nutrition Surveys, among whom 8365 consumed milk. Milk consumption, except when the eating episode involved preparation like cooking, were aggregated from all of an individual's eating occasions on the same day for this representation of milk consumption, giving, for a day at random, the estimated fraction of the population who are milk consumers and the daily amount of milk consumed. When milk forms an ingredient in a serving, an appropriate fraction of the food to represent the amount of milk included was derived or estimated (Table 4.5). Preparations using powdered milk and foods that included milk as an ingredient but that were processed before reaching the consumer were specifically excluded.

Table 4.5 Food commodities used to describe milk consumption frequency and amount consumed.

Food code	Food name	Milk (%)	Eating occasions	Average serving (g) per occasion
432	Instant breakfast, made with whole milk	90%	1	327.9
546	Milk, whole, fluid, producer, 3.7% B.F.	100%	196	123.8
547	Milk, fluid, partly skimmed, 2% B.F.	100%	10 225	122.2
548	Milk, fluid, partly skimmed with added milk solids, 2% B.F.	100%	2	38.4
549	Milk, fluid, partly skimmed, 1% B.F.	100%	2 292	151.2
550	Milk, fluid, partly skimmed with added milk solids, 1% B.F.	100%	1	230.6
551	Milk, fluid, skim with added milk solids	100%	15	122.4
552	Milk, fluid, buttermilk, cultured	100%	34	222.5
558	Milk, fluid, chocolate, whole	100%	4	412.6
559	Milk, fluid, chocolate, partly skimmed, 2% B.F.	100%	226	370.3
563	Milk shake, chocolate, thick	50%	1	105.7
593	Milk, fluid, skim	100%	2 388	151.9
600	Milk, fluid, whole, pasteurized, homogenized, 3.3% B.F.	100%	3 808	98.9
2918	Chocolate syrup, unenriched, + whole milk	90%	1	432.3
4001	Milk; cow, chocolate drink, fluid, commercial, lowfat, 1% fat	100%	8	372.8
11742	Potatoes, mashed, home-prepared, +whole milk +butter	10%	18	13.8
11899	Milk, fluid, homogenized, triple-milk	100%	27	30.5

NOTE: B.F. = butterfat

The simulated distributions constructed for annual meals and daily consumption amounts with respect to the Age × Gender groups' contributions to non-susceptible and susceptible populations were defined similarly to Miller, Whiting and Smith (1997). For the fraction of individuals that possess the same age and gender characteristics among Canadian adults 18–74 years of age, 15% (3.3 million) would fall into the susceptible group and 85% (18.7 million) would fall into the non-susceptible group.

Annual milk servings

Uncertainty about the point estimates for the estimated fraction of the population who are milk consumers is described by attributing a beta distribution to the proportion of the sample respondents that would consume pasteurized milk on a random day.

The simulated distribution for the number of days per year with milk consumption can be attributed to the gender and age groups that make up those populations. It is assumed that the daily consumption probability is the same on every day of the year for individuals in the same Gender × Age group, whether the individuals are in the non-susceptible population or the susceptible population, that days are independent, and that binomial sampling can be used to represent day-to-day variability (Table 4.6).

Amounts of milk consumed

The distribution in Table 4.7 was constructed by sampling from the Nutrition Survey data within the Age × Gender groups defined, collecting results into simulated milk consumption amounts distributions for non-susceptible and susceptible populations. The distribution that was constructed represents the proportion of the population groups that constitute the non-susceptible and susceptible populations.

Table 4.6 Selected quantiles (1%, 5%, 10%, 25%, 50%, 75%, 90%, 95% and 99% points) from simulated distribution of annual days with milk consumption among all individuals in non-susceptible and susceptible adult populations in Canada.

Population	Cumulative probability								
	.01	.05	.10	.25	.50	.75	.90	.95	.99
Non-susceptible	2.3×10^{9}	2.8×10^{9}	3.1×10^{9}	3.5×10^{9}	4.0×10^{9}	4.5×10^{9}	4.9×10^{9}	5.2×10^{9}	5.6×10^{9}
Susceptible	3.5×10^{8}	4.5×10^{8}	5.1×10^{8}	6.1×10^{8}	7.2×10^{8}	8.2×10^{8}	9.0×10^{8}	9.4×10^{8}	1.0×10^{9}

Table 4.7 Selected quantiles (1%, 5%, 10%, 25%, 50%, 75%, 90%, 95% and 99% points) from simulated distribution of daily amount (g) of milk consumption among milk consuming individuals in non-susceptible and susceptible populations.

Population	Cumulative probability								
	0.01	0.05	0.10	0.25	0.50	0.75	0.90	0.95	0.99
Non-susceptible	5.3 g	15.4 g	20.7 g	61.8 g	185.0 g	365.9 g	671.1 g	889.2 g	1 363 g
Susceptible	5.3 g	15.5 g	30.9 g	62.0 g	182.7 g	335.4 g	519.5 g	686.7 g	1 011 g

4.2.3.9 Simulated L. monocytogenes *in contaminated pasteurized milk at consumption*

The simulated distribution for the number of *L. monocytogenes* in a contaminated pasteurized milk serving (Table 4.8) is constructed from the serving size (Table 4.7) and from the distribution of concentrations at point of consumption (Table 4.4). Quantities vary among 16 simulations, each involving 32 000 iterations.

Table 4.8 Selected quantiles from simulated distributions of $\log_{10}$ number of *Listeria monocytogenes* organisms in contaminated milk servings at point of consumption.

$\log_{10}$ CFU in serving	Cumulative probability	
	Non-susceptible population	Susceptible population
0	0.004 (2.06×10^{-5} s.e.)	0.003 (1.26×10^{-5} s.e.)
1	0.055 (5.74×10^{-5} s.e.)	0.046 (5.83×10^{-5} s.e.)
2	0.298 (1.07×10^{-4} s.e.)	0.304 (1.28×10^{-4} s.e.)
3	0.686 (8.31×10^{-5} s.e.)	0.701 (1.06×10^{-4} s.e.)
4	0.884 (7.10×10^{-5} s.e.)	0.890 (8.36×10^{-5} s.e.)
5	0.957 (5.94×10^{-5} s.e.)	0.960 (5.62×10^{-5} s.e.)
6	0.987 (3.55×10^{-5} s.e.)	0.988 (4.12×10^{-5} s.e.)
7	0.996 (1.87×10^{-5} s.e.)	0.997 (2.40×10^{-5} s.e.)
8	0.9992 (8.95×10^{-6} s.e.)	0.9993 (9.32×10^{-6} s.e.)
9	0.99987 (3.06×10^{-6} s.e.)	0.99988 (3.31×10^{-6} s.e.)
10	0.999994 (7.81×10^{-7} s.e.)	0.999996 (6.62×10^{-7} s.e.)
11	1	1

NOTE: s.e. = standard error of the mean.

4.2.4 Risk characterization

4.2.4.1 Annual illnesses per 100 000 population

The simulated distribution for the number of illnesses per year per 100 000 population (Table 4.9) is developed using the probability of illness from consuming a contaminated serving and the number of contaminated servings per year as intermediate calculations. The distribution of annual contaminated milk servings accounts for variability and uncertainty associated with the average prevalence of contaminated servings and the distribution for the number of annual milk servings (Table 4.6). Critical to the development of risk characterization measures is the mean value of that simulated distribution, for individuals from the non-susceptible population and for individuals from the susceptible population (G. Paoli, pers. comm., 2001). The distribution for the probability of illness from consuming a contaminated milk serving is constructed from the distribution for the number of *L. monocytogenes* organisms in a contaminated serving (Table 4.8) and the dose-response function described in Section 4.1.4, an output of the hazard characterization.

Results are reported separately for a susceptible and a non-susceptible adult population, and for a mixed (entire) adult population that consists of approximately 85% non-susceptible adults and 15% susceptible adults. Summary statistics for the simulated distribution of annual illnesses per 100 000 population vary as shown among 16 simulations, each involving 32 000 iterations from the input distributions.

Table 4.9 Selected quantiles (1%, 5%, 50%, 95% and 99% points) and distribution mean from simulated distributions for annual illnesses per 100 000.

Cumulative probability	Annual illnesses per 100 000 population		
	Non-susceptible population	Susceptible population	Mixed population
.01	0.000	0.002 (0.0005 s.e.)	0.001 (0.0002 s.e.)
.05	0.000	0.027 (0.0018 s.e.)	0.007 (0.0004 s.e.)
.50	0.01 (0.0003 s.e.)	0.22 (0.009 s.e.)	0.04 (0.0015 s.e.)
.95	0.05 (0.002 s.e.)	1.37 (0.055 s.e.)	0.27 (0.011 s.e.)
.99	0.17 (0.005 s.e.)	4.93 (0.222 s.e.)	1.25 (0.063 s.e.)
Mean	0.016 (0.0005 s.e.)	0.519 (0.0312 s.e.)	0.091 (0.0047 s.e.)

NOTE: s.e. = standard error of the mean.

Table 4.10 Mean values from simulated distributions for number of illnesses per 1 000 000 servings.

	Illnesses per 1 000 000 servings		
	Non-susceptible population	Susceptible population	Mixed population
Mean	0.001 (0.0001 s.e.)	0.022 (0.0009 s.e.)	0.005 (0.0002 s.e.)

NOTE: s.e. = standard error of the mean.

4.2.4.2 Illnesses per 1 000 000 servings

The simulated distribution for the number of illnesses per 1 000 000 servings (Table 4.10) is developed using the prevalence of contaminated servings and the probability of illness from consuming a contaminated serving for individuals from non-susceptible and susceptible populations as intermediate calculations. The resulting distribution is concentrated at less than one illnesses per 1 000 000 servings, sometimes beyond the 99th percentile. Only mean values for the distributions are quoted for the results. Values vary as shown among 16 simulations, each involving 32 000 iterations from the input distributions.

4.2.5 Uncertainty and variability

A last step in this assessment for *L. monocytogenes* in milk examines the simulated results to consider how much the various inputs affect the outputs. As they are based on a simulation model, the risk characterization results are subject to uncertainty associated with a modelled representation of reality, involving assumed simple relationships among prevalence, concentration, consumption characteristics and adverse response to consumption of some number of *L. monocytogenes* organisms.

4.2.5.1 Effects of hazard characterization's dose-response

There is uncertainty in the hazard characterization's dose-response associated both with the form of the dose-response function used and with the parameterization. Describing distributions for the parameters captures how the response varies among individuals in a sub-population to the same pathogen dose. However, there is also uncertainty associated with the values assumed for the parameters.

4.2.5.2 Effects of estimated consumption frequency

Simulated milk consumption frequency is sensitive to the survey estimates of consumption frequency. Sample sizes, though, are large enough for the amount of uncertainty associated

with the point estimate to have only a minor influence. Among individuals from the non-susceptible population, defined to include only males and females less than 65 years of age, consumption frequency differences are small. So, simulated consumption frequency for the non-susceptible population is not sensitive to allocation of individuals based on gender and age. Consumption frequency in the susceptible population is sensitive to changes to the gender and age composition. Individuals from the 65–74-year-old age group dominate the characteristics of the susceptible population, but estimates of milk consumption probabilities are less precise than in other age groups. Therefore the uncertainty that would be associated with those estimates plays a more significant, although still minor, role. There is uncertainty associated with extrapolation of daily consumption characteristics to annual consumption for populations of individuals. There is also uncertainty associated with extrapolation of survey results from 1991–1995 to the present day.

4.2.5.3 Effects of estimated consumption amounts

Simulated distributions for milk consumption amounts are less sensitive to composition of non-susceptible and susceptible populations than for other parameters. The age, more so than the gender, of individuals contributes more to variability in the non-susceptible population. Gender, more so than age, of individuals contributes to variability in simulated consumption amounts for the susceptible population. Estimates of milk consumption have uncertainties, including errors associated with under- and over-reporting, estimation methods for the amount of milk consumed, the use of several food codes, and the derivation or estimation of an appropriate amount of milk to include when the milk was an ingredient in the meal. All of a respondent's identified milk amounts within a day were aggregated into a daily amount. That practice loses the distinction that one might wish to make among different eating occasions within the day, whether the milk was consumed alone or as part of a meal, and whether the milk was consumed at home or away from home. However, the practice does retain the variability in milk amount consumed among individuals in the population. As with consumption frequency there is uncertainty associated with extrapolation of daily consumption characteristics to annual consumption for populations of individuals as well as uncertainty associated with extrapolation of survey results from 1991–1995 to the present day.

4.2.5.4 Effects of L. monocytogenes *prevalence on risks of listeriosis*

Simulated distributions for *L. monocytogenes* prevalence in the milk portions that consumers eat depend on estimates of prevalence of the pathogen in packages of milk, here assumed to have been measured at retail, from studies reported in the literature and on inferences from those data about the variability of prevalence. Sensitivity to prevalence of *L. monocytogenes* in pasteurized milk at retail is nearly multiplicative. If the mean prevalence is reduced by a factor of 10, then simulated annual illnesses per 100 000 population and simulated illnesses per 1 000 000 milk servings are also reduced by approximately a factor of 10. Risk characterization results (Tables 4.9 and 4.10) assume that *L. monocytogenes* prevalence estimates are appropriately pooled using a beta mixing distribution, yielding an inference that the average prevalence is 3.50×10^{-3} [4.39×10^{-4}, 3.87×10^{-3}] 95% confidence interval. Alternatively, one can proceed under the assumption that all prevalence studies, regardless of source, have sampled the same phenomenon, namely a single, fixed prevalence, estimated to be 5.56×10^{-3} [2.88×10^{-3}, 9.70×10^{-3}] 95% confidence interval (12 samples positive for *L. monocytogenes* in 2157 samples). In either case, the inference describes uncertainty about

the prevalence of *L. monocytogenes* contamination in milk in a large number of servings. In the second case, there is less uncertainty about the mean prevalence, leading to a simulated distribution for risk characterization measures like the number of annual illnesses per 100 000 population that is less dispersed about the distribution mean. Mean values of the risk characterization results, though, are not sensitive to the different inferences.

4.2.5.5 Effects of L. monocytogenes *concentration at retail*

Simulated distributions for *L. monocytogenes* concentration in contaminated milk at the point of consumption (Table 4.4) depend very little on initial *L. monocytogenes* concentration (Table 4.2) in contaminated milk or at retail purchase, but are greatly dependent on the estimated maximum concentration at retail.

Simulated distributions for *L. monocytogenes* concentration in contaminated pasteurized milk at consumption showed levels that exceed 10^2 CFU/g in a small fraction of cases (Table 4.2). A set of simulations were done, setting maximum concentrations of *L. monocytogenes* in contaminated product at retail to levels $<10^2$ CFU/g at retail and to levels up to 10^3 CFU/g, still subject to growth under the same storage time and temperature (Table 4.3), to compare risk characterization measures (Table 4.11). Values vary as shown among 16 simulations, each involving 32 000 iterations from the input distributions.

Table 4.11 Comparison of simulated mean annual illnesses per 100 000 population associated with *Listeria monocytogenes* concentrations in contaminated pasteurized milk at retail from distributions with different assumed truncation points.

4.11a Concentrations at retail in contaminated pasteurized milk truncated to be <100 CFU/ml.

	Annual illnesses per 100 000 population		
	Non-susceptible population	Susceptible population	Mixed population
Mean	0.006 (0.0003 s.e.)	0.153 (0.0065 s.e.)	0.028 (0.0011 s.e.)

4.11b Baseline case, concentrations at retail in contaminated pasteurized milk modelled to be [100, 250] CFU/ml in approximately 1.6% of cases.

	Annual illnesses per 100 000 population		
	Non-susceptible population	Susceptible population	Mixed population
Mean	0.016 (0.0005 s.e.)	0.519 (0.0312 s.e.)	0.091 (0.0047 s.e.)

4.11c Concentrations at retail in contaminated pasteurized milk modelled to be [100, 1000] CFU/ml in approximately 1.6% of cases.

	Annual illnesses per 100 000 population		
	Non-susceptible population	Susceptible population	Mixed population
Mean	0.023 (0.0012 s.e.)	0.681 (0.0218 s.e.)	0.121 (0.0035 s.e.)

NOTE: s.e. = standard error of the mean.

4.2.5.6 Effects of higher storage temperatures

Simulated distributions for *L. monocytogenes* concentration in contaminated pasteurized milk are subject to pathogen growth that is modelled to depend on storage time and temperature (Table 4.3). To examine the effect of storage conditions on contamination levels, sets of simulations were done where storage temperatures were increased and where storage times were increased.

Storage temperature was simulated from the data that Johnson et al. (1998) reported from a survey of home refrigerator temperatures in the United Kingdom (Table 4.12a). Storage times were defined as a nominally Triangular(1, Uniform(4,6), Uniform(6,18)) distribution and then truncated to represent the effects of spoilage of milk held at temperatures described in Johnson et al. (1998). Daily growth was defined as Uniform(0.092, 0.434) ($\log_{10}$/day) at 5°C and adjusted to storage temperature. Total growth was constrained to respect maximum population densities at the storage temperatures, as explained earlier. Summary statistics for storage conditions and mean values (Table 4.12b) of simulated distributions for annual illnesses per 100 000 population vary as shown among 16 simulations, each involving 32 000 iterations.

Table 4.12 Comparison of mean annual illnesses per 100 000 population for pasteurized milk held at refrigerator storage temperatures simulated from different assumed distributions.

4.12a Selected quantiles (1%, 5%, 50%, 95% and 99% points) from simulated distributions of storage temperature and storage time.

Storage temperature (from Johnson et al., 1998)		**Storage time** (FDA/FSIS, 2001) truncated to respect pasteurized milk storage life	
Cumulative probability	Storage temperature quantile (°C)	Cumulative probability	Storage time quantile (days)
0.01	-0.1	0.01	1.54 (0.0004 s.e.)
0.05	1.7	0.05	2.21 (0.0004 s.e.)
0.50	6.2	0.50	4.91 (0.0004 s.e.)
0.95	8.5	0.95	8.57 (0.0018 s.e.)
0.99	10.3	0.99	11.07 (0.0056 s.e.)

4.12b Mean values from simulated distribution for annual illnesses per 100 000 population, with scenario of warmer storage temperatures compared to baseline case.

1. Storage temperatures from Johnson et al., (1998)

	Annual illnesses per 100 000 population		
	Non-susceptible population	Susceptible population	Mixed population
Mean	0.23 (0.012 s.e.)	6.41 (0.252 s.e.)	1.15 (0.045 s.e.)

2. Baseline case, storage temperatures from Audits International (2000)

	Annual illnesses per 100 000 population		
	Non-susceptible population	Susceptible population	Mixed population
Mean	0.016 (0.0005 s.e.)	0.519 (0.0312 s.e.)	0.091 (0.0047 s.e.)

NOTE: s.e. = standard error of the mean.

4.2.5.7 Effects of longer storage times

Storage temperature was simulated from the data that were reported (Audits International, 2000) from a survey of home refrigerator temperatures in the United States of America (Table 4.13a). Storage times defined as a nominally Triangular(1, Uniform(4, 6), Uniform(6, 18)) distribution were lengthened by 1 day and truncated to represent the effects of spoilage. Daily growth was defined as a Uniform(0.092, 0.434) distribution ($\log_{10}$/day) at 5°C and adjusted to storage temperature to complete the specification of growth conditions, and total growth was constrained to respect maximum population densities at the storage temperatures. Summary statistics for storage conditions and mean values (Table 4.13b) of simulated distributions for annual illnesses per 100 000 population vary as shown among 16 simulations, each involving 32 000 iterations.

Table 4.13 Effects of changes to storage time distribution on risk characterization measures.

4.13a Selected quantiles (1%, 5%, 50%, 95% and 99% points) from simulated distributions of storage temperature distribution and storage time distribution.

Storage temperature (from Audits International, 2000)		**Storage time** (FDA/FSIS, 2001) truncated to respect pasteurized milk storage life lengthened by 1 day	
Cumulative probability	Storage temperature quantile (°C)	Cumulative probability	Storage time quantile (days)
0.01	0.06	0.01	3.07 (0.0006 s.e.)
0.05	0.53	0.05	3.78 (0.0008 s.e.)
0.50	3.41	0.50	6.68 (0.0010 s.e.)
0.95	6.88	0.95	11.66 (0.0039 s.e.)
0.99	8.59	0.99	13.78 (0.0063 s.e.)

4.13b Mean values from simulated distribution for annual illnesses per 100 000 population, with scenario of longer storage time distribution compared to baseline case.

1. Storage time lengthened by 1 day

	Annual illnesses per 100 000 population		
	Non-susceptible population	Susceptible population	Mixed population
Mean	0.073 (0.0073 s.e.)	0.950 (0.0573 s.e.)	0.204 (0.0115 s.e.)

2. Baseline case

	Annual illnesses per 100 000 population		
	Non-susceptible population	Susceptible population	Mixed population
Mean	0.016 (0.0005 s.e.)	0.519 (0.0312 s.e.)	0.091 (0.0047 s.e.)

NOTE: s.e. = standard error of the mean.

4.2.5.8 Effects of growth

Risk characterization measures depend markedly on the amount of growth of the pathogen populations before consumption. Estimated amount of growth is modelled simply as the product of the daily growth rate at the storage temperature and the number of days of storage. That amount of growth is constrained by the maximum population density, which is modelled as a deterministic function of the storage temperature, but only seldom invoked, within the conditions modelled here.

If held under conditions under which no growth of *L. monocytogenes* occurs, with the same prevalence and level of contamination at retail (Table 4.2) and with the same consumption characteristics (Tables 4.6 and 4.7) as in the other cases examined, the simulated annual illnesses per 100 000 population and the illnesses per 1 000 000 servings decrease (Table 4.14).

Table 4.14 Risk characterization results for the pasteurized milk example, assuming no growth of *Listeria monocytogenes* in contaminated product.

4.14a Annual illnesses per 100 000 population.

	Non-susceptible population	Susceptible population	Mixed population
Mean	1.26×10^{-5} (6.72×10^{-8} s.e.)	3.76×10^{-4} (1.58×10^{-6} s.e.)	6.68×10^{-5} (2.42×10^{-7} s.e.)

4.14b Illnesses per 1 000 000 servings.

	Non-susceptible population	Susceptible population	Mixed population
Mean	5.87×10^{-7} (3.14×10^{-9} s.e.)	1.72×10^{-5} (7.50×10^{-8} s.e.)	3.64×10^{-6} (1.41×10^{-8} s.e.)

NOTE: s.e. = standard error of the mean.

4.3 EXAMPLE 2. ICE CREAM

4.3.1 Statement of purpose

The ice cream assessment begins with an estimation of the prevalence and concentration of *L. monocytogenes*, nominally at retail, in packages or containers of this RTE product, thus simulating the prevalence and levels of *L. monocytogenes* in consumed portions. Growth of the pathogen population in contaminated ice cream does not occur. Ancillary information is simulated for the frequency of consumption and the annual number of servings consumed by a large population of susceptible adults and non-susceptible adults. Among those annual servings are some contaminated ice cream portions, as estimated in the exposure assessment phase, which might lead to illness, as defined in the hazard characterization.

4.3.2 Hazard identification

The raw ingredients of ice cream and the processing environment may contain *L. monocytogenes*, which has been found in frozen dairy products. In the United States of America, there have been many recalls of ice cream, ice milk, sherbet and ice cream novelties

by the Food and Drug Administration in implementing its zero tolerance policy, at a cost of many millions of dollars. However, no illness has been conclusively linked with these types of products in that country (Ryser, 1999a). In 1986, the mother of an infected newborn had eaten ice cream sandwiches 3 days before delivery. In 1987, a cluster of 31 cases seemed to be epidemiologically linked to consumption of ice cream (Schwartz et al., 1989). In neither of these scenarios were any strains isolated from the implemented products. However, one case in an immunocompromised man was caused by *L. monocytogenes* serotype 4b infection arising from consumption of a commercially prepared ice cream in Belgium (Andre et al., 1990). The ice cream was found to contain 10^4 CFU/g, which probably arose because of post-pasteurization recontamination. The epidemiological and laboratory evidence indicates that contamination of ice cream occurs, but, with no opportunity for growth after production, levels are typically very low.

4.3.3 Exposure assessment results

4.3.3.1 Prevalence of L. monocytogenes *at retail*

For prevalence and concentration data, studies were selected based on the types of products – ice cream, ice cream mix and ice cream novelties – sampled from retail outlets, distribution centres or processing facilities. It is assumed that *L. monocytogenes* survives but does not grow at the temperatures appropriate for storing ice cream. So, any source of prevalence information, after final packaging of the product, should be appropriate for this exposure assessment. Thirteen studies contributed 24 separate prevalence estimates for *L. monocytogenes* contamination in ice cream. Extensive data are available from North America and Europe, but fewer studies have reported data collected from ice cream obtained in countries in Asia, Australia and South America. Prevalence estimates ranged from 0 to 8.3%, in studies involving from 5 to 48 520 samples; there were 191 461 samples in total (Table 4.15). Considered, but not included in the results, is the information from one study or data set, which reported prevalence but without stating a sample size (in Pitt, Harden and Hull, 1999). The study by Pitt, Harden and Hull (1999) gave a prevalence estimate of 0.139, which is the highest reported prevalence found in the literature (no other prevalence estimate exceeded 0.083). However, without knowing the sample size, it is difficult to know how much weight to give that individual point when determining an appropriate description of the variability in prevalence.

The stochastic structure of the collection of studies in Table 4.15 is represented by attributing binomial variability to the within-study estimates to account for their individual precision and attributing a Beta distribution to the between-study variability of the true study prevalences, π_i, from data y_i of n_i samples positive for *L. monocytogenes* in the i^{th} study, giving a two-stage hierarchical model $\boldsymbol{Y}_i|n_i,\pi_i \sim$ Binomial(n_i,π_i), $i = 1, \ldots, 24$ and $\pi_i \sim$Beta(α,β). This leads to the inference that average prevalence is 1.75×10^{-2} [8.31×10^{-3}, 0.042] at the 95% confidence interval when maximum likelihood estimates are $\hat{\alpha} = 0.42$ and $\hat{\beta} = 23.86$ (Figure 4.2).

Table 4.15 Data sets used to estimate prevalence of *Listeria monocytogenes* in ice cream.

Food	Stage	Country of study	Positive	Samples	Fraction	Ref.
Ice cream	NA	Austria	0	5	0	[1]
Ice cream	Retail	Canada	1	394	0.003	[2]
Ice cream mix	Retail	Canada	0	85	0	
Ice cream novelties	Retail	Canada	1	51	0.020	
Ice cream	Processing	Finland	4	603	0.007	[3]
Ice cream	Processing	Finland	0	188	0	
Ice cream	Processing	Finland	2	264	0.008	
Ice cream	Processing	Finland	0	74	0	
Ice cream	NA	Germany	1	2490	4.02×10^{-4}	[4]
Ice cream	NA	Germany	1	43	0.023	[5]
Ice cream, parfait	Retail or consumption	Hungary	1	15	0.067	[6]
Ice cream	Distribution	Korea	8	132	0.061	[7]
Ice cream (18 ewe milk; 1 goat milk; 131 cow milk)	Retail	UK	3	150	0.020	[8]
Ice cream	Retail	USA	23	659	0.035	[9]
Ice cream novelties	Retail	USA	29	351	0.083	
Ice milk	Retail	USA	0	42	0	
Ice cream	NA	USA	6	231	0.026	[10
Ice cream novelties	NA	USA	10	145	0.069	
Ice cream	Processing	various	48	48520	0.001	[11]
Ice cream	Processing	various	33	36661	0.001	
Ice cream	Processing	various	10	32078	3.12×10^{-4}	
Ice cream	Processing	various	11	36873	2.98×10^{-4}	
Ice cream	Processing	various	13	31407	4.19×10^{-4}	
Chocolate ice cream	NA	Australia	NA	NA	0.139	[12]

NOTE: NA = not available.

SOURCES: [1] From data submitted to FAO/WHO by the Austrian authorities, March 2000. [2] Farber, Sanders and Johnston, 1989. [3] Miettinen, Bjorkroth and Korkeala, 1999. [4] Hartung, 2000. [5] Steinmeyer and Terplan, 1990, cited in Klein, 1999. [6] Kiss et al., 1996. [7] Baek et al., 2000. [8] Greenwood, Roberts and Burden, 1991. [9] Kozak et al., 1996, citing unpublished 1987 data of Kozak. [10] US FDA, 1987, cited in Hitchins, 1996. [11] ICD, 2000. [12] [author not given] cited in Pitt, Harden and Hull, 1999.

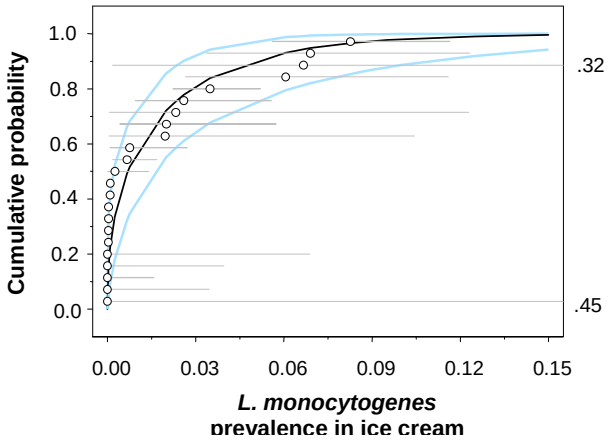

Figure 4.2 Empirical cumulative distribution function (open circles with 95% confidence intervals) for individual study prevalence estimates in Table 4.15, and fitted Beta distribution (solid line with shaded lines for 95% confidence limits) describe 2-stage hierarchical model for combining individual studies' estimates into an estimate for *Listeria monocytogenes* prevalence in ice cream.

4.3.3.2 Concentration of L. monocytogenes *at retail*

Kozak (1996, citing unpublished 1987 data from Kozak) provides the only information found to describe the concentration[1] of *L. monocytogenes* in contaminated ice cream (Table 4.16a). These data were used to construct a distribution with estimated minimum and maximum concentrations. Minimum concentration in positive samples was assumed to be 0.04 CFU/g and maximum concentration was assumed to be 100 CFU/g, based on the authors' judgment (Table 4.16b). Variability in *L. monocytogenes* concentrations in contaminated ice cream, at retail, was constructed by simulating concentrations in [0.04, 100] CFU/g, assuming that concentrations are block Uniform between the $\log_{10}$ quantiles in Table 4.16b.

4.3.3.3 Growth of L. monocytogenes *in ice cream*

No growth or die-off is modelled for *L. monocytogenes* in ice cream (FDA/FSIS, 2001).

4.3.3.4 Consumption characteristics for ice cream

Defining ice cream consumption

Selection of foods from Canadian Federal-Provincial Nutrition Surveys (CFPNS, 1992–1995) databases was intended to reflect both consumption frequency and the amount of ice cream consumed on eating occasions. Results are based on the reported consumption practices of

1. A comment on a late draft of this example exposure assessment pointed to further information in Stainer and Maillot (1996), which has not been incorporated here.

the 12 089 consumers who were respondents to the Nutrition Surveys, among whom 1409 consumed ice cream. Ice cream consumptions were aggregated from all an individual's eating occasions on the same day to give, for a single day at random, the estimated fraction of the population who consume ice cream and the daily amount of ice cream consumed. When ice cream was reported as an ingredient in a meal, an appropriate fraction of the food to represent the amount of ice cream included was derived or estimated. Some foods were used as surrogates for the amount of ice cream consumed in a serving, to enrich the database, but were not used to estimate frequency of ice cream consumption (Table 4.17).

The simulated distributions constructed for annual meals and daily consumption amounts respect the Age × Gender groups' contributions to a non-susceptible and a susceptible population defined as in Miller, Whiting and Smith (1997), attributing a fraction of individuals that possess the same age and gender characteristics to a susceptible population so that, among Canadian adults 18–74 years of age (71.5% of the population), for whom the consumption characteristics determined by the Nutrition Surveys apply, 15% (3.3 million) would fit into the susceptible group and 85% (18.7 million) would fit into the non-susceptible group.

Table 4.16 *Listeria monocytogenes* concentration in contaminated ice cream.

4.16a. Data set used to estimate *L. monocytogenes* concentration.

	<5 CFU/g	<15 CFU/g	Samples
Kozak, 1996	1	1	2

4.16b. Assumed cumulative distribution function for *L. monocytogenes* concentration in contaminated ice cream, with estimated minimum (0.04 CFU/g) and maximum (100 CFU/g) concentration.

CFU/g	$\log_{10}$ CFU/g	Cumulative probability [95% confidence interval]
0.04	-1.4	0
5	0.7	0.286 [0.013, 0.987]
15	1.18	0.714 [0.158, 1]
100	2	1

Table 4.17 Food commodities used to describe ice cream consumption frequency and amount consumed.

Food code	Food name	Respondent eating occasions	Average serving (g) per occasion
536	Ice cream, vanilla, regular, hardened, 10% B.F.(1)	1184	79.1
537	Ice cream, vanilla, rich, hardened, 16% B.F.	83	81.1
538	Ice milk, vanilla, hardened or soft serve	143	118.3
539	Sherbet, orange	24	96.2
563	Milk shake, chocolate, thick (2)	1	105.7
633	Yoghurt, frozen	56	111.1
11847	Light ice cream, vanilla, hardened, 7% B.F.	17	72.5
11848	Light ice cream product, vanilla, hardened, 1% B.F.	13	109.2

NOTE: (1) B.F. = butter fat. (2) Assumes 50% ice cream.

Annual ice cream servings

Uncertainty about the point estimates for the estimated fraction of the population who consume ice cream is described by attributing a beta distribution to the proportion of the sample respondents that would consume ice cream on a random day.

The simulated distribution for the number of days per year with ice cream consumption adds up the days per year with ice cream consumption in the gender and age groups that make up those populations. It is assumed that the daily consumption probability is the same on every day of the year for individuals in the same Gender × Age group, whether the individuals are in the non-susceptible population or the susceptible population, that days are independent, and that binomially sampling can be used to represent day-to-day variability (Table 4.18).

Amounts of ice cream consumed

The distribution in Table 4.19 was constructed by sampling from the Nutrition Survey data for Age × Gender groups defined, and collecting results into simulated ice cream consumption amounts distributions for non-susceptible and susceptible populations. The simulated distribution respects the gender and age proportions that make up the non-susceptible and susceptible populations.

Table 4.18 Selected quantiles (1%, 5%, 10%, 25%, 50%, 75%, 90%, 95% and 99% points) from simulated distribution of annual days with ice cream consumption among all individuals in non-susceptible and susceptible adult populations in Canada.

Population	**Cumulative probability**								
	0.01	0.05	0.10	0.25	0.50	0.75	0.90	0.95	0.99
Non-susceptible	7.4×10^{8}	1.0×10^{9}	1.2×10^{9}	1.5×10^{9}	1.9×10^{9}	2.2×10^{9}	2.6×10^{9}	2.9×10^{9}	3.3×10^{9}
Susceptible	1.2×10^{8}	1.7×10^{8}	2.0×10^{8}	2.7×10^{8}	3.5×10^{8}	4.5×10^{8}	5.3×10^{8}	5.9×10^{8}	6.9×10^{8}

Table 4.19 Selected quantiles (1%, 5%, 10%, 25%, 50%, 75%, 90%, 95% and 99% points) from simulated distribution of daily amount (g) of ice cream consumption among individuals in non-susceptible and susceptible adult populations in Canada.

Population	**Cumulative probability**								
	0.01	0.05	0.10	0.25	0.50	0.75	0.90	0.95	0.99
Non-susceptible	8.5 g	19.1 g	33.2 g	46.9 g	75.4 g	130.3 g	168.8 g	210.3 g	335.8 g
Susceptible	8.4 g	16.9 g	28.1 g	38.7 g	66.5 g	102.8 g	133.0 g	152.4 g	266.1 g

L. monocytogenes *in contaminated ice cream serving*

The simulated distribution for the number of *L. monocytogenes* organisms in a contaminated ice cream serving (Table 4.20) is constructed from the concentration (Table 4.16) and serving size (Table 4.19) distributions. Quantiles vary as shown among 16 simulations, each involving 32 000 iterations from the input distributions.

Only servings from contaminated ice cream will contain any *L. monocytogenes* organisms. Only a fraction of servings that contain very low levels of contamination will contain any of the pathogen. Assumptions about homogeneity or heterogeneity of the organisms in a contaminated foodstuff can have a great effect on the simulated results. Clustering of colonies of pathogens would introduce extra variability into the results (Haas, Rose and Gerber, 1999). Here, it is assumed that the organism is distributed homogeneously throughout the product in a Poisson distribution, but small variations in the number of *L. monocytogenes* organisms present in servings drawn from a packaged product with the same average concentration are ignored. It is assumed also that all organisms present would be in a part of the ice cream that would be consumed.

4.3.4 Risk characterization

4.3.4.1 Annual illnesses per 100 000 population

The simulated distribution for the number of illnesses per year per 100 000 population (Table 4.21a) is developed using the distribution for the probability of illness from consuming a contaminated serving and the distribution for the number of contaminated servings per year as intermediate calculations. The distribution of annual contaminated ice cream servings includes variability and uncertainty associated with the distribution for the average prevalence of contaminated servings and the distribution for the number of annual ice cream servings (Table 4.18). Critical to the development of risk characterization measures is the mean value of that simulated distribution for individuals from the non-susceptible population and individuals from the susceptible population (G. Paoli, pers. comm., 2001).

Table 4.20 Selected quantiles from simulated distributions of $\log_{10}$ number of *Listeria monocytogenes* organisms in contaminated ice cream servings at point of consumption.

Quantile ($\log_{10}$ CFU in serving)	**Cumulative probability**	
	Non-susceptible population	Susceptible population
0	7.23×10^{-4} (1.04×10^{-5} s.e.)	8.36×10^{-4} (7.99×10^{-6} s.e.)
1	0.019 (3.40×10^{-5} s.e.)	0.022 (4.22×10^{-5} s.e.)
2	0.147 (6.73×10^{-5} s.e.)	0.163 (7.93×10^{-5} s.e.)
3	0.630 (1.04×10^{-4} s.e.)	0.683 (7.98×10^{-5} s.e.)
4	0.993 (2.68×10^{-5} s.e.)	0.996 (2.27×10^{-5} s.e.)
5	1	1

NOTE: s.e. = standard error of the mean.

The distribution of the risk characterization result is concentrated at nil illnesses per 100 000 population, up to beyond the 99th percentile. Mean values for the distributions are quoted for the results. Results in Table 4.21a are reported separately for a susceptible and a non-susceptible adult population, and for a mixed (total) adult population that consists of approximately 85% non-susceptible adults and 15% susceptible adults. Summary statistics for the distributions vary as shown among 16 simulations, each involving 32 000 iterations from the input distributions.

4.3.4.2 Illnesses per 1 000 000 servings

The simulated distribution for the number of illnesses per 1 000 000 servings (Table 4.23b) is developed from the average prevalence of contaminated servings and the probability of illness from consuming a contaminated serving. The distribution of the risk characterization result is concentrated at less than one illness per 1 000 000 servings, up to beyond the 99th percentile. Mean values for the distributions are quoted for the results. Results for a non-susceptible and for a susceptible population vary as shown among 16 simulations, each involving 32 000 iterations from the input distributions.

Table 4.21 Risk characterization for ice cream.

4.21a Annual illnesses per 100 000 population

	Non-susceptible population	Susceptible population	Mixed population
Mean	2.10×10^{-5} (1.70×10^{-8} s.e.)	6.73×10^{-4} (4.24×10^{-7} s.e.)	1.18×10^{-4} (6.91×10^{-8} s.e.)

4.21b Illnesses per 1 000 000 servings.

	Non-susceptible population	Susceptible population
Mean	2.09×10^{-6} (1.19×10^{-9} s.e.)	6.08×10^{-5} (4.21×10^{-8} s.e.)

NOTE: s.e. = standard error of the mean

4.3.5 Uncertainty and variability

A last step in this assessment for *L. monocytogenes* in ice cream examines the simulation model to consider how much the various inputs affect the outputs. Based as they are on a simulation model, the risk characterization results are subject to uncertainty associated with a modelled representation of reality, involving assumed simple relationships among prevalence, concentration, consumption characteristics and adverse response to consumption of some number of *L. monocytogenes* organisms.

4.3.5.1 Effects of hazard characterization's dose-response

There is uncertainty in the hazard characterization's dose-response relationship used to relate the simulated distributions of the number of *L. monocytogenes* organisms in a serving to the measures that have been used to characterize the risk. There is uncertainty associated with the form of the dose-response function used and with the parameterization. Describing distributions for the parameters captures variability in the response to the same pathogen dose

among individuals in a subpopulation. However, there is uncertainty associated with the distributions assumed for the parameters.

4.3.5.2 Effects of estimated consumption frequency

Simulated ice cream consumption frequency for non-susceptible and susceptible populations (Table 4.18) is sensitive to the survey estimates of consumption frequency. Sample sizes are large enough that the amount of uncertainty associated with the point estimate has only a minor influence. There is uncertainty due to extrapolation of those results to the present day. Further, consumption characteristics were derived for non-susceptible and susceptible individuals by imputing characteristics associated with age and gender, a source of uncertainty. There is uncertainty and variability associated with extrapolation of daily consumption characteristics to annual consumption for populations of individuals. There is uncertainty associated with extrapolation of survey results from 1991–1995 to the present day.

4.3.5.3 Effects of estimated consumption amounts

Simulated distributions for ice cream consumption amounts are less sensitive to how the composition of the non-susceptible and susceptible populations is defined. Generally, the gender and age of individuals in the non-susceptible and susceptible populations have only minor influence on the simulated distribution for the amounts of ice cream consumed. Ice cream consumption amounts have uncertainty, including errors associated with under- and over-reporting, estimation methods for the amount of ice cream consumed, the representation of ice cream consumption using several food codes and the derivation or estimation of an appropriate amount of ice cream to include when the ice cream was an ingredient in the meal. All of a respondent's identified ice cream amounts within a day were aggregated into a daily amount for the respondent. That practice loses the distinction that one might wish to make among different eating occasions within the day, whether the ice cream was consumed alone or as part of a meal and whether the ice cream was consumed at home or away from home. There is uncertainty and variability associated with extrapolation of daily consumption characteristics to annual consumption for populations of individuals. There is uncertainty associated with extrapolation of survey results from 1991–1995 to the present day.

4.3.5.4 Effects of L. monocytogenes *prevalence*

Simulated numbers of ice cream servings with any *L. monocytogenes* contamination are influenced by the number of servings that the population consumes and the prevalence of *L. monocytogenes* in packages of ice cream. Prevalence is sensitive to correct inclusion and exclusion of data sets from literature, government surveillance reports and industry (Table 4.15). Sensitivity to prevalence of *L. monocytogenes* in ice cream at retail is nearly multiplicative. If the prevalence is reduced by a factor of 10, then simulated annual illnesses per 100 000 population and simulated illnesses per 1 000 000 servings are also reduced by approximately a factor of 10.

In point of fact, risk characterization results are sensitive to the nature of the inference that one makes from Table 4.15's data concerning the prevalence in a large number of servings. Risk characterization results (Table 4.21) are based on the assumption that true *L. monocytogenes* prevalence estimates in individual studies or data sets follow a beta distribution, yielding an inference that average prevalence is 1.75×10^{-2} [8.31×10^{-3}, 0.042]

at the 95% confidence interval. Alternatively, if one assumed that all prevalence studies have sampled the same phenomenon and pooled the studies' samples to provide an estimate for a single, fixed prevalence, then the inference about mean prevalence becomes 1.07×10^{-3} [9.29×10^{-4}, 1.23×10^{-3}] at the 95% confidence interval (205 samples positive for *L. monocytogenes* in 191 461 samples). Based on that inference about prevalence, risk characterization results, such as the number of annual illnesses per 100 000 population, are approximately 8% of the results in Table 4.21 (Table 4.22).

4.3.5.5 Effects of L. monocytogenes *concentration at retail*

Concentration of *L. monocytogenes* at consumption (Table 4.16) influences the simulated number of organisms in contaminated consumer servings (Table 4.20). Concentrations in consumer portions are simulated to be very low, but are based on data attributed to a single reference and are little influenced by departures from the estimated maximum concentration of 100 CFU/g in contaminated ice cream (Table 4.23).

Table 4.22 Comparison of simulated annual illnesses per 100 000 population under different inferences about the prevalence of *Listeria monocytogenes* in ice cream at retail.

4.22a Two-stage hierarchical model assuming Beta distribution as mixing distribution for prevalence estimates in the individual studies in Table 4.15.

	Annual illnesses per 100 000 population		
	Non-susceptible population	Susceptible population	Mixed population
Mean	2.10×10^{-5} (1.70×10^{-8} s.e.)	6.73×10^{-4} (4.24×10^{-7} s.e.)	1.18×10^{-4} (6.91×10^{-8} s.e.)

4.22b Pooled all studies' samples to estimate a single, fixed prevalence for all data sets in Table 4.15.

	Annual illnesses per 100 000 population		
	Non-susceptible population	Susceptible population	Mixed population
Mean	1.68×10^{-6}	5.54×10^{-5} (8.14×10^{-8} s.e.)	9.68×10^{-6} (1.21×10^{-8} s.e.)

NOTE: s.e. = standard error of the mean

Table 4.23 Annual illnesses per 100 000 population for ice cream under different inferences about the maximum concentration of *Listeria monocytogenes* in contaminated ice cream at retail.

4.23a Baseline case, assumed maximum *L. monocytogenes* concentration at retail = 100 CFU/g.

	Annual illnesses per 100 000 population		
	Non-susceptible population	Susceptible population	Mixed population
Mean	2.10×10^{-5} (1.70×10^{-8} s.e.)	6.73×10^{-4} (4.24×10^{-7} s.e.)	1.18×10^{-4} (6.91×10^{-8} s.e.)

4.23b Assumed maximum *L. monocytogenes* concentration at retail = 250 CFU/g.

	Annual illnesses per 100 000 population		
	Non-susceptible population	Susceptible population	Mixed population
Mean	2.67×10^{-5} (2.57×10^{-8} s.e.)	8.53×10^{-4} (8.79×10^{-7} s.e.)	1.50×10^{-4} (1.27×10^{-7} s.e.)

4.23c Assumed maximum *L. monocytogenes* concentration at retail = 1000 CFU/g.

	Annual illnesses per 100 000 population		
	Non-susceptible population	Susceptible population	Mixed population
Mean	4.19×10^{-5} (6.57×10^{-8} s.e.)	1.33×10^{-3} (1.93×10^{-6} s.e.)	2.34×10^{-4} (2.90×10^{-7} s.e.)

NOTE: s.e. = standard error of the mean

4.4 EXAMPLE 3. FERMENTED MEAT

4.4.1 Statement of purpose

This assessment aims to estimate the risk of listeriosis from fermented meat products (FMPs). In this assessment, fermented meats are taken to include those meat products in which reduction of water activity through addition of salt and drying and acidification due to the metabolic activity of microorganisms on added sugars are used to extend the shelf-life of meat. It does not consider risk in a specific nation, or specific regions within a nation, because consumption patterns vary widely, but it does attempt to calculate generic estimates of the risk of listeriosis per serving for any consumer of FMPs anywhere in the world. It should be noted that the data are representative of Western-style fermented meat products. The assessment begins with *L. monocytogenes* in fermented meat products after production.

4.4.2 Hazard identification

L. monocytogenes is widely distributed in raw meats. It has also often been detected in commercially produced FMPs (See Table A4.1 in Appendix 4). Investigation of an outbreak of listeriosis in Philadelphia in 1986/87 suggested that either ice cream or fermented meats were involved, based on the consumption records of victims (Schwartz et al., 1988, 1989). However, no documented cases of listeriosis have been directly attributed to FMPs (Lücke, 1995). Farber and Peterkin (1999) have reviewed the importance of *L. monocytogenes* in processed meats, including FMPs.

The acid tolerance of *L. monocytogenes* and its ability to grow at low water activity levels could allow survival or growth of the pathogen in fermented meat products. Many fermented meat products, however, do not support growth of *L. monocytogenes* in their final product form, although they can support its growth during the early stages of production.

4.4.3 Exposure assessment

4.4.3.1 Production and Consumption

Although this assessment is primarily concerned with estimating the *per serving* risk because of the paucity of national consumption data, available data describing national consumption of FMPs is presented in Table 4.24 below. The basis for the estimates is presented in Section A4.7 in Appendix 4.

Table 4.24 National population and national fermented meats consumption data used in the assessment.

Country	Population (million)[(1)]	Consumption (kg/person/year)	Number of 50-g servings per year
USA	271	0.295	6
Australia	19	0.4–1.68	8–34
Canada	31	0.912	18
Germany	81	0.723	14.5
Finland	5.2	3.1	62

NOTE: (1) Derived from NGS, 1999.

These data were modelled by a Triangular(6, 25, 62) distribution, empirically based on data in Table 4.24, to reflect the variability in mean national per capita consumption. While some reports indicate differences in frequency of consumption of FMPs by age and gender (e.g. CFPNS, 1992–1995; ABS, 1995), there is a lack of corresponding information on serving size. For that reason, and the inability to relate differences in susceptibility to listeriosis and the age-gender categories considered in those nutrition surveys, no attempt was made to incorporate these differences in this risk assessment.

4.4.3.2 Modelling exposure

Initial contamination

Initial contamination at production or retail was modelled using a discrete distribution (Analytica "Chancedist") based on the data presented in Section A4.1 of Appendix 4. The total number of positive results across all surveys is 13.65%. Conversely, 86.35% of samples were interpreted to have less than 1 CFU *L. monocytogenes* per 25 g, or, equivalently, <0.04 CFU *L. monocytogenes* per 1 g. Eight of the data sets included quantitative data that were used to estimate the proportion of all positive samples in three concentration ranges: $0.04 < X < 10$ CFU/g; $10 < X < 100$ CFU/g; and $100 < X < 10\,000$ CFU/g.

Other concentrations reported (see Section A4.1 of Appendix 4) had too few data to be used, or did not add any information because only two levels were specified. In these cases, the data were ascribed to the next highest concentration level with which they were consistent.

The distribution of final contamination levels at the point of production that was used in the model is shown in Table 4.25. In all cases, all samples in each range were presumed to be present at the highest level in that range – an inherently conservative decision. A non-conservative assumption, however, is also incorporated indirectly into the model because the contamination level data included data based on surveys conducted at retail. From the foregoing, it is anticipated that contamination levels will decline between the time of production and sampling at retail. The effects of this assumption are discussed in Section 4.4.5.

Table 4.25 Distribution of reported contamination levels of fermented meat products at retail.

% of samples in range[(1)]	Concentration of *L. monocytogenes*
86.35	<0.04 CFU/g
6.94	<10 CFU/g
6.10	<100 CFU/g
0.60	<10 000 CFU/g

NOTE: (1) Where several percentage data were available for a specific range, an average was calculated. Thus, the sum of the percentages ascribed to each range of "positive" results slightly exceeded the predicted 13.65%. Accordingly, each percentage value was adjusted in equal proportion so that the sum of the positive results was 13.65%.

Potential for Growth of L. monocytogenes *in Fermented Meat Products*

Product manufacture and composition

A description of fermented meats and their characteristics and methods of production is presented in Section A4.2 of Appendix 4.

FMPs have long shelf lives due to the combination of acidification (through fermentation of sugar added to the meat or to addition of an acidulant such as glucono-∂-lactone or encapsulated citric acid), removal of oxygen, and addition of compounds that favour the growth of desirable microbes while retarding the growth of others. Available water is typically limited through the addition of salt and the removal of water over an extended "maturation" period. These factors combine to produce products that are shelf stable and resistant to spoilage by bacteria.

Variables in the production of FMPs include:

- type of meat;
- amount of fat added;
- starter culture used (if used), and whether it produces bacteriocins;
- curing mix composition and concentration – nitrite or nitrate levels, salt concentration, spices, etc.;
- fermentation time and temperature;
- heating time and temperature (if applied);
- maturation time and temperature;
- sausage diameter;
- final pH;
- final water activity; and
- recommended storage temperatures.

The relevance of each of these variables to the microbiological safety of the product is discussed in Section A4.2 of Appendix 4.

Temperatures above 65°C are considered cooking temperatures. Such temperatures are listericidal, with D-values of a few minutes. A post-fermentation cooking step may be included with some FMPs; however, recontamination of the exterior of the product can occur.

Ecology of *L. monocytogenes* in uncooked fermented meat products.

Published literature sources indicate that *L. monocytogenes* does not grow in most FMPs once the fermentation is well underway and pH has fallen, nor does it grow during subsequent maturation (e.g. Schillinger, Kaya and Lücke, 1991; Campanini et al., 1993; Farber et al., 1993; Rödel, Stiebing and Kröckel, 1993; Samelis et al., 1998; Encinas et al., 1999; Laukova et al., 1999). Once fermentation has been established, *L. monocytogenes* is usually slowly inactivated as a result of the conditions present in shelf-stable FMPs. Some growth of *L. monocytogenes* may occur in the raw ingredients or during the initial phases of the fermentation, particularly if products are not inoculated with a starter culture (Campanini et al., 1993). Similarly, if fermentable carbohydrates are not added to the raw ingredients, the decline in pH and increase in salt concentration may be delayed. This could extend the time during which the product composition might allow growth of *L. monocytogenes*.

The extent of inactivation will depend on the time of storage, temperature and the characteristics of the FMPs, such as pH, organic acid concentration, salt concentration and presence of preservative compounds. When microorganisms cannot grow in an environment, they die at a rate governed by environmental factors, of which temperature appears to be most important (Buchanan et al., 1997; Ross and Shadbolt, 2001). The environmental limits to growth of *L. monocytogenes* are detailed in Table 3.1, and models describing them in Table A3.1. The final composition of an FMP dictates the survival of *L. monocytogenes* in the product during maturation and subsequent marketing. Composition and processing parameters for a variety of common FMPs types are shown in Appendix 4 (see Section A4.3). Each of those variables is regarded as contributing to the overall character of the product.

Change in contamination level

Gradual inactivation of *L. monocytogenes* is expected under conditions characteristic of mature FMPs, leading to an expected decline in *L. monocytogenes* levels during distribution and storage. The model of Tienungoon et al. (2000) for *L. monocytogenes* growth limits supports the belief that growth would not be expected in any FMPs that falls within the accepted pH and a_w specifications for stable FMPs (see Appendix 4, Section A4.3).

It was therefore assumed that growth of *L. monocytogenes* does not occur in the finished product. Conversely, it was assumed that inactivation would occur over time, therefore, inactivation of *L. monocytogenes* was modelled for the period that the product was held at retail and the period that the product was held in the consumer's home before consumption. It was also assumed that contamination data related to product sampled during retail storage.

Non-thermal inactivation model

The Buchanan, Golden and Phillips. (1997) model describing non-thermal inactivation of *L. monocytogenes* under reduced oxygen conditions and in response to temperature, water activity, pH, nitrite and salt concentration was selected. That model includes most factors considered relevant to inactivation of *L. monocytogenes* in FMPs, i.e. temperature (4–42°C), pH (3–7), lactic acid (0–2%), NaCl (0.5–19%) and sodium nitrate (0–200 µg/ml).

That model, however, predicts the time required under a given set of environmental conditions for a 10 000-fold reduction (t_{4D}) in *L. monocytogenes.* For the purpose of the current risk assessment, the t_{4D} value predicted by the model from the product composition data in each scenario was divided by four to generate the D-value (time for a 10-fold reduction). The storage times at retail and in the consumer's home were then divided by the modelled D-value to predict the $\log_{10}$ reduction in *L. monocytogenes* in the product at the point of consumption.

This simplification of the inactivation model of Buchanan, Golden and Phillips (1997) could be criticised because it could lead to an overprediction of inactivation. Buchanan, Golden and Phillips (1997) modelled t_{4D} rather than D-values because the inactivation kinetics observed in their experimental broth system were not always log-linear. However, Buchanan, Golden and Phillips (1997) compared the predictions of their model with published values for the inactivation of *L. monocytogenes* under analogous condition in foods. Model predictions typically over-estimated the observed t_{4D} by factors of 2–3, i.e. the predictions were inherently conservative, and it is probable that factors other than those included in the model are important in determining the rate of non-thermal inactivation. Evaluation of these values indicated that the simplification of the Buchanan, Golden and

Phillips (1997) model employed in the current assessment provides a reasonable estimate of the inactivation of *L. monocytogenes* in commercial FMPs.

Product composition data

The model of Buchanan, Golden and Phillips (1997) predicts the effect of salt concentration, pH, temperature, nitrite and undissociated lactic acid concentration on the inactivation of *L. monocytogenes*. Absolute ranges of those factors reported in FMPs were determined from the data presented in Appendix 4 (see Section A4.2), and approximations to the most probable values were made. The distributions used and their behaviour are shown in Table 4.26.

Table 4.26 Characteristics of distributions used to describe physico-chemical properties of fermented meat products that affect *Listeria monocytogenes* inactivation.

Characteristic of Variable Description		Product Parameter		
		pH	water activity[(1)]	nitrite (ppm)
Beta distribution		2.5, 6, 4.3, 6.6	15, 5, 0.73, 1.00	10, 90, 0, 200
Minimum estimate		4.31	0.811	3.5
Mean estimate		4.98	0.933	20
Maximum estimate		6.36	0.995	30.7
Percentiles (estimated)	5	4.49	0.887	11.1
	50	4.94	0.935	19.5
	95	5.6	0.97	53.8

NOTES: (1) a_w was converted to salt concentration using the following equation, obtained by fitting an empirical polynomial equation to a calibration curve of water activity and salt concentrations:

Salt concentration (% w/w) =√(((1000 × (1-Product water activity)+56.3904195) -7.50935546)/0.349337086)

Lactic acid concentrations were estimated from product pH data, as described in Appendix 4 (see Section A4.4).

Temperature and time of storage

Times and temperature of storage were divided into retail storage and home storage. It was assumed that the shelf-life of the product ranged between 1 and 180 days (FSIS, 1995; Ross et al., in press) using a Triangular(1, 60, 180) distribution. Some FMPs, e.g. soft spreadable types such as mettwurst, teewurst, or Braunschweiger, are considered to have a refrigerated shelf-life of only a few days. Other products, e.g. hard or dry sausages, have an indefinite unrefrigerated shelf-life. In the allocation of shelf-life, the relationship between product composition and shelf-life was not modelled.

It is assumed that the product is sold at any point in this shelf-life but, from the data shown in Appendix 4 (see Section A4.5), the probability is greatest that the sale will occur mid-way through the product's life, and no product was found available for retail sale with more than 66% of its shelf-life remaining. It is assumed that this period represents the time taken for product to reach point of sale. The data in Appendix 4 (Section A4.5) are modelled by a Triangular(33, 58, 100) distribution, representing the *percentage* of the entire shelf-life that the product is held at retail prior to sale.

The remaining shelf-life of the product is calculated from the difference between the total shelf-life and the shelf-life elapsed at retail. Of the shelf-life remaining available to the

consumer, it was also considered more likely that the consumer would consume the product relatively quickly. Thus, the time of consumption was modelled by multiplying the remaining shelf-life by a Triangular(0.0, 0.1, 1.0) distribution.

The model traces the flow of an individual serving from retail to consumption. The resulting distribution is considered to be representative of a serving, not necessarily a package of servings.

Fermented meat products may be stored under refrigeration or, more traditionally, at ambient temperature. In the United States of America, many FMPs are stored, distributed and displayed at refrigeration temperature (for marketing and product quality reasons), but in Europe it is more usual for these products to be held at ambient temperature (B. Tompkin, pers. comm., 2001). It was assumed that storage at retail would be at ambient temperature in 30% of cases, but that 85% of consumers would store their FMPs in the refrigerator. Based on the data of Audits International (2000) for processed meats (sold at the delicatessen counter or pre-packaged luncheon meats), temperatures during retail storage were approximated by a Triangular(0, 6, 17) distribution and domestic refrigeration temperatures by a Triangular(-1, 5, 12) distribution. Ambient temperatures at retail or in the home were described by a Triangular(5, 20, 35) distribution.

The overall structure of the model describing inactivation during retail and home storage is shown as an influence diagram in Figure 4.3.

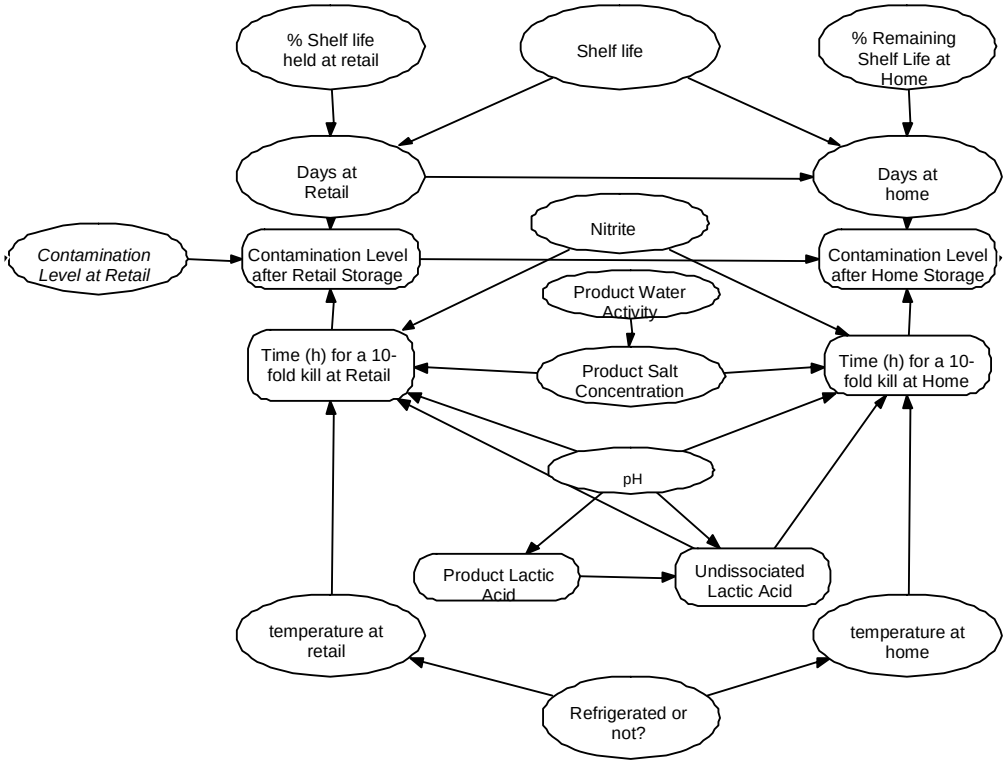

Figure 4.3 Influence diagram describing the modelled inactivation of *Listeria monocytogenes* during retail and home storage prior to consumption.

4.4.4 Risk characterization

The above elements were combined to estimate the risk to public health from *L. monocytogenes* in fermented meat products using stochastic modelling software (Analytica 1.1.1).

The overall model is shown schematically in Figure 4.4 and includes modules for:

- contamination level at retail, the effect of conditions during retail storage, home storage and point of consumption,
- modelled probability of illness per 1 million servings, and

modelled estimates of annual cases of listeriosis per 100 000 population.

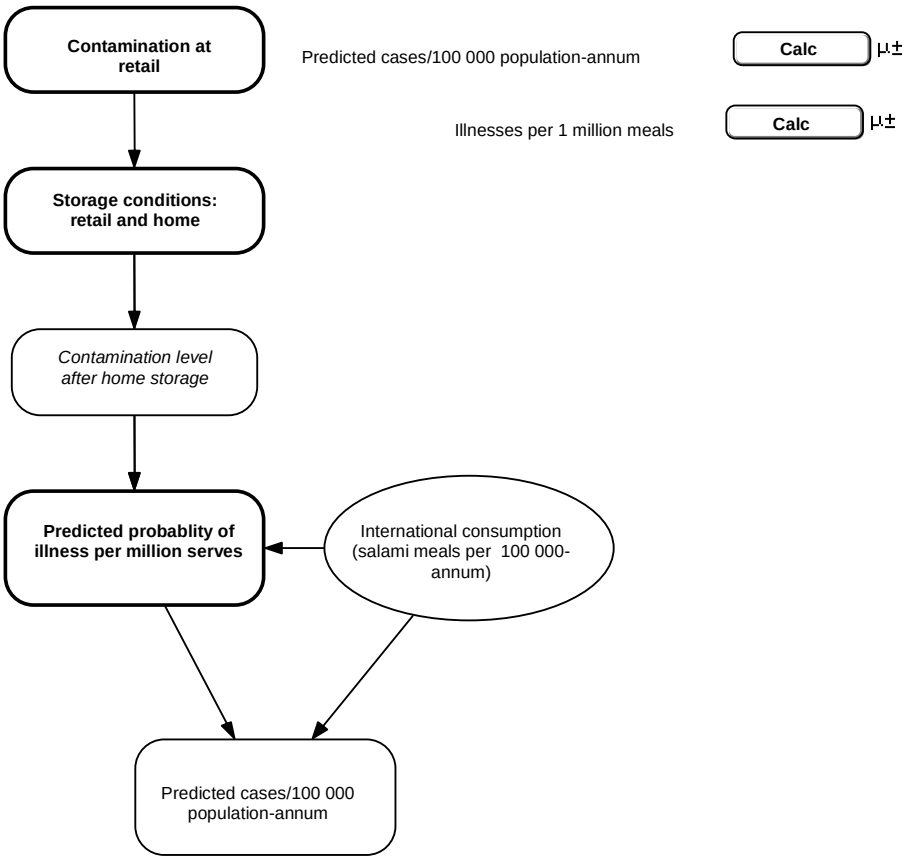

Figure 4.4 Overall structure of the conceptual model used to estimate the public health risk of listeriosis from fermented meat products.

Modelling listeriosis due to consumption of FMPs

The module modelling the relationship between the levels of *L. monocytogenes* in FMPs at the point of consumption and the corresponding prevalence of illness anticipated is shown as an influence diagram in Figure 4.5.

The module contains two sections. The first generates the dose-response relationship, assuming an exponential dose-response model developed and implemented as described in Section 4.1.4, by selecting a value for the parameter R in the exponential dose-response model as outlined in Section 4.1. In the second stage, the dose-response model is combined with serving size data and the modelled contamination level data to predict probability of illness per serving.

Serving size distribution is drawn from the United States of America data presented in FDA/FSIS (2001), and modelled by an empirically derived distribution (Beta(2, 8, 0, 270)). Using this, 80% of meals are predicted to be in the range 20–100 g per serving. A comparison of the original and empirical distribution showing the range of meal sizes is shown in Section A4.6 in Appendix 4.

From the contamination level estimate, serving size and r-value, the probabilty of illness per meal is then estimated.

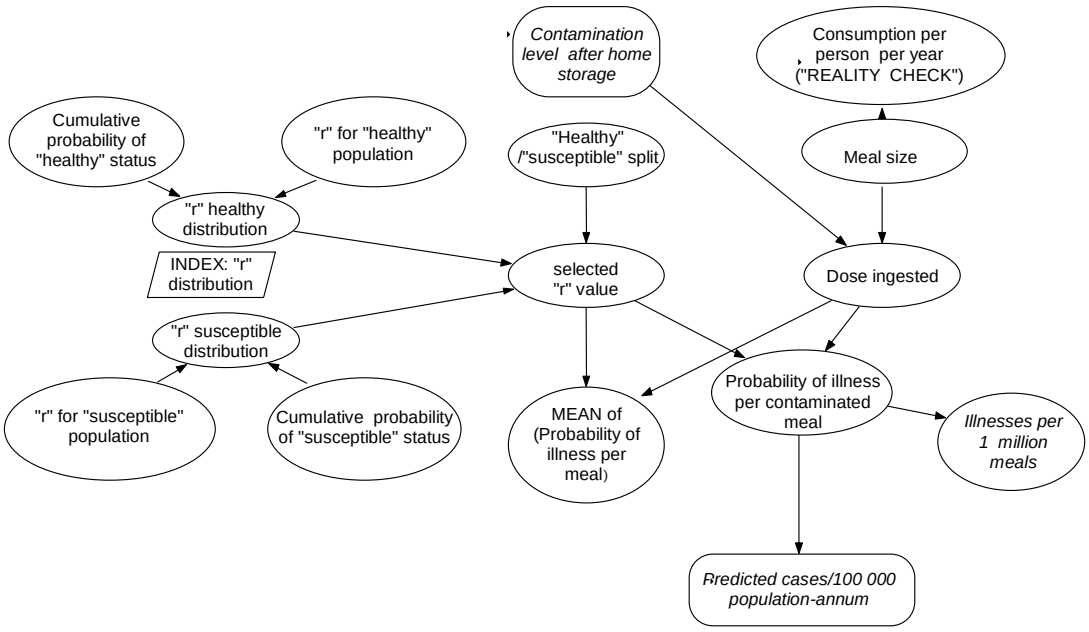

Figure 4.5 Influence diagram showing interplay of dose-response relationships for listeriosis and dose of *Listeria monocytogenes* ingested.

NOTES: Cum = Cumulative. R = r-value.

Calculations

As discussed in Section 4.1.4, the primary measure of risk estimated is the total number of cases per 1 000 000 meals based on the mean per-meal risk estimated from the 32 000 iterations. The final calculation multiplies the risk per serving by the estimate of the number of FMPs servings per annum in a range of nations. This is based on an estimate of the number of servings per person in each of the nations considered, described by a Triangular(6, 25, 62) distribution.

During the simulations it was noted that many calculations resulted in very low predicted concentrations of contamination such that the software was unable to carry through calculations. Accordingly, finite values of 1×10^{-12} CFU were added to intermediate values at some steps. Tests showed that this had no effect on the calculations of means, but did allow additional percentiles to be expressed.

The mean of the mean, minimum and maximum estimates of the results of the 16 trials are shown in Table 4.27, in addition to the standard deviation of the 16 estimates of the mean.

Table 4.27 Predicted risk of listeriosis from fermented meat products based on consumption data from several nations.

Summary statistics for 16 simulation runs	Cases per 100 000 consumers/year	Cases per 1 000 000 meals
Mean of Means	5.47×10^{-6}	2.11×10^{-6}
SD of Means(1)	8.59×10^{-6}	3.66×10^{-6}
Mean of Maxima	0.13	0.055
Mean of Minima	0.00	0.00

NOTE: (1) The Standard Deviation (SD) reported here does not measure the variability in the risk outcome distribution. Rather, it describes how much the estimator of a characteristic (i.e. in this case, the mean) of that risk outcome distribution varies from simulation run to simulation run.

As discussed in Section 4.1.4, no information was available to enable differentiation of consumption patterns of susceptible groups from those of the "normal" population. Accordingly, no attempt was made to differentiate risk for those groups. The total population risk estimate is based on the assumptions that between 15 and 20% of the total population is more susceptible to listeriosis, and the difference in susceptibility between those two broad groups is modelled by the use of two r-values in the modelling.

The simulation modelling predicted 2% of samples were contaminated at the time of serving at levels at >0.04 CFU *L. monocytogenes*, i.e. the threshold for many detection methods. From available survey data (see Table A4.1 in Appendix 4), the proportion of samples contaminated at >0.04 CFU *L. monocytogenes* at retail is 13.2%. This difference results from inclusion in the simulation model of inactivation of *L. monocytogenes* in fermented meats.

The annual observed incidence of listeriosis in the total population of many nations is in the range 0.3–0.5 cases/100 000 population although it has been suggested (Mead et al., 1999) that the true incidence may be twice as high. The above estimate (Table 4.27) suggests that consumption of fermented meat products probably contributes to only a very small proportion of those cases. The results are based on a number of assumptions, as discussed below.

4.4.5 Uncertainty and variability

The model draws together results from disparate studies and national cultures. As such, while the estimates are representative of that total population, they may not simulate very well the risk of listeriosis in any individual nation, because of differences in consumption patterns, for example. Different consumption behaviour between nations is expected and reported indirectly (Holdsworth et al., 2000) but practically no data quantifying national FMPs production were found. From limited data, per capita consumption patterns were estimated (see Section A4.4 in Appendix 4) to range from approximately 300 g to nearly 3 kg per year.

Such differences in estimated levels of consumption suggest that caution is required if using the results of this assessment for risk management actions within a specific nation. Rather, national consumption patterns should be used to estimate the public health risk from fermented meats in specific nations. Similarly, differences in predominant product types and processing methods, handling practices (e.g. storage temperature) would also be expected to lead to systematic differences in individual and population risks of listeriosis from this product type between nations. The model used does not discretely differentiate risk due to different product types.

Similarly, the model does not discretely predict the consequence of process failures (e.g. slow fermentation leading to growth of *L. monocytogenes* during preparation of the FMPs), except as they are represented in the contamination data used. The data used to model contamination levels, however, are sparse and it is unlikely that even low levels of process failure would be represented.

Also, as noted earlier, contamination level estimates were determined at various stages during the product's shelf-life, yet the model assumes that the levels reflect those initially present at the point of production or distribution to retail markets. The net effect of this assumption would be to somewhat underestimate the risk, because some inactivation would be expected to occur between production and the point of sampling at retail, i.e. the assumed starting levels in the modelling are probably lower than the "true" levels

The effect of this assumption was tested by re-running the model, as described above, after reducing the total storage time to 1 day. The effect was to increase the predicted incidence approximately 400-fold (±577 SD), to 6.47×10^{-4} cases per 100 000 per annum. The inference of this recalculation is that, even though the practical effect of sampling location is large, when a "worst-case" calculation is employed, the estimated relative risk of foodborne listeriosis associated with fermented meat products remains very low in comparison with observed international incidence of listeriosis. Another inference is that the model's outputs depend heavily on the validity of the inactivation model used. As indicated above, if no inactivation is modelled, the estimated risk increases several hundred-fold.

Finally, many of the parameter values of the distributions used do not include estimates of uncertainty in those values. As such, the uncertainty in the result is expected to be higher than implied by the spread from minima to maxima in Table 4.27.

Acknowledgements

The assistance of Meat and Livestock Australia for access to the report of Ross and Shadbolt (2001) and the Australian and New Zealand Food Authority for access to product composition data is gratefully acknowledged.

4.5 EXAMPLE 4: COLD-SMOKED FISH

4.5.1 Statement of purpose

Cold-smoked fish products are often found to be contaminated with *L. monocytogenes*. This has caused concern among regulatory agencies, particularly because vacuum-packed cold-smoked fish has a long shelf-life, is known to support the growth of *L. monocytogenes*, and there is indirect epidemiological evidence associating contaminated smoked fish and human cases of listeriosis. Others argue that despite the recognized hazard, the product has never been definitively linked to human systemic listeriosis.

This assessment aims to estimate the risk to a general consumer of vacuum-packed (VP) cold-smoked fish. It does not differentiate risk on the basis of nationality, consumption, age, gender or health status. Instead, it pools data from many nations to estimate a global risk of listeriosis due to consumption of cold-smoked fish. This assessment forms part of an overall assessment of the risk of listeriosis from foods that do, or do not, support the growth of *L. monocytogenes*.

4.5.2 Hazard identification

L. monocytogenes is frequently isolated from VP cold-smoked salmon and other fish products (see Table 4.28). Salmon is the fish type most commonly used for cold-smoked product, and comprises the majority of all cold-smoked fish production globally. The potential for *L. monocytogenes* to grow in RTE seafood products has been demonstrated by many authors (Hudson and Mott, 1993; Bell, Penny and Moorhead, 1995; Dalgaard and Jørgensen, 1998; Jørgensen and Huss, 1998; Thurette et al., 1998; Tienungoon, 1998).

Loncarevic, Tham and Danielsson-Tham (1998) compared *L. monocytogenes* isolates from human cases in Sweden with those isolated from trout and salmon. They found that three strains were isolated from both fish and human cases, suggesting that those fish products may be a source of infections. Similarly, the isolation of identical subclones of *L. monocytogenes* from both human patients and smoked seafoods in Norway (Rørvik et al., 2000) suggested that such products may have been possible sources for listeriosis cases. Conversely, Boerlin et al. (1997) found no such relationship among 47 human isolates and 72 isolates from fish products in Switzerland, nor Norton et al. (2001) between 275 human clinical isolates and 117 isolates from smoked fish and smoked fish processing plants in the United States of America. Kvenberg (1991) reported that there had been no cases of listeriosis in the United States of America that were linked to the consumption of seafood. Similarly, Bean et al. (1996) indicates that, of an average 500 *outbreaks* of foodborne listeriosis in the United States of America during the period 1988–1991, none were attributable to *L. monocytogenes* in seafoods. Recently, *gravad* trout, a lightly preserved (though not smoked) fish product was linked to a small outbreak of listeriosis (Ericsson et al.,

1997). More recently, Miettinen et al. (1999) reported five cases of febrile gastroenteritis (i.e. not invasive listeriosis) linked to cold-smoked trout.

Table 4.28 Incidence of *Listeria monocytogenes* contamination of smoked fish products.

Location	Product and species	No. of samples	% positive for *L. m.*	Levels	Most common serovars	Ref.
Europe	cold-smoked fish at 10 production sites	~340	overall 34–60[(1)]	*see* Table 4.29		[1]
USA	cold-smoked from plants with known problems	61	78.7			[2]
Norway	smoked salmon	13	33.0			[3]
Cyprus	smoked salmon (at retail)	–	28.6	<20 CFU/g		[4]
Italy	vacuum-packed sliced smoked salmon	100	20.0			[5]
Denmark	preserved fish products (not heated)	335	10.8			[6]
Sweden	gravad fish	58	21.0		1/2, 4	[7]
	cold-smoked fish	26	3.9			
Sweden	hot, cold and gravad					[8]
Canada	hot and cold-smoked fish at retail	258	27.9			[9]
Iceland	smoked salmon	–	29.0	Note (2)		[10]
England and Wales (UK)	smoked mackerel	116	7			[11]
	smoked salmon	86	2			
	other	1	3			
Germany	smoked salmon		7.1			[12]
Japan	smoked salmon	92	5.4		1/2a, b	[13]
USA	smoked finfish	1 210 [(3)]	12.0–16.3	<10 MPN/g		[14]
Australia	Smoked salmon at final product (one plant only)	285	0.4	Presence in 25 g		[15]
Australia	smoked fish and mussel products, retail, Canberra	49	4.1	4 MPN/g, 460 MPN/g		[16]
Australia	smoked fish	9	10.0	presence in 25 g		[17]
Finland	vacuum-packed cold-smoked salmon	30 (12 producers)	17.0	50%: >100/g	1/2a, 4b	[18]

NOTES: (1) Range of frequency of contamination from individual sites: 1.4–100. (2) 46% of generic *Listeria*-positive samples contained *L. monocytogenes.* (3) Over 6 years (1991–1996). (4) MPN = most probable number.

SOURCES: [1] Jorgensen and Huss, 1998. [2] Eklund et al., 1995. [3] Rørvik et al., 1997. [4] Data sumbmitted to FAO in 2000 by Director, State General Laboratory, Ministry of Health, Cyprus. [5] Cortesi et al., 1997. [6] Andersen and Nørrung, 1995. [7] Loncarevic, Tham and Danielsson-Tham, 1996. [8] Lindqvist and Westöö, 2000. [9] Dillon, Patel and Ratnam, 1994. [10] Hartemink and Georgsson, 1991. [11] McLaughlin and Nichols, 1994. [12] Teufel and Bendzulla, 1993. [13] Inoue et al., 2000. [14] Jinneman, Wekell and Eklund, 1999. [15] Garland, 1995. [16] Rockliff and Millard, 1996. [17] Dunn, Son & Stone, 1998. [18] Johansson et al., 1999.

4.5.3 Exposure assessment

4.5.3.1 Production and consumption of smoked fish products

Data were not readily available for total cold-smoked fish production. Instead, data for cold-smoked salmon production were used as a surrogate for total cold-smoked fish. Those data are presented in Appendix 5. In summary, the data suggest that, in the 15 nations considered, the mean consumption amount is approximately 60 g per serving, with average consumption frequency ranging from <1 to 18 servings per person per year. Mean annual consumption across those nations is estimated at about 144 g per person.

4.5.3.2 Contamination rates and levels

Numerous studies have demonstrated that smoked fish products are frequently contaminated with *L. monocytogenes* at rates varying from 0.4 to 78.7%, but more typically in the range 4 to 30%. Table 4.28 summarizes the results of many of those studies.

From the data in Table 4.28, an overall average contamination rate of 18.6% is estimated based on the mean (weighted according to sample size) of all surveys for which both contamination rate and sample size are available. The unweighted mean is 18.0%.

While several publications provide information on the level of contamination of cold-smoked salmon at retail (Teufel and Bendzulla, 1993; McLaughlin and Nichols, 1994; Jørgensen and Huss, 1998; Nørrung, Andersen and Schlundt, 1999; Inoue et al., 2000), this assessment begins at the point of completion of processing, prior to distribution to retailers. Only one data source (Jørgensen and Huss, 1998) provided information on levels of *L. monocytogenes* at the point of completion of processing. Those data are shown in Table 4.29.

Other reports support the general conclusions of Jørgensen and Huss (1998) that, at production, contamination levels are usually less than 10 CFU/g. Dalgaard and Jørgensen (1998) reported that most of the positive samples in their study had an average contamination of ≤ 8 MPN/g, for samples taken 4 to 12 days after production and held at 5°C. They noted, however, that some samples were in the range 10–100 MPN/g. Similarly, Pelroy et al. (1994) reported median levels at production of 0.5 - 11.7 CFU/g. Due to the paucity of data it was assumed for the purposes of this report that the results of Jørgensen and Huss (1998) are representative of all cold-smoked fish at the final point of processing, and those data were used as the initial contamination level in the modelling.

Table 4.29 Contamination levels at end of production for cold-smoked salmon in Denmark.

Point of Testing	Storage time at 5±1°C between initial and final analyses	No. (%) of 25-g samples positive	Contamination levels. No. of positive samples (% of total samples)				Total number of samples
			<10/g	10 – 100/g	100 – 1000/g	>1000/g	
Initial	0	64 (34)	53 (28)	9 (5)	2 (1)	0	190
Final	14–20 days	46 (40)	11(10)	23 (20)	10 (9)	2 (2)	115
Final	21–50 days	32 (43)	17 (23)	11 (15)	2 (3)	2 (3)	75

SOURCE: Data of Jørgensen and Huss, 1998

4.5.3.3 Time and temperature of storage

The nominal shelf lives for vacuum-packed smoked fish are in the range of 3 to 6 weeks at a storage temperature of 4–5°C. Several studies have assessed the *sensory* acceptability of smoked salmon (Truelstrup Hansen, Drewes Røntved and Huss, 1998; Jørgensen, Dalgaard and Huss, 2000; Leroi et al., 2001) and found that that sensory shelf-life at 5°C for cold-smoked salmon is highly variable (from 3 to 9 weeks) and that there is no single indicator for the onset of spoilage.

Storage temperature data were derived from Audits International (2000) data for refrigerated cabinets at retail used for storage of cold-smoked fish. The data is tabulated in Section A5.2 in Appendix 5. In the modelling, storage temperature was described by Beta(4, 7.5, -5, 22) which generated a mean temperature of 4.4°C, and ranged from ~ -4.5 to +19°C (from 32 000 iterations).

4.5.3.4 Physico-chemical parameters of cold-smoked fish

The physico-chemical composition of cold-smoked fish products are assumed to be similar to those for cold-smoked salmon reported in Ross, Dalgaard and Tienungoon (2000) who noted that the postmortem pH of the fish muscle drops to ~ 6.0–6.4 due to the catabolism of muscle glycogen resulting in lactic acid production. At that pH, the muscle contains from 65 to 130 mM lactic acid. Lower pH is correlated to higher lactate concentration, and the lactate present will enhance the inhibitory affect of the reduced pH.

Cold-smoked fish products typically have low salt levels, in the range of 1.5 to 4% (NaCl in the aqueous phase) and would be expected to have water activity in the range 0.977 to 0.99 (Dalgaard, 1997; Leroi et al., 2001).

Leroi et al. (2001) and Thurette et al. (1998) reported a wide variation in phenol levels in cold-smoked salmon (n = 13) produced in several countries and sampled in France. The levels ranged from 2.7 to 10.8 mg phenol/kg fish (i.e. 2.7 to 10.8 ppm), with an average of 5.5 ±1.5 (SD) mg phenol/kg. These levels are consistent with the levels (5 to 10 ppm) reported by Leblanc et al. (2000) and Eklund et al. (1995; 8 to 13 ppm). However, phenol concentration is not included directly in the modelling (see Section A5.3.1 in Appendix 5), but was included indirectly by manipulation of the "Other growth inhibiting factors" input.

4.5.3.5 Growth potential and microbial ecology of vacuum-packed products

Growth Rate Model

As shown in Table 4.29, *L. monocytogenes* can grow on cold-smoked fish products. FDA/FSIS (2001) collated results of published growth rate studies. In this study, a predictive model for growth rate of *L. monocytogenes* as a function of temperature, pH, water activity and lactic acid concentration is used to calculate growth rate. That model and its basis are discussed in Section A5.3 in Appendix 5. To assess its utility for cold-smoked salmon, the model's predictions for growth

Table 4.30 Comparison of model predictions of *Listeria monocytogenes* growth rate at 5°C on cold-smoked fish and those collated from published literature

	Growth rate estimate (logCFU/day)	
	Predictive model	FDA/FSIS (2001) literature collation
Mean	0.113	0.155
SD	0.055	0.100

rate at 5°C were compared with the growth rates collated by FDA/FSIS (2001). The results in Table 4.30 indicate that the two approaches produce consistent, though not identical, results at 5°C.

The effects of product parameters on the *potential* for growth was also considered, both on the basis of individual factor limits (see Table 3.1) and using the growth/no-growth model of Tienungoon et al. (2000) because in some iterations of the Monte Carlo simulation model the factor combinations sampled may, in fact, preclude growth. The implementation of this is described in Section A5.3 (in Appendix 5).

Effect of lag time and lactic acid bacteria on growth potential

Lag times and the effect of growth of lactic acid bacteria on the shelf-life of the product and the growth potential of *L. monocytogenes* in vacuum-packed cold-smoked fish were also considered explicitly and implemented in the modelling. Maximum population densities were also modelled. The importance of these factors, and their implementation in the modelling, is described in Section A5.3 (in Appendix 5).

4.5.3.6 Exposure assessment model

The overall structure of the exposure assessment model is shown in Figure 4.6. Each box in the diagram represents a module or sub-model. The structure of these sub-models is described below.

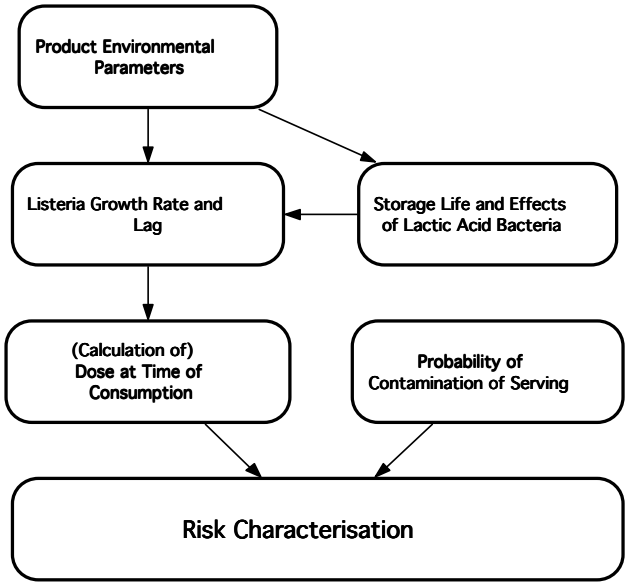

Figure 4.6 Exposure assessment model used shown as an influence diagram. The structure of each of the sub-models is described in the text.

Product environmental parameters

Product environmental parameters (water activity, pH and lactic acid) were modelled as described in Section A5.3 (in Appendix 5) Other components of the product, though not explicitly modelled (e.g. phenol, spices), were considered to reduce growth rate by 10% from that predicted on the basis of product parameters and temperature alone (see below). Storage temperature was modelled as described in Section A5.2 (in Appendix 5)

Listeria Growth Rate and Lag

The growth rate module is fully described in Section A5.3 (in Appendix 5). In summary, growth rate is predicted from storage temperature and product composition values sampled during each iteration. The conditions are first evaluated to determine whether the combinations of pH, temperature and water activity would permit growth, using the model of Tienungoon et al. (2000), or whether any individual parameter value is beyond the range that permits growth of *L. monocytogenes* (see Table 3.1). If growth is predicted to be possible, two growth rates are estimated, i.e. growth before lactic acid bacteria reach their stationary phase and, if appropriate, subsequent growth, but before the product is predicted to spoil at the sampled temperature. The time at which lactic acid bacteria reach stationary phase is predicted from the values in Product Environmental Parameters, as described below.

Storage life and effects of lactic acid bacteria

A nominal storage life of the product at 5°C is specified as described in Section 4.5.3.4. Nominal shelf-life is adjusted for other temperature scenarios using a square root type relative rate function as described in Appendices 2 and 3. Details are given in Section A5.3 in Appendix 5.

In each iteration of the model, the total possible growth of *L. monocytogenes* is calculated from the growth rate and storage time, and, after deducting the contribution of the lag time, is expressed as potential number of generations of growth.

Calculation of dose at the time of consumption

The dose at the time of consumption is calculated from the initial contamination level distribution (described in Section 4.5.3.2), to which is added the predicted growth as described above. The predicted concentration of *L. monocytogenes* in the serving is then compared with the maximum concentration level. If the predicted concentration exceeds the maximum concentration level ($10^{9.5}$ CFU/g) it is changed to the maximum concentration, otherwise the original modelled contamination level is used. That concentration level is combined with the serving size estimate (see Appendix 5 for details of modelling, and Section 4.5.3.1 for summary) to estimate the dose ingested by the consumer from that scenario. These interrelationships are depicted as an influence diagram in Figure 4.7.

Probability of contamination of serving

The probability of consuming a contaminated serving is derived from data presented in Table 4.28 and described empirically in the model as Beta(2, 6.6, 0.004, 0.787), which models a mean contamination rate of 18.6%, a minimum contamination rate of 0.46% and a maximum rate of 67.8%.

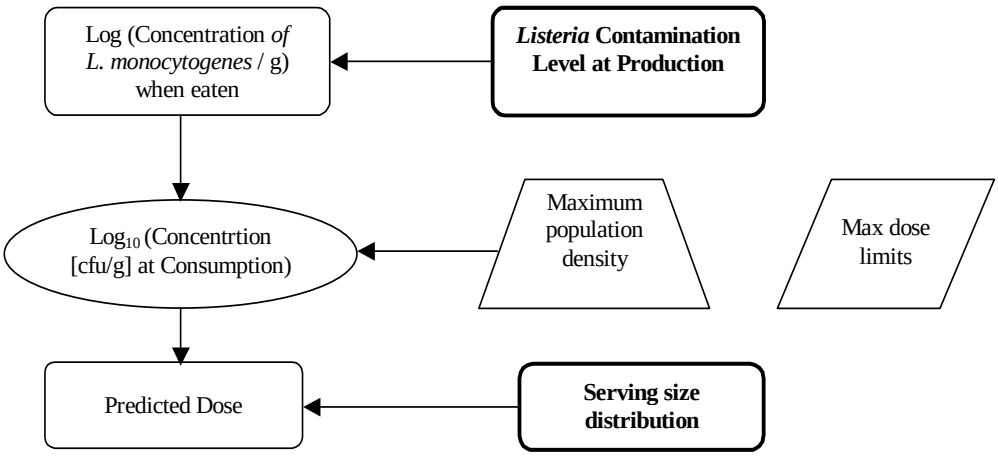

Figure 4.7 Influence diagram showing calculation of *Listeria monocytogenes* dose per contaminated serving of cold-smoked fish.

4.5.4 Risk characterization

4.5.4.1 Introduction

The risk characterization combines the dose-response model described previously (Section 4.1) with the exposure assessment model described above. Outputs of the model are as described in Section 4.1. Figure 4.8 depicts the risk characterization model as an influence diagram.

4.5.4.2 Assumed variables

The model was executed 16 times with 32 000 iterations per execution to generate a set of "baseline" values. Those values are based on a number of assumptions including that:

- the storage life of the product ranges from 1 to 42 days, with a most likely storage time of 28 days;
- the maximum population density of *L. monocytogenes* on the product is 3×10^9 CFU/g;
- the lag time ranges between the equivalent of 0 and 35 generation times, with a most likely lag time equivalent to three generation times at the storage temperature sampled and for the product parameters sampled; and
- when the lactic acid bacteria reach stationary phase, they will reduce the growth rate of *L. monocytogenes* by 85–100% (described in the model as the growth rate predicted as described above, multiplied by Uniform(0.00, 0.15)).

Consumption pattern is described in Section A5.1 in Appendix 5.

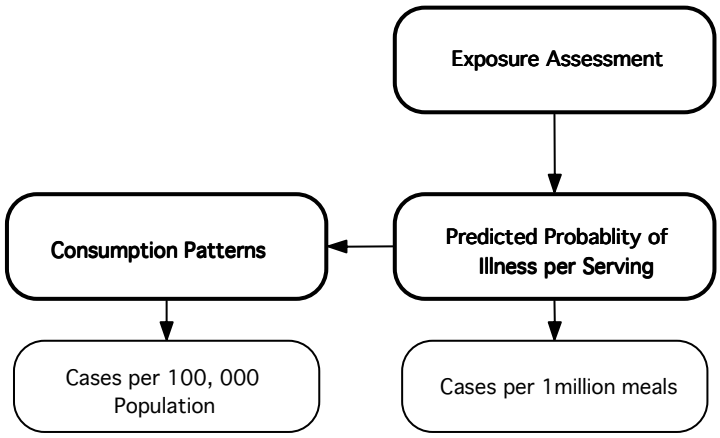

Figure 4.8 Influence diagram depicting the interrelationship of factors governing the risk estimates.

4.5.4.3 Results

The results, based on the assumptions considered above, are presented in Table 4.31. The results are the mean values of the mean and maximum values of the distribution of risk estimates for each of 16 runs of the Monte Carlo model. Maximum values are included to give some indication of the range of risk estimated. In both sets of estimates of the two measures of risk, the minimum value estimates were of the order of 10^{-11} to 10^{-12}.

Table 4.31a Cases per 100 000 consumers per year in cold-smoked fish.

Mean of Means	0.0163
SD of Means	0.0117
Mean of Maxima	186
SD of Maxima	253

Table 4.31b Cases per 1 000 000 meals in cold-smoked fish.

Mean of Means	0.0530
SD of Means	0.0355
Mean of Maxima	555
SD of Maxima	745

4.5.5 Uncertainty and variability

Uncertainty and variability concerning the dose-response component of the assessment are as discussed in the other three case studies.

4.5.5.1 Consumption

There are a number of uncertain variables specific to the exposure assessment component of this model, including the true level of consumption of cold-smoked fish, and factors affecting the level of contamination with *L. monocytogenes*.

From the data presented in Section A5.1 in Appendix 5, consumption was suggested to vary widely between nations, but it was also apparent that different estimates would arise

from different data sources. It was also shown that the model possibly overpredicted consumption by 50%. This inaccuracy is expected to contribute a relatively small error.

4.5.5.2 Effect of other microbiota

The effect of other microbiota (e.g. lactic acid bacteria) in vacuum-packed, cold-smoked fish is well known, but the magnitude of the effect could not be quantified with certainty. In the modelling presented, it was *assumed* that growth rate of *L. monocytogenes* was inhibited by between 85% and 100%, but there are no data to evaluate the validity of this assumed level of inhibition. To assess the significance of the assumption, the model was re-run ($16 \times 32\,000$ iterations) with four assumptions concerning the magnitude of growth rate inhibition due to the Jameson effect and one with no inhibition. The assumptions were:

- total inhibition of *L. monocytogenes* growth rate;
- 95% inhibition of *L. monocytogenes* growth rate;
- between 80% and 100% inhibition of *L. monocytogenes* growth rate;
- 70% inhibition of *L. monocytogenes* growth rate; and
- no inhibition of *L. monocytogenes* growth rate.

The results (means of mean values of 16 simulated distributions) are compared in Table 4.32, and indicate that the differences can be profound compared with the situation where no inhibition is modelled, indicating the importance of this aspect of the microbial ecology of VP RTE foods for the estimation of the risk of listeriosis. When inhibition was modelled, the differences in the risk estimates were about 2- to 5-fold. Further experimentation with the model, however, suggested that very large increases in risk occurred if inhibition less than 80% were assumed. For example, assuming that growth rate was reduced to 30% (i.e. 70% inhibition) resulted in risk estimates that were thousands of times higher than when 95% inhibition of growth rate was assumed.

Table 4.32 Effect of assumptions concerning the effect of growth rate inhibition of *Listeria monocytogenes* due to high levels of lactic acid bacteria on estimates of the risk of listeriosis in smoked fish.

	Assumed magnitude of *L. monocytogenes* growth rate inhibition				
	No Inhibition	Fixed at 70%	Variable (80–100%)	Fixed at 95%	Complete 100%
Cases per 100 000 popn.	366	5.65	0.010	0.004	0.002
Cases per million servings	1136	–	0.033	0.011	0.005

4.5.5.3 Reality check

There have been a handful of cases of listeriosis reported in the last decade that possibly have been related to cold-smoked fish. Production volumes of cold-smoked fish in the late 1990s were around 80 000 tonne/year. If it is assumed that this production level is representative of the last ten years, then those cases are due to ~800 000 tonnes, or 1.33×10^{10} servings (60 g each). Assuming a factor of 10 for cases of listeriosis due to smoked fish products that are not recognized, or not reported, one case would equate to approximately 0.008 cases per million meals. That estimate is closer to the low end of the estimates derived from the

simulation model in this assessment (~90% inhibition) and might suggest that the more stringent inhibition assumptions concerning the inhibition of growth of *L. monocytogenes* by other organisms present in VP cold-smoked fish products are more consistent with actual experience.

Varying the upper population limit (maximum population density – MPD) had no effect on the risk estimate, indicating that in virtually all cases other factors described in the model controlled growth of *L. monocytogenes* to the extent that it never reached MPD.

Not all parameters in the model included estimates of variability, e.g. variability in growth rates. Equally, the conversion of per-meal risk to risk per 100 000 is based on multiplying the mean of the per-serving risk by population estimates. While the mean risk estimate is unaffected, the model does not accurately portray the extent of variability in the estimates. As these risk assessment are all characterized by mean values of population or per-meal risk, the risk estimates are not affected. Note, however, that the standard deviations quoted in Table 4.31 reflect the variability in the simulation modelling procedure, i.e. between-run variation, not the variability in system being modelled.

Finally, because of the combination and pooling of data from many diverse sources, the risk estimates are not nation-specific, and so may not accurately represent the situation in any nation.

Table 4.33 Parameter values for Triangular distributions used for storage time scenarios tested for their effect on risk estimates.

Description	Days before consumption			Mean value of the distribution (days)
	Minimum	Most Likely	Maximum	
"Reduced"	1	14	21	12
"Good"	1	21	28	14
"Realistic"	1	28	42	24
"Product Abuse"	1	28	180	70

4.6 SUMMARY

The risk characterizations for all four examples are summarized in Table 4.34.

Table 4.34 Estimated risks of listeriosis per 100 000 population and per million servings for the four selected foods.

Food	Cases of listeriosis per 100 000 consumers	Cases of listeriosis per 1 million servings
Milk	0.091	0.005
Ice Cream	0.00012	0.000014
Cold-Smoked Fish	0.016	0.053
Fermented Meat Products	0.0000055	0.0000021