

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 homogeneously 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 Westö, 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.
↓		<i>volumetric changes:</i> mixing with other ingredients, changes due to dilution or concentration steps (e.g. evaporation, removal of whey)	cross-contamination, mixing with other bulk ingredients, splitting into smaller units for retail or food service
Processing		<i>growth or inactivation changes</i> brining, heating steps, holding times and temperatures,	
↓		time, temperature, product composition	
Transport and Storage		time, temperature, product composition, breakdown to smaller units	packaging and cross-contamination, portioning, breakdown to smaller units
↓		time, temperature, product composition	cross-contamination, combination with other foods
Retail Sale		heating; mixing with other components (e.g. vinegar in salads); breakdown to smaller units	breakdown to smaller units/serving portions
↓	frequency and amount consumed affected by: season, wealth, age, sex, culture/region, etc.		
Home/food service			
↓			
Consumption			

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.

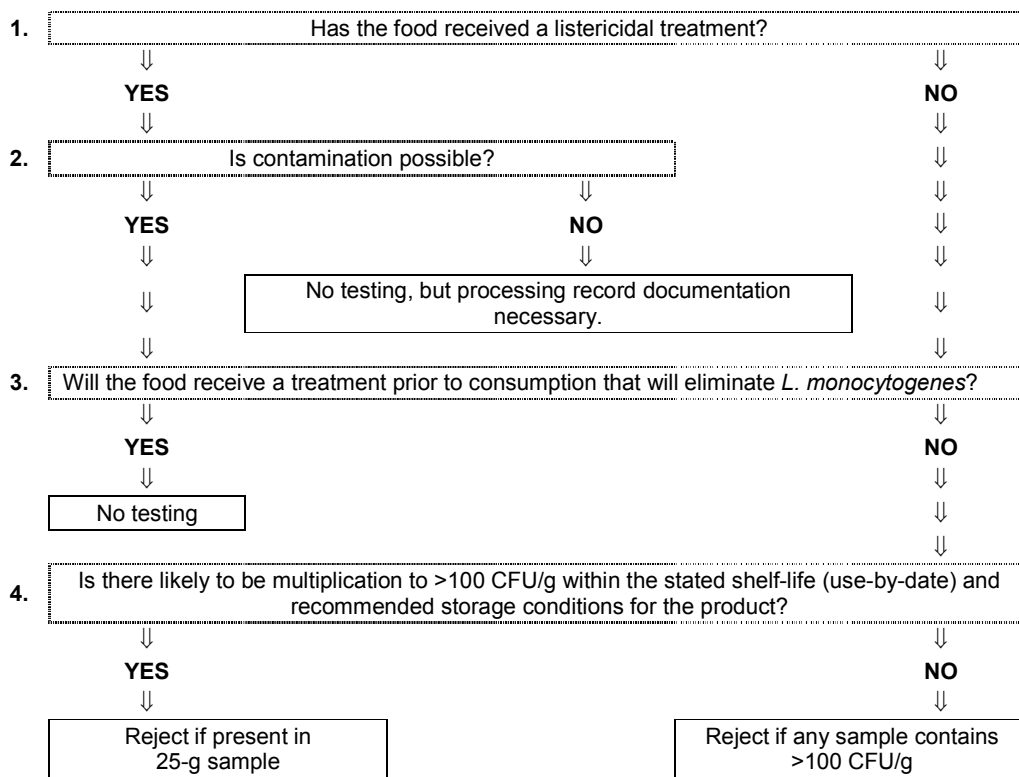


Figure 3.2 A decision tree to aid the management of the hazard of listeriosis from foods showing qualitative risk assessment decisions within a risk management scheme.

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).

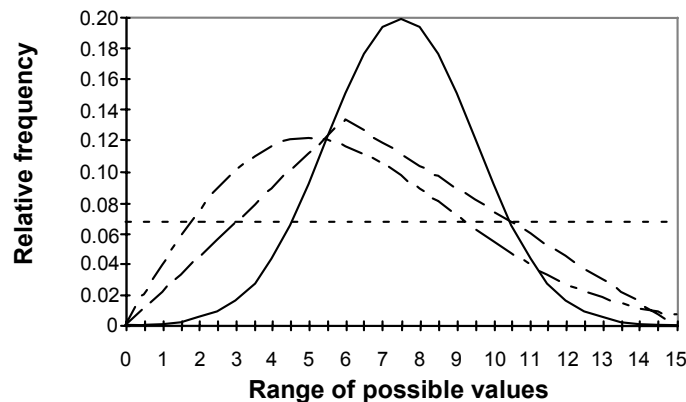


Figure 3.3 Some types of distributions used to describe ranges of values for observations.
Normal (————); triangular (— — — —); uniform (- - - -); Beta-Pert (— · · · · ·).

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) (and corresponding a_w)	<0.5 (0.91–0.93)	13 – 16 (> 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 μ M (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 in 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 yield¹ (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 (Ratkowsky 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 Program²) 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 CO₂ 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.

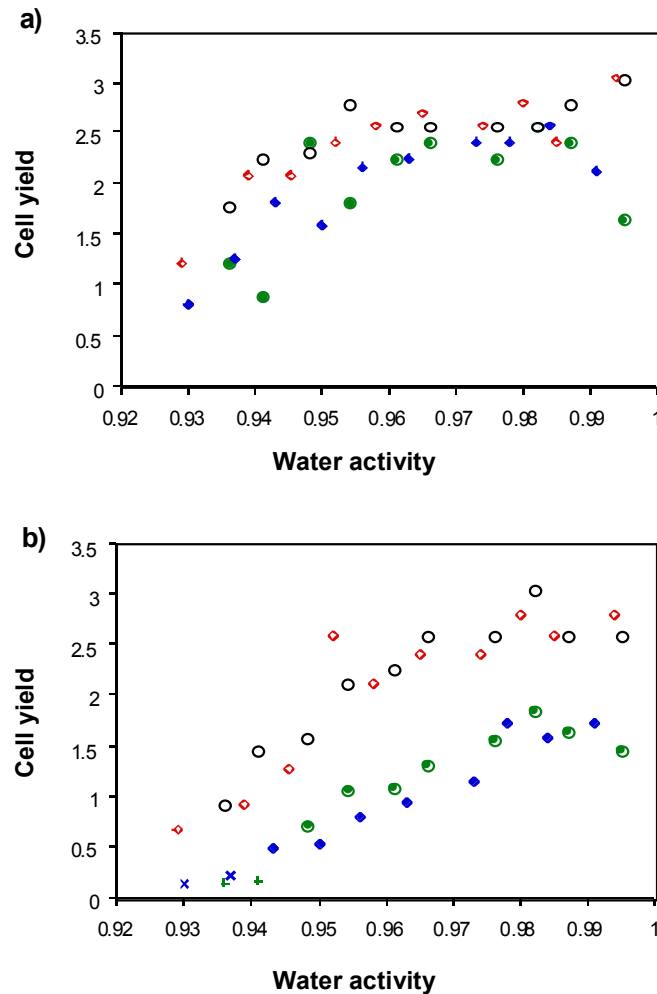


Figure 3.4 The observed cell yield of *Listeria monocytogenes* “corrected” for the non-linearity of the Optical Density (OD)-concentration relationship using the function of Dalgaard et al. (1994) and plotted against water activity (NaCl as humectant), demonstrating the influence of lactic acid, and 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”.

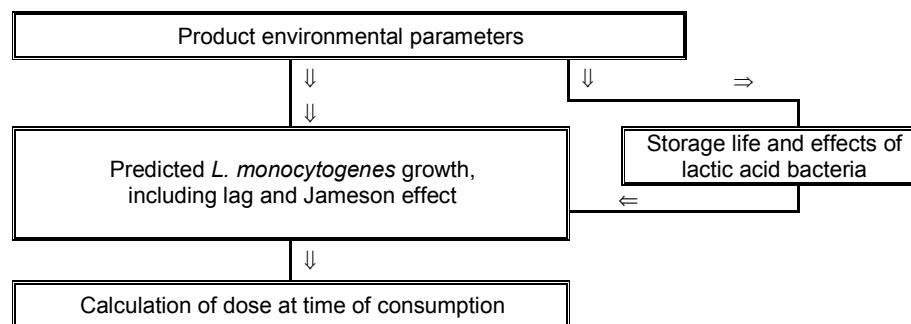


Figure 3.5 Overall model structure for the conceptual model and influence diagram for the interaction of factors governing the extent of growth of *Listeria monocytogenes* in ready-to-eat foods. Each of the boxes represents a “module” of calculations. Details of the predictive growth module are shown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

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.

