MICROBIOLOGICAL RISK ASSESSMENT SERIES

5

Risk assessment of *Listeria monocytogenes* in ready-to-eat foods

TECHNICAL REPORT

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FOREWORD

The Members of the Food and Agriculture Organization of the United Nations (FAO) and of the World Health Organization (WHO) have expressed concern regarding the level of safety of food both at national and international levels. Increasing foodborne disease incidence over the last decades seems, in many countries, to be related to an increase in disease caused by microorganisms in food. This concern has been voiced in meetings of the Governing Bodies of both Organizations and in the Codex Alimentarius Commission. It is not easy to decide whether the suggested increase is real or an artefact of changes in other areas, such as improved disease surveillance or better detection methods for microorganisms in foods. However, the important issue is whether new tools or revised and improved actions can contribute to our ability to lower the disease burden and provide safer food. Fortunately new tools, which can facilitate actions, seem to be on their way.

Over the past decade, Risk Analysis – a process consisting of risk assessment, risk management and risk communication – has emerged as a structured model for improving our food control systems with the objectives of producing safer food, reducing the numbers of foodborne illnesses and facilitating domestic and international trade in food. Furthermore, we are moving towards a more holistic approach to food safety, where the entire food chain needs to be considered in efforts to produce safer food.

As with any model, tools are needed for the implementation of the risk analysis paradigm. Risk assessment is the science-based component of risk analysis. Science today provides us with in-depth information on life in the world we live in. It has allowed us to accumulate a wealth of knowledge on microscopic organisms, their growth, survival and death, even their genetic make-up. It has given us an understanding of food production, processing and preservation, and of the link between the microscopic and the macroscopic world and how we can benefit from as well as suffer from these microorganisms. Risk assessment provides us with a framework for organizing all this data and information and to better understand the interaction between microorganisms, foods and human illness. It provides us with the ability to estimate the risk to human health from specific microorganisms in foods and gives us a tool with which we can compare and evaluate different scenarios, as well as to identify the types of data is necessary for estimating and optimizing mitigating interventions.

Microbiological risk assessment can be considered as a tool that can be used in the management of the risks posed by foodborne pathogens and in the elaboration of standards for food in international trade. However, undertaking a microbiological risk assessment (MRA), particularly quantitative MRA, is recognized as a resource-intensive task requiring a multidisciplinary approach. Yet foodborne illness is among the most widespread public health problems, creating social and economic burdens as well as human suffering, making it a concern that all countries need to address. As risk assessment can also be used to justify the introduction of more stringent standards for imported foods, a knowledge of MRA is important for trade purposes, and there is a need to provide countries with the tools for understanding and, if possible, undertaking MRA. This need, combined with that of the Codex Alimentarius for risk-based scientific advice, led FAO and WHO to undertake a programme of activities on MRA at the international level.

The Food Quality and Standards Service, FAO, and the Food Safety Department, WHO, are the lead units responsible for this initiative. The two groups have worked together to develop the area of MRA at the international level for application at both the national and international levels. This work has been greatly facilitated by the contribution of people from around the world with expertise in microbiology, mathematical modelling, epidemiology and food technology to name but a few.

This Microbiological Risk Assessment series provides a range of data and information to those who need to understand or undertake MRA. It comprises risk assessments of particular pathogen-commodity combinations, interpretative summaries of the risk assessments, guidelines for undertaking and using risk assessment, and reports addressing other pertinent aspects of MRA.

We hope that this series will provide a greater insight into MRA, how it is undertaken and how it can be used. We strongly believe that this is an area that should be developed in the international sphere, and have already from the present work clear indications that an international approach and early agreement in this area will strengthen the future potential for use of this tool in all parts of the world, as well as in international standard setting. We would welcome comments and feedback on any of the documents within this series so that we can endeavour to provide Member countries, Codex Alimentarius and other users of this material with the information they need to use risk-based tools, with the ultimate objective of ensuring that safe food is available for all consumers.

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ABBREVIATIONS USED IN THE TEXT

AIDS	Acquired Immunodeficiency Syndrome
a_w	Water activity
BHI	Brain-heart infusion
CCFH	Codex Committee on Food Hygiene
CDC	Centers for Disease Control and Prevention (USA)
CFU	Colony-forming units
CNS	Central nervous system
CSFII	Continuing Survey of Food Intakes by Individuals (USA)
EGR	Exponential growth rate
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration (USA)
FSIS	Food Safety and Inspection Service [USDA]
ID_{50}	Dose of an infectious organism required to produce infection in 50 percent of the experimental subjects or exposed population.
HIV	Human Immunodeficiency Virus
IV	Intravenous
LD_{50}	The amount of an infectious organism or toxic agent that is sufficient to kill 50 percent of the exposed population within a certain time.
LLO	Listeriolysin O
LMRA	*Listeria monocytogenes* Risk Assessment [FDA/FSIS]
MPD	Maximum population density
MPN	Most probable number
MRA	Microbiological risk assessment
MSE	Mean square error
NaCl	Sodium chloride
NHANES	National Health and Nutrition Examination Survey (USA)
RLT	Relative lag time
RTE	Ready-to-eat
USDA	United States Department of Agriculture
WHO	World Health Organization
WPS	Water phase salt

Executive Summary

This risk assessment on *Listeria monocytogenes* in ready-to-eat (RTE) foods was undertaken to (i) respond to the request of the Codex Committee on Food Hygiene (CCFH) for sound scientific advice as a basis for the development of guidelines for the control of *L. monocytogenes* in foods; and (ii) address the needs expressed by Member countries for adaptable risk assessments that they can use to support risk management decisions and to conduct their own assessments.

The risk assessment was tailored to address three specific questions posed by the 33rd session of the CCFH (CAC, 2000) namely:

1. Estimate the risk of serious illness from *L. monocytogenes* in food when the number of organisms ranges from absence in 25 grams to 1000 colony forming units (CFU) per gram or millilitre, or does not exceed specified levels at the point of consumption.
2. Estimate the risk of serious illness for consumers in different susceptible population groups (elderly, infants, pregnant women and immunocompromised patients) relative to the general population.
3. Estimate the risk of serious illness from *L. monocytogenes* in foods that support its growth and foods that do not support its growth at specific storage and shelf-life conditions.

By answering these questions, this risk assessment aims to assist risk managers in conceptualizing how some of the factors governing foodborne listeriosis interact, thereby assisting the development of strategies to reduce the rates of illness.

The risk assessment comprises the four steps of hazard identification, hazard characterization, exposure assessment and risk characterization. A quantitative approach was taken and mathematical modelling employed to estimate the risks per serving and risk to a population in a year from the selected foods. The risk assessment focused on four RTE foods in order to provide examples of how microbiological risk assessment techniques can be used to answer food safety questions at an international level. The study was limited to foods at retail and their subsequent public health impact at the time of consumption. The impact of post-retail factors that could influence the risk to a consumer, such as temperature and duration of refrigerated storage, was also examined. This was considered sufficient to address the questions posed by the CCFH within the time frame and resources available to the risk assessors, and also reflects the situation that most of the currently available exposure data for *L. monocytogenes* relate to the frequency and extent of contamination at the retail level.

HAZARD IDENTIFICATION

Foodborne listeriosis is a relatively rare but serious disease with high fatality rates (20–30%) compared with other foodborne microbial pathogens, such as *Salmonella*. The disease largely affects specific segments of the population who have increased susceptibilities. Basically, *L. monocytogenes* is an opportunistic pathogen that most often affects those with a severe underlying disease or condition (e.g. immunosuppression, HIV/AIDS, chronic conditions such as cirrhosis that impair the immune system); pregnant women; unborn or newly

delivered infants; and the elderly. *L. monocytogenes* is widely dispersed in the environment and foods. However, it was not until several large, common-source outbreaks of listeriosis occurred in North America and Europe during the 1980s that the significance of foods as the primary route of transmission for human exposure to *L. monocytogenes* was recognized (Broome, Gellin and Schwartz, 1990; Bille, 1990). An important factor in foodborne listeriosis is that the pathogen can grow to significant numbers at refrigeration temperatures when given sufficient time. Despite the fact that a wide variety of foods may be contaminated with *L. monocytogenes*, outbreaks and sporadic cases of listeriosis are predominately associated with RTE foods – a large, heterogeneous category of foodstuffs that can be subdivided in many different ways and vary from country to country according to local eating habits; availability and integrity of the chill chain; and regulations specifying, for example, the maximum temperature at retail level. Although listeriosis is a relatively rare disease, the severity of the disease and the very frequent involvement of industrially processed foods, especially during outbreaks, mean that the social and economic impact of listeriosis is among the highest of the foodborne diseases (Roberts, 1989; Roberts and Pinner, 1990). Listeriosis is mainly observed in industrialized countries and it is not known whether the differences in incidence rates between developed and developing countries reflect true geographical differences, differences in food habits and food storage, or differences in diagnosis and reporting practices.

HAZARD CHARACTERIZATION

The hazard characterization provides a description of the pathogen and host characteristics that contribute to an infection by *Listeria*, the public health outcomes of infection with this pathogen, the foods most commonly associated with listeriosis, and a description of the dose-response relationship. Various clinical manifestations are associated with listeriosis and these can be grouped in two categories: invasive listeriosis and non-invasive listeriosis. Invasive listeriosis are cases when initial infections of the intestinal tissue by *L. monocytogenes* leads to invasion of otherwise sterile body sites, such as the pregnant uterus, the central nervous system, or the blood, or combinations. Invasive listeriosis is characterized by a high case-fatality rate, ranging from 20 to 30% (Mead et al., 1999) and sequelae may follow listeriosis infections (McLauchlin, 1997), though their incidence is rarely estimated (Rocourt, 1996). Non-invasive listeriosis (referred to as febrile listerial gastroenteritis) has been observed during a number of outbreaks where the majority of cases developed symptoms of gastroenteritis, such as diarrhoea, fever, headache and myalgia, after a short period of incubation (Dalton et al., 1997; Salamina et al., 1996; Riedo et al., 1994; Aureli et al., 2000). These outbreaks have generally involved the ingestion of high doses of *L. monocytogenes* by otherwise healthy individuals. The incidence rate and factors that govern the onset of this non-invasive form are not known. As a result, this risk assessment only considered invasive listeriosis as the outcome of exposure.

Dose-response data from human volunteer studies with *L. monocytogenes* or from volunteer studies with a surrogate pathogen do not exist. Therefore dose-response relations have been developed and evaluated based on expert elicitations, epidemiological or animal data, or combinations of these. These dose-response relations, which were reviewed and summarized in this work, cover the spectrum of biological end-points, i.e. infection, morbidity and mortality, and have, to varying degrees of sophistication, been evaluated using human epidemiological data. All models assume that each microbial cell acts independently, and that a single bacterial cell has the potential to cause disease. However, none of the

available models were fully able to meet the needs of the current risk assessment in relation to the parameters examined and simplicity of calculation. For these reasons, alternative approaches were developed and evaluated for this risk assessment.

The approach used took advantage of the epidemiological data and detailed exposure assessment available in the *Listeria* risk assessment developed in the United States of America (FDA/FSIS, 2001). The model contains one parameter, r, which is the probability that a single cell will cause invasive listeriosis. This parameter was estimated from the pairing of population consumption patterns (exposure) with epidemiological data on the number of invasive listeriosis cases in the population. The estimated r-value, which will vary with the data sets used and the assumptions made, was then used in the exponential model to estimate specific risks given the number of *L. monocytogenes* consumed.

EXPOSURE ASSESSMENT

A full farm-to-fork risk assessment was not required to address the questions posed by the CCFH. Thus, the focus of the exposure assessment models was to account for changes in the frequency and extent of contamination in the food between retail marketing and the point of consumption. This simplified the modelling and reduced the model uncertainties, thereby decreasing the ranges around the final risk estimates. The models developed describe the growth or decline of *L. monocytogenes* between the time of purchase and consumption, using information and models for the growth rate and the lag time of *L. monocytogenes* as affected by storage temperature and food composition, the maximum growth of *L. monocytogenes* supported by the food, and the distribution of retail and home storage times and temperatures. Calculating the numbers of *L. monocytogenes* actually consumed also required consideration of how much of and how often the food is eaten (i.e. the size and the number of servings).

RTE foods are a broad and diverse food category, prepared and stored in different ways and under different conditions, some of which support growth of *L. monocytogenes* and others that do not support growth at specific storage and shelf-life conditions. As it was therefore not possible to consider all RTE foods, four foods – pasteurized milk, ice cream, fermented meat and cold smoked fish – were selected to illustrate how the different factors mentioned above interact to affect the risk of acquiring listeriosis. Pasteurized milk is a food that is widely consumed, has very low frequencies and levels of contamination with *L. monocytogenes* but allows growth of the organism during storage. Ice cream is similar to milk but does not permit growth of *L. monocytogenes* during storage. Fermented meat products are often contaminated with *Listeria* and are produced without any lethal processing step, but their final composition prevents growth of the microbe during storage. Cold-smoked fish is frequently contaminated with *L. monocytogenes*, has no lethal processing step and permits growth during an extended storage period.

Several “what-if” scenarios were also considered in the case of milk and smoked salmon. These hypothetical scenarios have specific changes made to one or more of the exposure factors to demonstrate how the factors interact to affect the risk. In conducting the exposure assessments for these four foods, different databases were available and the modellers used slightly different techniques. These techniques are explained in the main risk assessment document and illustrate that there are numerous approaches that may be taken depending on the available data and the judgment of the risk assessors.

The outputs from the exposure assessment included a distribution of *L. monocytogenes* in the food at the point of consumption (frequency of contamination) and also the amount consumed (number of servings per year and size of servings).

RISK CHARACTERIZATION

The outputs from the exposure assessment were fed into the dose-response model to develop the risk characterization portion of the risk assessment to calculate the probability of contracting listeriosis. The outputs are described in terms of estimates of risk per million servings for the healthy and susceptible populations. The risk per serving and number of servings were used to estimate the number of illnesses in a specified population per year.

The mean risk estimates of the number of illnesses per 10 million people per year and the risk per serving for pasteurized milk, ice cream, fermented meats and smoked fish are shown in Table 1. For milk, for example, the risk per serving was low (5.0×10^{-9} cases per serving), but the very high frequency of consumption resulted in milk making substantial contributions to the total number of predicted cases of illness. In contrast, for smoked fish the risk per serving was estimated to be high (2.1×10^{-8} cases per serving). However, consumption of this product is modest (1 to 18 servings per year), and consequently the total number of cases of listeriosis was moderate.

Table 1

The mean risk estimates of the number of illnesses per 10 million people per year and the risk per serving for four ready-to-eat foods.

Food	Cases of listeriosis per 10 million people per year	Cases of listeriosis per 1 million servings
Milk	9.1	0.005
Ice cream	0.012	0.000014
Smoked fish	0.46	0.021
Fermented meats	0.00066	0.0000025

RESPONSE TO QUESTIONS POSED BY THE CCFH

These risk assessments were used to address the specific questions posed by the 33rd session of the CCFH. The replies to these questions are summarized below.

Question 1: *Estimate the risk of serious illness from* L. monocytogenes *in food when the number of organisms range from absence in 25 g to 1000 colony forming units (CFU) per gram or millilitre, or does not exceed specified levels at the point of consumption.*

Two approaches were taken: (i) the predicted risk per serving and predicted number of cases of listeriosis annually were estimated for a "worst-case" scenario by assuming that all servings had the maximum level being considered (0.04, 0.1, 1, 10, 100 and 1000 CFU/g); (ii) a more realistic, but also more complex, approach was to use a distribution of the levels of *L. monocytogenes* in foods when consumed rather than an absolute value to estimate the risk per serving and the predicted number of cases of listeriosis annually.

Comparisons between these two approaches indicated that there were vast differences in the estimated number of cases when one considers the worst-case scenario as opposed to a

scenario that attempts to also consider the frequency and extent of contamination actually encountered in RTE foods. These two scenarios demonstrated that as either the frequency of contamination or the level of contamination increases, the risk and the predicted number of cases also increase. These scenarios assume that ingestion of a single cell has the possibility to cause illness. Thus, if all RTE foods went from having 1 CFU/serving to 1000 CFU/serving, the risk of listeriosis would increase 1000-fold (assuming a fixed serving size). Conversely, the effect of introducing into the food supply 10 000 servings contaminated with *L. monocytogenes* at a level of 1000 CFU/g would, in theory, be compensated by removing from the food supply a single serving contaminated at a level of 10^7 CFU/g.

In interpreting these results and the actual effect of a change in the regulatory limits for *L. monocytogenes* in RTE foods, one also has to take into account the extent to which non-compliance with established limits occurs. Based on data available for the United States of America, where the current limit for *L. monocytogenes* in RTE foods is 0.04 CFU/g, the estimated number of cases for listeriosis for that population was 2130 (baseline level used in the United States *Listeria* risk assessment). If a level of 0.04 CFU/g was consistently achieved, one could expect less than 1 case of listeriosis per year. This, in combination with available exposure data, suggests that a portion of RTE food contains a substantially greater number of the pathogen than the current limit and that the public health impact of *L. monocytogenes* is almost exclusively a function of the foods that greatly exceed the current limit. Therefore it could be asked if a less stringent microbiological limit for RTE foods could be beneficial in terms of public health if it simultaneously fostered the adoption of control measures that resulted in a substantial decrease in the number of servings that greatly exceeded the established limit.

To examine this concept further, a simple "what-if" scenario was developed describing the impact on public health of the level of compliance to a microbiological limit. Two often discussed limits, 0.04 CFU/g and 100 CFU/g, were examined in conjunction with different "defect rates" (a defect rate is the percentage of servings that exceed the specified limit). To simplify the model, a single level of *L. monocytogenes* contamination, 10^6 CFU/g, was assumed for all "defective" servings. This assumption focuses the scenario on the group of defective servings that is responsible for the majority of listeriosis cases. Data demonstrate that at 100% compliance, the number of predicted cases is low for both limits, with an approximate 10-fold difference between them, that is 0.5 cases versus 5.7 cases. As expected the number of cases increases with an increasing frequency of defective servings. However, it is possible that public health could be improved if an increase in the regulatory limit in RTE foods resulted in a substantial decrease in the number of servings that greatly exceeded the established limit, i.e. if the rate of compliance increased.

To summarize, the risk assessment demonstrates that the vast majority of cases of listeriosis result from the consumption of high numbers of *Listeria*, and foods where the level of the pathogen does not meet the current criteria, whatever they may be (0.04 or 100 CFU/g). The model also predicts that the consumption of low numbers of *L. monocytogenes* has a low probability of causing illness. Eliminating higher levels of *L. monocytogenes* at the time of consumption has a large impact on the number of predicted cases of illness.

Question 2: Estimate the risk of serious illness for consumers in different susceptible population groups (elderly, infants, pregnant women and immunocompromised patients) relative to the general population.

These results showed that the probability of becoming ill from ingesting *L. monocytogenes* was higher for susceptible populations (immunocompromised; elderly; and perinatal) than the general population. The probability of becoming ill was also shown to vary between the sub-groups of the susceptible population. Based on susceptibility information available from the United States of America, it was determined that the elderly (60 years and older) were 2.6 times more susceptible relative to the general healthy population, while perinatals were 14 times more susceptible. Conditions that compromise the immune system also affect susceptibility to varying extents (Table 2). These results are consistent with the physiological observation that, as an individual's immune system is increasingly compromised, the risk of listeriosis at any given dose increases.

Table 2 Relative susceptibilities for different sub-populations based on French epidemiological data.

Condition	Relative susceptibility
Transplant	2584
Cancer-Blood	1364
AIDS	865
Dialysis	476
Cancer-Pulmonary	229
Cancer-Gastrointestinal and liver	211
Non-cancer liver disease	143
Cancer-Bladder and prostate	112
Cancer-Gynaecological	66
Diabetes, insulin dependent	30
Diabetes, non-insulin dependent	25
Alcoholism	18
Over 65 years old	7.5
Less than 65 years, no other condition	1

Question 3: Estimate the risk of serious illness from L. monocytogenes *in foods that support its growth and foods that do not support its growth at specific storage and shelf-life conditions.*

The risk assessment provides three approaches for answering the question: (i) the general consideration of the impact of the ingested dose on the risk of listeriosis; (ii) a comparison of four foods that were selected (according to diversity of prevalence and level of contamination, food composition and consumption patterns), in part, to evaluate the effect of *L. monocytogenes* growth or non-growth on risk; and (iii) the ability to conduct "what-if scenarios" for the evaluated foods that support growth of *L. monocytogenes.*

The results of the risk assessment show that the potential for growth of *L. monocytogenes* strongly influences risk, though the extent to which growth occurs is dependant on the characteristics of the food and the conditions and duration of refrigerated storage. Using the selected RTE foods, their ability to support the growth of *L. monocytogenes* appears to increase the risk of listeriosis 100- to 1000-fold on a per-serving basis. While it is not possible to present a single value for the increased risk for all RTE foods, because of the divergent properties of the foods, the ranges of values estimated in the risk assessment

provide some insight into the magnitude of the increase in risk that may be associated with the ability of food to support the growth of *L. monocytogenes.* Control measures that focus on reduction of both frequency and levels of contamination have an impact on reducing rates of listeriosis. Controlling growth post-processing is one of these measures.

KEY FINDINGS

The most important key findings of the risk assessment as a whole are:

- The probability of illness from consuming a specified number of *L. monocytogenes* is appropriately conceptualized by the disease triangle, where the food matrix, virulence of the strain and susceptibility of the consumer are all important factors.
- The models developed predict that nearly all cases of listeriosis result from the consumption of high numbers of the pathogen.
- Based on the available data, there is no apparent evidence that the risk from consuming a specific number of *L. monocytogenes* varies for the equivalent population from one country to another. Differences in manufacturing and handling practices in various countries may affect the contamination pattern and therefore the risk per serving for a food. The public health impact of a food can be evaluated by both the risk per serving and the number of cases per population per year.
- Control measures that reduce the frequencies of contamination will have a proportional reduction in the rates of illness, provided the proportions of high contaminations are reduced similarly. Control measures that prevent the occurrences of high levels of contamination at consumption would be expected to have the greatest impact on reducing rates of listeriosis.
- Although high levels of contamination at retail are relatively rare, improved public health could be achieved by reducing these occurrences at manufacture and retail in foods that do not permit growth. In foods that permit growth, control measures such as better temperature control or limiting the length of storage periods will mitigate increased risk due to increases in *L. monocytogenes*.
- The vast majority of cases of listeriosis are associated with the consumption of foods that do not meet current standards for *L. monocytogenes* in foods, whether that standard is zero tolerance or 100 CFU/g.

LIMITATIONS AND CAVEATS

- The risk assessment focuses on four RTE foods and only examines them from retail to consumption.
- The risk characterization results are subject to uncertainty associated with a modelled representation of reality involving simplification of the relationships among prevalence, cell number, growth, consumption characteristics and the adverse response to consumption of some number of *L. monocytogenes* cells. However, the modelling is appropriate to quantitatively describe uncertainty and variability related to all kinds of factors and attempts to provide estimates of the uncertainty and variability associated with each of the predicted levels of risk.
- The amount of quantitative data available on *L. monocytogenes* contamination was limited and restricted primarily to European foods.
- Data on the prevalence and number of *L. monocytogenes* in foods came from many different sources, which adds to uncertainty and variability. Also, assumptions had to be made with regard to distribution of the pathogen in foods.
- The data used for prevalence and cell numbers may not reflect changes in certain commodities that have occurred in the food supply chain during the past ten years.
- The consumption characteristics used in the risk assessment were primarily those for Canada or the United States of America.
- The r-values and their distributions were developed using epidemiological data on the current frequency of *L. monocytogenes* strain diversity observed, with their associated virulence. If that distribution of virulence were to change (as reflected by new epidemiological data), the r-values would have to be re-calculated.
- There is uncertainty associated with the form of the dose-response function used, and with the parameterization. Also, the dose-response section of the hazard characterization is entirely a product of the shape of the distribution of predicted consumed doses in the exposure assessment component of the *Listeria* risk assessment undertaken in the United States of America (FDA/FSIS, 2001). Therefore its validity is dependant on the validity of the FDA/FSIS exposure assessment, and changes to that exposure assessment should lead directly to changes in the parameter, r.
- Predictive modelling was used to model the growth of *L. monocytogenes* in RTE foods, between the point of retail and the point of consumption, and the exposure assessment was based on information derived from those models. It is known that models may overestimate growth in food, and so reliance on such a model can result in an overestimation of the risk.

CONCLUSION

This risk assessment reflects the state of knowledge on listeriosis and on contamination of foods with *L. monocytogenes* when the work was undertaken, in 2002. New data is constantly becoming available, but in order to complete this work it was not possible to incorporate the very latest data in the risk assessment. A future iteration of the work would incorporate such new data.

The risk assessment provides an insight into some of the issues to be addressed in order to control the problems posed by *L. monocytogenes*, and approaches for modelling a system to evaluate potential risk management options. It addresses the specific questions posed by the CCFH and provides a valuable resource for risk managers in terms of the issues to be considered when managing the problems associated with *L. monocytogenes*, and alternative or additional factors or means to consider when addressing a problem. For example, if a limit is being established, then the technical feasibility of achievable levels of compliance must also be considered. While the available data were considered adequate for the current purposes, the risk assessment could be improved with additional data of better quality for every factor in the assessment. For example, quantification provides new perspectives on the risk posed by exposure to different doses of *L. monocytogenes*. The gaps in the database have been identified and could be used as a basis for establishing priorities for research programmes. The risk assessment improves our overall understanding of this issue and can therefore pave the way for risk management action to address this problem at the international level.

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Part 1.

Hazard Identification

1.1 HISTORICAL

Early reports suggest that *Listeria monocytogenes* may have been isolated from tissue sections of patients in Germany in 1891, from rabbit liver from Sweden in 1911, and from spinal fluid of meningitis patients in 1917 and again in 1920 (Reed, 1958; McCarthy, 1990). However, it was not until 1926 that the microorganism was fully described, when Murray, Webb and Swann (1926) isolated a small, Gram-positive rod bacterium that had caused an epizootic outbreak in 1924 among rabbits and guinea pigs. They named the organism *Bacterium monocytogenes*. This was a year after listeriosis in sheep was recognized in Germany as a disease syndrome, though the causative agent had not been isolated. At approximately the same time, Pirie (1927) isolated and described the same organism from gerbils in South Africa. He named the bacterium *Listerella hepatolytica*, and subsequently recommended in 1940 that the name be changed to *Listeria monocytogenes* (Reed, 1958; McCarthy, 1990). The first report of human listeriosis was in 1929, and the first perinatal case was reported in 1936 (Gray and Killinger, 1966). The microorganism has been reported to cause disease in a wide range of wild and domestic animals, and has been isolated from numerous species of mammals, birds, amphibians, fish, crustaceans, insects and reptiles (Hird and Genigeorgis, 1990; McCarthy, 1990; Ryser and Marth, 1991).

It is now widely recognized that human listeriosis is largely attributable to foodborne transmission of the microorganism. However, the first case of foodborne listeriosis was not reported until 1953, when the stillbirths of twins was linked to consumption by the mother of raw milk from a cow with listerial mastitis (Potel, 1953). It was not until several large, common-source outbreaks of listeriosis occurred in North America and Europe during the 1980s that the significance of foods as the primary route of transmission for human exposure to *L. monocytogenes* was recognized (Broome, Gellin and Schwartz, 1990; Bille, 1990). While the modes of transmission for *L. monocytogenes* can include vertical (mother to child), zoonotic (contact with animal to man), and nosocomial (hospital acquired), it is generally considered that most cases of human listeriosis involve foodborne transmission.

1.2 CHARACTERISTICS OF *LISTERIA MONOCYTOGENES*

L. monocytogenes is a Gram-positive, facultatively anaerobic, non-sporeforming rod, which expresses a typical tumbling motility at 20–25°C, but not at 35°C. The organism is psychrotrophic and grows over a temperature range of 0° to 45°C, with an optimum around 37°C. *L. monocytogenes* can grow at pH levels between 4.4 and 9.4, and at water activities ≥0.92 with sodium chloride (NaCl) as the solute (Miller, 1992). The effects of temperature, pH, water activity, oxygen availability and antimicrobials on the growth of *L. monocytogenes*

have been studied extensively in both model systems and foods, and there are a number of mathematical models available for describing the interaction of these factors with the growth rate (Buchanan and Phillips, 2000).

L. monocytogenes is widely distributed in the environment and has been isolated from a variety of sources, including soil, vegetation, silage, faecal material, sewage and water. The bacterium is resistant to various environmental conditions, such as high salinity or acidity, which allows it to survive longer under adverse conditions than most other non-sporeforming bacteria of importance in foodborne disease (McCarthy, 1990; Ryser and Marth, 1991). *L. monocytogenes* occurs widely in food processing environments (Ryser and Marth, 1991, 1999), and can survive for long periods in foods, in processing plants, in households, or in the environment, particularly at refrigeration or frozen storage temperatures. The ability of *L. monocytogenes* to survive in foods and model systems has been studied extensively, and mathematical models are available that describe the effect of various environmental parameters on the microorganism's survival (Buchanan and Golden, 1994, 1995, 1998; Buchanan, Golden and Whiting, 1993; Buchanan et al., 1994; Buchanan, Golden and Phillips, 1997).

Although frequently present in raw foods of both plant and animal origin, it is also present in cooked foods due to post-processing contamination if the cooked food is handled post-cooking. *L. monocytogenes* has been often isolated from food processing environments, particularly those that are cool and wet. *L. monocytogenes* has been isolated in foods such as raw and pasteurized fluid milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw meat and cooked sausages, raw and cooked poultry, raw meats, and raw and smoked seafood (Buchanan et al., 1989; Farber and Peterkin, 1991; FDA/FSIS, 2001; Ryser and Marth, 1991, 1999). Even when *L. monocytogenes* is initially present at a low level in a contaminated food, its ability to grow during refrigerated storage means that its levels are likely to increase during storage of those foods that can support the growth of the microorganism. A survey of a wide variety of foods from the refrigerators of listeriosis patients in the United States of America found *L. monocytogenes* in at least one food specimen in 64% of the patient's refrigerators. Food in 33% of the refrigerators had the same strain as the patient strain (Pinner et al., 1992). However, because the frequency at which people are exposed to *L. monocytogenes* is much higher than the incidence of listeriosis, there has been a public health debate about the significance of ingesting low levels of the pathogen, particularly for the portion of the population who are not immunologically compromised (Farber, Ross and Harwig, 1996; ICMSF, 1994).

1.3 OVERVIEW OF LISTERIOSIS

Listeriosis is a relatively rare disease. The reported yearly incidence of human listeriosis ranges from 0.1 to 11.3 cases per million persons (references cited in Notermans et al., 1998), with for example 0.3 to 7.5 cases per million people in Europe (EC, 2003), and 3 cases per million people in Australia. The data from the U.S. Centers for Disease Control and Prevention (CDC) active food surveillance programme, FoodNet, for the years from 1996 to 1998 indicate that there were about 5 reported cases of listeriosis per 1 000 000 population annually. Using the CDC 1996–97 surveillance data (CDC, 1998) and extrapolating to the 1997 total United States of America population, Mead et al. (1999) estimated that there were 2493 cases, including 499 deaths, due to foodborne listeriosis Although listeriosis is a relatively rare foodborne illness (Table 1.1), its severe nature makes it likely that individuals

will seek medical care. In the United States of America, where listeriosis is a "reportable" disease, CDC estimates that it recognizes and identifies approximately half of all listeriosis cases, as compared to the 3% identification rate for most other foodborne pathogens (Mead et al., 1999).

Table 1.1. Estimated incidence of foodborne disease from epidemiological surveillance.

Pathogen	Cases per 1 000 000 population
Vibrio	3
Listeria	5
Yersinia	10
E. coli O157:H7	28
Shigella	85
Salmonella	124
Campylobacter	217
All bacterial pathogens	472

SOURCE: FoodNet data for 1997 (CDC, 1998).

One of the difficulties in characterizing the hazard associated with foodborne listeriosis is that there are no clear definitions for infection or cases in humans. In general, most cases that are reported to medical authorities are severe infections requiring medial intervention. Thus, for the purposes of the current hazard characterization, an infection in humans will be based on the colonization of the host, i.e. attachment and growth, which can include individuals that are asymptomatic, displaying febrile gastroenteritis, or suffering from severe symptoms or death. The terms "severe infection" or "invasive listeriosis" will be used to describe those infected individuals with life-threatening, systemic infections, such as perinatal listeriosis, meningitis or septicaemia, and where *L. monocytogenes* is present in normally sterile body tissues.

Invasive *L. monocytogenes* infections can be life threatening, with fatality rates of 20 to 30% being common among hospitalized patients. In 2000, the CDC (2000) reported that, of all the foodborne pathogens tracked by CDC, *L. monocytogenes* had the second-highest case fatality rate (21%) and the highest hospitalization rate (90.5%).

L. monocytogenes causes invasive listeriosis by penetrating the lining of the gastrointestinal tract and establishing infections in normally sterile sites within the body. Once *L. monocytogenes* penetrates the intestinal tissue it is taken up by cells of the immune system, the phagocytes. However, inside the phagocyte it is capable of escaping from the phagosome and subsequently growing. Phagocytes appear to be the means by which the bacterium can be transported to various parts of the body (Shelef, 1989; Farber and Peterkin, 1991).

The likelihood that *L. monocytogenes* will invade the intestinal tissue depends upon a number of factors, including the number of organisms consumed, host susceptibility, and virulence of the specific isolate (Gellin and Broome, 1989). Incubation periods can be long, e.g. typically 2-3 weeks, and up to three months (Gellin and Broome, 1989). *L. monocytogenes* can produce a wide range of symptoms. In non-pregnant adults, disease syndromes most commonly linked to *L. monocytogenes* include bacteraemia, meningitis and encephalitis (Rocourt and Cossart, 1997). In pregnant women, *L. monocytogenes* often causes an influenza-like bacteraemic illness, which leads to amnionitis and infection of the fetus and results in abortion, stillbirth or premature birth. Listeriosis occurs most often either very early in life or after 60 years of age. Figure 1.1 shows listeriosis incidence by age, using 1997 FoodNet data. The incidence of listeriosis in males and females is approximately equal.

As more information became available linking listeriosis with food consumption, food control agencies and private industry developed programmes to reduce the incidence of

foodborne listeriosis. Industry initiated HACCP programmes and increased sanitation efforts to eliminate contamination. Food control agencies expanded programmes to prevent contaminated foods from entering commerce. There were also consumer education campaigns that focused on food safety. In the United States of America, a reduction in listeriosis from 7.9 per million in 1989 to 4.4 per million in 1993 was observed (Tappero et al., 1995). Rates of listeriosis simultaneously declined in the United Kingdom after the British government issued health warnings regarding *L. monocytogenes* (Fyfe et al., 1991; McLauchlin et al., 1991). Similar declines have been reported as a result of public health initiatives in other parts of Europe and in Australia (Jacquet et al., 1999). For example, it is reported that preventative measures implemented by the French food industry played a substantial role in the 68% reduction observed in France between 1987 and 1997 (Goulet et al., 2001a). However, since that time, the incidence of listeriosis has remained relatively constant (CDC, 2000). The reported yearly incidence of human listeriosis in Europe ranges from 0.1 to 11.3 cases per 10^6 persons (references cited in Notermans et al., 1998). A more recent study within the European Union indicates a slight decrease, with the reported yearly incidence of listeriosis for 2000-2001 ranging from 0.3 to 7.8 cases per million persons (de Valk et al., 2003). However, the accuracy of these values is dependent on the vigour with which individual countries conduct national surveillance programmes for listeriosis.

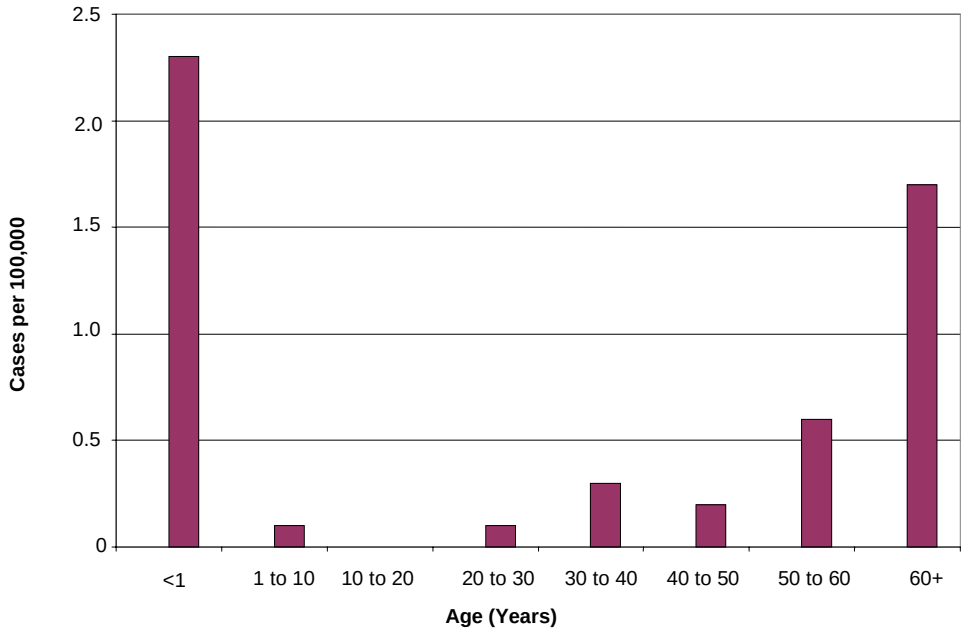

Figure 1.1. Estimated rate of listeriosis by age.
SOURCE: FoodNet 1997 data (CDC, 1998).

1.4 STATEMENT OF PROBLEM AND SCOPE OF RISK ASSESSMENT

Foodborne listeriosis represents a relatively rare but clinically serious disease that largely affects specific higher-risk segments of the population. The microorganism is widely dispersed in the environment and foods and it appears to be ingested in low numbers by consumers on a routine basis. Despite the fact that a wide variety of foods may be contaminated with *L. monocytogenes*, outbreaks and sporadic cases of listeriosis appear to be predominately associated with ready-to-eat (RTE) products. A number of risk assessments and related evaluations of foodborne listeriosis have been conducted by investigators and national governments. The current risk assessment was undertaken to determine, in part, how previously developed risk assessments done at a national level could be adapted or expanded to address concerns related to *L. monocytogenes* in RTE foods at an international level. This included an international team conducting a risk assessment to answer questions posed by an international organization. Data from different countries were used. This does not imply that the risk assessment reflects the global food supply or that the specific results are universally applicable, as different countries will have differences in contamination levels, processing and consumption patterns that are not addressed in this risk assessment. In addition, after initiation of the risk assessment, the risk assessment developers were asked by the Codex Committee on Food Hygiene (CCFH), through FAO/WHO, to consider three specific points related to RTE foods in general, namely:

- Estimate the risk of serious illness from *L. monocytogenes* in food when the number of organisms ranges from absence in 25 grams to 1000 colony forming units (CFU) per gram or millilitre, or does not exceed specified levels at the point of consumption.
- Estimate the risk of serious illness for consumers in different susceptible population groups (elderly, infants, pregnant women and immunocompromised patients) relative to the general population.
- Estimate the risk of serious illness from *L. monocytogenes* in foods that support its growth and foods that do not support its growth at specific storage and shelf-life conditions.

Considering the resources available and the time constraints placed on the risk assessment developers, it was impossible to consider all RTE foods that could be contaminated with *L. monocytogenes*. Accordingly, it was decided to limit the risk assessment to a small number of RTE foods selected to represent various classes of product characteristics. These foods were selected to provide realistic examples of how microbiological risk assessment techniques could be used to evaluate food safety questions at an international level. This educational component is a stated goal of the FAO/WHO microbiological risk assessment programme under the auspices of which the current risk assessment was developed. It was also decided to limit the scope of the risk assessment to foods at retail and their subsequent public health impact at time of consumption. This was done for two reasons. The first is that such a scope was sufficient to address the charge provided by the requestors of the risk assessment within the time frames and resources made available to the developers. Second, most of the exposure data for *L. monocytogenes* that are currently available are frequencies and extents of contamination at the retail level. More detailed examination of factors contributing to the levels found at retail as a result of manufacturing parameters would have either restricted evaluation to a much smaller range of foods, or required that substantially

greater resources and data be made available. Accordingly, the assessment does not evaluate the risks associated with different means of manufacturing the products selected. However, the risk assessment does consider several post-retail factors that could influence the consumers' risk of acquiring foodborne listeriosis, such as the temperature and duration of refrigerated storage.

Part 2.

Hazard Characterization

2.1 LISTERIOSIS

Most cases of human listeriosis appear to be sporadic, although a portion of these cases may represent previously unrecognized common-source clusters (Broome, Gellin and Schwartz, 1990; Farber and Peterkin, 1991). The source and route of infection is usually unknown, but contaminated food is considered to be the principal route of transmission, and estimated to be the source in as high as 99% of the cases (WHO, 1988; Mead et al., 1999).

L. monocytogenes appears to be a frequent transitory resident of the intestinal tract in humans. The proportion of individuals whose faecal samples have been positive for *L. monocytogenes* range from a low 0.5% to a high 29% (Farber and Peterkin, 1991). On average, 2 to 10% of the general population are carriers of the organism without any apparent adverse consequences (Farber and Peterkin, 1991; Rocourt and Cossart, 1997; Skidmore, 1981; Slutsker and Schuchat, 1999; Mascola et al., 1992; Schuchat, Swaminathan and Broome, 1991). Because of the high rate of clinically healthy carriers, Farber and Peterkin (1991) suggested that the presence of *L. monocytogenes* in the faeces is not necessarily an indication of infection. The role of healthy carriers is not clear, but investigations during an outbreak in California in 1985 suggested that community-acquired outbreaks might be amplified through secondary transmission by stool carriers (Rocourt, 1996). Pregnancy, while predisposing to listeriosis, does not seem to predispose women to carriage of the organism (Lamont and Postlethwaite, 1986). Healthy pregnant women may be carriers of *L. monocytogenes* and still give birth to healthy infants.

2.1.1 Manifestations of listeriosis

The pregnant uterus, the central nervous system (CNS) or the blood are the locations where bacteria are most often found when initial infections of the intestinal tissue by *L. monocytogenes* leads to invasion of otherwise sterile body sites. A summary of 782 cases of listeriosis reported from 20 countries in 1989 showed that 43% were perinatal (prenatal + neonatal) infections, 29% were septicaemic infections, 24% were CNS infections and 4% were atypical forms (Rocourt, 1991). However, changes in the epidemiology of listeriosis over the past ten years have been noted. For example, more recent data from England and Wales, France, Denmark and the United States of America show that the proportion of pregnancy related cases ranges from 11 – 31%: 17% in England and Wales in 1995-99 (Smerdon et al., 2001), 11% in Denmark in the period 1999-2000, 24% in France in 1999 (Goulet et al., 2001b) and 31% in the United States of America in 1993 (Tappero et al., 1995). In the non–pregnancy related group, the proportion of bacteraemic forms has increased and represents at least two thirds of the cases. This form nearly always occurs in

patients with an underlying disease, whereas CNS infection also occurs in previously healthy persons. Sequelae may follow listeriosis infections (McLauchlin, 1997), but their incidence is rarely estimated (Rocourt, 1996). Up to 11% of neonates and 30% of survivors of CNS infection suffer from residual symptoms, and psychiatric sequelae have also been reported (references cited in Rocourt, 1996). However, for survivors of CNS infection 30% is considered unusually high and the rate of occurrence of sequelae is normally lower. For example, in a study of 225 patients in France in 1992, neurological sequelae were observed in 12% of patients and 15% of survivors (Goulet and Marchetti, 1996).

A classification scheme has been proposed for differentiating the manifestations of syndromes associated with *L. monocytogenes* that takes into consideration host status, route of transmission, severity and incubation period (Table 2.1). It has been estimated that as much as 20% of the population may belong to groups with a greater risk for developing listeriosis (Buchanan et al., 1997; Lindqvist and Westöö, 2000). These higher-risk people can be divided into non-perinatal and perinatal groups. When severe infection occurs in adults and children, listeriosis is usually superimposed on another illness (Lorber, 1990; Broome, Gellin and Schwartz, 1990; Schuchat, Swaminathan and Broome, 1991; Shelef, 1989; Gray and Killinger, 1966; Linnan et al., 1988; WHO, 1988.). The high-risk cases primarily consist of persons with chronic debilitating illnesses that impair their immune system, such as cancer, diabetes or alcoholism; HIV/AIDS; persons taking immunosuppressive medication (e.g. immune suppressors taken by transplant patients); and persons over the age of 60–65, particularly individuals with pre-existing, debilitating medical conditions. Healthy children and immunocompetent adults have a low risk of severe infection from *L. monocytogenes*.

There have also been a number of outbreaks where the majority of cases developed mild symptoms (Dalton et al., 1997; Salamina et al., 1996; Riedo et al., 1994; Aureli et al., 2000), such as diarrhoea, fever, headache and myalgia. These outbreaks have generally involved the ingestion of high doses of *L. monocytogenes* by otherwise healthy individuals and these gastroenteritis symptoms generally self-resolve within a few days.

A summary of epidemiological information from some foodborne listeriosis outbreaks is shown in Table 2.2.

2.1.1.1 Systemic listeriosis

Non-perinatal

In non-pregnant humans, systemic listeriosis usually presents as either CNS infections, with or without bacteraemia, or bacteraemia alone. Cases of bacteraemia alone are often confined to the immunocompromised or elderly (McLauchlin, 1996).

In addition to these clinical manifestations, less common manifestations include peritonitis (Polanco et al., 1992; Nguyen and Yu, 1994), hepatitis and liver abscess (Bourgeois et al., 1993; Braun et al., 1993), endocarditis (Gallagher and Watanakunakorn, 1988), arterial infections (Gauto et al., 1992), myocarditis (Stamm et al., 1990), lung and pleural fluid infection (Mazzulli and Salit, 1991), septic arthritis and osteomyelitis (Louthrenoo and Schumacher, 1990; Ellis et al., 1995), and chorioretinitis, endophtalmitis and corneal ulcer (Ballen, Loffredo and Painter, 1979; Huismans 1986; Holland et al., 1987).

Table 2.1 Classification of illness caused by *Listeria monocytogenes*.

Type of Listeriosis	Mode of transmission	Severity	Time to onset
Occupational infection	Primary cutaneous listeriosis after direct contact with infected animal tissues.	Usually mild and self-resolving.	1–2 days.
Neonatal infection	Infection of newborn babies from infected mother during birth or due to cross-infection from one neonate in the hospital to other babies.	Can be extremely severe, resulting in meningitis and death.	1–2 days (early onset), usually from congenital infection prior to birth. 5–12 days (late onset), following cross-infection from another infant.
Infection during pregnancy (prenatal)	Acquired following consumption of contaminated food.	Mild flu-like illness or asymptomatic in the mother, but serious complications for unborn infant, including spontaneous abortion, fetal death, stillbirth and meningitis. Infection is more commonly reported in third trimester.	
Infection of non-pregnant adults (non-perinatal)	Acquired following consumption of contaminated food.	Asymptomatic or mild illness, which may progress to CNS infections such as meningitis. Most common in immunocompromised or elderly.	Illness may occur within 1 day or up to 3 months, but commonly within 20–30 days.
Listeria food poisoning (febrile gastroenteritis)	Consumption of food with exceptionally high levels of *L. monocytogenes*, > 10^7/g.	Vomiting and diarrhoea, sometimes progressing to bacteraemia but usually self-resolving.	<24 h after consumption.

SOURCE: Modified from Bell and Kyriakides, 1998, as described in EC, 1999.

Despite the fact that infections can be treated successfully with antibiotics, between 20 and 40% of cases are fatal (Gellin and Broome, 1989; McLauchlin, 1996). In severely immunocompromised patients, the case-fatality rate may approach 75% (Nørrung, Andersen and Schlundt, 1999).

Perinatal (prenatal/neonatal) infections

The perinatal group consists of pregnant women and their fetuses or newborns. About two-thirds of *L. monocytogenes*-infected pregnant women will present with a prodromal influenza-like illness, which includes fever, chills and headache. About three to seven days after the onset of prodromal symptoms, a woman may abort the fetus or have premature labour (Gellin and Broome, 1989). Sepsis or fever is reported in about 30% of pregnant women with listeriosis (Gellin and Broome, 1989). Women may get listeriosis at any time during pregnancy, but most cases are reported in the third trimester (Slutsker and Schuchat, 1999). In the first trimester, listeriosis may result in spontaneous abortion. In later stages of pregnancy the result may be stillbirth or a critically ill newborn. Listeriosis is rarely severe or life threatening to the mother and is not known to cause increased risk in subsequent pregnancies (Skidmore, 1981; Farber and Peterkin, 1991). The epidemiological records for prenatal cases are incomplete in that the rate of occurrence during early pregnancy and recovery of fetuses from infection are unknown. Neonatal cases of listeriosis are better documented and the rate of prenatal listeriosis was estimated to be 1.5 times that of neonates (FDA/FSIS, 2001).

Table 2.2 Summary of epidemiological information from some published foodborne listeriosis outbreaks.

Country	Number of cases				Number of deaths			Percentage of Manifestations					Source
	Total (exposed)	Healthy	Materno -fetal	Immunocom-promised	Total (%)	Adults	Perinatal	Septi-caemia	Meningitis	Other CNS	Other	GI	
Australia	9	–	–	–	6 (67)	–	–	–	–	–	–	–	[10]
Australia	4	4	0	0	0	0	0	–	–	–	100	75	[11] [12]
Australia	5	0	0	5	1 (20)	1	0	100	–	–	–	–	[24]
Denmark	26	10	3	13	6 (23)	–	–	26(4)	65(4)	–	–	–	[9]
Canada	41	7	34	0	18 (44)	2	16	14(4)	86(4)	–	–	–	[3]
Finland	25	–	0	24	6 (24)	6	0	80	16	–	4	–	[27]
France	279	62	92	125	88 (32)	59	29	–	–	–	–	–	[15] [16] [17] [28]
France	36(7)	8	18	19	9 (25)	4	5	22	67(4)	11(4)	–	–	[23] [18]
France	38	2	31	5	11 (29)	1	10	28(4)	57(4)	14(4)	45–93(1)	3(1)	[18] [19]
France	10	1	3	6	3 (30)	2	1	4	57(4)	43(4)	–	–	[29]
France	32	12	9	11	9 (28)	5	4	7	30(4)	70(4)	–	–	[29]
Italy	1566 (2930)	–	0	–	0	0	0	0	0	0	6-82	19-72	[25]
Italy	18 (39)	18	0	0	0	0	0	–		–	22–100	78	[20]
New Zealand	22	0	22	0	6 (27)	0	6	27(2), 55(1)	28(2)	–	82(1)(3)	45(1)	[2]
New Zealand	4	0	4	0	2 (50)	0	2	–	25(2)	–	–	–	[13] [14]
Sweden	8	0	3	5	2 (25)	1	1	50	25	–	–	–	[22]
Switzerland	122	33/17(5)	65	24/40(5)	34 (28)	18	16	21(4)	40(4)	39(4)	56(6)	46(4)	[6] [7]
UK	>350	–	–	–	–	–	–	–	–	–	–	–	[8]
USA	20	10	0	10	5 (25)	5	0	90	50	30	–	65	[1]
USA	49	0	7	42	14 (29)	12	2	69(4), 29(2)	31(4), 42(2)	–	–	–	[4]
USA	142	1	93	48	48 (34)	18	30	52(1), 71(4)	0(1), 14(4)	–	–	–	[5]
USA	45 (60)	44	1	0	0	0	0	–	–	–	3-72	79	[21]
USA	101	–	–	–	21	–	–	–	–	–	–	–	[26]

KEY: CNS = central nervous system. GI = gastrointestinal. – information not available

NOTES: (1) refers to the pregnant women; (2) refers to the fetus or the baby; (3) flu like illness or urinary tract symptoms; (4) refers to adults (not including pregnant women); (5) Including age >65 year as predisposing factor; (6) Including meningismus and altered mental status; (7) information given on only 20 cases.

SOURCES: [1] Ho et al., 1986. [2] Lennon et al.,1984. [3] Schlech et al., 1983. [4] Fleming et al., 1985. [5] Linnan et al., 1988. [6] Bille, 1990. [7] Büla, Bille and Glausser, 1995. [8] McLauchlin et al., 1991. [9] Jensen, Frederiksen and Gerner-Smidt, 1994. [10] Kittson, 1992. [11] Mitchell, 1991. [12] Misrachi, Watson and Coleman, 1991. [13] Baker et al., 1993. [14] Brett, Short and McLauchlin, 1998. [15 Rocourt et al., 1993. [16] Salvat et al., 1995. [17] Jacquet et al., 1995a. [18] Jacquet et al., 1995b. [19] Goulet et al., 1998. [20] Salamina et al., 1996. [21] Dalton et al., 1997. [22] Ericsson et al., 1997. [23] Goulet et al., 1995. [24] Hall et al., 1996. [25] Aureli et al., 1998. [26] Mead, 1999. [27] Lyytikäinen et al., 2000. [28] Goulet, 1995. [29] de Valk et al., 2001.

Neonates may present with an early-onset or late-onset form of listeriosis. Early onset (infected in utero) is defined as a case of listeriosis (Granulomatosis Infantisepticum) in a neonate less than 7 days old. Early-onset listeriosis is characterized by premature birth, respiratory distress and circulatory failure. Most early-onset cases present with sepsis and about 20% have meningitis. Late-onset is defined as listeriosis in a neonate between 8 to 28 days of life. Usually, late-onset neonates are born healthy and at full term. Meningitis is more common in late-onset babies (Farber, 1991). The mothers of late-onset babies usually had an uneventful pregnancy without prodromal illness. *L. monocytogenes* is rarely isolated from the mother and the source of listeriosis is often not identified in late-onset cases (Farber and Peterkin, 1991; Slutsker and Schuchat, 1999). While a number of alternative sources of *L. monocytogenes* could be hypothesized for the purposes of the current hazard characterization, it will be assumed that neonatal infections are the result of *in utero* exposure. About 25% of neonates with listeriosis die (Gellin and Broome, 1989; McLaughlin, 1990a), with the mortality rate being 15–50% in early-onset listeriosis and 10–20% in late-onset listeriosis (Farber and Peterkin, 1991).

2.1.1.2 Febrile gastroenteritis

Typical signs and symptoms associated with febrile listerial gastroenteritis include chills, fever, diarrhoea, headache, abdominal pain and cramps, nausea, vomiting, fatigue, joint and muscle pain, and myalgia. *L. monocytogenes* infection manifestation may be limited to these symptoms in otherwise healthy individuals. Although mild symptoms associated with listeriosis have been reported in several countries, and a variety of foods have been implicated as the vehicle of infection, there is a high potential for underreporting of mild illness due to *L. monocytogenes* because of the general nature of the symptoms. Table 2.3 summarizes reported outbreaks where most of the cases reported only mild symptoms (Aureli et al., 2000; Miettinen et al., 1999; Heitmann, Gerner-Smidt and Heltberg, 1997; Dalton et al., 1997; Salamina et al., 1996; Riedo et al., 1994). The reports from Italy (1997), Denmark (1996) and the United States of America (1994) are of particular note because they show that listeriosis can be limited to mild symptoms even if blood cultures are positive for *L. monocytogenes*.

Table 2.3 Reports of mild illness associated with *Listeria monocytogenes.*

Location	Year	Cases	Vehicle	Ref.
Denmark	1996	3	Unknown	[1]
Finland	1999	5	Smoked rainbow trout	[2]
Italy	1997	1 566	Maize and tuna salad	[3]
Italy	1993	18	Rice salad	[4]
USA	1994	45	Chocolate milk	[5]
USA	1989	10	Shrimp	[6]

SOURCES: [1] Heitmann, Gerner-Smidt and Heltberg, 1997. [2] Miettinen et al., 1999. [3] Aureli et al., 2000. [4] Salamina et al., 1996. [5] Dalton et al., 1997. [6] Reido et al., 1994.

There are insufficient data available about the incidence of the milder symptoms to allow the impact of this biological end point on public health to be assessed in the current exercise.

2.1.2 Foods associated with foodborne listeriosis

Food is the principal route of transmission of listeriosis (WHO, 1988). Listeriosis cases are observed in conjunction with both common-source outbreaks and individual sporadic cases. Foods of most concern include RTE products that (i) support growth of *L. monocytogenes*, (ii) have a long refrigerated shelf-life, and (iii) are consumed without further listericidal

treatments (Pinner et al., 1992; Rocourt, 1996; FDA/FSIS, 2001; Nørrung, Andersen and Schlundt, 1999). This includes products that receive a listericidal treatment but are subject to post-processing recontamination. This also includes cross-contamination in both the retail and home setting. For example, in the French outbreak in 1992, cross-contamination was suspected at the distribution level (Rocourt, 1996). Similar cross-contamination is likely to occur in the home (Schwartz, Pinner and Broome, 1990).

Common-source outbreaks have been associated or linked epidemiologically with the consumption of Hispanic-style soft cheeses (*queso fresco*); soft, semi-soft and mould-ripened cheeses; hot dogs; pork tongue in jelly; processed meats; paté; salami; pasteurized chocolate-flavoured milk; pasteurized milk; unpasteurized milk; butter; cooked shrimp; smoked salmon; maize and rice salad; maize and tuna salad; potato salad; raw vegetables; and cole slaw (see FDA/FSIS, 2001). In addition, sporadic cases have been linked to the consumption of raw milk; unpasteurized ice cream; ricotta cheese; goat, sheep and feta cheeses; soft, semi-soft and mould-ripened cheeses; Hispanic-style cheese; salami; hot dogs; salted mushrooms; smoked cod roe; smoked mussels; undercooked fish; pickled olives; raw vegetables; and cole slaw.

In general, the levels of *L. monocytogenes* in the implicated food have been greater than 10^3 CFU/g (EC, 1999), but there have been instances where the observed level of *L. monocytogenes* in the implicated food has been substantially lower. However, there is a great deal of uncertainty concerning these estimates because the actual level of the pathogen in a serving of food consumed by an individual could have varied considerably from that observed in other portions of the food during a subsequent investigation.

2.2 DOSE-RESPONSE RELATIONS

2.2.1 Characterization of severity and the selection of appropriate biological end points to be modelled

The severity of a hazard can be evaluated by qualitative, semi-quantitative and quantitative approaches. Roberts, Ahl and McDowell (1995) summarized approaches used to rank or prioritize different foodborne illnesses in terms of their severity or consequences. Different criteria used to evaluate severity included:

- The number of acute illness cases.
- The number of deaths.
- The number of chronic illness cases.
- The quality-adjusted life-years lost due to the illness.
- The damage to society in terms of medical costs and loss of productivity.
- The willingness of the society to pay for reducing the risk of illness (Roberts, Ahl and McDowell, 1995).

Other work to assess or describe the severity of microbial hazards has tried to relate the dose to the severity of the disease (Glynn and Bradley, 1992). Due to the difficulty of obtaining the relevant information during outbreaks, case fatality rate and hospitalization rate have been used for assessing severity, while attack rate, incubation period, amount of contaminated food and the vehicle involved have been used as proxy measures of infecting dose. At least for *Salmonella*, there appears to be an association between the dose and the

incubation period. A correlation between dose and severity was found also for some of the food poisoning salmonellae, but not for S*almonella. typhi* (Glynn and Bradley, 1992; Glynn and Palmer, 1992). No similar evaluation for the relation between the dose and severity of illness, or the dose and incubation period for *L. monocytogenes* was found in the literature.

In this risk assessment, characterization of the severity of listeriosis is limited to a description of the manifestations of the disease and a summary of epidemiological information from outbreaks. The quantitative relationship between the dose and the severity is addressed by selection of the biological end-points to be modelled, i.e. infection, morbidity or mortality. However, this is complicated because infection has been differently defined and estimated in different studies, e.g. faecal-positive versus an infected spleen. Similarly, the morbidity endpoint may cover the whole range from mild to severe manifestations. If the probability of morbidity after an infection were low, the use of the infection endpoint would be excessively conservative for a risk assessment model. The shape of the dose-response relationship, and thus the appropriate dose-response model, for these two biological end-points may also be different (FDA/FSIS, 2001).

2.2.2 Factors that affect dose-response relations for *L. monocytogenes*

The response of a human population to exposure of a foodborne pathogen is highly variable. This reflects the fact that the incidence of disease is dependent on a variety of factors, such as the virulence characteristics of the pathogen, the numbers of cells ingested, the general health and immune status of the host, and any attributes of the food that alter microbial or host status. Thus, the likelihood that any individual will become ill due to an exposure to a foodborne pathogen depends on the integration of host, pathogen and food matrix effects. These interactions are often referred to as the infectious disease triangle. Each of these classes of factors and how they affect the dose-response relations for *L. monocytogenes* will be discussed briefly.

2.2.2.1 Virulence of L. monocytogenes *isolates*

The traditional taxonomic scheme for the genus *Listeria* differentiates the species *L. monocytogenes* and *Listeria innocua* based on their ability to produce listeriolysin. Otherwise, the two species have nearly identical cultural and biochemical characteristics. Listeriolysin is a haemolysin (i.e. an enzyme capable of lysing red blood cells) produced by *L. monocytogenes* that is associated with the microorganism's ability to cause disease. Thus taxonomically, all *L. monocytogenes* were presumed to be pathogenic. However, the relative virulence of individual *L. monocytogenes* isolates can vary substantially (at least in animal models), presumably from different forms of other virulence factors (Hof and Rocourt, 1992). This variability influences the number of microorganisms required to produce an infection, the potential for an infection to become symptomatic, the severity or manifestations of illness, and the population at greatest risk.

Invasive listeriosis is characterized by bacterial dissemination to the CNS and the feto-placental unit, due to the capacity of *L. monocytogenes* to cross the intestinal barrier, the blood-brain barrier and the feto-placental barrier. Recent advances in the study of virulence factors have improved our understanding of the steps in the infection process at the cellular level, although much remains unknown (Lecuit et al., 1999; Vazquez-Boland et al., 2001). *L. monocytogenes* are facultative intracellular parasites and one important feature of this bacterium is its ability to induce its own internalization into cells that are normally non-

phagocytic. *L. monocytogenes* readily invades many types of cells *in vitro*, which suggests that there may be multiple routes by which the bacterium invades the host, but animal experiments suggest that the small intestine acts as the primary site of invasion (McLauchlin, 1997). Two invasion proteins of *L. monocytogenes* have been characterized – internalin A (InlA) and B (InlB) – that mediate entry into different cell types. The bacterial surface protein InlA is necessary for *L. monocytogenes* entry into human gut epithelial cells in the small intestine through binding to a human host receptor, a protein called E-cadherin (Lecuit et al., 2001). This appears to be a host-specific process and it was recently shown that InlA interacts with human and guinea pig E-cadherin, but not with mouse and rat E-cadherin (Lecuit et al., 1999, 2001). Thus, *L. monocytogenes* readily invades human and guinea pig gut epithelial cells but not mouse and rat epithelial cells. Instead, in mice it has been demonstrated that *L. monocytogenes* may colonize the Peyers´s patches of the host through the M cells (Vazquez-Boland et al., 2001). These results indicate that the mouse, which has been the most widely used animal model for the study of listeriosis, is inappropriate to study specific features of human listeriosis, i.e. the crossing of the intestinal barrier following exposure via the oral route. This is in contrast to the guinea pig (Lecuit et al., 1999) or a newly developed transgenic mouse model expressing human E-cadherin (Lecuit et al., 2001). The impact of this limitation for other aspects of listeriosis studied in a mouse or rat model is unclear at present, but this example illustrates the complexity of the pathogenesis of many bacterial diseases and the necessity for careful evaluation of results from surrogate animal studies.

After invasion of gut epithelial cells, *L. monocytogenes* bacteria are carried to the lymph nodes and then other tissues, including the spleen and the liver, by dendritic cells, phagocytes or as free cells (Pron et al., 2001). Based on experimental infection of mice via the intravenous route, it appears that most of the *L. monocytogenes* bacteria accumulate in the liver and that most of the ingested bacteria are killed by resident macrophages in the spleen and liver (Vazquez-Boland et al., 2001). However, surviving *L. monocytogenes* cells start multiplying in the liver, the principal site being the hepatocytes (Vazquez-Boland et al., 2001). In the majority of individuals, *L. monocytogenes* invasion may be successfully cleared, but if the infection is not controlled by an adequate immune response, proliferation of *L. monocytogenes* may result in the release of bacteria into the circulation system and a successive invasion of other sites, such as the uterus, fetus or CNS (McLauchlin, 1997; Vazquez-Boland et al., 2001).

From a mechanistic perspective, the virulence of *L. monocytogenes* has been studied extensively. Most studies of *L. monocytogenes* virulence have used genetically inbred mouse varieties as the surrogate animal model, and have, of necessity, been conducted using well-characterized strains of *L. monocytogenes* selected – or in some cases genetically modified – for the presence or absence of the specific virulence genes. These studies have discovered a large number of virulence determinants involved in the entry and colonization of host tissue. Examples of steps in the infection process include internalization by eucaryotic cells, lysis of the resulting phagosome, replication as well as movement within the host cytoplasm, direct cell-to-cell spread, and lysis of a double-membrane vacuole when entering neighbouring cells (Brehm et al., 1996). As discussed above, internalin is required for *L. monocytogenes* entry into epithelial cells (Lebrun et al., 1996). The production of superoxide dismutase by *L. monocytogenes* may aid in the survival in the macrophages (Farber and Peterkin, 1991). Bacteria that survive or are in a non-activated phagocyte then dissolve the phagosome by means of listeriolysin O (LLO) or possibly by phospholipase C (McLauchlin, 1997). *Listeria*

strains lacking LLO are avirulent, failing to colonize liver or spleen in gastric infection studies in mice (Gaillard, Berche and Sansonetti, 1986; Roll and Czuprynski, 1990; Tabouret et al., 1991; Erdenlia, Ainsworth and Austin, 2000). The production of the enzyme phospholipase C by virulent *L. monocytogenes* is important for its ability to survive the early host neutrophil-mediated defence mechanism (Conlan and North, 1992). The listerial surface protein ActA is required for actin polymerization and confers intracellular mobility and enables the bacterium to invade an adjacent host cell (Kocks et al., 1992). The surface-bounded protein actin A mediates the contact to the actin filament system of the host cell. The cell-to-cell spread is also mediated by phospholipase and lecithinase (Schwarzkopf, 1996). Most virulence genes are activated by the transcriptional regulator *prfA* (Mengaud et al., 1991; Renzoni, Cossart and Dramsi, 1999). The expression of *pfrA* and *prfA*-dependent proteins is under the control of several environmental parameters, such as temperature, pH, stress conditions and composition of the medium (Brehm et al., 1996).

While the use of tightly defined systems (i.e. clonal bacteria and genetically identical hosts) is needed to study the pathogen's virulence mechanisms, the frequency of naturally occurring strains that are deficient in one or more virulence markers appears to be relatively rare among populations of foodborne isolates of *L. monocytogenes*. Various cell cultures have been proposed as a means for differentiating virulent and non-virulent isolates of *L. monocytogenes*. While the methods have had varying degrees of success, most have indicated that the majority of isolates from foods have a complete array of virulence-associated genes and are virulent (del Corral et al., 1990; Pine et al., 1991; Wang et al., 1998). Accordingly, it is generally assumed that, except for atypical isolates such as listeriolysin-deficient mutants, all *L. monocytogenes* isolates are potentially pathogenic (Rocourt, 1996).

Testing with surrogate animal models (mice) has demonstrated substantial variation among isolates in relation to the differential levels of the microorganisms needed to induce morbidity or death after oral or intraperitoneal administration. For example, del Corral et al.

Table 2.4 The lethality of *Listeria monocytogenes* food and clinical isolates for immunocompromised mice.

Strain	LD_{50} (CFU)	Source
MF2-L-P	6	Food
V3-VT	13	Food
GV2-VS	29	Food
F3-VJ-G	31	Food
HO-V6-G	31	Food
LG4-VS	42	Food
VS2-VJ	74	Food
Scott A	93	Clinical
H4-V-G	100	Food
GVG-VS	110	Food
GLB1-LS	200	Food
CCR8-V-G	1 000	Food
S9-VJ-G	1 400	Food
F-4259	2 000	Clinical
GVN4-VG	3 100	Food

SOURCE: Adapted from del Corral et al., 1990.

(1990) found a 3-log range for LD_{50} values when immunocompromised mice were administered *L. monocytogenes* by an intraperitoneal route (Table 2.4). However, virtually all listeriolysin-positive clinical and food isolates were pathogenic for immunocompromised mice (del Corral et al., 1990; Pine, Malcolm and Plikaytis, 1990; Notermans et al., 1998). The level of the pathogen required to produce infections and morbidity declined by several orders of magnitude when the mice were immunocompromised (Golnazarian et al., 1989). Inhibition of gastric acid production also decreases the levels of *L. monocytogenes* needed to produce infections and morbidity after oral administration (Golnazarian et al., 1989). Ribotyping in combination with allelic analysis of virulence genes and DNA sequencing has identified disease-associated sub-types of *L. monocytogenes* (Wiedman et al., 1997). Resistance to arsenite has been reported to occur at a higher rate in clinical isolates of *L. monocytogenes* (Buchanan et al., 1991; McLauchlin, 1997). Thus, a substantial heterogeneity in virulence has been observed in several *in vivo* (mice) and *in vitro* (cell culture) studies. However, no consistent pattern of increased virulence associated with any specific serotype or subtype in animal or *in vitro* studies has emerged (Pine et al., 1991; Tabouret et al., 1991; Hof and Rocourt, 1992; Wiedman et al., 1997) and none of the present methods have consistently identified strains that are non-pathogenic or less virulent (McLauchlin, 1997).

Nevertheless, there is evidence for variation in virulence among foodborne isolates of *L. monocytogenes*. Although human listeriosis may be caused by all 13 serotypes of *L. monocytogenes*, most listeriosis cases are associated with a restricted number of serotypes: 1/2a (15–25%); 1/2b (10–35%); 1/2c (0–4%); 3 (1–2%); 4b (37–64%); and 4 not b (0–6%) (McLauchlin, 1990b; Farber and Peterkin, 1991). The frequency of serotype 4b was significantly greater in pregnancy cases, whereas serovar 1/2b was most commonly associated with non-pregnancy cases (McLauchlin, 1990b). However, the frequency with which these serotypes can be isolated from foods does not closely parallel the disease distribution (Pinner et al., 1992). Contamination of hot dogs by two serotypes of *L. monocytogenes* (1/2a and 4b) resulted in disease associated only with the 4b serotype, which was present at apparently much lower concentrations (FDA/FSIS, 2001). This suggests that the 4b isolate was either more virulent, better able to survive transport through the stomach or grew at a greater rate in the food.

The difference in the distribution of strains isolated from foods and human clinical cases, does not necessarily reflect a difference in virulence, but may also be a reflection of the adaptations by this bacterium to different ecological niches (Boerlin and Piffaretti, 1991). It may also be a reflection of the methodology used. MacGowan et al. (1991) investigated faeces from different categories of patients and detected more than one *Listeria* species or serovar in 40% of the positive samples. Similarly, a direct plating method recovered two serovars from a gravad rainbow trout (Loncarevic, Tham and Danielsson-Tham, 1996).

The observed variability in virulence of different *L. monocytogenes* isolates reflects the number of microorganisms required to produce an infection, the potential for an infection to become symptomatic, the severity or manifestations of illness, and which individuals in the population are at greatest risk. In addition to the factors that directly influence the ability of *L. monocytogenes* to produce a systemic infection, the microorganism's virulence can also be influenced by characteristics that increase its likelihood of reaching the intestinal tract. For example, *L. monocytogenes* does have adaptive acid-resistance mechanisms that, when

induced, increases the likelihood that it will survive passage through the stomach (Kroll and Pratchett, 1992; Buchanan et al., 1994). This will be discussed more fully below.

2.2.2.2 Host susceptibility

Human populations are highly diverse in their response to infectious agents, reflecting the population's diversity in genetic background, general health and nutrition status, age, immune status, stress level and prior exposure to infectious agents. For certain foodborne diseases, it appears that prior exposure to the agent renders the individual resistant to subsequent exposures to the pathogen (e.g. for *Cyclospora cayetanensis*). However, for many infectious and toxico-infectious foodborne pathogens, immunity is of limited importance, due to either the presence of the pathogen being restricted to the intestinal tract (e.g. enterohaemorrhagic *Escherichia coli*), great diversity of serotypes (e.g. *Salmonella*), or mechanisms for avoiding or overcoming the host's defences (e.g. *L. monocytogenes*).

Severe listeriosis most often affects those with severe underlying illness, the elderly, pregnant women and both unborn or newly delivered infants (McLauchlin, 1996). Infection in healthy adults is typically asymptomatic. The rate of *L. monocytogenes* carriage among these asymptomatic individuals is not known (Slutsker and Schuchat, 1999). The majority of human cases of severe listeriosis occur in individuals who have an underlying condition that suppresses their T-cell mediated immunity (Farber and Peterkin, 1991; Rocourt, 1996). A summary of listeriosis cases in 1989 from 16 countries showed that 31% of the cases occurred in patients older than 60 years, and 22% occurred in patients younger than 1 month (Rocourt, 1991). In addition to age (elderly and the neonates) and pregnancy, risk factors include cancer and immunosuppressive therapy, AIDS, and chronic conditions such as cardiovascular disease, congestive heart failure, diabetes, cirrhosis and alcoholism (Nieman and Lorber, 1980; McLauchlin, 1990a; Paul et al., 1994; Goulet and Marchetti, 1996; Rocourt, 1996). A review of 98 cases of non-pregnancy associated sporadic listeriosis in the United States of America revealed that 98% of individuals involved had at least one underlying condition (Schuchat et al., 1992). Most, but not all, of these were associated with probable immunosuppression. Antacid therapy (Ho et al., 1986) and iron overload (Lorber, 1990) were also reported as risk factors. However, in surveillance data from France for 1999 no identified immunosuppressive condition was noted in up to 15% of cases (Goulet et al., 2001b). Thus, individuals without any of the risk factors mentioned above have occasionally become severely infected.

As discussed earlier, *L. monocytogenes*, at high numbers, can cause febrile gastroenteritis in healthy persons (Salamina et al., 1996; Miettinen et al., 1999; Aureli et al., 2000). The course of the disease appears to be similar to more classical foodborne pathogens, such as *Salmonella*, where infections are generally limited to gastroenteritis, but, for a small percentage of the population, particularly those with an underlying condition, systemic, life threatening infections may occur.

For the purposes of this hazard characterization, the elderly will be considered to be individuals aged 60 years or older, and the very young are ≤ 28 days of age.

While susceptibility in these groups is thought to be related primarily to an impaired or undeveloped immune function, another physiological parameter thought to be relevant to susceptibility is a reduced level of gastric acidity. As previously mentioned, antacid use has been identified as a risk factor for severe listeriosis. Reduced gastric acidity may be

associated with aging or with drug treatment for gastric hyperacidity. An increasing portion of the population suffers from achlorhydria as age increases above 50 years. Two dose-response studies dealing with this issue involved treatment of mice or rats with the acid suppressor Cimetidine concurrent with oral infection with *L. monocytogenes*. The mouse study showed no significant effect with the drug treatment (Golnazarian et al., 1989), while the rat study showed increased infectivity of *L. monocytogenes* at the lowest dose (Schlech, Chase and Badley, 1993). Another factor that can reduce gastric acidity is infection with *Helicobacter pylori*. Basal gastric acidity was found to be increased in individuals following successful eradication of *H. pylori* compared with subjects whose infection persisted after antibiotic therapy (Feldman et al., 1999). The subjects in this study were asymptomatic for *H. pylori* infection, as are the majority of infected individuals. While this population may be more at risk for infection with *L. monocytogenes* (and other pathogenic bacteria) by reduction of the stomach acid barrier, no studies were found that focused on this relationship.

With respect to immune function, specific human dose-response information must be gleaned from surveillance data. However, much of our understanding of the effect of immune status on the pathogenicity of *L. monocytogenes* comes from research with surrogate animals. Thus, an underlying assumption is that human and animal resistance mechanisms are similar. The mouse is the most thoroughly characterized with respect to the role that specific immune defects have on susceptibility to *L. monocytogenes*. Host resistance mechanisms against *L. monocytogenes* have been studied primarily using a variety of immunocompromised mouse models. These models include gene knockout models, depletion of cytokines or immune cells with monoclonal antibodies, and mouse strains with genetic defects related to macrophage-mediated killing of *L. monocytogenes* (Czuprynski, Theisen and Brown, 1996; Stevenson, Rees and Meltzer, 1980; Cheers and McKenzie, 1978*).*

Within some susceptible human populations, immune system defects that correlate with resistance in mouse models have been identified. In pregnancy, there is a characteristic inhibition of natural killer (NK) cell activity in the placenta (Schwartz, 1999). During the early phase of resistance in the mouse, NK cells, stimulated by interleukin 12, are the primary source of gamma-interferon, a key component of resistance (Unanue, 1997; Tripp et al., 1994). Pregnancy is also associated with development of a Th-2 cytokine environment that favours the production of interleukins 4 and 10 (Schwartz, 1999). Using gnotobiotic pregnant mice, Lammerding et al. (1992) observed cellular immune response in the mother's liver and spleen, but a similar response was not observed in the placenta or fetus. Immune defects in the mouse that reflect these changes have a negative effect on resistance (Nakane et al., 1996; Genovese et al., 1999) while cytokines characteristic of a Th-1 response (e.g. gamma-interferon) are critical for resistance (Unanue, 1997; Tripp et al., 1994; Huang et al., 1993). Listeriosis symptoms in pregnancy are often mild (Slutsker and Schuchat, 1999), suggesting that pregnancy may not predispose mothers to more severe illness. However, it is possible that immunosuppression as a consequence of pregnancy results in increased likelihood that even small numbers of *Listeria* in the circulation can colonize placental tissues, increasing the chances of fetal exposure. The consequences of fetal exposure are severe, often resulting in stillbirth or neonatal infection.

At the extremes of age – neonates and the elderly – changes in both innate and acquired immunity have been observed. Numerous biomarkers of immune responsiveness have been measured in the elderly, including decreased gamma-interferon production and NK cell activity, and increased IL-4 and IL-10 production (Rink, Cakman and Kirchner, 1998;

Mbawuike et al., 1997; Di Lorenzo et al., 1999). The effects on IL-4 and IL-10 are suggestive of a predominant Th-2 versus Th-1 response. A similar imbalance, characterized by decreased gamma-interferon production and down regulation of IL-10, may occur in neonates (Lewis, Larsen and Wilson, 1986; Genovese et al., 1999). Thus, in pregnancy, as well as in elderly and neonatal immune systems, there are changes in the immune system and biomarkers can be documented in mouse models that correlate with decreased resistance. Relatively few mouse studies investigated dose-response in an oral infection model in immunocompromised mice (Czuprynski, Theisen and Brown, 1996; Golnazarian et al., 1989).

Because the experimental studies summarized above all involve highly controlled manipulation of the immune system, it is very difficult to interpret the results with respect to a highly variable human population. Furthermore, the results of studies involving knockout mice or treatment with monoclonal antibodies reflect a nearly complete abrogation of the immune parameter in question – a condition that is probably seldom the case in humans. In addition, most studies were not conducted using oral administrations of *L. monocytogenes* that might have an impact on targeted immune mechanisms locally in the gut.

2.2.2.3 Food matrix effects

Traditionally, food had been viewed as a neutral vehicle for the pathogen, and as such had little impact on dose-response relations. However, recently there has been increasing awareness of the impact that the food matrix can have on the likelihood of disease. Much of the focus has been on the impact that microbial adaptation has on the acid resistance of enteric pathogens. The stomach acts as the body's first defence against foodborne pathogens via their inactivation by the pH of gastric fluids (Gianella, Broitman and Zamcheck, 1973; Peterson et al., 1989). The key factors influencing the extent of inactivation of ingested pathogens by this barrier are the pH of the stomach, the residence time of the bacteria in the stomach, and the pathogen's inherent acid resistance. Since the inactivation of *L. monocytogenes* due to adverse pH values follows first order kinetics (Buchanan, Golden and Phillips, 1997), the extent of inactivation will also be dependent on the initial numbers of bacterial cells (i.e. dose) ingested. Exposure times of between 15 and 30 minutes were required to achieve more than a 5-log inactivation of three strains of *L. monocytogenes* in simulated gastric juice (Roering et al., 1999). Anything that reduces the contact between bacteria and gastric acid could potentially have the effect of reducing the number of bacterial cells needed to produce an infection. For example, outbreaks of salmonellosis involving water and other liquids have often been associated with low levels of the pathogen. Mossel and Oei (1975) demonstrated that a liquid bolus of less than 50 ml could pass rapidly through the stomach because the pyloric sphincter fails to constrict when challenged with such a small bolus.

Fatty food vehicles can protect bacteria from the gastric acid during passage through the stomach (Blaser and Newman, 1982). This was illustrated by an outbreak of *Salmonella* Typhimurium present in chocolate at very low levels (Kapperud et al., 1990). However, a reduced intestinal colonization and diarrhoea in rats fed milk with a high fat content as opposed to milk with a low fat content was recently reported for *L. monocytogenes* (Sprong, Hulsterin and Van der Meer, 1999), whereas *Salmonella* Enteritidis infection was apparently unaffected. *L. monocytogenes* were killed mainly in the stomach by free fatty acids and monoglycerides resulting from digestion of fat, whereas the Gram-negative cell wall was

suggested as protecting the *Salmonella* (Sprong, Hulsterin and Van der Meer, 1999). In agreement with these results, Schlech (1993) reported a lower proportion of infection in rats arising from *L. monocytogenes* grown in Brain Heart Infusion (BHI) broth and administered in milk than when administered in BHI alone, which suggested that milk might have an inhibitory effect on the number of organisms available for colonization. In mice, however, Notermans et al. (1998) reported that the ID_{50} was the same when *L. monocytogenes* was orally administered in water or in milk with 1% or 3% fat. However, given that two different animal models were used (rats and mice), the proper interpretation for humans of these findings is difficult.

Food vehicles with high buffering capacity may also protect bacteria from gastric acid, although the gastric response to exogenous buffers may be complex (Blaser and Newman, 1982). Volunteers either quickly secreted more acid to overcome the effect of the buffer or experienced a prolonged buffering effect. Volunteers with a prolonged buffering had a higher attack rate for *Vibrio cholerae* than those who overcame the effect (references cited in Blaser and Newman, 1982).

While there is a clear indication that food matrix effects could influence dose-response relations associated with *L. monocytogenes*, there are insufficient data to allow this to be considered currently as a variable within the hazard characterization.

2.2.2.4 Interaction of pathogen, host and matrix variables

Based on the observation that serovars 1/2a, 1/2b and 4b dominate among the strains isolated from human cases, whereas a wider range of serovars have been isolated from foods, it has been suggested that this is a reflection of their different potential for causing disease. Schlech (1991) suggested that the sporadic nature of outbreaks is more consistent with changes in the virulence of strains than in host susceptibility, since the population at risk may not vary greatly. In fact, an indirect vaccination due to the presence of strains with reduced or no virulence has been suggested as an explanation for the low incidence of listeriosis, despite the frequent exposure due to contaminated food (Chakraborty et al., 1994; Schwarzkopf, 1996). McLauchlin (1996), in contrast, commented that the explanation for the wide variation observed in incubation periods after oral ingestion is unknown but it may be dose dependent, strain dependent, or perhaps reflect unknown differences in host susceptibility.

One of the adaptive mechanisms in *L. monocytogenes* is its ability to develop acid resistance (Buchanan et al., 1994; Patchett et al., 1996; Phan-Thank and Montagne, 1998). An acid-tolerant mutant demonstrated an increased lethality in mice following intraperitoneal inoculation (O'Driscoll, Gahan and Hill, 1996). Conversely, a mutant that was acid-tolerant deficient had decreased lethality to mice (Marron et al., 1997). Acid adaptation was reported to lead to an enhanced resistance to a number of other environmental stresses, including heat treatment (Farber and Pagotto, 1992), lactoperoxidase (Ravishankar, Harrison and Wicker, 2000), bacteriocins (van Schaik, Gahan and Hill, 1999) and other preservatives (Lou and Yousef, 1997), and to increased survival in acidic foods (Gahan, O'Driscoll and Hill, 1996).

The effect of growth temperature on the subsequent pathogenicity of *L. monocytogenes* has been examined by several investigators. Growth at 4°C was reported to increase the virulence in mice infected by the intravenous route but not by the intragastric route (Czuprynski, Brown and Roll, 1989; Stephens et al., 1991). The effect was suggested to be dose dependent because it was observed only at levels greater than 10^4 viable

L. monocytogenes cells (Stephens et al., 1991). This suggested that there might be variants within the population with increased virulence as a result of the non-optimal growth conditions. Clinical strains were demonstrated to be more resistant to cold storage in terms of lag phase duration and the degree of pathogenicity to chick embryos compared with strains isolated from meat (Avery and Buncic, 1997). Cells grown at 10°C were less acid resistant than cells grown at 30°C (Patchett et al., 1996) and as such would be less likely to survive passage through the stomach. Differences in pathogenicity of cells grown at 5° and 10°C were not observed when the pathogen was grown in crabmeat or microbiological media (Brackett and Beuchat, 1990).

An apparent variation in the virulence of *L. monocytogenes* can also reflect a change in the health status of the host. Clinical and epidemiological investigations of an outbreak of listeriosis in 1987 in Philadelphia in the United States of America led to the suggestion that individuals colonized by *L. monocytogenes* but previously asymptomatic for listerial infection became symptomatic because of a co-infecting disease (Schwartz et al., 1989; Rocourt, 1996).

2.2.3 Approaches to modelling dose-response relations

2.2.3.1 General approaches and limitations to modelling dose-response relations for foodborne pathogens

When modelling dose-response relations, the number of microorganisms entering the digestive tract per exposure may be expressed as a mean number of functional particles of the pathogenic organism, CFU, spores, oocysts, etc. (Teunis et al., 1996; Vose, 1998). This is the dose, a quantitative measure of the intensity of the exposure. At a certain dose, certain effects in the host occur. The frequency within the exposed population of hosts at which this occurs constitutes the response. The response may be more or less well defined, but generally there will not be a one-to-one relationship between the size of the dose and the specific kind and frequency of the biological effect it produces. Furthermore, pathogenic microorganisms generally produce an array of effects or conditions within an affected host. Thus, instead of a single dose-effect relation there will be a series of dose-response relations that describe the relationship between the various biological effects and the magnitude of the dose (Teunis et al., 1996). The effects commonly considered, which are also referred to as biological end points, include infection (for *L. monocytogenes* this is often measured by the presence of bacteria in the spleen or the liver of an animal model), various forms of morbidity, or death (Vose, 1998).

The response of a human population to an exposure to a foodborne pathogen is highly variable, in terms of both the duration and the severity of the symptoms observed. The variability is a reflection of the dependency of the frequency and extent of disease on a variety of factors, such as the virulence characteristics of the pathogen, the number of bacterial cells ingested, the general health and immune status of the host, and attributes of the food that may alter microbial or host status. Thus, the relationship between the dose and the response is a function of the *L. monocytogenes* strain in terms of its virulence properties and its survival characteristics, the food in which it resides, and the susceptibility of the host. A mathematical relationship between the dose and the response would ideally be able to describe the interactions between all these factors. It is important to note that such

mathematical relations describe the dose-response relationship on a population basis and cannot describe the likelihood of illness for any specific individual.

Sources of data and general considerations

An appreciation of the factors described above is critical to a scientifically rigorous consideration of dose-response relations. Equally as important is an appreciation of the uncertainty and variability associated with the different sources of dose-response data.

Human volunteer feeding studies

The primary source of microbiological dose-response data for other pathogens has been human volunteer feeding studies. Such trials provide the most direct measure of human response to pathogens and have been the data of choice for quantitative microbial risk assessments. However, these data do have limitations that must be considered when these dose-response relations are used to estimate the susceptibility of the entire population. Volunteers for these studies have been almost exclusively limited to healthy adult males. Information on the susceptibility of higher-risk populations or potential gender effects is generally not available. Of necessity, volunteer studies are limited to foodborne diseases that are not considered life threatening for the test subjects. Thus, volunteer feeding studies are unlikely to be conducted for pathogens or diseases that are either life threatening (e.g. enterohaemorrhagic *E. coli*) or that almost exclusively affect higher-risk populations (e.g. *L. monocytogenes*). Volunteer studies have often been conducted in conjunction with vaccine trials, which tend to focus on higher dose levels. Typically, there are relatively few test subjects per dose, and because of the small size of the test population, dose levels are used that produce relatively high rates of infection or morbidity. It is usually not possible to evaluate doses that are directly pertinent to the pathogen levels most often associated with human exposures via food. Thus, most dose-response determinations rely on extrapolations of the dose-response relations based on high doses. This leads to a high degree of uncertainty at the low-dose levels. Human volunteer feeding studies are not available for *L. monocytogenes.*

Surrogate animals

Because *L. monocytogenes* primarily affects specific, high susceptibility populations, human feeding studies are ethically not feasible. Animal models have been used as the primary alternative means of studying its dose-response relations. The successful use of animal models is dependent on a number of factors, not the least of which is the need for a "conversion factor" that allows the quantitative relations observed in the animal to be correlated with human response to the pathogen. Success is highly dependent on the selection of an appropriate animal model. This can be a significant challenge with many foodborne pathogens. It assumes that the pathogen causes disease by the same mechanism of pathogenicity in both the human and surrogate animal, that the animal's physiological and immune responses are similar to that of humans, and that quantitative relationships between infectivity, morbidity and mortality are similar for the two species. Further, animal feeding studies have many of the same limitations as human volunteer studies. For example, most studies are conducted using only healthy animals that are similar in age and weight. In fact, most laboratory animals are so highly inbred that genetic diversity among the animals is negligible. This reduces the variability associated with the testing but brings into question the data's applicability to the general population.

While the disease characteristics of *L. monocytogenes* have been examined in a wide range of animals, the primary animal model for dose-response studies with this microorganism has been the mouse, with death being the primary biological end point measured. Care must be taken in reviewing these studies since the dose-response relations vary substantially depending on both route of entry and the variety of mouse employed as the surrogate for humans. This caution is reinforced by the recent finding, discussed in the section on virulence of *L. monocytogenes*, that the mouse may not be an appropriate model for study of at least some aspects of human listeriosis (Lecuit et al., 1999).

Epidemiological approaches

Potentially, epidemiological investigations could be a source for human dose-response information, particularly for outbreaks involving RTE foods that do support the growth of the pathogenic bacterium. However, to be useful for risk assessments, the investigations would have to be expanded beyond their usual scopes. In addition to detailed information about who became ill, the investigations also have to acquire information about a variety of other factors such as who consumed the food and did not become ill, the amounts of food consumed by both groups, and the frequency and extent of contamination. Regrettably, few epidemiological investigations have been conducted in a manner that provided such data.

An alternative approach for pathogens that are not appropriate for human volunteer feeding studies have been suggested by Buchanan et al. (1997). These authors proposed using data on the annual national incidence for a disease, and food survey data on the frequency and extent of contamination of an RTE food, to produce an estimate of the microorganism's dose-response relationship. Assuming that all cases of listeriosis were due to a single food, this approach was used to generate a conservative estimate of the dose-response relations for *L. monocytogenes* in higher-risk populations.

Mathematical models

The relation between ingestion of a certain number, N, of a pathogenic microorganism and the possible outcomes has been quantitatively described in a number of ways (Table 2.5).

Models may be classified or distinguished in different ways. Depending on the assumptions and parameter values chosen, some models may be special cases of other models (Haas, 1983; Holcomb et al., 1999). One important distinction is between models describing infection as a deterministic or a as stochastic process (Haas, 1983). The deterministic view assumes that for each microorganism there is an inherent minimum dose, i.e. there is a threshold level, below which no response is seen. Thus, in a deterministic threshold model, the risk below the threshold is zero. However, the threshold level (i.e. the minimum dose) may potentially vary across individuals in a population. If this variation is incorporated into the model it becomes a stochastic model. The stochastic view on infection holds that the actions of individual cells of pathogenic microorganisms are independent from other cells and that a single microorganism has the potential to infect and provoke a response in the individual, i.e. a single-hit, non-threshold model (Haas, 1983).

Table 2.5 Summary of some dose-response models used for foodborne pathogens. The table is adapted and modified from Holcomb et al. (1999) and other sources.

Model name	Function Probability (P) =	Parameter definitions	Comments and sources
Log-Normal	$\phi[b_0 + b_1 * \log_{10}(N)]$	ϕ = cumulative normal distribution function b_0 = intercept b_1 = log10(dose) slope parameter	[1]
Log-Logistic	$\beta/1+[(1-p)/p] * e^{-\varepsilon\{\log_{10}(N) - \chi\}}$	β = Asymptotic value of probability of infection as dose approaches ∞. β =1 in Holcomb et al. (1999). χ = Predicted dose at specified value of p where p = P ε = Curve rate value affecting spread of curve along dose axis	[2]
Simple Exponential	$1- e^{- r*\log_{10}(N)}$	r = Reflects host/microorganism interaction probability	[3] Note (1)
Flexible Exponential	$\beta * [1-p*e^{-\varepsilon\{\log_{10}(N) - \chi\}}]$	β = Asymptotic value of probability of infection as dose approaches ∞. β=1 in Holcomb et al. (1999). χ = Predicted dose at specified value of p where p = 1 – P ε = Curve rate value affecting spread of curve along dose axis	[2]
Beta-Poisson	$1 - (1 + N/\beta)^{-\alpha}$	α, β = Parameters affecting the shape of the curve	[4] Note (2)
Beta-Binomial	$1-(1-P_I(1))^N$	$P_I(1)$ = probability of illness from exposure to one organism. $P_I(1)$ assumed to be Beta(α, β) distributed	[5]
Weibull-Gamma	$1 – [1 + (N)^b/\beta]^{-\alpha}$	α, β, b = parameters affecting shape of curve	[6]
Gompertz	1 – exp[-exp(a + bf(x))]	a = model (intercept) parameter; b = model (slope) parameter; f(N) = function of dose.	[7]

KEY: N = Ingested dose of microorganisms; P = Probability of infection.

NOTES: (1) Rose, Haas and Regli (1991) used the form $1- e^{- r *dose}$.
(2) See Vose (1998) for a discussion on the interpretation of α, β.

SOURCES: [1] Dupont et al., 1972. [2] Levine et al., 1973. [3] Rose, Haas and Regli, 1991. [4] Haas, 1983. [5] Cassin et al., 1998. [6] Todd and Harwig, 1996. [7] Coleman and Marks, 1998.

Models can also be differentiated on the basis of whether they are mechanistic or empirical. Buchanan, Smith and Long (2000) suggested that most dose-response models used currently are empirical and are limited because they attempt to extrapolate beyond the limits for which there are data. Potentially, mechanistic models would be more flexible since they focus on specific physiological or chemical attributes; however, there have been few attempts to develop such models. Buchanan, Smith and Long (2000) encouraged the development of mechanistic dose-response models, and outlined a simple three-compartment dose-response model for foodborne salmonellosis. The model compartments were survival in the stomach; attachment and colonization in the intestine; and invasion of body tissues or production of toxins. No mechanistic dose-response models are currently available for *L. monocytogenes*.

Threshold models assume a minimum threshold dose before the response occurs. In a given population, the variation in the minimal dose can be described by a distribution. For instance, the Log-Normal model (Probit model) assumes that the minimal dose is

lognormally distributed (Haas, 1983), and the Log-Logistic model assumes that $\log_{10}$ of the infectious dose follows a logistic distribution (Holcomb et al., 1999).

Threshold models

Marks et al. (1998) compared a Beta-Poisson model with a combined Beta-Poisson model that also employed a threshold level (3 bacteria) in a risk assessment for *E. coli* O157 in hamburgers. The introduction of a threshold means that, at low doses, the location of the dose-response curve is shifted along the x-axis by the threshold amount. The differences between these models were significant only in the low dose range. The resulting estimates of risk were 100- to 1000-fold larger, depending on cooking temperature, using the non-threshold model. The authors concluded that the two-parameter Beta-Poisson model appeared insufficient for describing the complexity of dose-response interactions and that it was inadequate as a default model for microbial risk assessment, especially in cooked foods (Marks et al., 1998). They also concluded that the consideration of threshold models as alternative dose-response models is of great importance and that additional research was needed in this area.

Stochastic – single-hit models

Other researchers have favoured the use of single hit models, which in many instances have described data quite well (Haas, 1983; Teunis et al., 1996). For instance, dose-response data for protozoan parasites can be well described by the exponential model (Teunis, 1997), and bacterial infection data are generally well described by Beta-Poisson models (Teunis, 1997; Teunis, Nagelkerke and Haas, 1999), or by the Weibull-Gamma model (Holcomb et al., 1999). The same model may not be equally effective for all biological end points caused by the pathogen. For example, the FDA/FSIS *L. monocytogenes* risk assessment reported that the exponential model did not fit mouse infection data (i.e. isolation of *L. monocytogenes* from the spleen and liver), but was among the best models for describing the relationship between dose and the frequency of death (FDA/FSIS, 2001).

Exponential model

In the derivation of this model it is assumed that all of the ingested organisms have the same probability, r, of being individually capable of causing an infection to a specific consumer. Further, the probability of a single-hit, r, is independent of the size of the inoculum. Then the probability of infection after ingesting N organisms is the probability of one or more hits:

$$P_{inf} = 1 - (1-r)^N$$

Assuming that the distribution of organisms follows a Poisson process, with a mean number of organisms N per portion, the exponential dose-response relation follows (Haas, 1983; Vose, 1998):

$$P = 1 - \exp^{-r*N}$$

In a few cases, notably for the pathogenic protozoa *Cryptosporidium parvum* and *Giardia lamblia*, this model provides an acceptable fit, but the slope of the model is generally steeper than what is observed from data (Teunis et al., 1996). Holcomb et al. (1999) modified the form of the exponential model and termed it the Simple Exponential model by using the $\log_{10}$ of the dose instead of the dose directly (Table 2.5), the reason being that the Simple Exponential model fitted more of the investigated data sets. Holcomb et al. (1999) also used a

model similar to the Simple Exponential model, which they termed the Flexible Exponential model (Table 2.5). According to the authors, the benefit of this model was that it could be applied to experimental data where doses much greater than 1 still resulted in zero percent infection.

Beta-Poisson model

In this model, heterogeneity in the microorganism–host interaction is introduced. The r-value, the probability of an organism initiating infection given a successful introduction into the host, is assumed to follow a Beta-distribution. Haas (1983) suggested that this variation reflected the variation in virulence of the individual pathogens or in the sensitivity of the host, or both. In contrast, Vose's (1998) interpretation was that the Beta-distribution characterized by its α and β values describes the expected probability of each of the consumed microorganisms causing infection, averaged over all volunteers.

A complex function results from the derivation of this model. However, assuming that β is much larger than both α and 1, the following approximation can be used:

$$P = 1- [1 + N/\beta)]^{-\alpha}$$

In some cases the use of this model to fit dose-response data has not fulfilled this condition (Teunis et al., 1996). Initially the authors proposed using an approximated function in all cases because the influence of using the more rigorous function was considered relatively insignificant. However, Teunis and Havelaar (2000) recently showed that the discrepancies between the models were largest in the low-dose region, which is the region of interest for many risk applications, and that errors may become very large in the results of uncertainty analysis or when the data contain little low-dose information. Vose (1998) criticised using α and β just as fitted parameters without any consideration of their interpretation in the beta distribution. For instance, values between 0 and 1 of these parameters mean that the distribution for the probability of infection will peak at both 0 and 1. This could be interpreted as a partition among volunteers into susceptible and non-susceptible populations. Teunis et al. (1996) concluded that the Beta-Poisson model appears to fit most available dose-response data well and has the desired property of being conservative when extrapolated to low doses.

Beta-Binomial model

Cassin et al. (1998) developed a Beta-Binomial dose-response model to assess the risk of *E. coli* O157:H7 in hamburgers. The model reflected the same assumptions used in the original Beta-Poisson model. However, the Beta-Binomial model yields variability for probability of illness from a particular dose in contrast to the original model, which only specifies a mean population risk.

$$P = 1- (1-P_I(1))^N$$

$P_I(1)$ is the probability of illness from ingestion of one microorganism, and this probability was assumed to be Beta-distributed with parameters α and β. By fitting the model to data from human feeding studies with *Shigella*, it was possible to generate a dose-response curve showing the estimated uncertainty in the average probability of illness verses the ingested dose. The variability between feeding studies was used as a proxy for the uncertainty in the parameters α and β.

Weibull-Gamma model

This model was chosen by Farber, Ross and Harwig (1996) because of its flexibility, i.e. it is possible to accommodate the available qualitative dose-response information for *L. monocytogenes* and to adapt to both healthy and higher-risk groups. The starting point for the derivation is the Weibull model:

$$P = 1 - e^{-a*N\wedge b}$$

where N is the dose ingested and *a* and *b* are parameters. The parameter *a* is related to the probability of illness given exposure to a single organism and *b* determines the shape of the individual dose-response curve. In this model, host–pathogen heterogeneity is considered by assuming that *a* follows a Gamma distribution characterized by the parameters α and β. The resulting equation, the Weibull-Gamma model becomes:

$$P = 1 - [1 + (N^b)/\beta]^{-\alpha}$$

Depending on the parameter values, the Weibull-Gamma model can be reduced to both the Beta-Poisson and the Log-Logistic models (Farber, Ross and Harwig, 1996; Holcomb et al., 1999).

Gompertz model

Recognizing that a number of empirical models may fit observed data adequately, Coleman and Marks (1998), in addition to Logistic and Beta-Poisson models, used a Gompertz model to describe the results of human feeding studies:

$$P = 1 - \exp[-\exp(a + bf(N))]$$

where *a* is a model (intercept) parameter, *b* is a model (slope) parameter, and f(N) is a function of dose.

Choice of dose-response model

The issue of which functional form truly describes reality, i.e. the interactions between the pathogen, the food vehicle and the host, remains an open question needing additional research. For example, an equally good fit for *Shigella* dose-response data was provided by a Gompertz function as by the Beta-Poisson model. However, outside the data range, the predictions differed greatly (Marks et al., 1998). The choice of dose-response model may depend on its applicability, e.g. how well it fits the available data, the simplicity of the model in relation to parsimony in the number of parameters used, and the range of conditions over which the model gives good predictions (Holcomb et al., 1999). Holcomb et al. (1999) emphasized the flexibility of the dose-response model to fit data from different organisms, thereby allowing direct comparisons of infectious doses for use in risk assessment. In their comparison of how well the models in Table 2.5 fitted different experimental data, they reported differences of up to nine orders of magnitude in the predicted dose affecting the most sensitive 1-percentile of the population. This illustrates the difficulty of extrapolating from high to low doses. They also concluded that the three-parameter Weibull-Gamma model was the only model capable of describing all data sets. However, this flexibility is achieved through the use of three parameters, which generally increases the extent of uncertainty in the prediction.

Although results of dose-response experiments fit a single-hit model well, e.g. the Beta-Poisson model, some serious shortcomings have been noted (Teunis, 1997; Teunis, Nagelkerke and Haas, 1999). The models ignore the incubation period and there is no opportunity for generalization with regard to microorganism, host and vector. Furthermore, the experimental evidence suggests that the probability of illness changes with dose in a manner that differs from that of the probability of infection. For instance, a decrease in the probability of illness was noted with a higher dose of *Campylobacter jejuni* (Black et al., 1988). Teunis, Nagelkerke and Haas (1999) developed a hazard function for the probability of illness, given successful infection, occurring in the time between onset and clearing of the infection. The duration of the infection period was assumed to follow a Gamma distribution. The scale parameter, λ, representing the time scale for the primary events responsible for clearing the infection (a Poisson process) was the authors' primary choice for dose dependence. Three possible scenarios were modelled: increased likelihood of illness with dose; decreased likelihood of illness with dose; and the likelihood of illness being independent of dose. Examples of each of these possible scenarios were illustrated using volunteer data from the literature. The different alternatives were suggested to reflect the balance of the interactions between the pathogen and the host (Teunis, Nagelkerke and Haas, 1999).

In their risk assessment of *L. monocytogenes*, FDA/FSIS (2001) assumed that there is no *a priori* means of determining which is the "correct" model to fit to a data set. Accordingly, they employed an alternative approach, namely that of fitting several of the dose-response models described above to dose-response data. An integrated dose-response relationship was then derived by combining the individual dose-response curves after weighting for how well each model fitted the data and for the parsimony of each model. The differences in the response value at any single dose that were predicted by the individual models were used as a means of estimating the uncertainty related to model selection.

2.2.3.2 Listeria monocytogenes *dose-response models developed from epidemiological data and expert elicitations*

The models of Farber, Ross and Harwig (1996) and of Bemrah et al. (1998)

Citing the lack of volunteer feeding studies and the tenuous extrapolation of animal data to the human situation, Farber, Ross and Harwig (1996) evaluated different dose-response models to determine which had the flexibility to use qualitative data. They proposed the Weibull-Gamma model as having advantageous attributes. As a means of demonstrating how the model would be used, they estimated, based on the literature (Farber and Peterkin, 1991; McLauchlin, 1993), that the ID_{10} and ID_{90} for *L. monocytogenes* are 10^7 and 10^9 CFU for healthy adults and 10^5 and 10^7 CFU for high-risk individuals, respectively. Farber, Ross and Harwig (1996) used this dose-response relationship to estimate the probability of illnesses based on data for both the consumption of soft cheeses and their contamination by *L. monocytogenes*. However, in so doing they did not clearly define the case definition for infection and assumed that all individuals that become infected also become symptomatic. This is an assumption that is not supported by work with surrogate animals. The results did demonstrate how these techniques could be used to develop quantitative microbial risk assessments. However, the study also demonstrated that care must be taken in ensuring that the advice of experts provides estimates of dose-response relations that are realistic in terms of the incidence of disease in the population and are consistent with the biological end point

of concern. This initial estimate of the dose-response relationship is generally considered to predict substantially higher rates of illness than actually occur in human populations. Farber, Ross and Harwig (1996) also assumed that only 1% to 10% of *L. monocytogenes* strains are pathogenic, an assumption that is not in keeping with the observations described earlier, namely that most isolates are pathogenic.

Based on the work of Farber, Ross and Harwig (1996), Bemrah et al. (1998) used the Weibull-Gamma model for the dose-response relations for the risk assessment of human listeriosis from the consumption of soft cheese made from raw milk. They used values of $\alpha = 0.25$ and $b = 2.14$ for both the general population and the more highly susceptible population, and β values of $10^{15.26}$ and $10^{10.98}$ for these two groups, respectively. Based on these parameter values, the doses that would lead to 50% of the general and of the more highly susceptible populations becoming ill would be 48 000 000 CFU and 480 000 CFU, respectively.

The models of Buchanan et al. (1997) and of Lindqvist and Westöö (2000)

Epidemiological investigations of listeriosis outbreaks have not generally been useful in elucidating dose-response relations. This is because the levels of *L. monocytogenes* in the suspect food and the percentage of the individuals that consumed that food but that did not become ill are seldom quantified adequately. As an alternative approach to using epidemiological data, Buchanan et al. (1997) explored whether a purposefully conservative dose-response relation for *L. monocytogenes* could be developed using the annual incidence of listeriosis in combination with food survey data. Taking advantage of the fact that the exponential model is a single-parameter model, as discussed above, they used data for the incidence of listeriosis in Germany and the levels of *L. monocytogenes* in smoked fish as a means of deriving the r-value for the exponential model. Based upon *L. monocytogenes* prevalence and food consumption data, smoked fish was the likely source for most of the illnesses in Germany. They further assumed that symptomatic cases of listeriosis were largely restricted to that portion of the population that was immunocompromised. Based on this, Buchanan et al. (1997) estimated that the dose that would be expected to produce severe illnesses in half of a population of immunocompromised individuals was 5.9×10^9 CFU, based on a r-value of 1.179×10^{-10}. The validity of this approach relies on several assumptions, including the percentage of individuals susceptible to severe *L. monocytogenes* infections, the uniformity of consumption patterns, the suitability of the exponential model to describe the pathogen's dose-response relationship in humans, and the accuracy of the statistics on the annual rate of severe listeriosis cases. The model was purposefully precautionary in relation to each of its underlying assumptions. However, the approach has several advantages, including "anchoring" the dose-response relationship to values that are based upon observed incidences of disease and using data based on the entire population instead of the small sample in human volunteer studies.

Using data for the consumption of smoked fish in Sweden and the incidence of listeriosis in that country, Lindqvist and Westöö (2000) used a similar approach to derive an r-value for *L. monocytogenes*. They reported an r-value of 5.6×10^{-10} for the 20% of the population at greater risk of listeriosis. Lindqvist and Westöö (2000) subsequently compared the exponential model of Buchanan et al. (1997) and the Weibull-Gamma model of Farber, Ross and Harwig (1996), assuming that this product was the primary source of listeriosis in that population. If all *L. monocytogenes* were assumed to be equally pathogenic, the exponential

model predicted 168 cases and the Weibull-Gamma predicted 95 000. The reported number of cases per year in Sweden was 37. If it was assumed that only 1% to 10% of the *L. monocytogenes* isolates were pathogenic, the predicted number of cases was 9 and 5200 for the exponential and Weibull-Gamma models, respectively.

Evaluation of outbreak data

While epidemiological investigations of listeriosis outbreaks have generally been insufficient to allow calculation of dose-response relations or even attack rates, the US FDA/FSIS *L. monocytogenes* risk assessment (FDA/FSIS LMRA) team (FDA/FSIS, 2001) did acquire sufficient information related to two outbreaks that permitted a more detailed evaluation. These were the outbreak associated with Hispanic-style cheese that occurred in the United States of America in 1985, and the outbreak associated with butter among patients at a hospital in Finland in 1998–99. These evaluations and similar considerations of outbreaks of febrile gastroenteritis were used in the current document to estimate dose-response curves using the exponential model.

FDA/FSIS LMRA estimates for attack rates and dose ranges: Pregnant females and United States of America Hispanic-style cheese outbreak

Archival data from the Hispanic-style cheese outbreak in Los Angeles County, California, in 1985 were re-examined to determine if the attack rate and dose range could be estimated. The original report did not contain information on the amount consumed by individuals or the attack rate. Fortunately, consumption data by individuals had been collected and records from the outbreak were saved such that an attack rate could be estimated (FDA/FSIS, 2001).

The strategy used to estimate the dose-response for pregnant females was to assume a very high percent of pregnant Hispanic females ate the implicated cheese. Using the outbreak odds ratio table, the number of controls that were exposed to the implicated food was derived. This number of exposed controls, divided by the total number of controls results in a quotient (Q = 11/31 = 35%) that is an estimate of the proportion of the population that consumed the implicated food. The population giving rise to the cases was identified from the outbreak data and the common feature for cases and controls in this outbreak was pregnancy in Hispanic females. The total number of pregnant Hispanic females within the cheese marketing area during the time interval of interest provides (P). The proportion (Q) of the population that consumed the implicated food was multiplied by the total number of people (P), and this product, $Q \times P$, is an estimate of the number in the population of interest that ate the implicated food.

Laboratory data provided the total number of food samples qualitatively tested (T = 85) and the number of samples that were positive (T+ = 22). An estimate of the proportion of food contaminated was obtained by dividing the number of positive tests by the total number of tests (T+/T = 22/85 = 0.26). Multiplication of $(T+/T) \times Q \times P$ ($0.26 \times 11\,775 = 3061$) provided an estimate of the total number of exposed persons in the population. Based on 21 cases answering a questionnaire on the frequency and amount of Hispanic cheese consumed, an average of 219 servings during the critical time of 20 weeks for a pregnant woman was estimated (R.C. Whiting, pers. comm., 2001). There were several brands of Hispanic cheese on the market. Assuming that 50% of the servings were the outbreak brands, an average of 110 servings of the implicated brand of cheese were consumed per person. From the total number of exposed persons and the number of servings, the attack rate was estimated. A

second estimate of T+/T was based on 56 qualitatively-positive samples of 665 samples tested (56/665 = 0.084).

The cases caused by the implicated food were defined as those cases infected with the outbreak phage type. The estimated attack rate then equalled the number of cases that were infected with the outbreak phage type divided by the total number of exposed persons in the population. The proportion of actual cases that were identified during the outbreak was then estimated. Using this strategy, the estimated attack rate (i.e. the percentage of exposed pregnant women (or their fetuses) who became cases during the pregnancy) during the Hispanic-style cheese outbreak was between a low of 2.1–2.7% and a high of 6.4–8.5% pregnant Hispanic females with the epidemic phage type. Sample calculations used by the FDA/FSIS LMRA are shown in Table 2.6.

The dose of microorganisms consumed was based on the most likely consumption of cheese for each case, multiplied by the estimated number of organisms (CFU/g) of food. From the outbreak records, the estimated one-day consumption of the implicated cheese by 39 of 63 pregnant Hispanic females infected by the epidemic phage of *L. monocytogenes* serotype 4b ranged from 0.5 ounces/day [15 g/day] to 21 ounces/day [650 g/day] (median about 5.5 ounces/day [170 g/day]). In addition to reporting consumption for one day, about 38% of the females reported their usual consumption of cheese for more than one day. Contamination of the cheese by *L. monocytogenes* has been reported to be 10^3 to 10^4 *L. monocytogenes* CFU/g (NACMCF, 1991) and 140 000 to 500 000 *L. monocytogenes* CFU/g (Ryser and Marth, 1999). Thus, about 2.1–8.5% of pregnant Hispanic females that consumed between 1.5×10^4 and 5.0×10^7 *L. monocytogenes* serotype 4b organisms in a single day became ill. The effect of cumulative doses on the attack rate and pathogenesis was not estimated.

Table 2.6 FDA/FSIS LMRA (2001) estimates of attack rates and dose ranges: Pregnant women and United States of America Hispanic-style cheese outbreak.

Hispanic births (January – June, 1985), LA County	33 628
Hispanic fetal and neonatal deaths (January – June, 1985)	+ 350
Proportion of multi-gestational births (1%)	- 336
Population giving rise to cases (Total Hispanic pregnant females, January – June, 1985)	33 642
Total Hispanic pregnant females that ate the implicated cheese (based on an estimate that 35% of controls ate the implicated cheese)	11 775
High estimate of Hispanic pregnant females that ate contaminated cheese (based on an estimate of 26% product contamination × 11 775)	3 061
Average number of servings consumed	110
Total listeriosis cases among Hispanic pregnant females	81
Cases with outbreak phage type	63
Attack rate if all cases were identified (63/(3 061 × 110))	1.9×10^{-4}
Attack rate if 75% of cases identified (63/(3 061 × 110))/0.75	2.5×10^{-4}
Low estimate of Hispanic pregnant females that ate contaminated cheese (based on an estimate of 8.4% product contamination × 11 775)	989
Total listeriosis cases among Hispanic pregnant females	81
Cases with outbreak phage type	63
Attack rate per serving if all cases were identified (63/989 × 110)	5.8×10^{-4}
Attack rate per serving if 75% of cases identified (63/989 × 110)/0.75	7.7×10^{-4}

FDA/FSIS LMRA estimates for attack rates and dose ranges: Immunocompromised individuals and Finnish butter outbreak

The strategy used to calculate the Finland butter outbreak attack rate and dose range was similar to the strategy described above to calculate the attack rate and dose range for the Hispanic-style cheese outbreak (FDA/FSIS, 2001). Between December 1998 and February 1999, an increase in cases of listeriosis due to *L. monocytogenes* serotype 3a in Finland was recognized (O. Lyytikäinen, pers. comm., 1999; Lyytikäinen et al., 2000; Maijala et al., 2001). Review of national laboratory surveillance data from 1 June 1998 to 31 March 1999 identified a total of 25 *L. monocytogenes* serotype 3a cases, including six deaths. Cases of listeriosis were identified by cultures of blood, cerebrospinal fluid and samples from other sterile sites. Most of the cases were haematological or organ transplant patients. The median age of cases was 53 years (range 12–85). There were ten males and no pregnant females or newborns. The median hospital stay within 70 days prior to positive *Listeria* culture was 31 days for cases, and 10 days for controls.

Butter was implicated, and the isolates of *L. monocytogenes* serotype 3a from the butter and from 15 of the cases were indistinguishable. In the tertiary-care hospital where most cases occurred the implicated butter brand was the only brand consumed during the outbreak period. The hospital is the only site for organ transplantation and is also where most bone marrow transplants are performed. The total number of butter samples obtained at the hospital kitchen was 13 (pooled samples of 7-g packages), with an additional 139 samples (packages of 25 kg, 500 g, 7 g and 10 g) obtained from the dairy and from retail outlets. In addition, there were three estimates of the proportion of product contamination. There were 13 positives in 13 samples from the hospital kitchen (100%), and 4 positives in 5 samples (80%) and 3 positives in 5 samples (60%) from the retail outlets. In all positive hospital kitchen samples, the number of *L. monocytogenes* was <100 CFU/g (range 7–79). One wholesale supplier butter sample from a 7-g package contained 11 000 CFU/g.

It was possible to estimate butter consumption for five patients. The estimated consumption was divided by 31 days (median hospital stay) to estimate daily butter consumption. To determine the most likely dose range, the minimum butter consumption (1.1 g/day) was multiplied by the minimum contamination level for the hospital kitchen samples (7 CFU/g), and the maximum butter consumption (55 g/day) was multiplied by the maximum contamination level for the hospital samples (79 CFU/g). Using quantitative levels from the hospital samples, the consumed dose would be 8×10^0 to 4.3×10^3 CFU/day. If the maximum contamination level found in the wholesale samples (11 000 CFU/g) was the contamination actually consumed by those who became ill, then the daily dose consumed would range between 1.21×10^4 and 6.05×10^5 CFU/day.

Table 2.7 shows the attack rate calculations for the 1999 Finland butter outbreak. Approximately 6.4–10.7% of the haematological and transplant patients at the hospital that consumed between 8×10^0 and 4.3×10^3 *L. monocytogenes* serotype 3a organisms in a single day developed listeriosis. It is assumed that hospitalized patients ate 2.5 servings per day of the implicated butter on each of 31 days (median hospital stay), implying a total of 77.5 servings while hospitalized. The majority of the illnesses were associated with severe symptoms. The effect of cumulative doses on the attack rate and pathogenesis was not estimated.

Table 2.7 FDA/FSIS LMRA calculation of attack rate for an outbreak of *Listeria monocytogenes* serotype 3a infections from butter in Finland for haematological and transplant patients.

Annual number of new diagnoses for acute leukaemias or lymphomas plus annual number of kidney or liver transplants performed at the hospital.	410
Total persons at risk (time interval × annual new diagnoses; time interval was June 1998 to February 1999 = 9/12 months)	308
Estimated number of haematological and transplant patients in the population that ate the butter (proportion of controls that ate implicated butter, 76%)	234
Average number of servings during hospital stay	77.5
Number of cases during the outbreak	25
Number of cases with the same phage type	15
High estimate of product contamination (100%)	
Total number of contaminated servings ($1 \times 234 \times 77.5$)	18 135
Attack rate per serving (15/18135)	8.3×10^{-4}
Mid-estimate of product contamination (80%)	
Total number of contaminated servings ($0.8 \times 234 \times 77.5$)	14 508
Attack rate per serving (15/14 508)	1.0×10^{-3}
Low estimate of product contamination (60%)	
Total number of contaminated servings ($0.6 \times 234 \times 77.5$)	10 881
Attack rate per serving (15/10 881)	1.4×10^{-3}

SOURCE: FDA/FSIS, 2001.

Dose-response relation for L. monocytogenes *based on attack rates from the Hispanic-style cheese and butter outbreaks*

While the attack rate calculations for the Hispanic-style cheese and butter outbreaks were not used to generate a dose-response model in the FDA/FSIS LMRA (FDA/FSIS, 2001), for this risk assessment an attempt was made to use that information to estimate a relationship. For the Hispanic-style cheese outbreak, it was assumed that (1) the attack rate was the average of the estimates (4.5×10^{-4}) (see Table 2.6); (2) the contamination level was the higher estimate of 5×10^5 CFU/g; and (3) a median portion size was 34 g (ca 1 ounce). These dose and response values were then used in combination with the exponential model. The derived r-value was 2.6×10^{-11}. This r-value, in turn, leads to an estimate that if a dose of 1×10^6 CFU was consumed by a population of pregnant women, 0.0026% of their perinates/neonates would acquire listeriosis ($P = 2.6 \times 10^{-5}$). The FDA/FSIS LMRA team (FDA/FSIS, 2001) used a significantly more sophisticated dose-response model (see section 2.3.3.4 below). Their dose-response curve estimated that a 1×10^6 dose would lead to an attack rate of 1.6×10^{-6} for neonates (or 4.0×10^{-6} for all pregnancy-associated cases). These dose and attack rate values would yield an r-value of 4.0×10^{-12}. Considering the uncertainties regarding the numbers of *L. monocytogenes* that were consumed, the one-log difference between the calculated r-values from the Hispanic cheese outbreak and the FDA/FSIS model suggests the models were in reasonable agreement.

In the Finnish outbreak, the estimated attack rates varied between 8.3×10^{-4} and 1.3×10^{-3}, with a median of 1.0×10^{-3}. The estimated dose ranged from 8×10^0 to 4.3×10^3 CFU (hospital samples), and 1.21×10^4 to 6.05×10^5 CFU (wholesale samples, see earlier). Obviously, the large uncertainty in the estimation of the dose consumed leads to a large uncertainty in the estimated r-value for the exponential model. It was assumed that the attack rate was 1.03×10^{-3} (median attack rate), and the dose was 8.2×10^3 CFU (the median of the

dose range values). The derived r-value was 3.15×10^{-7}, which leads to an estimate that if a dose of 1×10^{6} CFU were consumed by haematological and organ transplant patients, 27% of them would acquire listeriosis.

Although the virulence of this strain has not been compared to other strains, the susceptibility of these patients to listeriosis is clearly greater than other groups. An estimate based on French epidemiological data of relative risk of transplant patients versus people less than 65 years old with no immunosupression was 2854 to 1. Estimates based on the FDA/FSIS data for the relative risk for immunocompromised persons within the intermediate age group versus the rest of the intermediate age group was 1584 to 1.

Dose-response relation for L. monocytogenes *based on attack rates from two outbreaks of febrile gastroenteritis among immunocompetent individuals*

- Chocolate milk - United States of America: Gastroenteritis in healthy adults

  An outbreak of gastroenteritis and fever occurred among persons who had attended a picnic in Elizabeth, Illinois, in the United States of America in 1994 (Dalton et al., 1997; Proctor et al., 1995). By both epidemiological and laboratory findings, the outbreak was linked to consumption of contaminated chocolate milk. None of those attending the picnic were reported to have an underlying chronic illness or immunodeficiency. Forty-five of the 60 people (75%) who consumed chocolate milk at the picnic reported illness that met the case definition, compared with none of the 22 people who did not drink chocolate milk. Nine other people who consumed chocolate milk had an illness in the week after the picnic that did not meet the case definition. This indicates that the attack rate was between 75% and 90%.

  Based on laboratory investigations of milk containers and the estimated consumption, the median dose was estimated to be 2.9×10^{11} CFU per person.

- Maize-tuna salad – Italy: Febrile gastroenteritis in immunocompetent students and staff

  Of those interviewed, 72% reported symptoms and 18.6% of those individuals had been hospitalized. The symptoms included headache, abdominal pain, diarrhoea, nausea, vomiting and joint and muscular pain, but no sepsis or deaths were reported. The investigation indicated that sweet corn and tuna salad were associated with the highest relative risk. The food-specific attack rate for sweet corn and tuna salad was reported to be 83.9%.

  The level of *L. monocytogenes* contamination in the salad was $>10^{6}$ CFU/g (Aureli et al., 2000). No estimate of consumption among the students was reported. Assuming that the average consumption was 100 g of sweet corn-tuna salad and the level of *L. monocytogenes* was 10^{6} CFU/g, the estimated dose becomes 10^{8} CFU.

The estimated ingested dose and attack rates of these outbreaks were then used in combination with the exponential model. The derived r-values were estimated to be 5.8×10^{12} (chocolate milk) and 1.8×10^{-8} (sweet corn-tuna salad), respectively.

Using the r-values described above, the exponential model dose-response curves derived from epidemiological data are shown in Figure 2.1 and compared with the Weibull-Gamma model developed using expert estimates for the low risk group (Farber, Ross and Harwig, 1996). It should be emphasized that while the end-points in these relationships are the same,

namely morbidity, they are based on data reflecting a wide range of symptoms in terms of severity.

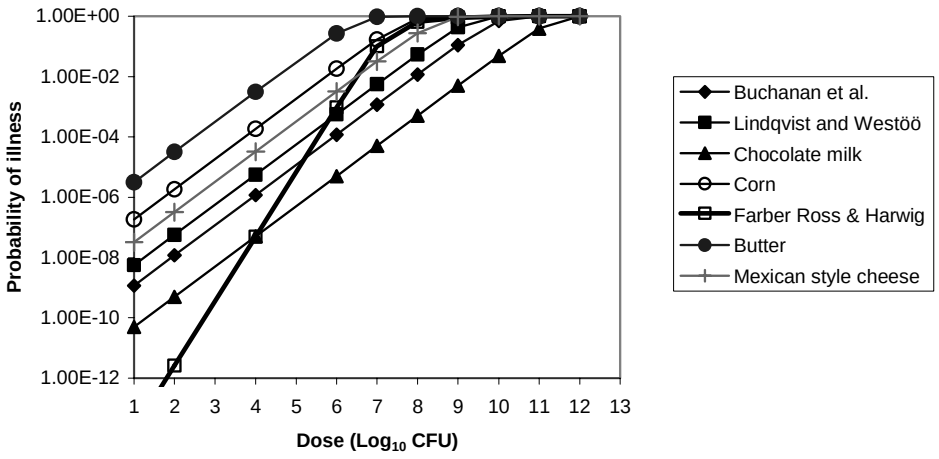

Figure 2.1 A comparison of the dose-response curves for morbidity derived from epidemiological data or expert elicitations. The models include outbreaks where the primary symptoms included serious illness (smoked fish: Buchanan et al., 1997; smoked fish: Lindqvist and Westöö, 2000, and Farber, Ross and Harwig, 1996; butter, current study), perinatal/neonatal infections (Hispanic-style cheese, current study), and febrile gastroenteritis (sweet corn-tuna salad and chocolate milk, current study).

2.2.3.3 Listeria monocytogenes *dose-response models developed from data derived from surrogate pathogens or surrogate animals*

Surrogate pathogens

L. monocytogenes is unusual among foodborne pathogens in terms of its pathogenicity, susceptible populations and clinical manifestations. As such, there is no microorganism that can serve as a surrogate for *L. monocytogenes* in relation to its ability to cause disease. The genus *Listeria* is differentiated into two very closely related species, *L. monocytogenes* and *L. innocua*. The differentiation is based on the ability to produce listeriolysin, a protein that causes lysis of red blood cells. This toxin is considered a key virulence determinant for *L. monocytogenes*. The original goal of the taxonomic scheme was to differentiate between pathogenic and non-pathogenic isolates. However, listeriolysin does not appear to be the only difference between these species, as shown in the recent comparison of the genomes of *L. monocytogenes* and *L. inoccua,* which indicated the presence of 270 and 149 strain-specific genes, respectively (Glaser et al., 2001). *L. innocua* is non-pathogenic and as such has been used extensively as a surrogate microorganism to study the growth and survival characteristics of *L. monocytogenes* in foods.

Surrogate animal data

Various feral, domestic and laboratory animals are susceptible to infections by *L. monocytogenes*, but rabbits, mice and rats have been used most extensively since these animals die within 1 to 7 days following intravenous (IV) or intraperitoneal inoculation (McLauchlin, 1997). Using these inoculation routes, the LD_{50} for mice ranged from 10^2 to 10^9 CFU (Table 2.8), depending on the strain of *L. monocytogenes*, the strain of mouse and the route of inoculation (Audurier et al., 1980; Mainou-Fowler, MacGowan and Postlethwaite, 1988; Golnazarian et al., 1989; Notermans et al., 1998).

Mice are susceptible to oral infection although the LD_{50} ratios for oral and intraperitoneal administration are not consistent among different strains (Pine, Malcolm and Plikaytis, 1990). However, the results of Lecuit et al. (1999) indicate that the specific mechanism for crossing of the intestinal barrier is not the same as in human listeriosis since mice lack E-cadherin, the receptor for internalin A. Conflicting results appear in the literature concerning the levels of *L. monocytogenes* necessary to induce infection or mortality by an oral route, and reported LD_{50} values range from 50 to $>10^9$ CFU (Pine, Malcolm and Plikaytis, 1990; Audurier et al., 1980; Notermans et al., 1998). Notermans et al. (1998) concluded that an intestinal barrier and a specific immune defence mechanism act independently in protecting mice inoculated orally with *L. monocytogenes* from infection. These results mean that mouse data must be used with caution when making inferences for humans.

The approximate LD_{50} for mice was not statistically different for five *L. monocytogenes* strains when grown in milk or suspended in milk, compared with suspension in phosphate-buffered saline (Pine, Malcolm and Plikaytis, 1990). Similarly, growth in milk did not enhance the virulence of *L. monocytogenes* for Sprague-Dewley rats but instead was suggested to have an inhibitory effect on the number of bacteria available for colonization (Schlech, 1993). The ID_{50} for these rats following gastric inoculation with *L. monocytogenes* was 10^6 CFU. This was not affected by pregnancy, although the invasive infection led to abnormal reproductive outcomes (Schlech, 1993).

The pathogenicity of a *L. monocytogenes* isolate injected intraperitoneally into mice did not differ when grown in crabmeat or tryptose phosphate broth (Brackett and Beuchat, 1990). The ID_{50} of pregnant mice inoculated orally with *L. monocytogenes* appeared to be lower than for normal control mice, although the difference was not statistically significant due to the small number of mice used in the trial (Golnazarian et al., 1989). The authors also compared the ID_{50} of normal mice and mice immunocompromised other than by pregnancy. The immunocompromised mice were beige mutants (deficient in lysosome production within their monocytes and granulocytes), cimetidine-treated mice (decreases gastric acidity) and hydrocortisone acetate-treated mice. With the exception of treatment with hydrocortisone acetate (an observed response with 2.5 mg/day but not with 0.25 mg/day), the responses of predisposed mice were not significantly different from the response of normal mice (Golnazarian et al., 1989). Similarly, decreasing gastric acidity with an antacid had no substantial effect on the infective dose in a non-human primate model (Farber et al., 1991). Immunosuppression by cyclosporin A did not alter the ID_{50} but led to more prolonged infections (Schlech, Chase and Badley, 1993). In contrast to the results of Golnazarian et al. (1989), treating rats with cimetidine lowered the infective dose significantly (Schlech, Chase and Badley, 1993). However, the interpretation of the effects of these treatments should be

treated with caution since the animals did not have the underlying physiological condition that required this cimetidine treatment (Golnazarian et al., 1989).

In the absence of human clinical data, various animal and *in vitro* (e.g. tissue culture) surrogates have been used to acquire experimental dose-response data. For foodborne listeriosis, the model with the greatest apparent similarity to human infections comes from dose-response studies that use oral infection of mammals. The primary animal surrogate used has been the mouse.

Table 2.8 Summary of some *Listeria monocytogenes* dose-response studies using animal models.

Animal model	Route	ID_{50} (CFU)	LD_{50} (CFU)	Other	Source
Monkey	IG			10^5, shedding 2 days 10^7, shedding 3 wks 10^9, shedding 3 wks, symptoms	[1]
Outbred mice	AS		$10^{3.1}$ –$10^{5.5}$		[2]
	GI			$10^{10,}$ 20–100% mortality	
Mice	IV	$<2.7x10^2$	$2.6x10^5$		[3]
	SC	$<2.1x10^2$	$>2.1x10^8$		
	O	$9.9x10^6$	$7.0x10^9$		
C57BL/6 mice	IV		$0.8–6.2x10^6$		[4]
BALB/c mice	IV		$0.04–0.6x10^6$		[4]
Mice	IP		$10^{2.57}$; $10^{2.69}$; $10^{4.96}$; $10^{5.08}$; $10^{5.75}$; $10^{5.91}$		[5]
	IG		$10^{5.47}$		[5]
Mice	IP		$10^{2.68}$; $10^{3.62}$; $10^{4.56}$; $10^{4.57}$; $10^{4.73}$; $10^{4.95}$; $10^{5.47}$; $10^{6.00}$; $10^{6.23}$; $10^{8.88}$; $10^{9.70}$		[6]
S-D Rats	O	10^6			[7]
Stelma	IP		$10^{6.04}$; $10^{6.80}$; $10^{7.28}$; $10^{7.30}$; $10^{7.54}$		[8]
Mice	IP	$10^{3.2}$	$10^{4.79}$; $10^{4.52}$		[9]
	O	$10^{4.57\ (1)}$; $10^{4.00\ (2)}$; $10^{3.30\ (3)}$; $10^{2.48\ (4)}$	$10^{4.77}$; $10^{4.24}$; $10^{0.94\ (1)}$		[9]
Mice	IP		$10^{0.77}$ **; $10^{1.11}$ **; $10^{1.46}$ **; $10^{1.49}$ **; $10^{1.49}$ **; $10^{1.62}$ **; $10^{1.87}$ **; $10^{1.97}$ **; $10^{2.00}$ **; $10^{2.04}$ **; $10^{2.30}$ **; $10^{3.00}$ **; $10^{3.15}$ **; $10^{3.30}$ **; $10^{3.49}$ **		[10]
Mice	IV	$10^{1.8}$; $10^{5.6}$*; $10^{1.0}$**	$10^{3.2}$; $10^{5.8}$*; $10^{2.3}$**		[11]
	O	$10^{6.5}$; $>10^{9.0}$*; $10^{6.3}$**	$>10^{9.0}$; $>10^{9.0}$*; $>10^{8.0}$**		[11]

KEY: IG = Intragastric. IV = Intravenous. IP = intraperitoneal. AS = Aerosol. SC = Subcutaneous, GI = Gastric intubation. O = Oral. * = Previously exposed to *L. monocytogenes.* ** = Immunosuppressed by carrageenan. S-D = Sprague-Dewley.

NOTES: (1) = Hydrocortisone acetate treated. (2) = Lysosome deficient. (3) = Cimetidine treated. (4) = Pregnant.

SOURCES: [1] Farber et al., 1991. [2] Bracegirdle et al., 1994. [3] Audurier et al., 1980. [4] Mainou-Fowler, MacGowan and Postlethwaite, 1988. [5] Pine, Malcolm and Plikaytis, 1990. [6] Pine et al., 1991. [7] Schlech, 1993. [8] Stelma et al., 1987. [9] Golnazarian et al., 1989. [10] del Corral et al., 1990. [11] Notermans et al., 1998.

Endpoints in studies with animal surrogates have usually been infection or death. Because infection in mice is based on the recovery of *L. monocytogenes* from normally sterile internal organs (e.g. spleen, liver), it is difficult to relate this to data for humans where infection has been assessed largely on the basis of the colonization of the intestinal tract by the microorganism.

One study that determined both endpoints following oral dosing of inbred mice (Golnazarian et al., 1989) is useful for determining the relationship between these endpoints. No dose-response studies of *L. monocytogenes* in animal surrogates that used host physiological endpoints or biomarkers other than infection or lethality appeared to have been reported. Other animal surrogates, such as rats (Schlech, Chase and Badley, 1993) and primates (Farber et al., 1991), have also been used for oral dose-response studies, but are not as developed as the mouse system, lacking the extensive genetic and immunological tools that are available in the mouse model. A study with pregnant primates was underway in the United States of America, but results from this investigation were not yet available at the time of preparing this report. There is also a paucity of human data to directly correlate relevant biomarkers of exposure in mice to the frequency and severity of listeriosis in humans.

The work of Notermans et al. (1998)

Notermans et al. (1998) examined dose-response relations for a single strain of *L. monocytogenes* in normal and immunocompromised (i.e. carrageenan-treated) mice using both intravenous and oral dosing (Table 2.9). Both infection and lethality were used as biological end points. They also tested mice previously exposed to *L. monocytogenes* in order to elicit immune protection. Both infectivity and lethality were greater when the pathogen was administered by intravenous injection, and lethality was not observed with any of the orally dosed animals. Immunosuppression decreased the ID_{50} when *L. monocytogenes* was administered by intravenous injection, but did not affect the oral ID_{50}. Prior exposure of the mice decreased the infectivity and lethality of the *L. monocytogenes* isolate.

Table 2.9 Effect of immunosuppression, route of entry and prior exposure on the ID_{50} and LD_{50} values for mice exposed to *Listeria monocytogenes* strain EGD (serotype 1/2a).

Condition of mice	ID_{50}		LD_{50}	
	IV[1]	Oral	IV	Oral
Normal Immunocompetency, naive (Unexposed)	1.8[2] (1.10×10^{-2})[3]	6.5 (2.00×10^{-7})	3.2 (4.37×10^{-4})	>9.0
Normal Immunocompetency, protected (Prior exposure)	5.6 (1.70×10^{-6})	>9.0	5.8 (1.10×10^{-6})	>9.0
Immunosuppressed (carrageenan-treated), naive (Unexposed)	1.0 (6.93 × 10-2)	6.3 (3.00×10^{-7})	2.3	>8.0
Immunosuppressed (carrageenan-treated), protected (Prior exposure)	0.8 (1.10×10^{-1})	7.9 (8.73×10^{-9})	3.2 (4.37×10^{-4})	>8.0

NOTES: (1) IV = intravenous. (2) Log_{10} CFU. (3) r-value from fitted exponential model.
SOURCE: Adapted from Notermans et al., 1998.

Notermans et al. (1998) found the slope of the various dose-response curves to be steep, with both the infection and lethality data being described well using the exponential model. Using an oral LD_{50} value of $\log_{10} = 8.0$, the minimum value for immunosuppressed, protected mice, in conjunction with the yearly human exposure estimates of Notermans et al. (1998), the number of human cases predicted by the exponential dose-response model is <2054 deaths per 1 000 000 persons. This incidence is substantially higher than the 4 to 7 cases per 1 000 000 actually observed. Moreover, these models have limited usefulness since the data for oral administration did not actually establish a dose-response relation for lethality, and intravenous administration is questionable in relation to dose-response relations in humans.

The work of Haas et al. (1999)

The dose-response relation for *L. monocytogenes* infectivity was evaluated by Haas et al. (1999) using the data of Audurier et al. (1980) and Golnazarian et al. (1989). Both data sets represent mice that were orally administered *L. monocytogenes*. The data were fitted using the exponential model and the Beta-Poisson model. The exponential model did not adequately fit the data, whereas the Beta-Poisson model could describe the data sets. In comparing the dose-response curves for strains 10401 (serovar 4b) and F5817 (serovar 4b), used by Audurier et al. (1980) and Golnazarian et al. (1989), respectively, Haas and co-workers concluded that there were significant differences in the strains' infectivity, and that one strain could not be used to describe the dose-response relation of the other. The α and ID_{50} values were 0.17 and 2.1×10^6 CFU for strain 10401, and 0.25 and 2.76×10^2 for strain F5817. Haas and co-workers speculated that the difference in the infectivity of the strains might reflect the method and vehicle of administration. Golnazarian et al. (1989) dosed animals by gavage using milk, whereas Audurier et al. (1980) dosed through drinking water.

Using these models, Haas et al. (1999) compared predicted values with the burden of disease, both in relation to annual incidence and for several outbreaks of febrile gastroenteritis. They concluded that the model based on the data of Golnazarian et al. (1989) greatly over-predicted the infectivity of *L. monocytogenes* when compared with the attack rates reported for outbreaks associated with rice salad (Salamina et al., 1996) and chocolate milk (Dalton et al., 1997). Predictions based on the dose-response model from the Audurier et al. (1980) data were more realistic in comparison with the observed data. When the two dose-response models were used to evaluate the exposure estimates of Notermans et al. (1998), Haas et al. (1999) concluded that the predicted infection rate was unrealistically high.

These observations provide a good example of the care that must be exercised in developing and interpreting dose-response models based on surrogate animal data. First, care must be taken to ensure that the models are based on the same biological end point as the disease's manifestation in humans. It is not surprising that a dose-response model based on infectivity does not provide predictions that match the data on reported cases of illness. The public health data is based largely on cases of meningitis, septicaemia and other severe symptoms, rather than febrile gastroenteritis. Lethality would be a more consistent biological end point and there is a relatively constant ratio in human cases between severe cases and fatalities (i.e. approximately 20% to 30% of hospitalized patients die). The dose differential between the LD_{50} and ID_{50} for the Audurier et al. (1980) and Golnazarian et al. (1989) studies was approximately 1000-fold and 10-fold, respectively. Second, there is no assurance that the dose-response relations for mice and humans are the same. There is a need to correlate or "anchor" the response in a surrogate animal with that in humans. Traditionally, this has been

done with human volunteer feeding studies. However, this is not possible with *L. monocytogenes*, so an alternative means is needed, such as annual disease statistics.

2.2.3.4 Listeria monocytogenes *dose-response models developed from data derived from a combination of surrogate animal and epidemiological data*

The FDA/FSIS LMRA team (FDA/FSIS, 2001) employed an approach that combined the use of surrogate animal data with epidemiological findings. The exposure assessment provided an estimate of the frequency and distribution of consuming *L. monocytogenes*. Surrogate animal data were used to establish the shape of the dose-response curve and the epidemiological data to anchor the results, i.e. the results were constrained so that the predicted incidence of disease approximated the incidence of severe infections noted in a population. A dose-response adjustment factor was created so the exposure data and dose-response model would calculate the estimated number of cases per year in the United States of America. The dietary consumption surveys did not indicate any major difference between population groups. Separate dose-response models were calculated for three populations: pregnant women and their unborn; the elderly (> 60 years); and intermediate-aged (everyone else). The numbers of deaths per year from the epidemiological surveillance were 50 neonatal, 250 elderly and 200 intermediate-aged. The estimated total number of deaths per year for the entire perinatal group (prenatal and neonatal) was 125.

The risk assessment examined two different biological end points, infection (defined as serious illness) and lethality. The incidences of infection, i.e. the incidence of cases requiring hospitalization, were derived from the lethality data using the established ratios between human infection and lethality. The models were developed to account for the variability and uncertainty both in the biological phenomena being modelled and the modelling approaches employed. Accordingly, the dose-response models were designed to run as Monte Carlo simulations. The models factored-in the differences in the virulence of *L. monocytogenes* isolates and the differences in the susceptibility among the three groups of humans (i.e. the general population, the elderly, and perinates/neonates).

Dose-response model based on studies with mice

The relationship between the number of *L. monocytogenes* consumed and the occurrence of either infection (serious illness) or death (mortality) was modelled using data obtained for immunocompetent mice orally administered *L. monocytogenes* F5817. Because of the effects of strain variation, host susceptibility and the differences between a surrogate animal in a controlled environment versus humans in an uncontrolled environment, the mouse model served primarily to establish the shape or steepness of the dose-response curve. In actuality, with the added uncertainties and the linear shape of the curve in the probability of illness range of interest (log dose-log probability plot), the shape of the mouse curve had relatively little influence on the final dose-response curve.

The data used to develop a dose-response curve for mortality was taken from Golnazarian et al. (1989). Data were fitted to six different models using an iterative, least-squares curve-fitting procedure. The best four models (Beta-Poisson, exponential, logistic and Gompertz-Log) were used to characterize the uncertainty in the shape of the dose-response curve. The Gompertz-Log and Weibull-Gamma models were discarded for lack of fit. The Exponential model provided the best fit and received the most weight (Figure 2.2). Notermans et al. (1998) also reported the exponential model to be effective for depicting the relationship

between dose and lethality in mice. The resultant FDA/FSIS LMRA dose-response relations for infection and mortality in mice are summarized in Table 2.10.

Because mortality is a more consistent measure than infection or illness in mice and human data, the FDA/FSIS LMRA used lethality as their primary model to define the *L. monocytogenes* dose-response relations. The number of serious illnesses that did not lead to death was estimated to be four times the number of deaths based upon epidemiological data. An exception to this was the dose-response relation for perinatal infections, where the primary public health impact was considered to be death. The frequency of perinatal deaths was estimated to be 1.5 times the observed frequency of neonatal deaths. For the United States of America in recent years, this was approximately 500 deaths and 2000 additional cases of serious illness.

Modelling the variability in virulence among strains of L. monocytogenes

Since there appears to be substantial variability in the pathogenicity of *L. monocytogenes* strains, based on animal model data, the FDA/FSIS LMRA included a model for variability in virulence. The model is based on data acquired using mice. Specifically, the range of LD_{50} values observed in mice was also used to characterize the range of variation expected in humans. Adjustment of the dose-response relationship relative to the LD_{50} presumes that the shape of the population dose-response function is the same for different strains.

The data used was from three studies (Stelma et al., 1987; Pine, Malcolm and Plikaytis, 1990; Pine et al., 1991) wherein *L. monocytogenes* was administered to immunocompetent mice by intraperitoneal injection (Table 2.11). The LD_{50} values encompassed a range of over 7 orders of magnitude. Although some of the strains were obtained directly from food, most of the strains tested were clinical isolates, which may have biased the model towards strains that are more virulent. Conversely, while a range of strains were used, there are no definitive studies that have attempted to examine the relative virulence of foodborne *L. monocytogenes* strains on the basis of their relative occurrence.

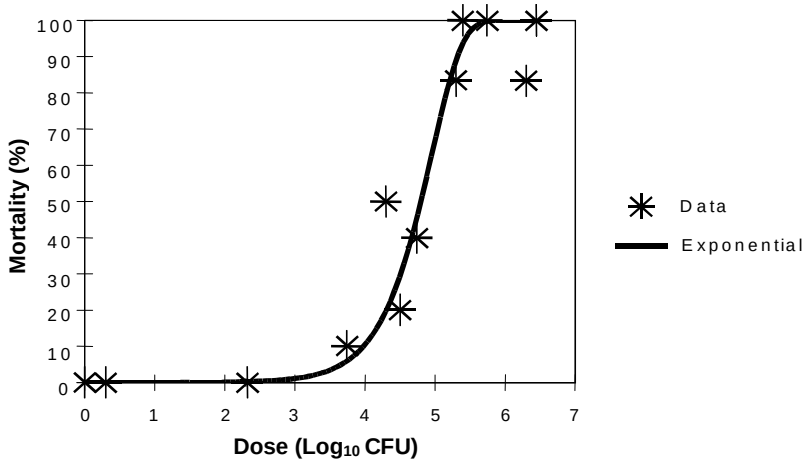

Figure 2.2 Dose versus frequency of mortality in mice administered *Listeria monocytogenes.*
SOURCE: Adapted from FDA/FSIS, 2001.

The strains examined in the laboratory investigations were selected to provide a wide virulence range, and not on the basis of their relative occurrence in foods. Because most food isolates do not appear to cause outbreaks, the array of strains in Table 2.11 could have a disproportionate number of strains with reduced virulence. No large or obvious trends in the LD_{50} values relative to either serotype or strain source were apparent.

Because the three studies used to estimate the variability in virulence among *L. monocytogenes* strains were based on studies using intraperitoneal administration and the dose-response model was based on orally dosed mice, there was concern that this could introduce a bias. To avoid this potential source of error, the FDA/FSIS LMRA developed a correction factor for adjusting the virulence model. The correction factor was based on a study of Pine, Malcolm and Plikaytis (1990) that compared the LD_{50} values for *L. monocytogenes* strains administered both by intragastric gavage and by intraperitoneal injection.

Table 2.10 Dose-response functions for infection and mortality in mice resulting from oral exposure to *Listeria monocytogenes* strain F5817.

Effective Dose (Log_{10} CFU)	Infection	Mortality
0	9.11%(1) (0.87%, 9.38%)(2)	0.001% (0.000%, 0.007%)
0.5	12.2% (2.6%, 12.5%)	0.003% (0.000%, 0.018%)
1	16.4% (7.3%, 16.5%)	0.011% (0.000%, 0.045%)
1.5	21.7% (17.1%, 21.8%)	0.035% (0.000%, 0.117%)
2	28.4% (28.4%, 31.3%)	0.110% (0.000%, 0.301%)
2.5	36.7% (36.5%, 46.0%)	0.347% (0.002%, 0.775%)
3	46.4% (46.2%, 58.7%)	1.09% (0.06%, 1.99%)
3.5	57.4% (57.1%, 68.7%)	3.42% (1.06%, 5.11%)
4	68.8% (68.6%, 76.3%)	10.4% (8.3%, 12.9%)
4.5	79.6% (79.5%, 82.1%)	30.8% (29.4%, 32.0%)
5	88.6% (86.5%, 88.6%)	66.7% (63.6%, 67.6%)
5.5	94.8% (89.8%, 95.0%)	95.2% (90.7%, 96.9%)
6	98.2% (92.3%, 98.4%)	100% (98%, 100%)
6.5	99.6% (94.2%, 99.7%)	100% (100%, 100%)
7	99.9% (95.6%, 100.0%)	100% (100%, 100%)
7.5	100% (97%, 100%)	100% (100%, 100%)
8	100% (97%, 100%)	100% (100%, 100%)
8.5	100% (98%, 100%)	100% (100%, 100%)
9	100% (99%, 100%)	100% (100%, 100%)
9.5	100% (99%, 100%)	100% (100%, 100%)
10	100% (99%, 100%)	100% (100%, 100%)
10.5	100% (99%, 100%)	100% (100%, 100%)
11	100% (100%, 100%)	100% (100%, 100%)
11.5	100% (100%, 100%)	100% (100%, 100%)
12	100% (100%, 100%)	100% (100%, 100%)

NOTES: (1) Median estimate. (2) Confidence intervals representing the 5th and 95th percentiles of the uncertainty distribution.

SOURCE: Based on data from Golnazarian et al. (1989).

Table 2.11 LD_{50} values for various *Listeria monocytogenes* strains administered by intraperitoneal injection to immunocompetent mice.

Strain	Serotype	Source	**LD_{50}** (Log_{10} CFU)	Study
G9599	4	Clinical	*2.57*	[1]
G1032	4	Clinical	*2.69*	[1]
G2618	1/2a	Food	*2.89*	[2]
F4244	4b	Clinical	3.62	[2]
F5738	1/2a	Clinical	3.67	[1]
F6646	1/2a	Clinical	4.49	[1]
15U	4b	Clinical	4.56	[2]
F4246S	1/2a	Clinical	4.57	[2]
F7208	3a	Clinical	4.61	[1]
G2228	1/2a	Clinical	*4.66*	[1]
F2381	4b	Food	4.73	[2]
G2261	1/2b	Food	*4.95*	[2]
F2380	4b	Food	*4.96*	[1]
F2392	1/2a	Clinical	5.08	[1]
1778+H1b	1/2a	Clinical	*5.47*	[2]
F7243	4b	Clinical	*5.75*	[1]
F7245	4b	Clinical	*5.91*	[1]
SLCC 5764	1/2a	Clinical	6.00	[2]
V37 CE	–	Food	6.04	[3]
F7191	1b	Clinical	6.23	[2]
V7	–	Food	6.80	[3]
Brie 1	–	Food	7.28	[3]
Murray B	–	Clinical	7.30	[3]
Scott A	4b	Clinical	7.54	[3]
G970	1/2a	Clinical	8.88	[2]
NCTC 5101	3a	Clinical	9.70	[2]

NOTE: (1) The italicized LD_{50} values are averages from multiple experiments. (2) The original studies were [1] Pine, Malcolm and Plikaytis, 1990. [2] Pine et al., 1991. [3] Stelma et al., 1987.

SOURCE: FDA/FSIS, 2001: Table IV-2.

Table 2.12 Effect of administration route (intraperitoneal vs intragastric gavage) on mouse LD_{50} values.

Strain	Serotype	Source	**Log_{10} ratio (intragastric/intraperitoneal)**
F2380	4b	Food	-1.81
F7243	4b	Clinical	-0.75
F7245	4b	Clinical	-0.47
G2228	1/2a	Clinical	0.00
G2261	2/1b	Food	0.00
NCTC 7973	1/2a	Food	0.04
F6646	1/2a	Clinical	0.21
F2380	4b	Food	0.71
G9599	4	Clinical	0.96
G1032	4	Clinical	1.60
F5738	1/2a	Clinical	1.81
G2618	1/2a	Food	2.00

Source: FDA/FSIS, 2001: Table IV-3., based on data from Pine, Malcolm and Plikaytis (1990).

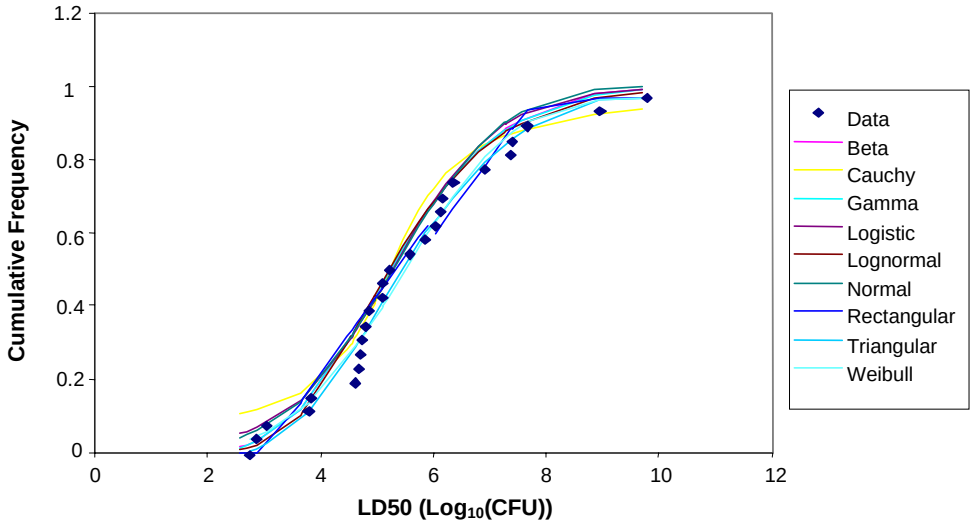

Figure 2.3 Variation in *Listeria monocytogenes* Strain Virulence: Nine Distributions.

SOURCE: FDA/FSIS, 2001. Figure IV-2.

Table 2.13 Model output for *Listeria monocytogenes* strain virulence

Percentile	**LD_{50}** (Log_{10} CFU)
1st	2.55 (0.97, 2.80)
5th	3.12 (2.47, 3.32)
10th	3.53 (3.18, 3.66)
25th	4.28 (4.20, 4.39)
Median	5.25 (5.15, 5.34)
75th	6.35 (6.23, 6.48)
90th	7.45 (7.25, 7.67)
95th	8.06 (7.84, 8.54)
99th	9.47 (8.52, 10.59)

NOTE: Values in parentheses are the 5th and 95th percentiles for the uncertainty about the distribution in virulence.

SOURCE: FDA/FSIS, 2001: Table IV-4.

Although there was up to a 100-fold difference in the LD_{50} values by the two routes, the intragastric or the intraperitoneal route was the most effective depending upon the strain (Table 2.12). The median value of the ratio between the LD_{50} determined using the intragastric and intraperitoneal routes, respectively, was greater than 1.0 (i.e. [LD_{50}oral/ LD_{50}ip]), indicating that the correction factor for virulence could overestimate the virulence of *L. monocytogenes* (by approximately half a log).

In Table 2.12, a $\log_{10}$ ratio of 0 indicates that the LD_{50} by the two routes were identical. A negative number indicates a lower LD_{50} by the intragastric route, while a positive number indicates a greater LD_{50} by the intragastric route. The data in Table 2.12 were modelled by fitting nine distributions. The best five were used to characterize model uncertainty

associated with distribution (Figure 2.3). The resulting distribution in LD_{50} is given in Table 2.13. This distribution was used to describe the extent of virulence variability in determining dose-response. Because the virulence was estimated from the distribution of intraperitoneal administered doses, the estimated LD_{50} was increased by zero to one log (uniform uncertainty range) to adjust the virulence value to more accurately predict the estimated oral LD_{50}.

Dose-response model for mortality in humans

Intermediate-Age Population

The FDA/FSIS LMRA considered three age-related populations: perinatal cases (mothers, and fetus and newborns from 16 weeks of gestation to 30 days of age); elderly cases (>60 years of age); and an intermediate age group (>30 days, <60 years). Considering that it appears that humans are commonly exposed to low levels of foodborne *L. monocytogenes*, direct application of the mouse dose-response model would greatly overestimate the incidence of lethal infections in humans from *L. monocytogenes*. The LD_{50} in the mouse study from which the curve was derived was about $\log_{10} = 4.3$, or about 20 000 CFU. The periodic exposure of humans to such numbers of *L. monocytogenes* is frequent (FDA/FSIS, 2001; Buchanan et al., 1997; Notermans et al., 1998). Based on the described consumption, contamination and growth data, it was estimated that if the mouse dose-response model were used directly, it would overestimate the number of illnesses and deaths due to listeriosis by a factor of over 10^9. If the estimates of the occurrence of *L. monocytogenes* in food (developed in the exposure assessment) are reasonable, then human beings are much less susceptible than laboratory mice to *L. monocytogenes*. Therefore, the mouse-derived models had to be adjusted to reflect human susceptibility. A dose-response adjustment factor was applied that allowed the models to predict serious illness and death occurrences roughly consistent with surveillance data reported to FoodNet. Thus, while the shape of the curve was initially derived from mice, the curve's position on the dose scale is determined by the human surveillance record. Because of large differences in the behaviour of the dose-response model at low doses, the magnitude of the adjustment factor was model-dependent (Tables 2.14 and 2.15).

Table 2.14 Model-dependence of dose-response adjustment factor for intermediate-age populations.

Model	**Dose Adjustment** (Log_{10} CFU)	
	Minimum	Maximum
Logistic	11.85	12.35
Exponential	11.85	12.35
Gompertz-Log	11.85	12.35
Probit	11.95	12.20
Multihit	11.95	12.45

SOURCE: FDA/FSIS, 2001: Table IV-5a.

Table 2.15 Model-dependence of the *Listeria monocytogenes* dose-response adjustment factor ranges for the three human populations.

Population	**Dose-Response Adjustment Factor Range** (Log_{10} CFU)	
	Minimum	Maximum
Intermediate-Age	11.85	12.45
Neonatal (1)	7.8	8.4
Elderly	11.85	11.45

NOTE: (1) An adjustment to account for total perinatal deaths (prenatal and neonatal) is in the risk characterization section of FDA/FSIS, 2001.

SOURCE: FDA/FSIS, 2001: Table IV-5b.

After applying the virulence distribution (Table 2.13) to the normal mouse dose-response mortality curve (Table 2.10), the dose-response adjustment factor was shifted using iteration

techniques, moving the curve towards the higher doses necessary for lethality estimates to agree with surveillence data. Figure 2.4 depicts the results of applying this factor to the intermediate-aged population. The distribution considered four sources of uncertainty and variability: strain virulence, host (human) susceptibility, uncertainty in the exposure, and dose-response adjustment factor.

In two subsequent dose-response curves (Figures 2.5 and 2.6), adjustments are made that reflect increased susceptibility in perinatal and elderly populations (see next section). The intermediate-age population includes higher-risk individuals not explicitly included in the perinatal and elderly groups, such as AIDS, cancer and transplant patients. These individuals probably make up a disproportionate number of the cases of serious listeriosis within this population; however, it was considered that insufficient data to further distinguish these populations were available when FDA/FSIS conducted their dose-response modelling. Because the portion of the intermediate-age population at higher risk for listeriosis is small in comparison with entire population, this leads to dose estimates at high response rates (e.g. LD_{10}, LD_{50}) that are unrealistic in terms of the number of bacteria that could be consumed by an individual. Doses greater than 10^{12} or 10^{13} CFU should be considered notional and be interpreted as indicating that a substantial segment of the population is not susceptible.

Modelling dose-response relations for perinatal and elderly populations

The FDA/FSIS LMRA adjusted the dose-response model to account for the increased susceptibility of neonates and the elderly, in order to make predicted results consistent with both the number of cases reported from surveillance data (CDC, 2001) and the range of sensitivity encountered in studies with immunocompromised mice. These models employed a susceptibility adjustment distribution for each sub-group. This included adjusting the number of servings consumed by the size of the population relative to the total population.

For neonates, the population size was adjusted for an annual birth rate corresponding to 1.8% of the total population and a distribution period for *in utero* exposure with a range of 1 to 30 days prior to birth, with an average value of 10 days. The dose-response relations were initially modelled only for neonates because the epidemiology data were for that group. Conversion to perinatal case rates (prenatal and neonatal combined) was done after dose-response simulation. Perinatal deaths were estimated at 2.5 times the neonatal deaths, based on Los Angeles County historical data. Figure 2.5 depicts the neonatal dose-response curve, based on the dose, when consumed maternally, required to produce death due to *in utero* exposure of the neonate.

For the elderly, the census estimated that 13% of the current United States of America population was aged 60 or over. Figure 2.6 depicts the elderly population dose-response curve.

The dose-response relations for mortality rate on a per-serving basis for the three population groups are summarized in Table 2.16. In general, the risk of a fatal listeriosis infection was 10 to 100 times greater for the elderly and neonate populations compared with the intermediate-age population.

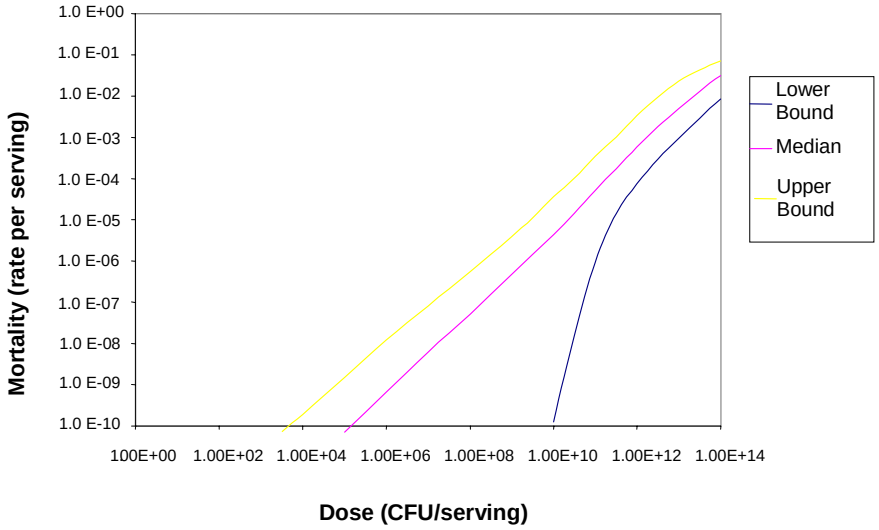

Figure 2.4 Dose response with variable strain virulence for the intermediate-age population.

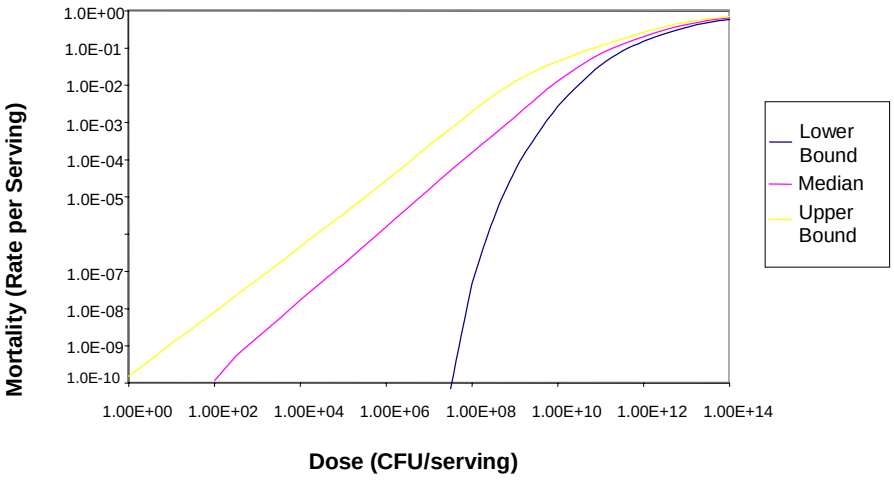

Figure 2.5 Dose response with variable strain virulence for neonates.

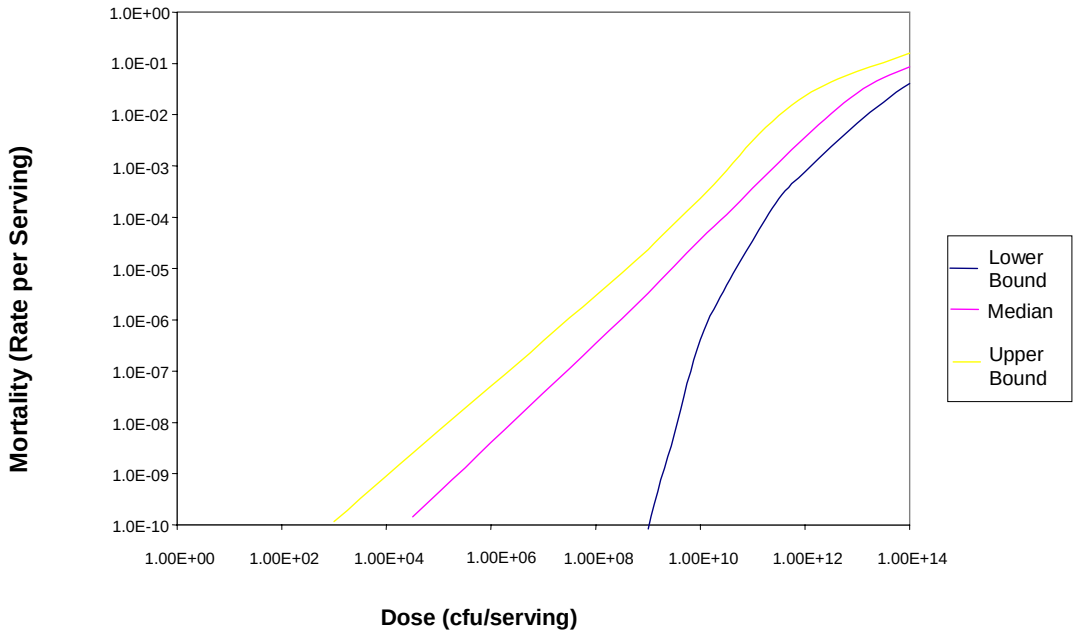

Figure 2.6 Dose response with variable strain virulence for the elderly

Table 2.16 Dose response with variable *Listeria monocytogenes* strain virulence for three age-based populations.

Dose (CFU per serving)	Median mortality rate per serving [1]		
	Intermediate-age	Neonatal[2]	Elderly
1	1.6×10^{-15} (1.9×10^{-134}, 2.7×10^{-13})	1.7×10^{-15} (7.5×10^{-84}, 1.5×10^{-10})	6.2×10^{-15} (4.3×10^{-112}, 3.9×10^{-13})
10^3	1.6×10^{-12} (3.9×10^{-83}, 7.9×10^{-11})	1.7×10^{-9} (1.3×10^{-44}, 6.2×10^{-8})	5.2×10^{-12} (2.2×10^{-65}, 1.2×10^{-10})
10^6	1.3×10^{-9} (1.2×10^{-43}, 2.9×10^{-8})	1.6×10^{-6} (2.1×10^{-18}, 2.8×10^{-5})	4.0×10^{-9} (8.6×10^{-32}, 5.0×10^{-8})
10^9	1.0×10^{-6} (7.1×10^{-18}, 1.2×10^{-5})	1.4×10^{-3} (5.0×10^{-5}, 1.3×10^{-2})	3.3×10^{-6} (3.6×10^{-11}, 2.3×10^{-5})
10^{12}	1.1×10^{-3} (3.5×10^{-5}, 5.7×10^{-3})	2.0×10^{-1} (1.5×10^{-1}, 2.6×10^{-1})	3.6×10^{-3} (7.8×10^{-4}, 2.3×10^{-2})

NOTES: (1) The 5th and 95th percentiles from the uncertainty are in parentheses.

SOURCE: FDA/FSIS, 2001: Table IV-9.

The median dose-response curves depicted in Figures 2.4, 2.5 and 2.6 approach the exponential model in shape. Using the median values for the 10^{12} CFU dose from Table 2.16, the current study used the response probability to estimate r-values for the intermediate-age, neonate and elderly population. The r-values were 8.5×10^{-16}, 5.0×10^{-14} and 8.4×10^{-15}, respectively. This allows a direct comparison of the three FDA/FSIS LMRA dose-response curves with the dose-response curves derived using only epidemiological data (Figure 2.7). All three of the FDA/FSIS LMRA models indicate a lower median probability of response at a specified dose compared with the other dose-response relations. This probably reflects the fact that the FDA/FSIS model was (1) based on mortality, not morbidity, and (2) the other models are based on strains with known high virulence, whereas the FDA/FSIS model considers the distribution of virulence that is likely to be encountered with *L. monocytogenes* isolates from foods. The predicted risk of serious listeriosis would be 5 times that for mortality.

The models include outbreaks where the primary symptoms included serious illness (including deaths) (smoked fish: Buchanan et al., 1997; smoked fish: Lindqvist and Westöö, 2000, and Farber, Ross and Harwig, 1996; butter: current study), perinatal and neonatal infections (death) (Hispanic-style cheese: current study; FDA/FSIS-neonates: FDA/FSIS, 2001), febrile gastroenteritis (sweet corn-tuna salad and chocolate milk: current study), death (general population) (FDA/FSIS, 2001), and death (elderly) (FDA/FSIS, 2001).

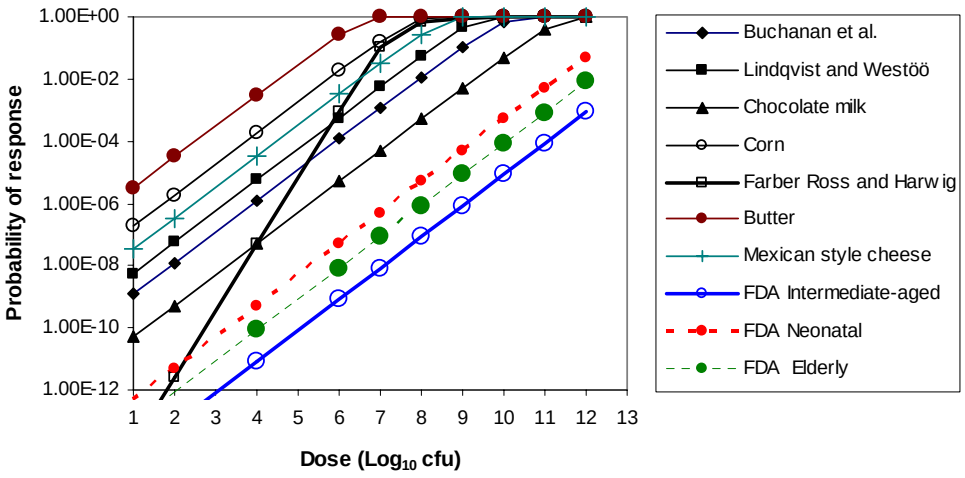

Figure 2.7 A comparison of the FDA/FSIS LMRA dose-response models for mortality with those derived earlier for morbidity based on epidemiological data or expert elicitations.

SOURCES: Buchanan et al., 1997; Lindqvist and Westöö, 2000; Farber, Ross and Harwig, 1996; FDA/FSIS, 2001.

2.3 OPTIONS FOR HAZARD CHARACTERIZATIONS TO BE USED FOR MODELLING THE PUBLIC HEALTH IMPACT OF *L. MONOCYTOGENES* IN READY-TO-EAT FOODS

Dose-response data from human volunteer studies with *L. monocytogenes* or from volunteer studies with a surrogate pathogen do not exist. Instead, dose-response relations have been developed and evaluated based on expert elicitations, epidemiological or animal data, or combinations of these. These dose-response relations, which were reviewed and summarized in the preceding sections, cover the spectrum of biological end-points, i.e. infection, morbidity and mortality, and have, to varying degrees of sophistication, been evaluated using human epidemiological data. The potential effects of the food matrix on the dose-response relation have not been considered as a variable within any of the models due to insufficient data. Available models, categorized by the end-point being modelled, include:

Infection:

- Farber, Ross and Harwig, 1996; Bemrah et al., 1998 – Weibull-Gamma model.
- Notermans et al., 1998; Haas et al., 1999 – Exponential model.
- Haas et al., 1999 – Beta-Poisson model.

Morbidity:

- Buchanan et al., 1997; Lindqvist and Westöö, 2000 – Exponential model.
- FDA/FSIS, 2001 – FDA/FSIS model.

Mortality:

- FDA/FSIS, 2001 – FDA/FSIS model.
- Notermans et al., 1998 – Exponential model.

The predictions of these models show wide variation, and some appear to be more conservative than others (See Figure 2.7).

The absence of human feeding trial data, incomplete epidemiological information, difficulties in extrapolating from animal data to humans, absence of information on strain virulence, and lack of mechanistic models are all limiting factors that contribute to the uncertainty in the description of the dose-response relationship. The approach taken in the FDA/FSIS LMRA is noteworthy since it addresses several of these limitations, but it will need further evaluation and development. It would be revealing to attempt to validate the model with health surveillance data and exposure estimates for another country.

While there are substantial differences in the dose-response relations that have been developed by different investigators (Figure 2.7), it appears that for those based on epidemiological data, a substantial part of the variability may reflect a combination of the biological endpoint being examined and the size and the characteristics of the population being considered. For example, the dose-response relation that was developed based on the outbreak of listeriosis in a hospital in Finland suggested that the LD_{50} for humans was approximately 10^6 CFU. This was based on 15 cases from a population of 234 individuals, all of who were highly immunocompromised. However, if this group were considered in relation to the entire population of individuals that had consumed the butter, this would have a great affect on the calculated dose-response. For example, if the dose-response model had

been based on the entire population of Finland (5.2×10^6 individuals) having consumed the butter, but with only the hospital patients becoming seriously ill, the dose leading to a 50% serious infection rate in the population would be approximately 1×10^{11} CFU.

The calculated dose-response model will be influenced strongly by the numbers and the characteristics of the individuals included within that population. This is particularly important when epidemiological data is used to determine the dose-response relation. In part, the selection of the population to be considered will be a risk management decision related to the degree of conservatism that is to be built into the model and the degree to which even the most at-risk individuals are to be protected. However, by selecting a dose-response relation based on a specific higher-risk sub-population, any hazard characterization based on that relation would exaggerate the risk faced by the population in general.

At present there are only limited criteria on which to base the selection of the dose-response model, and better tools are needed to compare different models. Available criteria include the recommended use of non-threshold dose-response models that are linear in the low-dose region, and which have a biological basis and biologically interpretable parameters (*Hazard Characterization for Pathogens in Food and Water: Guidelines* (FAO/WHO, 2003)). However, the choice of which models to use will also depend on factors such as the purpose of the risk assessment and the level of resources and sophistication available to the risk assessors. This requires that the basis for the various dose-response relations and their impact on the overall risk assessment be adequately communicated to the risk managers who request the assessment. The use of several dose-response model relationships to frame the risk estimates is one approach to addressing the uncertainty related to current gaps in knowledge. A second approach, which has been used by at least one group of risk assessors, is the simultaneous use of several dose-response model relationships (FDA/FSIS, 2001). However, the latter choice requires a high degree of modelling sophistication, a requirement that could influence negatively the goal of providing a risk assessment that could be adapted by FAO/WHO for use internationally, where the level of risk assessment resources and sophistication varies substantially. On this basis, the risk assessment team, with the concurrence of an international panel of experts in foodborne disease, opted to develop a set of simpler dose-response models based on the use of the exponential model.

2.3.1 Exponential dose-response model used in the present risk assessment

The preceding sections discussed the various dose-response relations that have been developed and described their strengths and weaknesses. However, none of the available models were fully able to meet the needs of the current risk assessment in relation to the parameters examined and the requirement for simplicity of calculation. For these reasons, alternative approaches based on the exponential model were developed and evaluated.

The general approach was to estimate the single parameter r in the exponential model, i.e. the probability that a single cell will cause invasive listeriosis, by pairing population consumption patterns (exposure) with epidemiological data on the number of invasive listeriosis cases in the population. This was done in a manner similar to that described in Buchanan et al. (1997) and Lindqvist and Westöö (2000), but it was possible to refine their approach with the new epidemiological data and the detailed exposure assessment from the recently published draft FDA/FSIS *L. monocytogenes* risk assessment [see FDA/FSIS, 2001].

The validity of this approach is dependent on several assumptions or sources of information, or both: the percentage of individuals susceptible to severe *L. monocytogenes* infections; the appropriateness of the exponential model for describing the pathogen's dose-response relation in humans in the dose range of interest; the exposure assessment and numbers of *L. monocytogenes* consumed; and the accuracy of the statistics on the annual rate of severe listeriosis cases. The approach is based on mean population characteristics, i.e. the estimated exposure of the human population to a distribution of different strains, resulting in a number of illnesses. Consequently, variability in virulence is considered in the sense that the data, and therefore r-values, reflect the mean characteristics of many strains of *L. monocytogenes*, including frequency of occurrence and virulence. Similarly, the biological end point (response) used for the dose-response relationships is listeriosis. As indicated earlier, that term refers to "severe infection" or "invasive listeriosis" and includes those infected individuals suffering from life-threatening, systemic infections such as perinatal listeriosis, meningitis or septicaemia. Since the annual incidence of listeriosis included the entire designated population, the variability among individuals exposed to the pathogen is also inherently considered in this approach to dose-response modelling.

2.3.1.1 Overview of the estimation of parameter r in the exponential dose-response model

Specific r-values were derived for the less susceptible (healthy) and more susceptible populations as inputs to the current risk assessment, on the assumption that the overall consumption of *L. monocytogenes* was similar in these groups. The dietary consumption surveys did not indicate any major differences between population groups included in the draft FDA/FSIS (2001) risk assessment. Derivation of the r-values was achieved using the consolidated food contamination distribution from the FDA/FSIS 2001 draft exposure model in conjunction with the CDC annual estimated number of listeriosis cases (Mead et al., 1999) as a percentage of the total population of either more or less susceptible groups within the United States of America population. This provided values for P and N in the exponential model so that the r-value could be calculated by re-arranging the equation and solving.

Mathematically, the r-value is considered to be a constant parameter for a specified population. However, the accuracy of the estimate of the r-value is dependent on the size and inclusiveness of the population being considered, the accuracy of the annual disease statistics, and the reliability of data on the frequency and extent of *L. monocytogenes* contamination in foods. The uncertainty associated with the r-value included uncertainty estimates in the data used to derive the constant. Uncertainty estimates for the percentage of the population who are at increased risk range from 15 to 20% of the total population. The uncertainty estimates in the percentage of total cases in the annual disease statistics associated with the increased susceptibility population was estimated to range from 80 to 98%, and the uncertainty range in the total number of listeriosis cases in the United States of America was assumed to be from 1888 to 3148 cases (2518 cases ±25%). The derived r-values with estimated uncertainties were then determined by Monte Carlo simulation. Thus, although the r-value is mathematically a constant, due to the uncertainty in its estimation, the actual values used in the calculation of the dose-response curve were a distribution based on the estimated uncertainties.

In the FDA/FSIS 2001 draft risk assessment the total number of servings at each of five different dose levels for a number of RTE foods was estimated. The upper bound of the

highest dose level, i.e. the maximum level of *L. monocytogenes* in an individual serving is uncertain and may vary for the different types of foods. Limitations in the contamination databases do not permit resolution of this issue. However, the maximum levels of *L. monocytogenes* encountered in individual servings of the different foods have a large impact on the calculated mean ingested doses. This, in turn, affects the derived r-value and the resulting dose-response curve. Consequently, this assumption was evaluated in detail. In the present study, the effect on the estimated r-value was considered by assuming different maximum contamination levels and calculating values for four point estimates of the maximum doses of 7.5, 8.5, 9.5 and 10.5 $\log_{10}$ CFU. In the studies by Buchanan et al. (1997) and Lindqvist and Westöö (2000), the maximum dose was assumed to be 5.7–6.0 $\log_{10}$ CFU, whereas the FDA/FSIS (2001) risk assessment allowed growth in a food to reach over 10 $\log_{10}$ CFU per serving. Assuming a lower maximum contamination level produces a more conservative or cautious estimation of the r-value. The lower the maximum dose assumed, the larger is the estimated r-value. The larger the r-value, the greater is the assumed virulence of the *L. monocytogenes*. In addition to using point estimates for the maximum levels of *L. monocytogenes*, r-values for the susceptible and healthy populations were also calculated using Monte Carlo simulation techniques, wherein the uncertainty in the maximum dose was addressed by combining all the previous dose levels into a discrete uniform distribution.

A schematic overview of the model used to estimate the r-value is shown in Figure 2.8. The uncertainty of the r-value due to the uncertainties in the assumed maximum dose levels in the different food categories, the size of the population of interest, and the number of cases in this population were calculated using the routine illustrated in Table 2.22 (at end of Part 2), where the input data employed and the Excel spreadsheet routine used for developing the model are indicated. From the FDA/FSIS exposure assessment, a summary table of the total number of servings at each of five different dose levels (<1; 1–$10^{3;}$ 10^3–10^6; 10^6–10^9; >10^9 CFU) was extracted (Figure 2.8 and Table 2.22, at end of Part 2). These data were fitted to an empirical cumulative frequency distribution. The equation was used to generate a new frequency distribution at closer dose intervals. The newly generated frequency distribution did not differ significantly from the original distribution (Figure 2.9). The data on the number of servings at different dose levels from the FDA/FSIS risk assessment and the number of United States of America cases of severe listeriosis were the basis for estimating the r-value for the population of interest. Finally, r-values were calculated in manners analogous to those described by Buchanan et al. (1997), for two scenarios: (1) assuming that all cases were attributable to servings from the maximum dose level only; and (2) assuming that all dose levels contributed to causing listeriosis. The first approach is based on the observation that the exponential model is generally steep, which results in the highest exposure levels having the greatest impact on the probability of disease within the dose ranges of interest.

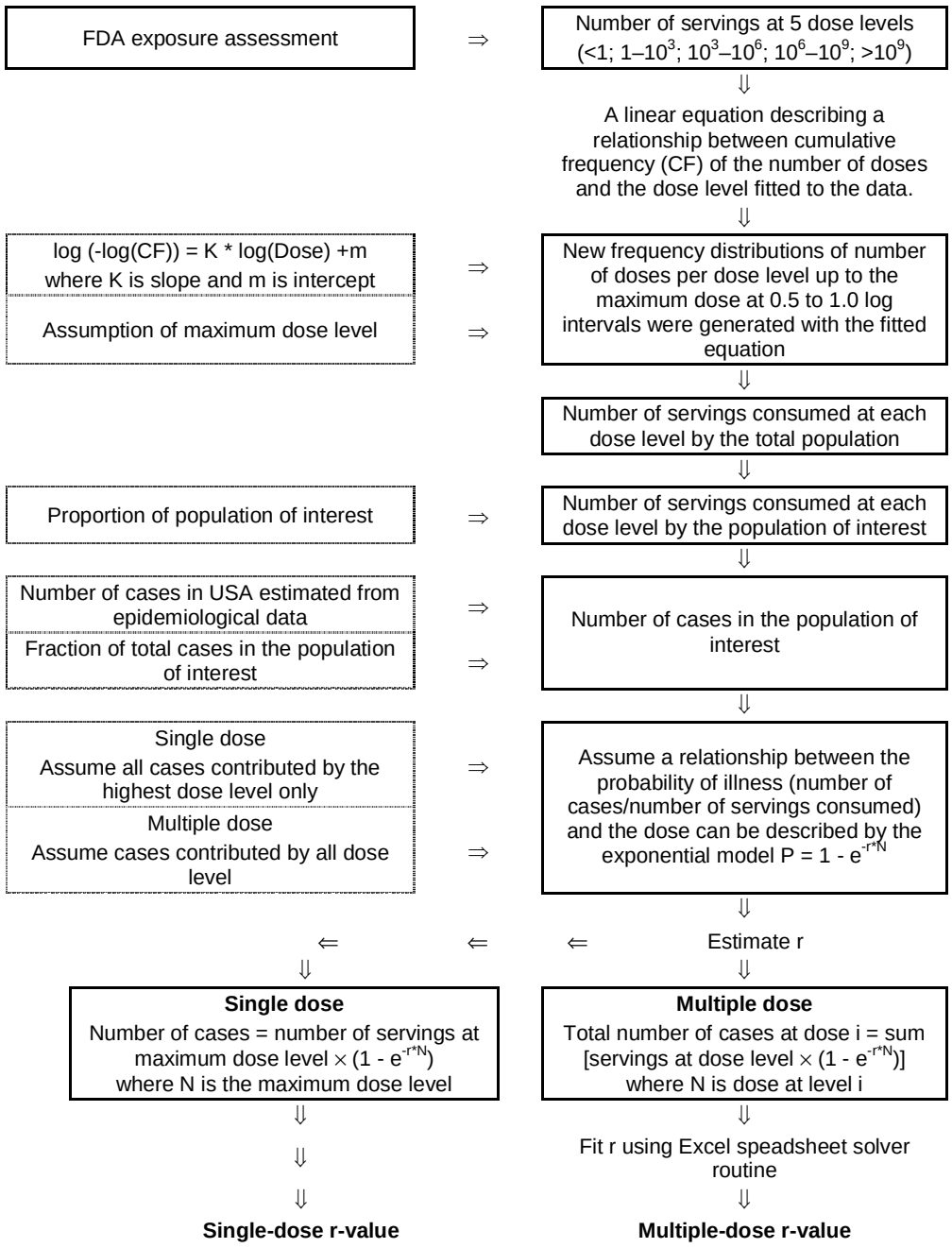

Figure 2.8 Schematic overview of the model used to estimate r in the exponential dose-response model.

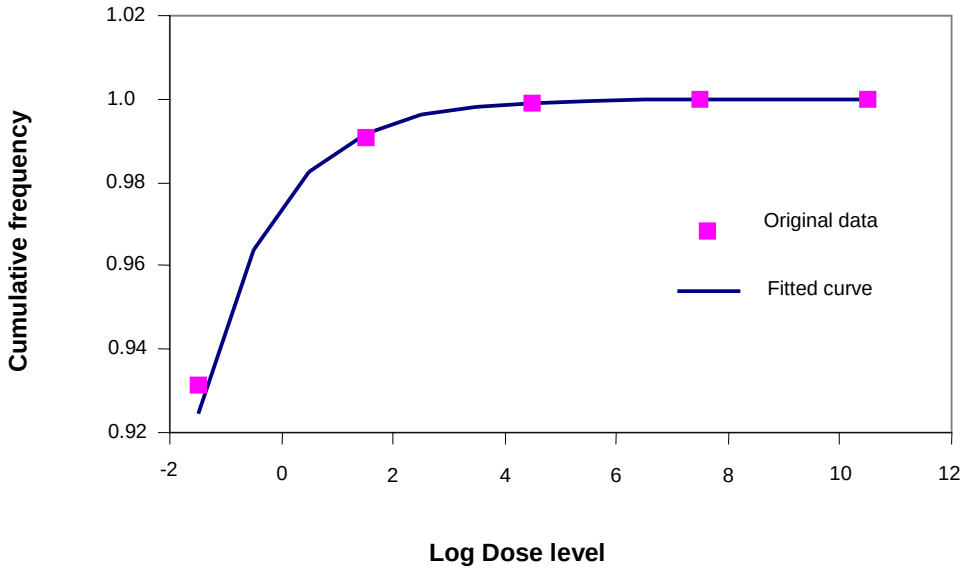

Figure 2.9 The cumulative frequency distribution of servings with different dose levels of *L. monocytogenes*. The squares represent the original data from the draft FDA/FSIS risk assessment (2001) describing the number of servings at each of five dose levels. The curve represent the resulting equation when these data were fitted to an empirical cumulative frequency distribution. This curve was used to generate a new frequency distribution at closer dose intervals.

2.3.2 Dose-response models for healthy and susceptible population

Dose-response models for the susceptible population and the healthy population were calculated in three different ways, as described below. In each case, the impact of the assumed maximum level of contamination that *L. monocytogenes* reaches in foods was considered.

The first approach was to assume that cases of severe listeriosis are due overwhelmingly to those servings of foods that have the highest level of contamination. In addition, uncertainty associated with three parameters that influence the dose-response relations was evaluated by assuming that (i) the percentage of the population with increased susceptibility to *L. monocytogenes* varied between 15% and 20%; (ii) that the percentage of cases of total severe listeriosis cases associated with this increased susceptibility population ranged from 80% to 98%; and (iii) the estimates of the total number of cases (2518) has a degree of uncertainty of 25%. The equation was then solved using Monte Carlo techniques, with 5000 iterations being run to obtain the median r-value and its 5% to 95% confidence interval for maximum assumed contamination levels per serving of 7.5, 8.5, 9.5 and 10.5 $\log_{10}$ CFU. In addition, the maximum contamination level was also considered as a variable using a discrete uniform distribution [RiskDuniform(7.5; 8; 8.5; 9; 9.5; 10; 10.5)]. The r-values obtained are presented in Table 2.17.

The second approach was to again assume that the incidence of severe listeriosis was due to the highest contamination level. However, in this case, point estimates were used for the percentage of the population in the more susceptible group (17.5%), the percentage of cases associated with the more susceptible population (83%), and the total number of severe listeriosis cases (2518). The r-values obtained at the various maximum dose levels considered are presented in Table 2.18.

The third approach was to assume that all doses contribute to the overall incidence of severe listeriosis, in accordance with the level of *L. monocytogenes* per serving and the number of servings consumed. The influence of maximum contamination level per serving was again considered for these multiple-dose derived r-values and compared with the corresponding r-values derived considering only the highest dose level (Table 2.18). The same point estimates for percentage of population with increased susceptibility, percentage of listeriosis cases associated with that population, and the total number of listeriosis events were used, so the single-dose derived and multiple-dose derived r-values could be compared.

The r-value for *L. monocytogenes* increased approximately 30-fold when the maximum assumed $\log_{10}$ dose level was decreased from 10.5 to 7.5 (Table 2.17). The 5% to 95% confidence interval for the r-value was typically small ($\log_{10}$ differential of approximately 0.2 to 0.3) within a maximum assumed dose per serving level. When the maximum assumed $\log_{10}$ dose level was treated as a variable having a discrete uniform distribution, the 5% to 95% confidence range increased substantially, spanning a $\log_{10}$ differential of approximately 1.6. This indicated that the impact of the assumed maximum level of contamination in food had a substantially greater effect than the other parameters considered.

The multiple-dose derived r-values (Table 2.18) were consistently lower than, but not very different from, the corresponding r-value based on maximum-dose derivation, i.e. the assumption that all cases were due only to servings contaminated by the maximum dose level that would be encountered. The maximum-dose assumption simplifies calculation but in reality all dose levels may contribute to the incidence of foodborne listeriosis. This is depicted as a function of the maximum assumed level of contamination per serving in Table 2.19. The estimated number of cases does not increase monotonically going from a lower to a higher dose level. This is because the number of cases contributed by food in a specific dose category depends not only on that dose, but also on the number of servings in that dose category.

Table 2.17 The effect of the assumed maximum individual dose level on the calculated r-values for *Listeria monocytogenes* for the fraction of the population with increased susceptibility. Estimations assume all cases of severe listeriosis to be due to ingestion of servings only at the highest dose level.

Maximum Log Dose per Serving	Median Maximum-Dose Derived r-value	5th Percentile r-value	95th Percentile r-value
7.5	8.61×10^{-12}	6.38×10^{-12}	1.13×10^{-11}
8.5	2.09×10^{-12}	1.53×10^{-12}	2.76×10^{-12}
9.5	5.59×10^{-13}	4.14×10^{-13}	7.47×10^{-13}
10.5	2.79×10^{-13}	2.06×10^{-13}	3.70×10^{-13}
7.5 to 10.5(2)	1.06×10^{-12}	2.47×10^{-13}	9.32×10^{-12}

NOTE: (1) Values were obtained by Monte Carlo simulation techniques assuming that (i) the percentage of the population with increased susceptibility to *L. monocytogenes* varied between 15 and 20%; (ii) the percentage of cases of total severe listeriosis cases associated with this increased susceptibility population ranged from 80 to 98%; and (iii) the uncertainty of the estimates of the total number of cases is ±25%.
(2) Uncertainty of maximum dose level described by RiskDuniform (7.5; 8.0; 8.5; 9.0; 9.5; 10.0; 10.5) distribution.

Table 2.18 The effect of the assumed maximum individual dose level on the calculated r-values for *Listeria monocytogenes* for the fraction of the population with increased susceptibility. The estimations are based on calculations using the single-value estimates of the maximum (maximum-dose derived r-values) or using the entire range of maximum dose values in deriving a single r-value (multiple-dose derived r-values). These estimations assume that all cases of severe listeriosis are due to ingestion of servings only at the highest dose level (see Note 1).

Maximum Log Dose per Serving (CFU)	Maximum-Dose Derived r-value	Multiple-Dose Derived r-value
7.5	8.05×10^{-12}	5.85×10^{-12}
8.5	1.95×10^{-12}	1.45×10^{-12}
9.5	5.24×10^{-13}	3.72×10^{-13}
10.5	2.61×10^{-13}	1.34×10^{-13}

NOTE: (1) Point estimates were used for (i) the percentage of the population in the more susceptible group (17.5%); (ii) the percentage of cases associated with the more susceptible population (83%); and (iii) the total number of severe listeriosis cases (2518).

The exponential dose-response model is a non-threshold model. Consequently, there is no dose value other than zero that results in a prediction that there is no risk of illness. As mentioned previously, the r-value in the exponential model can be viewed as the probability that a single cell of *L. monocytogenes* would cause an illness. Table 2.19 indicates that when the most conservative assumption for the numbers of *L. monocytogenes* consumed in a serving ($10^{7.5}$ maximum CFU/serving) was used, over 99% of the cases arise from the consumption of servings that contain $10^{5.5}$ CFU per serving. If the maximum number in a serving was assumed to be $10^{10.5}$, over 99% of the cases of listeriosis arise from 10^8 CFU or more per serving. Only a very small fraction of the 3.66×10^{11} United States of America servings need to achieve these levels of *L. monocytogenes* to account for 2500 cases of severe listeriosis. The estimated number of servings for each dose category can be found in spreadsheet cells F82 to F98 in Table 2.22 (at the end of Part 2). The distribution is shown in Figure 2.9.

The second set of r-values was developed for the remainder of the population that did not have increased susceptibility for *L. monocytogenes*. The same types of calculations were performed as for the susceptible population. The parameters were (i) the portion of the population with decreased susceptibility for *L. monocytogenes* was 80% to 85% (point estimate = 83%) of the total population; and (ii) this portion of the population is associated with 2–20% of the cases (point estimate = 11%). It was again assumed that the total number of cases of severe listeriosis was 2518 ± 25%. The effect of the maximum assumed level of contamination on maximum-dose derived r-values based on Monte Carlo simulations with estimates of uncertainty for size of this population, the fraction of listeriosis cases with which it is associated, and the total number of listeriosis cases is summarized in Table 2.20. The corresponding comparison of the maximum-dose derived values and the multiple-dose derived r-values is presented in Table 2.21.

The r-values for the less susceptible portion of the population were 1 to 2 orders of magnitude smaller than the corresponding r-values for the more susceptible population. In general, the uncertainty associated with the less susceptible population (Table 2.20) was greater than that for the more susceptible population (Table 2.17). Like the more susceptible population, the multiple-dose derived r-values for the less susceptible population was consistently smaller (i.e. less conservative) than the corresponding maximum-dose derived r-

values (Table 2.21). It should be noted that calculating morbidity$_{50}$ values for r-values of the magnitude observed in Table 2.21 should be considered notional, since the doses required are higher than achievable in a food serving. It is highly unlikely than any individual would ever encounter a dose in foods greater than 10^{10} or 10^{11} CFU per serving. In such instances, the dose-response curve would most appropriately be interpreted as indicating that a large portion of the population would not acquire severe listeriosis even in the presence of extremely high doses. This also indicates why most cases of listeriosis are sporadic.

Table 2.19 The effect of assumed maximum individual dose level per serving on the number of cases contributed per dose level per serving of food. The predictions are for the susceptible population and were based on the exponential dose-response model, the distribution of servings per dose level and the multiple-dose derived r-values in Table 2.18.

Log Dose	**Estimated number of cases with different presumed maximum $\log_{10}$ doses**			
	7.5	8.5	9.5	10.5
-1.5	<1[(1)]	<1	<1	<1
-0.5	<1	<1	<1	<1
0.5	<1	<1	<1	<1
1.5	<1	<1	<1	<1
2.5	1	<1	<1	<1
3.5	2	1	<1	<1
4.5	12	3	1	<1
5.5	56	14	3	1
6.5	265	64	16	6
7.0	235	57	14	5
7.5	1 519	123	31	11
8.0		268	68	25
8.5		1 561	149	53
9.0			323	116
9.5			1 483	252
10.0				547
10.5				1 073
Total cases[(2)]	2 090	2 090	2 090	2 090
Multiple-dose derived r-value used	5.85×10^{-12}	1.45×10^{-12}	3.72×10^{-13}	1.33×10^{-13}

NOTE: (1) Predicted number of cases attributed to a specific dose. Total cases based on the assumption behind the r-values in Table 2.18 that 83% of total cases (2518) are in the susceptible group; $0.83 \times 2518 = 2090$ cases

Table 2.20 The effect of assumed maximum individual dose level on the calculated r-values for *Listeria monocytogenes* for the population with decreased susceptibility. The estimations asssume that all cases of severe listeriosis are due to ingestion of servings only at the highest dose level (see Note (1)).

Maximum log dose per serving (CFU)	**Median Maximum-Dose Derived r-value**	**5th Percentile r-value**	**95th Percentile r-value**
7.5	2.23×10^{-13}	5.82×10^{-14}	4.22×10^{-13}
8.5	5.34×10^{-14}	1.42×10^{-14}	1.02×10^{-13}
9.5	1.45×10^{-14}	3.75×10^{-15}	2.74×10^{-14}
10.5	7.18×10^{-15}	1.85×10^{-15}	1.15×10^{-14}
7.5 to 10.5[(2)]	2.37×10^{-14}	3.55×10^{-15}	2.70×10^{-13}

NOTES: (1) Values were obtained by Monte Carlo simulation techniques, assuming that (i) the percentage of the population with increased susceptibility to *L. monocytogenes* varied between 80 and 85%, (ii) that the percentage of cases of total severe listeriosis cases associated with this increased susceptibility population ranged from 2 to 20%, and (iii) the uncertainty of the estimates of the total number of cases is ±25%. (2) Uncertainty of maximum dose level described by RiskDuniform(7.5; 8.0; 8.5; 9.0; 9.5;10.0; 10.5) distribution.

Table 2.21 The effect of assumed maximum individual dose level on the calculated r-values for *Listeria monocytogenes* for the population with decreased susceptibility: The estimations are based on calculations using the single-value estimates of the maximum dose (maximum-dose derived r-values) or using the entire range of maximum dose values in deriving a single r-value (multiple-dose derived r-values), assuming that all cases of severe listeriosis are due to ingestion of servings only at the highest dose level (see Note (1)).

Maximum log dose per serving (CFU)	Maximum-dose derived r-value	Multiple-dose derived r-value
7.5	2.25×10^{-13}	1.64×10^{-13}
8.5	5.45×10^{-14}	4.07×10^{-14}
9.5	1.47×10^{-14}	1.07×10^{-14}
10.5	7.27×10^{-15}	3.73×10^{-15}

NOTES: (1) Point estimates were used for (i) the percentage of the population in the more susceptible group (17.5%), (ii) the percentage of cases associated with the more susceptible population (83%), and (iii) the total number of severe listeriosis cases (2518).

2.3.3 Differences in susceptibility to listeriosis for different human populations.

In addition to developing dose-response models for the entire more-susceptible population, the Codex Committee for Food Hygiene also requested estimates of the relative susceptibility of different sub-populations that have specific chronic diseases. These had not been developed in previous risk assessments, so a means of fulfilling this request had to be developed. The approach taken was to estimate the relative susceptibility based on detailed epidemiological data and to estimate the dose-response relations in conjunction with the exponential dose-response model (*see* Section 5.2).

2.4 r-VALUES FOR RISK CHARACTERIZATION

As explained in the preceding sections, the available contamination and epidemiological data do not permit an unequivocal choice of the most appropriate r-values for different populations. Accordingly, the risk assessment team, in consultation with the international panel of experts, used the following r-values to illustrate various attributes associated with the risk assessment and to address the CCFH questions.

- For CCFH Question 1, on the risk from consuming different numbers of *L. monocytogenes*, an r-value of 5.85×10^{-12} was used for the susceptible population. This was the most conservative dose-response curve used in the current risk assessment and was calculated on the assumption that the maximum individual dose was 7.5 $\log_{10}$ CFU per serving (Table 2.18).
- To illustrate how to estimate r-values based on the relative risks for different susceptible sub-populations in CCFH Question 2, an r-value of 5.34×10^{-14} was selected as the reference value for the general healthy population. This r-value was derived based on an assumption of an intermediate level of maximum individual dose, 8.5 $\log_{10}$ CFU per serving, in food (Table 2.20).
- For the food examples described in the risk assessment and CCFH Question 3, the r-values used were based on the use of Monte Carlo simulation techniques in combination with a discrete uniform distribution wherein the maximum number of *L. monocytogenes* consumed varied from 7.5 to 10.5 $\log_{10}$ CFU per serving. For the

population with increased susceptibility, the median r-value used with its distribution was 1.06×10^{-12} (Table 2.17). For the healthy population, the median r-value used with its distribution was 2.37×10^{-14} (Table 2.20).

Table 2.22. Spreadsheet-based exponential *Listeria monocytogenes* dose-response model (See following pages).

Exponential Lm Dose-response model

For the population of interest, R is estimated based on the U.S. FDA/FSIS assessment of the annual exposure to different doses of Lm and the annual number of listeriosis cases. The estimated uncertainty of R due to the uncertainties in the assumed maximum dose levels in the different food categories, the size of the population of interest, and the number of cases in this population is calculated. Blue indata, Red outdata, Green results calculated and used in the spreadsheet model

Assumptions

* The same as FDA/FSSIS Exposure assessment.
* Maximum dose levels: vary between 7.5 and 10.5
* The total number of listeriosis cases 2518 +/-25%
* The fraction of cases within each of the subgroups were based on outbreak data shown in worksheet proportion susceptible (T. Ross), and estimates from the U.S. Risk assessment (98% cases belonging to the susceptible group, R. Whiting)

	Input data	Input	Formula			Output	Formula
	Population of interest	Susceptible			**Estimated R**	2.80E-13	G4 = E106
	Fraction of total population	1.75E-01	C4 = RiskUniform(0.15;0.2)				
	Total # of listeriosis cases	2.52E+03	C5 = RiskUniform(1888;3148)				
	Fraction of listeriosis cases	8.90E-01	C6 = RiskUniform(0.8;0.98)				
	# listeriosis cases in this population	2.24E+03	C7 = C5*C6				
	Assumed maximum log dose level	10.5	C8 = Input data				

Dose (CFU) as % of serves at point of consumption

Food category	**Total consumption (Servings)**	**< 1 g**	**1 to 1000**	**10^3 to 10^6**	**10^6 to 10^9**	**> 10^9**	**TOTAL %**
Smoked Seafood	2.05E+08	70.64%	14.29%	11.06%	3.42%	0.20%	0.996
Raw Seafood	1.82E+08	92.07%	6.66%	1.21%	0.07%	0.00%	1.000
Preserved Fish	1.05E+08	84.77%	10.42%	3.89%	0.49%	0.04%	0.996
Cooked RTE Shellfish	5.52E+08	94.50%	4.01%	1.28%	0.20%	0.05%	1.000
Vegetables	1.17E+11	91.11%	7.23%	1.54%	0.07%	0.00%	0.999
Fruits	5.03E+10	81.37%	18.49%	0.13%	0.00%	0.00%	1.000
Soft mold-ripened	2.44E+08	92.81%	3.21%	3.34%	0.67%	0.01%	1.000
Goat/Sheep etc cheese	2.55E+08	92.18%	6.24%	1.48%	0.07%	0.00%	1.000
Fresh Soft Cheese	1.34E+08	89.72%	3.20%	4.31%	2.51%	0.19%	0.999
Heated and Processed	1.82E+10	98.20%	1.71%	0.08%	0.01%	0.00%	1.000
Aged Cheese	1.38E+10	98.07%	1.82%	0.03%	0.00%	0.00%	0.999
Pastuerised Milk	8.72E+10	99.20%	0.74%	0.05%	0.00%	0.00%	1.000
Raw Milk	4.36E+08	91.87%	7.56%	0.55%	0.01%	0.00%	1.000
Ice Cream	1.49E+10	99.08%	0.53%	0.02%	0.00%	0.00%	0.996
Miscellaneous Dairy	2.81E+10	98.26%	1.64%	0.07%	0.00%	0.00%	1.000
Frankfurters	6.52E+09	92.40%	6.08%	1.37%	0.21%	0.02%	1.001
Dry/Semi-Dry	1.79E+09	90.27%	6.83%	2.40%	0.10%	0.00%	0.996
Deli Meats	2.07E+10	90.66%	5.40%	3.29%	0.70%	0.12%	1.002
Pâté	1.18E+08	91.52%	4.01%	2.87%	1.06%	0.22%	0.997
Deli Salads, Non-	5.63E+09	86.30%	8.77%	3.98%	0.80%	0.03%	0.999
Total Servings	3.66E+11	SUMMA(B12:B31)					

Formula
B12:G31 = Input data
H12:H31 = Sum(C12:G12)

	A	B	C	D	E	F	G	H	I	J	K	L	M
34													
35					Predicted Number of Doses at indicated dose level								
36	**Food category**	**< 1 g**	**1**	**10^3**	**10^6 to**	**> 10^9**	**Formula**						
37	Smoked Seafood	1.45E+08	2.93E+07	2.27E+07	7.01E+06	4.20E+05	B37:F56 = C12 * $B12						
38	Raw Seafood	1.68E+08	1.21E+07	2.20E+06	1.24E+05	5.46E+03	B57:F57 = Sum(B37:B56)						
39	Preserved Fish	8.90E+07	1.09E+07	4.09E+06	5.19E+05	4.27E+04	G57 = Sum(B57:G57)						
40	Cooked RTE Shellfish	5.22E+08	2.21E+07	7.05E+06	1.13E+06	2.59E+05	B58:F58 = SUM(B57:B57)/SUM(B57:F57)						
41	Vegetables	1.07E+11	8.46E+09	1.80E+09	7.80E+07	0.00E+00							
42	Fruits	4.09E+10	9.30E+09	6.49E+07	1.34E+06	0.00E+00							
43	Soft mold-ripened	2.26E+08	7.83E+06	8.15E+06	1.62E+06	2.20E+04							
44	Goat/Sheep etc cheese	2.35E+08	1.59E+07	3.76E+06	1.84E+05	0.00E+00							
45	Fresh Soft Cheese	1.20E+08	4.28E+06	5.77E+06	3.36E+06	2.60E+05							
46	Heated and Processed	1.79E+10	3.12E+08	1.50E+07	1.05E+06	3.03E+05							
47	Aged Cheese	1.35E+10	2.51E+08	3.84E+06	0.00E+00	0.00E+00							
48	Pastuerised Milk	8.65E+10	6.43E+08	4.24E+07	3.63E+06	1.60E+06							
49	Raw Milk	4.01E+08	3.30E+07	2.39E+06	5.38E+04	1.02E+04							
50	Ice Cream	1.48E+10	7.83E+07	2.38E+06	7.45E+04	0.00E+00							
51	Miscellaneous Dairy	2.76E+10	4.61E+08	1.88E+07	1.40E+06	4.92E+05							
52	Frankfurters	6.02E+09	3.96E+08	8.95E+07	1.40E+07	1.30E+06							
53	Dry/Semi-Dry	1.62E+09	1.22E+08	4.30E+07	1.82E+06	5.37E+04							
54	Deli Meats	1.88E+10	1.12E+09	6.80E+08	1.45E+08	2.41E+07							
55	Pâté	1.08E+08	4.73E+06	3.39E+06	1.25E+06	2.57E+05							
56	Deli Salads, Non-	4.86E+09	4.94E+08	2.24E+08	4.51E+07	1.84E+06							
57	**Total servings at each dose level**	3.41E+11	2.18E+10	3.05E+09	3.07E+08	3.10E+07	3.662E+11						
58	**Cumulative frequency**	0.931297	0.990762	0.999077	0.999915	1.000000							
59													
60													
61	**Empirical cum freq distribution. The max log dose (A66) is variable between 8 and 10.5**						**log no**	**Formula**					
62	**Log Dose levels**	**No of servings**	**Cum no servings**	**Cum Freq**	**Log Cum Freq**	**log(-log(CF))**	**servings**						
63	-1.5	3.41E+11	3.41E+11	9.313E-01	-3.091E-02	-1.509874594	11.53286444	A63:A67 = Input data					
64	1.5	2.18E+10	3.63E+11	9.908E-01	-4.031E-03	-2.39464095	10.33804167	B63:B67 = B57					
65	4.5	3.05E+09	3.66E+11	9.991E-01	-4.011E-04	-3.396742519	9.483611912	C63:C67 = C63 + B64					
66	7.5	3.07E+08	3.66E+11	9.999E-01	-3.678E-05	-4.434399463	8.487262531	D63:D67 = C63/($B$68)					
67	10.5	3.10E+07	3.66E+11	1.000E+00	0.000E+00		7.491574159	E63:E67 = Log (D63)					
68	**Total**	3.662489E+11						F:63:F67 = Log (-E63)					
69				4.92E+00				G63:G67 = Log (B63)					
70													
71	**Fitted curve to Empirical cum freq distribution**												
72	**Log Dose level**	**Log (-log(CF))**			**Comment**	**Formula**							
73	-1.5	-1.509874594	slope	-0.32585587	Linear regression	A73:A76 = Input data							
74	1.5	-2.39464095	intercept	-1.95634676	Log(dose) vs Log (	B73:B76 = F63							
75	4.5	-3.396742519			Log(CF))	D73 = Slope(B73:B76;A73:A76)							
76	7.5	-4.434399463				D74 = Intercept(B73:B76;A73:A76)							
77													

	A	B	C	D	E	F	G	H	I	J	K	L	M
77													
78	**Generation of new frequency distribution data at closer intervals depending on the simulated**												
79	**maximum dose levels and using the fitted curve derived above**												
80													
81	**Log Dose level**	**log(-log(CF))**	**Cum Frequency**	**Frequency**	**From original data**	**Meals consumed in each category**	**# Meals consumed by population**	**Formula**					
82	-1.5	-1.467562955	0.924538301	0.9245383	9.313E-01	3.39E+11	5.93E+10	A82:A98 = Input data					
83	-0.5	-1.793418828	0.963627242	0.03908894		1.43E+10	2.51E+09	B82:B91 = $D$74+$D$73*A82					
84	0.5	-2.1192747	0.982656078	0.01902884		6.97E+09	1.22E+09	B92:B98 = IF($C$8>=7.5;$D$74+$D$73*A92;MISSING())					
85	1.5	-2.445130573	0.991772038	0.00911596	9.91E-01	3.34E+09	5.84E+08	C82:C98 = 10^(-(10^B82))					
86	2.5	-2.770986445	0.996106122	0.00433408		1.59E+09	2.78E+08	C92:C98 = IF($C$8>=7.5;10^(-(10^B92));MISSING())					
87	3.5	-3.096842318	0.998159341	0.00205322		7.52E+08	1.32E+08	D82:D98 = C83-C82					
88	4.5	-3.42269819	0.999130382	0.00097104	9.99E-01	3.56E+08	6.22E+07	D92:D98 = IF($C$8=A92;$B$100-C91;IF($C$8>A92;C92-C91;M					
89	5.5	-3.748554063	0.999589255	0.00045887		1.68E+08	2.94E+07	E82:E98 = Input data					
90	6.5	-4.074409935	0.999806017	0.00021676		7.94E+07	1.39E+07	E92:E98 = IF($C$8=7.5;1;D66)					
91	7.0	-4.237337872	0.999866694	6.0677E-05		2.22E+07	3.89E+06	F82:F98 = D82*$B$68					
92	7.5	-4.400265808	0.999908393	4.1698E-05	9.99915E-01	1.53E+07	2.67E+06	F92:F98 =IF($C$8>=7.5;D92*$B$68;					
93	8.0	-4.563193744	0.999937048	2.8655E-05		1.05E+07	1.84E+06	G82:G98 = F82*$C$4					
94	8.5	-4.72612168	0.99995674	1.9692E-05		7.21E+06	1.26E+06	G92:G98 = IF($C$8>=7.5;F92*$C$4;"")					
95	9.0	-4.889049617	0.999970272	1.3532E-05		4.96E+06	8.67E+05	F99 = SUM(F82:F98)					
96	9.5	-5.051977553	0.999979572	9.2992E-06		3.41E+06	5.96E+05	G99 = SUM(G82:G98)					
97	10.0	-5.214905489	0.999985962	6.3903E-06		2.34E+06	4.10E+05						
98	10.5	-5.377833425	0.999990353	3.9656E-06	1.00E+00	1.45E+06	2.54E+05						
99					**Total**	3.662452E+11	6.41E+10						
100	Fitted cum fr (10.5)	0.999989927573											
101													
102	**Collation of Annual US National Exposure at each Dose Level**												
103													
104	**Estimation of R**			**Solving for R**			**Input for calculation of attack rate**						
105	**Assumption**	**Endpoint**	**# Meals Max Dose**	**Attack rate**	**R**		**Max Log Dose**	**# Meals**					
106	Cases caused by highest dose level only	Listeriosis	2.54E+05	8.82E-03	2.80E-13		7.5						
107							8.0						
108		**Formula table**					8.5						
109		H106:H112 = IF($C$8=G110;G96;"")					9.0						
110		C106 = HLOOKUP($C$8;G106:G112;H106:H112)					9.5						
111		D106 = C7/C106					10.0						
112		E106 = -(LN(1-D106))/10^$C$8					10.5	2.54E+05					
113													
114													