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

INTERPRETATIVE SUMMARY

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INTERPRETATIVE SUMMARY

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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

CAC	Joint FAO/WHO Codex Alimentarius Commission
CCFH	Codex Committee on Food Hygiene
FAO	Food and Agriculture Organization of the United Nations
WHO	World Health Organization
CDC	Centers for Disease Control and Prevention (USA)
RTE	Ready-to-eat
CFU	Colony-forming unit

Executive Summary of the Main Report

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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1. Introduction

Quantitative risk assessment of microbiological hazards in foods is currently a priority area of work for the Codex Alimentarius Commission (CAC). At its 32nd Session, the Codex Committee on Food Hygiene (CCFH) identified a list of pathogen-commodity combinations for which it required expert risk assessment advice. In response to this and the needs of their member countries, the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) jointly launched a programme of work with the objective of providing expert advice on risk assessment of microbiological hazards in foods.

The FAO/WHO programme of activities on microbiological risk assessment aims to serve two customers – the CAC, and FAO and WHO member countries. The CAC, and in particular its subsidiary committee the CCFH, has requested sound scientific advice as a basis for the development of guidelines and recommendations for the management of risks posed by microbiological hazards in food, and has identified 21 pathogen-food commodity combinations of concern (CAC, 1999a). Member countries have further expressed the need for adaptable risk assessments that they can use to conduct their own assessments. In particular, they have indicated the desirability of modules that can be directly applied to their national situation. Taking these needs into account FAO and WHO initiated work on a number of pathogen-commodities combinations, one of them being *Listeria monocytogenes* in ready-to-eat (RTE) foods.

1.1 Scope and objectives of the risk assessment

The invasive form of foodborne listeriosis represents a relatively rare, but serious, disease, with high fatality rates (20–30%) compared with other foodborne microbial pathogens, such as *Salmonella enterica*. The disease largely affects specific segments of the population who have increased susceptibility. *L. monocytogenes* is widely dispersed in the environment and foods. 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 (FDA/FSIS, 2001). RTE food is a large, heterogeneous category of foodstuffs and can be subdivided in many different ways. According to the Codex definition (CAC, 1999b), RTE include any food (including beverages) that is normally consumed in its raw state, or any food handled, processed, mixed, cooked or otherwise prepared into a form in which it is normally consumed without further processing. RTE foods differ in different countries, according to local eating habits, availability and the integrity of the chill chain and regulations specifying, for example, the maximum temperature at retail level.

The current risk assessment was undertaken, in part, to determine how previously developed risk assessments done at the national level could be adapted or extended to address concerns related to *L. monocytogenes* in RTE foods at an international level. In addition, after initiation of the risk assessment, the risk assessors were asked by the 33rd Session of CCFH, through FAO and WHO, to consider three specific questions related to RTE foods in general.

These questions were:

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.
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 is intended to assist risk managers in conceptualizing how some of the factors governing foodborne listeriosis interact, thereby assisting the development of strategies to reduce rates of illness.

2. Approaches

Considering the resources available and time constraints placed on the risk assessors, it was impossible to consider all RTE foods that could be contaminated with *L. monocytogenes.* Accordingly, it was decided to limit the risk assessments to a finite range of RTE foods that were selected to represent various classes of product characteristics in order to determine if the risk of these foods serving as a vehicle for human foodborne listeriosis could be estimated. These foods were selected to provide examples of how microbiological risk assessment techniques could be used to answer food safety questions at an international level.

In its request to FAO and WHO in 1999 for expert risk assessment advice (CAC, 1999a), CCFH indicated that a farm-to-table risk assessment would provide the broadest range of management options. However, it was decided to limit the study to foods at retail and their subsequent public health impact at the time of consumption. This was for two reasons. First, this was sufficient to address the charge provided by the CCFH within the timeframe and resources available to the risk assessors. Second, most of the exposure data for *L. monocytogenes* that is currently available relate to the frequency and extent of contamination at the retail level. Therefore, the risk assessment does not evaluate the risk associated with different methods of manufacturing the products selected as examples of RTE food classes. However, it does consider several post-retail factors that could influence the risk to a consumer of acquiring foodborne listeriosis, such as temperature and duration of refrigerated storage. Additional risk assessments for specific foods or product categories would be necessary if pre-retail considerations are to be addressed.

In addition to the serious, invasive listeriosis, *L. monocytogenes* can also cause mild febrile gastroenteritis in otherwise healthy individuals. The public health significance of this type of listeriosis is uncertain at this time and is not considered in the current risk assessment.

For the most part, a stochastic approach, as opposed to a deterministic approach, was used in this risk assessment to estimate the risks per serving and risk to a population per annum from the selected foods. Stochastic means that inputs for the mathematical model used to estimate the risk are obtained by sampling from frequency or probability distributions. This allows uncertainty (which can be reduced if more data are gathered) and variability (the naturally occurring differences that occur among members of a population) to be estimated in the model's output. Deterministic approaches – estimates, interval modelling, worst-case, etc. – have their own advantages, but are generally less effective for demonstrating the impact of uncertainty and variability. For the purposes of this risk assessment, after the stochastic models were developed, a simplified deterministic approach was used in conjunction with the stochastic model to effectively answer CCFH Questions 1 and 2.

When a stochastic modelling approach is employed, the factors in the model (e.g. contamination, growth rate, storage time) are represented by distributions that describe the range of values associated with those factors. Because the factors considered in the risk assessment model have uncertainty distributions, the calculated results (e.g. the risk per serving) will also have uncertainty distributions. To make these calculations, Monte Carlo techniques, implemented using Analytica® software, were employed where the model is

calculated many times (iterations). In each iteration, values are selected from each input distribution and an output value is calculated. Each iteration, therefore, has a different set of input values and a different output value. The model is iterated many times, yielding a set of output values that create a distribution. In the exposure assessment phase of the risk assessment, the output values represent the averages of 16 sets of simulations, each set containing 32 000 iterations. The resulting distributions are described in various ways, including their mean, standard deviation, median (50^{th} percentile value) and 5^{th} and 95^{th} percentiles. This stochastic modelling approach, where the results are expressed as distributions, provides a more complete description of the process being modelled than would a single point or deterministic calculation.

3. Hazard Identification

It is now widely recognized that listeriosis is largely attributable to foodborne transmission of the microorganism. Most cases of human listeriosis are sporadic or involve outbreaks that are typically diffuse in terms of time or geographical location, or both. While the modes of transmission for *L. monocytogenes* can include vertical (mother to child), zoonotic (animal to human) or nosocomial (hospital acquired), 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 Schwarz, 1990; Bille, 1990). Foods most often associated with human listeriosis include industrially processed RTE foods that (i) support growth of *L. monocytogenes*, (ii) have a long recommended refrigerated shelf-life, and (iii) are consumed without further listericidal treatments, e.g. cooking (Pinner et al., 1992; Rocourt, 1996; FDA/FSIS, 2001; Nørrung, Andersen and Schlundt, 1999). Due to the severity of the disease and the very frequent involvement of industrially processed foods, especially during outbreaks, the social and economic impact of listeriosis is among the highest of the foodborne diseases (Roberts, 1989; Roberts and Pinner, 1990).

L. monocytogenes is an opportunistic pathogen that most often affects those with severe underlying conditions (such as immunosuppressive therapy, AIDS, and chronic conditions such as cirrhosis that impair the immune system), pregnant women, unborn or newly delivered infants, and the elderly. *L. monocytogenes* infections can be life threatening, with fatality rates of 20 to 30%. All strains of *L. monocytogenes* appear to be pathogenic. However, the relative virulence of individual *L. monocytogenes* isolates can vary substantially (Hof and Rocourt, 1992), and virulence, as defined in experimental animal studies, may vary up to 1000-fold. Similarly, there is evidence for variation in virulence among foodborne isolates 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, 1990; Farber and Peterkin, 1991). 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; Weidman et al., 1997), and none of the present methods have consistently identified strains that are non-pathogenic or less virulent (McLauchlin, 1997).

Following invasion of the intestinal tissue, *L. monocytogenes* most often spreads to the blood, liver, the pregnant uterus, or the central nervous system. Manifestations of invasive listeriosis include, but are not limited to, bacteraemia, central nervous system infections (meningitis, encephalitis, meningoencephalitis), prodromal illness in pregnant women, miscarriage, premature birth, stillbirth, and neonatal disease. Incubation periods before individuals become ill can be long: typically two to three weeks, and occasionally up to three months (Gellin and Broome, 1989).

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), 0.3 to 7.5 cases per million people in Europe (EC, 2003), 4.4 cases per million people in the

United States of America (Mead et al., 1999) and 3 cases per million people in Australia; however, the accuracy of these values is dependent on the vigour with which individual countries conduct national surveillance programmes for listeriosis. The severe nature of listeriosis makes it likely that individuals will seek medical care, and in the United States of America, the Centers for Disease Control and Prevention (CDC) estimates that 90% of all listeriosis cases are hospitalized and that approximately half of all cases are reported to the CDC, as compared to the 3% identification rate for most other foodborne pathogens (Mead et al., 1999). Listeriosis is mainly observed in industrialized countries. What is not known is whether these differences in incidence rates between developed and less developed countries reflect true geographical differences, differences in food habits and food storage, or differences in diagnosis and reporting practices.

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. There is evidence to suggest that it is a transitory resident of the intestinal tract of humans, with 2 to 10% of the general population, as assessed by examination of faecal samples, being carriers of the organism without any apparent adverse health consequences (Farber and Peterkin, 1991; Rocourt and Cossart, 1997; Skidmore, 1981; Slutsker and Schuchat, 1999; Mascola et al., 1992; Schuchat et al., 1991). An important factor in foodborne listeriosis is that the pathogen can grow to significant numbers at refrigeration temperatures when given sufficient time. *L. monocytogenes* is more resistant to various environmental conditions than many other non-spore forming foodborne pathogenic bacteria, which allows it to survive longer under adverse conditions (McCarthy, 1990; Ryser and Marth, 1991). *L. monocytogenes* is present in many food processing environments (Ryser and Marth, 1991, 1999), and can survive for long periods in foods, in processing plants, in households and food service establishments, or in the environment, particularly at refrigeration or frozen storage temperatures. The ability of *L. monocytogenes* to grow and 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 growth and survival.

L. monocytogenes is frequently present in raw foods of both plant and animal origin and can become endemic in food processing environments. It is also present in cooked foods due to post-processing contamination or insufficient heat treatment. *L. monocytogenes* has been isolated from such foods 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). A survey of a wide variety of foods from the refrigerators of listeriosis patients in the United States of America found that food isolates of *L. monocytogenes* indistinguishable from the patient strain could be isolated from 33% of the refrigerators (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 (CCFH, 1999; EC, 1999; Farber, Ross and Harwig, 1996; ICMSF, 1994).

4. Hazard Characterization
[Dose-response relationship]

4.1 Severity of listeriosis

Characterization of the severity of listeriosis was limited to a description of the manifestations of the disease, a summary of epidemiological information from outbreaks, and a consideration of case fatality rates. A more detailed quantification of the severity of the disease was beyond the scope of the risk assessment and not necessary to address the questions posed by CCFH. Briefly, various clinical manifestations are associated with listeriosis that can be grouped in two categories: invasive listeriosis and non-invasive listeriosis.

Invasive listerioses are cases when initial infections of the intestinal tissue by *L. monocytogenes* leads to invasion of otherwise sterile body sites. The organs most often infected are the pregnant uterus, the central nervous system, and the blood. A summary of 782 cases of listeriosis reported from 20 countries showed that 43% were pregnancy-related infections, and 57% were non-pregnancy-related cases, which could be further categorized as 29% septicaemic infections, 24% central nervous system infections and 4% atypical forms (Rocourt, 1991). In addition to the unusual severity of clinical manifestations, listeriosis is characterized by a high case-fatality rate, ranging from 20 to 30% (Mead et al., 1999). 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 central nervous system infection suffer residual symptoms, and psychiatric sequelae have also been reported (references cited in Rocourt, 1996). The usual epidemiological feature of invasive listeriosis is a relatively frequent occurrence of sporadic cases and the occasional recognition of outbreaks. Most cases of listeriosis appear to be sporadic, although a portion of these sporadic cases may be unrecognized common-source clusters. A recent study indicated that 95% of these sporadic cases are foodborne (Mead et al., 1999). A number of foodborne outbreaks have been described since 1981, and some of them have involved large numbers of patients over a long period: 122 patients in Switzerland in 1985–1987, approximately 300 patients in the United Kingdom in 1987–1989, and 279 patients in France in 1992 (Rocourt and Cossart, 1997).

Non-invasive listeriosis (referred to as febrile listerial gastroenteritis) has been observed mainly 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 for sporadic cases are not known.

Because the public health impact of non-invasive listeriosis is uncertain and there are insufficient data available about the incidence of the milder symptoms, the impact of this

biological end point on public health was not assessed in the current exercise. Thus, in the current assessment, the term listeriosis relates implicitly to invasive listeriosis.

4.2 Foods associated with listeriosis

Common-source outbreaks have been associated with or linked epidemiologically with the consumption of Hispanic-style soft cheeses (*queso fresco*); soft, semi-soft and mould-ripened cheeses; hot dogs (frankfurters); pork tongue in jelly; processed meats; pate; salami; pasteurized chocolate flavoured milk; pasteurized milk; unpasteurized milk; butter; cooked shrimp; smoked mussels; smoked fish; potato salad; raw vegetables and coleslaw.

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 coleslaw.

In general, the levels of *L. monocytogenes* in the implicated food have exceeded 10^3 CFU/g (EC, 1999; FSA/FSIS, 2001), 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 the serving of food consumed by an infected individual could have varied considerably from that observed in other portions of the food during a subsequent investigation.

4.3 Review of existing dose-response relationships for *L. monocytogenes*

The responses of a human population to exposures to a foodborne pathogen are highly variable, reflecting the fact that the incidence of disease is dependent on a variety of factors, including the virulence characteristics of the pathogen, the numbers of cells ingested, the general health and immune status of the host, and the attributes of the food matrix that alter microbial or host status. It is also unknown what role prior exposure to foodborne *L. monocytogenes* has on the host's immune response, as presumably most individuals routinely have some exposure to this pathogen. Thus, the likelihood that any individual will become ill due to an exposure to a foodborne pathogen is dependent on the integration of host, pathogen and food matrix effects. These interactions are often referred to as the infectious disease triangle. Each of these categories and how they affect the dose-response relations for *L. monocytogenes* are addressed in the Technical Report (FAO/WHO, 2004). A mathematical relationship between the dose and the response would ideally be able to describe the interactions between all these factors. However, due to insufficient data, the potential effects of the food matrix on the dose-response relation were not considered explicitly as a variable in any of the models. The influence of host factors both in the available models and in the models developed in the present risk assessment (see Section 4.4) was addressed by developing different relationships for different susceptible and non-susceptible populations. 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.

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 are reviewed and summarized in the Technical Report, 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 cell acts independently, and that a single bacterial cell has the potential to cause disease (the minimum infectious dose is one bacterium). With the exponential model, the probability that a single bacterium causes illness is assumed to be the same for all ingested *L. monocytogenes*, and this probability is expressed by a single parameter, the "r-value". The two-parameter Beta-Poisson model introduces heterogeneity in the pathogen–host interaction and r is assumed to be variable. The Weibull-Gamma model is a three-parameter model that, in addition to addressing pathogen–host heterogeneity, also includes a parameter that modifies the shape of the dose-response curve. Each of these dose-response models has specific characteristics and limitations (see summary in FAO, 2000). The available models, categorized by the biological end-point considered and the type of model used, are summarized in Table 1.

The empirical relationships described in Table 1 give widely differing predictions in the dose region corresponding to levels of *L. monocytogenes* commonly found in food. As an illustration, the predicted dose-response curves for some of the relationships in Table 1, developed based on epidemiological data or expert elicitations, are presented in Figure 1. The differences reflect the fact that different data sets, mathematical models and biological end-points have been used to describe the likelihood that an exposure to *L. monocytogenes* could lead to disease.

With current knowledge, it was not possible to endorse a single, previously available dose-response model (Table 1). Animal data can not be used directly because the susceptibility of laboratory mice, for example, is orders of magnitude greater than that of humans. Because of the severity of listeriosis, no human volunteer studies have been, or will be, conducted. A complete investigation of an outbreak has been done in only a few instances because of the extended period between consumption of a contaminated food and onset of illness. The sporadic nature of listeriosis also makes investigations very difficult. The outbreaks in Los Angeles in pregnant women who consumed Hispanic cheese, and in Finland in hospitalized transplant patients who consumed contaminated butter, were the only outbreaks with relatively complete documentation. These two outbreaks were used to evaluate the exponential dose response models developed for these risk groups in the present study (see the response to Codex Question 2, in Section 6). Thus, incomplete epidemiological information, uncertain extrapolations from animal data to humans, the absence of human feeding trial data, and a lack of mechanistic models are, together with insufficient understanding of strain variation and food matrix effects, limiting factors that contribute to the uncertainty in the description of the dose-response relationship.

Table 1. Summary of *Listeria monocytogenes* dose-response models reviewed in the current risk assessment.

Model and study	Biological end point	Comments
Exponential[(1)] (Buchanan et al., 1997)	Morbidity (serious listeriosis). Based on annual disease statistics and food survey data.	Based on an estimate of immunocompromised individuals. It is purposefully conservative (i.e. biased towards overestimating risk) and assumed that all cases were caused by a single food category. Predicted $Morbidity_{50} = 5.9 \times 10^9$ CFU.
Exponential[(1)] (Lindqvist and Westöö, 2000)		Based on an estimate of immunocompromised individuals. It is purposefully conservative (i.e. biased towards over estimating risk) and assumed that all cases were caused by a single food category. Predicted $Morbidity_{50} = 1.2 \times 10^9$ CFU.
Weibull-Gamma[(1)] (Farber, Ross and Harwig, 1996)	Serious infection in humans. Based on expert elicitation.	The dose estimated for 50% of the population to become infected: High Risk: 4.8×10^5 CFU; Low Risk: 4.8×10^7 CFU. The model is of limited usefulness due to over-prediction of the number of serious illnesses and a general lack of transparency regarding how the various assumptions were reached.
Exponential[(1)] Butter (current study; FDA/FSIS, 2001)	Morbidity (Serious listeriosis). Analysis of outbreak data.	Based on an outbreak in Finland caused by butter. The affected population was a group of highly immunocompromised individuals in a hospital setting. Predicted $Morbidity_{50} = 6.8 \times 10^4$ CFU.
Exponential[(1)] Hispanic-style cheese (current study; FDA/FSIS, 2001)	Morbidity (Perinatal listeriosis). Analysis of outbreak data.	Based on an outbreak in pregnant women in the United States of America caused by Hispanic-style cheese. Predicted $Morbidity_{50} = 1.9 \times 10^6$ CFU.
FDA/FSIS-General[(2)] (FDA/FSIS, 2001)	Mortality. All three models based on combination of animal (mice) lethality and human fatality statistics.	Model includes individuals between the ages of 30 days and 60 years. See Note (3). The number of cases of serious listeriosis was estimated by multiplying predicted fatalities by a factor of 5.
FDA/FSIS-Neonates[(2)] (FDA/FSIS, 2001)		The model includes foetuses and neonates less than 30 days of age. It assumed that exposure is *in utero*.
FDA/FSIS-Elderly[(2)] (FDA/FSIS, 2001)		The model includes individuals over 60 years of age. See Note (3). The number of cases of serious listeriosis is estimated by multiplying predicted fatalities by a factor of 5.
Exponential Model[(1)] Notermans-IV, normal (Notermans et al., 1998)	Mortality in mice.	It is based on mice injected intravenously with *L monocytogenes*. Mice that were not previously exposed were more susceptible to *L. monocytogenes*. The use of mortality in mice without correction for the apparent decreased susceptibility of humans for *L monocytogenes* led to a substantial overestimation of mortality in humans.
Beta-Poisson and Exponential (no fit)[(1)] Haas et al. (1999)	Infection in mice.	Using infection in mice without correction for the apparent decreased susceptibility of humans for *L. monocytogenes* led to a substantial overestimation of the incidence of infection in humans. The selection of the end point of infection of normally sterile sites in mice is difficult to correlate with human disease.

NOTES: (1) See Section 4.3 in the main Report for descriptions of the exponential, Beta-Poisson and Weibull-Gamma models.
(2) Original model based on weighted, multiple mathematical models. FDA/FSIS model used surrogate experimental animal data to establish the shape of the dose-response curve. United States of America epidemiological data estimates 2500 cases and 500 deaths per year. The dose-response curve was fitted to the *L. monocytogenes* contamination at consumption distribution so it would calculate the number of cases from the epidemiological data.
(3) It includes consideration of distributions for strain virulence. It is based on mouse lethality data "anchored" so that the model provides prediction consistent with incidence of lethal *L. monocytogenes* infections reported in FoodNet – The US Foodborne Diseases Active Surveillance Network.

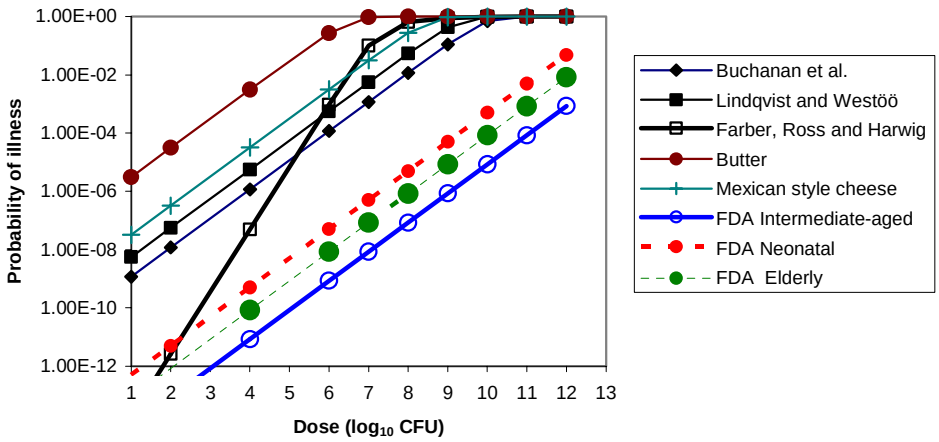

Figure 1. A comparison of dose-response curves for morbidity derived from epidemiological data or expert elicitations. The models are based on illness cases where the primary symptoms included serious illness (smoked fish, Buchanan et al., 1997; smoked fish, Lindqvist and Westöö, 2000; Farber Ross and Harwig, 1996; butter, Finland, see FDA/FSIS, 2001), or perinatal/neonatal infections (Hispanic-style cheese, see FDA/FSIS, 2001). See Table 1 here for a description of the models.

NOTE: The points on the curves are only for legend purposes and do not represent data points. This figure is included for illustrative purposes and caution should be used in interpreting these curves since they are based on different endpoints, types of data, etc., and, in general, the predictions based on the models show a high degree of uncertainty and variation.

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 that have a biological basis and biologically interpretable parameters (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. 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. Another approach that 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 negatively affect 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. Also, 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 simplicity of calculations. For these reasons, 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.

4.4 Exponential dose-response models developed for the present risk assessment

4.4.1 Principle

The general approach used was to take advantage of the epidemiological data and detailed exposure assessment available in the FDA/FSIS risk assessment. The modelling was simplified by describing the dose-response relations using an exponential dose-response model in manner similar to that described in Buchanan et al. (1997) and in Lindqvist and Westöö (2000).

The use of the exponential model in conjunction with food survey data and annual disease statistics to develop a dose-response model was first proposed by Buchanan et al. (1997), based on an analysis of food contamination and epidemiological data from Germany. The exponential model by Lindqvist and Westöö (2000) was based upon food survey data and annual disease statistics from Sweden. Additionally, the FDA/FSIS (2001) risk assessment described dose-response models based on several mathematical forms, including the exponential model. These uses of the exponential model were all based on inferring the dose-response relationship based on the annual incidence of listeriosis and exposure data for one or more foods. The models were based upon similar epidemiological data for the occurrences of listeriosis in healthy and susceptible populations. They also presumed similar ratios of susceptible and healthy people and that the consumption of the foods was similar in the two populations. However, they differed in the extent of exposure assumed, with the models of Buchanan et al. (1997) and Lindqvist and Westöö (2000) relating exposure to a single food and the FDA/FSIS (2001) considering a wider range of RTE foods. The models also differed in the estimate of the highest numbers of *Listeria* consumed. The Buchanan et al. (1997) and Lindqvist and Westöö (2000) modelling assumed that the highest levels found in the foods were 10^4 CFU/g. In contrast, the FDA/FSIS (2001) models assumed that when 10^8 to 10^{10} servings per year are considered, the maximum levels would, on the rare occasions leading to listeriosis, be several orders of magnitude greater (10^7 CFU/g). These differences in assumed maximum individual doses lead to substantial differences in the derived dose-response relationships, such that *L. monocytogenes* is estimated to be significantly less virulent in the FDA/FSIS model (Figure 1).

The validity of this approach depends on several assumptions and sources of information: 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 in this present study 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 magnitude of virulence. Similarly, the biological endpoint (response) used for the dose-response relationships is listeriosis, implying that that term refers to "severe infection" or "invasive listeriosis", and includes those infected individuals suffering from life-threatening, invasive 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.

The exponential dose-response model was chosen because of its acknowledged applicability (i.e. fit) for modelling severe listeriosis, its simplicity as a single-parameter model, and its linear nature when extrapolated to the low dose ranges of interest. The equation is:

$$P = 1 - e^{-r*N}$$

where P is the probability of severe illness, N is the ingested dose (the number of *L. monocytogenes* consumed), and r is the probability that a single cell causes illness, which defines the dose-response relation for the population being considered.

The exponential model is a non-threshold model that implies there is no "minimum infectious dose". Instead the model assumes that a single *L. monocytogenes* cell has a very small but finite probability of causing illness. A key attribute of the model is its linearity or proportionality between dose and probability of illness at low doses. This implies that if the dose is reduced ten-fold, the probability of illness is reduced ten-fold. In addition, it implies that, except at very high doses, 1 000 servings with a specified level of contamination has the same public health impact as 10 000 servings with ten-fold less organisms. Another advantage of using the single-parameter exponential model is that a set of r-values for different susceptible populations can be calculated from relative risks derived from epidemiological studies.

As inputs to the current risk assessment, specific r-values were derived for the less susceptible (healthy) and more susceptible populations, based on the assumption that the overall consumption of *L. monocytogenes* was similar in these groups. This 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, so that the r-value could be calculated by re-arranging the above equation and solving (see Response to Question 2, in Section 6).

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 dose. This, in turn, affects the derived r-value and the resulting dose-response curve. Consequently, this assumption was evaluated in detail. The r-values were estimated for four point estimates of the maximum doses of 7.5, 8.5, 9.5 and 10.5 $\log_{10}$ CFU, respectively. The lower the maximum dose assumed, the larger the estimated r-value. The larger the r-value, the greater 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.

4.4.2 r-values for Risk Characterization and the CCFH questions

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 r-values presented in Table 2 to illustrate various attributes associated with the risk assessment and to address the questions posed by CCFH.

For CCFH Question 1, addressing 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" (i.e. the greatest assumed virulence for *L. monocytogenes*) dose-response curve used in the current risk assessment and was calculated assuming that the maximum individual dose in the FDA/FSIS (2001) exposure assessment was 7.5 $\log_{10}$ CFU per serving.

For illustrating how to estimate r-values based on relative risks for different susceptible sub-populations in 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 calculated based on an assumption on an intermediate maximum individual dose, 8.5 $\log_{10}$ CFU per serving, in food.

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 (see previous section) wherein the maximum number of *L. monocytogenes* consumed varied from 7.5 to 10.5 $\log_{10}$ CFU per serving. These dose-response curves and their confidence intervals are depicted in Figure 2.

In summary, an exponential dose-response model was used in the risk assessment. The model contains one parameter, r, which is the probability that a single cell will cause invasive listeriosis. This parameter (r-value) 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 N, the number of *L. monocytogenes* consumed.

Table 2. r-values used in the risk assessment to address CCFH questions.

Used for question	Population	Median	5% percentile	95% percentile
1	Susceptible[1]	5.85×10^{-12} [4]		
2	Healthy[2]	5.34×10^{-14} [4]		
3 and the four food examples	Susceptible[3]	1.06×10^{-12}	2.47×10^{-13}	9.32×10^{-12}
	Healthy[3]	2.37×10^{-14}	3.55×10^{-15}	2.70×10^{-13}

Notes: (1) calculated assuming of a maximum dose of 7.5 $\log_{10}$ CFU per serving. (2) Calculated assuming a maximum dose of 8.5 $\log_{10}$ CFU per serving. (3) The actual maximum dose level of *L. monocytogenes* in food was assumed to vary uniformly between 7.5 and 10.5 $\log_{10}$ CFU per serving. (4) Used as point estimates.

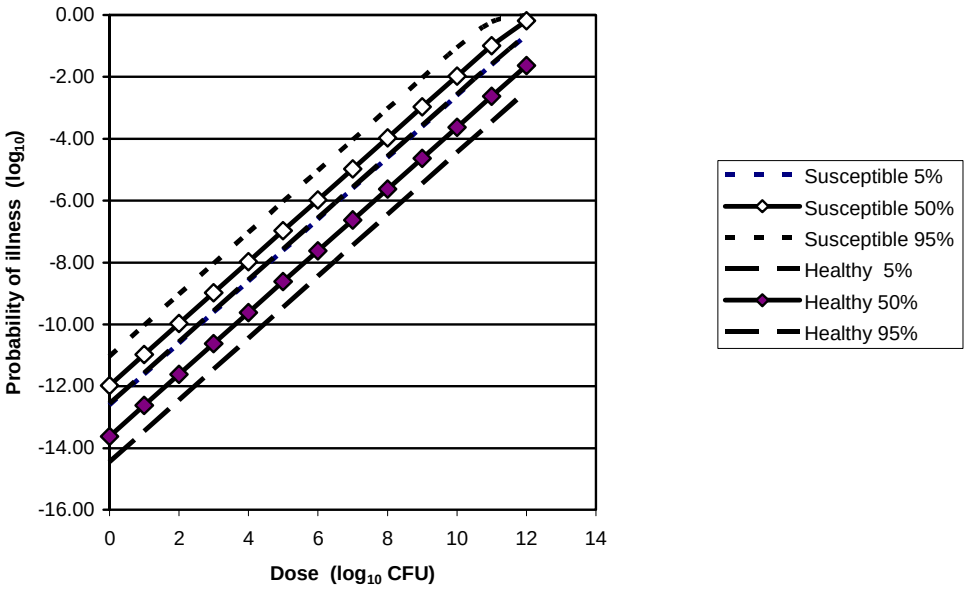

Figure 2. Comparison of the dose-response curve for susceptible and healthy populations. The median (50%) and the 5% and 95% uncertainty levels are shown.

NOTE: The Susceptible 5% and Healthy 95% lines are indistinguishable.

5. Sample risk assessments of selected RTE foods

5.1 Exposure Assessment

The risk management questions posed by CCFH were broad in nature and did not require a full consideration of the products from production-to-consumption. 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 about 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 of *L. monocytogenes* as affected by storage temperature and food composition, the lag time 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 the range of serving sizes and how often the food is eaten (i.e. number of servings).

The third question posed by CCFH for the risk assessment was to estimate the risk from *L. monocytogenes* in foods that support growth and foods that do not support growth at specific storage and shelf-life conditions. Four foods were selected that illustrate how the different factors mentioned above interact to affect the risk of listeriosis per one million servings and the risk per 100 000 people per year in a country. The latter estimate takes into account the impact that the levels of consumption of different foods have on the public health risk.

Pasteurized milk is a food that is widely consumed, has very low frequencies and levels of contamination, but allows growth during storage. Ice cream is similar to milk, but does not permit growth during storage. Fermented meat products are often contaminated and are produced without any lethal processing step. The final composition, however, prevents growth during storage. Cold-smoked fish is frequently contaminated, has no lethal processing step and permits growth during an extended storage period.

In addition to estimating the baseline risks for milk and smoked salmon, which represent the current situation, several “what-if” scenarios were calculated. These hypothetical scenarios have specific changes made to one or more of the exposure factors to demonstrate how the factors interact to affect the risks. In conducting the risk assessments for these four foods, different databases were available and modellers used slightly different techniques. These techniques are explained in the main risk assessment document and illustrate that there are numerous approaches that might be taken depending on the available data and the judgement of the risk assessors.

5.1.1 Contamination at retail

Data from published scientific papers, government surveys and the US FDA/FSIS draft risk assessment (FDA/FSIS, 2001) up to the year 2001 were collected by the risk assessment team. Data from all countries and years that were found in the literature were included in this risk assessment because of the paucity of directly relevant data. This means that a variety of conditions of manufacture and storage and changes with time are reflected in the data set. The majority of the data were prevalence, i.e. presence or absence determinations based on an analytical sensitivity of 0.04 *L. monocytogenes* per gram (1 microorganism per 25-g sample). An estimate of uncertainties about presence-absence data was made with a Beta distribution, thereby including the effect of the number of samples in a data set. Only a portion of the available data sets provided the actual levels of *L. monocytogenes* per gram in positive samples. These quantitative data were arrayed as a cumulative frequency distribution. In pasteurized milk, for example, 5% of the samples had levels ≤-1.18 $\log_{10}$ CFU/g; 50% ≤-0.58 $\log_{10}$ CFU/g; 95% ≤ 0.23 $\log_{10}$ CFU/g; and 99% ≤ 2.15 $\log_{10}$ CFU/g. After assigning uncertainty ranges, these distributions were used to estimate the levels of *L. monocytogenes* in the foods evaluated at the point of purchase.

5.1.2 Growth before consumption

A survey of 939 home refrigerators conducted by Audits International in the United States of America in 1999 (Audits International, 2000) provided data for considering the impact of home storage temperatures on the levels of *L. monocytogenes* at consumption. A cumulative distribution of the data was used without any model fitting. The 5^{th}, 50^{th} and 95^{th} percentile temperatures were 0.5, 3.4 and 6.9°C. This distribution had approximately 1.4% of the refrigerators operating above 10°C, where growth of *L. monocytogenes* would be relatively rapid. The distribution of refrigerator temperatures may differ in important ways between different countries. Thus European temperatures appear to average 6.6°C (Nauta, 2001).

No surveys of storage times at retail or in homes were available. After consulting various sources for "expert advice," the risk assessment team assigned storage time values based on what they felt were reasonable estimates. Triangular distributions for the variation in storage times were defined by minimum, most frequent and maximum times. For example, milk was given values of 1, 5 and 12 days, respectively. To further emphasize and explore the uncertainty associated with storage time values, the most likely and maximum values were described by uniform distributions. Again using milk as an example, an uncertainty range of 4 to 6 days was assigned.

The risk assessors considered storage times and temperatures to be not independent, and so these two factors were linked in the Monte Carlo modelling. Spoilage would severely limit the higher-temperature storage times, e.g. it would be unlikely that milk would be consumed after storage at 10°C for 12 days. In the smoked salmon risk assessment, the effect of indigenous lactic acid bacteria on the growth rate and maximum populations of *L. monocytogenes* and on the shelf-life of vacuum-packed products was specifically modelled. The section for each food details the method used to set the allowable combinations of times and temperatures (see Technical Report (FAO/WHO, 2004)).

The storage time and temperature data were used in combination with information on the growth rates of *L. monocytogenes* to estimate how the levels of the microorganism in the food were likely to change between point of purchase and time of consumption. Most of the growth rates in the selected foods were from published inoculated pack studies, where foods

with their normal spoilage flora were inoculated with *L. monocytogenes*. Only a limited amount of data was available on naturally contaminated products. Much of the information was obtained from the data collection in the United States of America for the FDA/FSIS draft risk assessment (FDA/FSIS, 2001). In the studies cited, the inoculated foods were stored at various temperatures, sampled over time, bacterial loads enumerated and the exponential growth rate determined. Except for ice cream, predictive models were used to estimate the growth rates, inactivation rates and growth limits of *L. monocytogenes* in foods. Because different studies were conducted at different storage temperatures, a mathematical relationship (square root model (Ratkowsky et al., 1982)) was used to convert the growth rates to what the expected value would be at 5°C. The means and standard deviations of the adjusted growth rates were calculated from the set of studies available for each food. The model was then used to estimate the growth rate at other temperatures by relating it to the growth rate at 5°C. Whenever possible, the growth model also considered the effect of temperature on the maximum colony density. This was done because, characteristically, *L. monocytogenes* does not reach as high a density of growth when grown at temperatures close to its lower limit for growth. Thus, when the microorganism is grown at higher refrigeration storage temperatures (e.g. 6 to 8°C) the maximum population density is in the range of 7 to 9 $\log_{10}$ CFU/g, while at lower temperatures (2 to 5°C) the level is 4 to 6 $\log_{10}$ CFU/g.

5.1.3 Consumption

The size of servings and frequency of consumption were either taken from the Canadian Federal-Provincial Nutrition Survey's databases (CFPNS, 1992-1995) or estimated globally from national consumption statistics as noted in individual product exposure assessments. The serving size for an individual was combined for all occasions in a day if multiple servings were consumed, including similar foods such as whole and skim milk. The serving size was described by a cumulative frequency distribution. Milk, for example, had 50^{th} and 95^{th} percentile values of 182 and 687 g respectively, for the susceptible population.

The frequency of consumption was calculated as both the probability of consumption during a day and the total number of servings per year for 100 000 people. For milk consumed by the non-immunocompromised Canadian population, the 50^{th} and 95^{th} percentiles of consumption were 0.75 and 0.79 servings per day, respectively. The respective percentile numbers of annual servings for 100 000 non-immunocompromised people were 4.0×10^9 and 4.9×10^9.

Because most data were not collected for use in risk assessments and because different risk assessments have different objectives, often data must be used that do not exactly meet the needs of a specific risk assessment. An example from the exposure assessments for milk and ice cream illustrate this, as follows. The frequency distributions for servings came from the Canadian Federal-Provincial Nutrition Survey (CFPNS, 1992-1995) that collected information from one day's consumption by 10 162 people between 18 and 74 years of age in 5 of 12 Provinces and Territories. This data would not show whether seasonal patterns existed: a summer survey might overestimate consumption of ice cream for the entire year, and *vice versa*. More critically, the database omitted children, a group who probably have more frequent consumption of milk and ice cream than the adult population. One approach to correcting this shortcoming would be to find additional information from other sources, e.g. surveys from other countries, or industry marketing data, and combine the sources into one estimate for the entire population. This would have required considerable time and effort

on the part of the risk assessors. Alternatively, the risk assessment could use the available data and interpret its shortcomings in the risk characterization. The latter was done in this risk assessment because of the knowledge that children between 1 and 18 years of age are not at greater risk of listeriosis than healthy adults (see Figure 2.1 in the Technical Report(FAO/WHO, 2004)). Therefore the risk per serving should not be significantly affected by this omission, within the overall uncertainties of the estimates. Because children probably consume ice cream more frequently than adults, the number of cases per 100 000 people would probably be slightly underestimated for the healthy population and slightly overestimated for the entire population. However, if the primary interests of the risk assessment were to compare milk and ice cream, which used the same consumption data, or to compare different storage scenarios for milk, this data shortcoming would have minimal effect.

5.1.4 Output from the exposure assessment

The outputs from the exposure assessment were fed into the dose-response model. It described the distribution of *L. monocytogenes* in the food at the point of consumption and also the amount consumed. The distribution at consumption was characterized as a cumulative frequency of $\log_{10}$ CFU/serving of contaminated food. The 5^{th}, 50^{th} and 95^{th} percentiles for contaminated milk, for example, were 1.0, 2.5 and 4.8 $\log_{10}$ CFU, respectively. Uncertainty estimates accompanied each percentile value to provide an estimate on the confidence in the accuracy of the percentiles. Other output values were the Beta distribution for frequency of contamination, the number of servings per year and size of servings.

5.2 Risk Characterization

Exposure assessment outputs and dose-response relationships were combined in the risk characterization portion of the risk assessment to calculate the probability of contracting listeriosis. The distributions of prevalence and level for *L. monocytogenes* in contaminated food at consumption and the dose-response relationships lead to estimates of the 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 illness per 100 000 people per year.

5.2.1 Case studies

Because of the effort necessary to calculate the risks for any single food, four foods were selected with diverse contamination, storage and consumption patterns. The four foods were modelled with the same general model structure: contamination frequency and level at retail; growth or inactivation until consumption using storage temperatures, storage times, exponential growth rates or death rates, lag phases, maximum growth and consideration of spoilage; frequency and amount of consumption; and dose-response relationship for the healthy and susceptible populations. However, the available data were not always the same for each of the four foods and details of the modelling process also differed. The previous discussion of the Canadian dietary consumption data illustrates this difficulty. The *L. monocytogenes* risk assessment Technical Report and its Appendices (FAO/WHO, 2004) provides more comprehensive details of the data and modelling techniques used for each food.

The mean of the estimates for the risks per million servings and cases per 100 000 people are shown in Table 3. As noted in Section 3, above, the annual incidence of listeriosis is reported as 0.1 to 11.3 cases per million persons in Europe. In addition to a baseline model that represents the best estimate of the actual process, many hypothetical scenarios could be

tested that involve shifts in either direction in the entire distribution, truncations, or changes in the shape of the distribution. Different scenarios would have different consequences for the estimated values of the risks depending upon the specific changes and the impact of that factor on the risks. The purpose of these scenario analyses might be to estimate what effect proposed changes in a process would have on the risks, or they might be intended to demonstrate for the risks the relative importance of different factors.

5.2.1.1 Milk

Milk represents a food that is consumed with very high frequencies in many Western nations, and in large quantities per serving. As a raw farm commodity, it is frequently contaminated, but proper pasteurization effectively eliminates the microorganism. It is assumed that controls are in place so that no unpasteurized milk is packaged for distribution, but that infrequent recontamination with low numbers occurs during the packaging operation. Milk has a moderate shelf-life when refrigerated and the microorganism can grow during storage at a relatively rapid rate. The shelf-life and growth rate can permit growth to high population densities. The risk per serving was estimated to be low (5.0×10^{-9} cases per serving), however, the very high frequency of consumption resulted in milk making substantial contributions to the total number of predicted annual cases of listeriosis (0.09 cases per 100 000 people).

5.2.1.2 Ice cream

Ice cream shares many characteristics with milk but, being a frozen food, *L. monocytogenes* can not grow during storage. Ice cream is consumed in high frequencies and in relatively large quantities per serving. The ice cream mix may be contaminated but the pasteurization eliminates the microorganism. Infrequent re-contamination with low numbers of *L. monocytogenes* may occur during mixing, freezing and packaging. Pathogens may also be introduced if the product contains additional ingredients, such as nuts, chocolate or fruit. No growth occurs during frozen storage and the contamination at consumption is the same as the contamination at production. The risk per serving was estimated to be very low (1.4×10^{-11} cases per serving) and the high frequency of consumption was not sufficient to make ice cream a substantial contributor to the total annual number of cases of listeriosis in a population (0.00012 cases per 100 000 people).

Table 3. Mean estimates of listeriosis per 100 000 population per year and per million servings for the four selected foods.

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

5.2.1.3 Cold-smoked fish

Smoked fish – of which the major part is cold-smoked salmon – is frequently contaminated and occasionally has high numbers of *L. monocytogenes*. Consumption varies widely in different countries. Consumption is very frequent in some Northern European countries, while North American consumption is relatively low. Serving sizes are moderate (ca. 60 g).

L. monocytogenes can grow in smoked seafood at a moderate rate when stored at refrigeration temperatures. The storage times can be long for smoked fish, potentially allowing significant growth to occur in contaminated samples. Cold smoking is the process most frequently employed. The impact of different methods of smoking on the contamination is not obvious, but evidence exists that the inactivation of *L. monocytogenes* during hot smoking is often balanced by additional recontamination. The risk per serving was estimated to be high (5.3×10^{-8} cases per serving). Globally, however, consumption is moderately frequent (1 to 18 servings per year), therefore, the total annual number of cases of listeriosis was moderate (0.016 cases per 100 000 people). Countries where the consumption is much greater, such as Northern Europe, would have a similar risk per serving, but would be expected to have a greater number of annual cases per 100 000 people.

5.2.1.4 Fermented meat products

Fermented meat products – typically fermented and dry or semi-dry sausages – have moderate rates of consumption in many countries. Serving sizes are also moderate. While there is a diversity across the world in the processing and composition of these products, they are primarily represented by products like salami and pepperoni. These products contain lactic acid, salt and nitrite that prevent the growth of *L. monocytogenes* and, in fact, cause inactivation of the pathogen during storage, particularly storage at room temperature. Some manufacturers include a thermal pasteurization step between fermentation and drying, but the traditional process does not have a lethal processing step. Because of the contamination of the raw meat ingredients, these products have moderate contamination rates at retail. Storage times can be very lengthy. However, because growth does not occur and inactivation is likely during storage, the contaminated packages usually experience a decrease in the numbers of *L. monocytogenes*, leading to a very low risk per serving (2.1×10^{-12}). The global number of annual cases per 100 000 people was calculated to be only 0.0000055.

6. Questions from CCFH that were specifically addressed by the risk assessment

6.1 Introduction

This section addresses the three risk questions posed by CCFH in 2001 in relation to the risk from *L. monocytogenes* in RTE foods. The specific question addressed is given in each case.

6.2 Question 1

Estimate the risk from L. monocytogenes *in food when the number of organisms range from absence in 25 grams to 1000 colony forming units per gram or millilitre, or does not exceed specified levels at the point of consumption.*

The question posed by the CCFH primarily requires a consideration of how the relative risk of acquiring listeriosis is affected by the level of *L. monocytogenes* present in a serving of food at the time of consumption. The ability to answer this question is dependent on the ability to articulate and interpret dose-response relationships for *L. monocytogenes*. However, there are a number of potentially confounding factors that could influence the approach taken and the complexity of the answer provided. In view of the generic nature of the CCFH question and the fact that this is one of the first microbial risk assessments requested by CCFH, it was decided that the response to this question should focus on communicating the key risk assessment concepts. It is also important to note that this question implies a series of comparisons based on relative risks and does not require the much more daunting task of calculating absolute risk. Accordingly, consideration of potential confounding factors was limited and a detailed consideration of uncertainty and variability was not undertaken in addressing this question. An introduction to issues related to the uncertainty and variability associated with dose-response models is provided in the hazard characterization section of this document and detailed in the Technical Report (FAO/WHO, 2004). In addition to not explicitly addressing uncertainty and variability, a number of simplifying assumptions were made in developing the examples used to answer the question posed by CCFH. For instance, to calculate the ingested dose, knowledge of the size of the serving is needed. A fixed serving size of 31.6 g was assumed for convenience to simplify the calculations because it approximates a typical serving size and because dose levels were estimated in 0.5 $\log_{10}$ increments ($10^{0.5} = 3.16$). To calculate the concentrations for other serving sizes in the tables that follow, the dose levels would have to be divided by the serving size.

As discussed in the hazard characterization, the exponential model was selected to describe the relationship between the dose of *L. monocytogenes* ingested and the probability of developing invasive listeriosis. Dose-response curves were developed for both the healthy population and the susceptible population and include the entire range of ingested doses (i.e. not restricted to 1000 CFU/g food). These curves are population based and describe the average dose-response relationship. In a specific outbreak situation involving a strain with high virulence or an unusually susceptible population, a significant number of cases may still

result from food containing comparatively low numbers of *L. monocytogenes*. For the purposes of the example, only the dose-response curve for the susceptible population was used, and it was assumed that all cases of listeriosis were restricted to that population. The specific dose-response curve selected was the one where the maximum level to which *L. monocytogenes* could grow in a food was assumed to be $10^{7.5}$ CFU/serving. The end result of these assumptions is that the most "conservative" dose-response model was used, i.e. the maximum virulence of *L. monocytogenes* was assumed. The r-value for this relationship was 5.85×10^{-12} (Table 2). The dose ingested is a function of the level of the microorganism in the food (CFU/g) multiplied by the size of the serving. Thus, the equation for calculating the probability of listeriosis was:

$$P = 1 - e^{(5.85 \times 10^{-12})(31.6g \times n)}$$

where *n* is the number of *L. monocytogenes* per gram. By then substituting different values for *n*, the likelihood of listeriosis at levels between 0.04 (1 CFU/25 g) and 1000 CFU/g were calculated.

The overall impact on the number of cases of listeriosis was estimated by multiplying the likelihood of listeriosis per serving by the total number of servings. For this calculation, the total number of RTE servings was assumed to be 6.41×10^{10} servings, i.e. the estimated total number of annual servings in the United States of America for the 20 classes of RTE food considered in the FDA/FSIS draft risk assessment (FDA/FSIS, 2001). The corresponding number of listeriosis cases for the susceptible population was considered to be 2130 (FDA/FSIS, 2001), and will be used to represent the current incidence of listeriosis when comparing the effect of changes to incidence under different theoretical scenarios.

As a simple, "worst case" scenario, the predicted risk per serving and predicted number of annual listeriosis cases were estimated by assuming that all 6.41×10^{10} servings had the maximum level of contamination being considered (0.04, 0.1, 1, 10, 100 and 1000 CFU/g) (Table 4).

A more realistic approach would be to employ a distribution of *L. monocytogenes* levels in foods when consumed. As a means exploring that more complex approach, the overall distribution of *L. monocytogenes* levels in 20 classes of RTE foods from the FDA/FSIS risk assessment (FDA/FSIS, 2001) was used (see Table 5). This distribution was then used to calculate the probability of listeriosis and the predicted number of listeriosis cases. At each maximum *L. monocytogenes* level considered, the number of servings from the distribution that were above the designated value was added to that maximum level. For example, for an upper limit of 1000 CFU/g, the number of servings was 6.23×10^7 (servings originally predicted to be at 1000 CFU/g) + 2.94×10^7 (servings originally predicted to be at 10 000 CFU/g) + 1.39×10^7 (servings originally predicted to be at 10^5 CFU/g) + 3.88×10^6 (servings originally predicted to be at $10^{5.5}$ CFU/g) + 8.55×10^6 (servings originally predicted to be at $>10^6$ CFU/g) = 1.18×10^8 servings. The predicted annual number of listeriosis cases was then calculated and summed. The predicted number of listeriosis cases for each maximum level is provided in Table 6.

Table 4. Probability of illness per serving for the susceptible population estimated for different levels of *Listeria monocytogenes* at the time of consumption and the estimated number of cases per year in the United States of America if all RTE meals were contaminated at that level.

Level (CFU/g)	Dose[1] (CFU)	Log_{10} dose (log_{10} CFU/serving)	Probability of illness per serving	Relative risk[2]	Estimated number of cases per year[3]
< 0.04	1	0	7.39×10^{-12}	1	0.54
0.1	3	0.5	1.85×10^{-11}	2.5	1
1	32	1.5	1.85×10^{-10}	25	12
10	316	2.5	1.85×10^{-9}	250	118
100	3160	3.5	1.85×10^{-8}	2500	1185
1000	31600	4.5	1.85×10^{-7}	25000	11850

NOTES: (1) Serving size of 31.6 g. (2) Using the risk from a dose of 1 CFU as reference. (3) A total of 6.41×10^{10} servings per year assumed.

Table 5. Predicted distribution of levels of *Listeria monocytogenes* occurring in RTE foods.

Level of *L. monocytogenes* in a food at consumption (CFU/g)	Number of servings assumed at the specified dose
<0.04	6.18×10^{10}
0.1	1.22×10^{9}
1	5.84×10^{8}
10	2.78×10^{8}
100	1.32×10^{8}
1000	6.23×10^{7}
10000	2.94×10^{7}
100000	1.39×10^{7}
316000	3.88×10^{6}
>1000000	8.55×10^{6}
Total	6.41×10^{10}

SOURCE: FDA/FSIS, 2001.

Table 6. Predicted annual number of listeriosis cases in the susceptible population when the level of *Listeria monocytogenes* was assumed not to exceed a specified maximum value and the levels in *L. monocytogenes* in the food are distributed as indicated in Table 5.

Level (CFU/g)	Maximum dose (CFU/serving)[1]	Cumulative percentage of servings when maximum level[2]	Estimated number of listeriosis cases per year[3]
0.04	1	100	0.5
0.1	3	3.6	0.5
1	32	1.7	0.7
10	316	0.8	1.6
100	3160	0.4	5.7
1000	31600	0.2	25.4

NOTES: (1) Serving size of 31.6g. (2) Number of servings in the highest *L. monocytogenes* level assumed, divided by 6.41×10^{10} times 100. (3) Levels of *L. monocytogenes* per serving used to calculate predicted number of cases based on the overall distribution from the FDA/FSIS risk assessment (2001) (see Table 5). A total of 6.41×10^{10} servings per year was assumed.

Comparisons between Table 4 and Table 6 show that there are vast differences in the estimated number of cases for the worst-case answer to the question (Table 4) compared with that estimated when an attempt is made to consider the frequency and extent of contamination actually encountered in RTE foods. While either set of predictions can be challenged on the basis of the assumptions used, such scenarios are useful in framing the extent of the risk likely to be encountered.

These two scenarios (Table 4 and Table 6) demonstrate that, when dealing with an infectious agent and where a non-threshold model is assumed, then as either the frequency of contamination (percentage of contaminated samples) or the extent of contamination (levels of *L. monocytogenes* in a contaminated food) increases, so does the risk and the predicted number of cases. Thus, if all RTE foods went from having 1 CFU/serving to 1000 CFU/serving (Table 4), the risk of listeriosis would increase 1000-fold (assuming a fixed serving size). Conversely, the likelihood of illness from introducing into the food supply 10 000 servings contaminated with *L. monocytogenes* at a level of 1000 CFU/g would, in theory, be compensated for by removing from the food supply a single serving contaminated at a level of 10^7 CFU/g.

In interpreting these results and in attempting to predict 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 deviations from established limits occur. The current example is based on data from the United States of America, where the current allowable limit for *L. monocytogenes* in RTE foods is effectively 0.04 CFU/g (1 CFU/25 g), a level that if consistently achieved would be expected to result in less than one case of listeriosis per year in the United States of America. However, the baseline level for the United States of America population was 2130 cases (Mead et al., 1999). Both the current risk assessment and the United States of America FDA/FSIS draft risk assessment (2001) indicate that a portion of RTE food contain a substantially greater number of the pathogen than the stated limit and that the public health impact of *L. monocytogenes* is, most probably, almost exclusively a function of the foods that greatly exceed the current limit. Thus, in addressing the question posed by CCFH, the current risk assessment indicates that increasing the level of *L. monocytogenes* in RTE foods from 0.04 to 1000 CFU/g would increase the risk of foodborne listeriosis, provided that the current rate of deviations above the established limit remained proportionally the same. However, it could also be asked whether public health could be improved if a less stringent microbiological limit for RTE foods resulted in a substantial decrease in the number of servings that greatly exceeded the established limit, e.g. if the change encouraged manufacturers to routinely screen for *L. monocytogenes* in the plant environment and to take appropriate remedial actions. Models developed during the current risk assessment could be used estimate the extent of control over deviations from established limits that would be needed to improve public health if regulatory limits were relaxed, provided that sufficient data on the rate and extent of deviations were available for individual RTE foods.

To examine this concept further, a simple hypothetical "what-if" scenario was developed based on the calculations provided in Tables 5 and 6. It examines the impact that the level of compliance to a microbiological limit (i.e. "defect rates") has on public health. In this what-if scenario, two potential and often-discussed limits, 0.04 CFU/g and 100 CFU/g, were examined in conjunction with different defect rates, i.e. the percentage of servings that exceed the specified limit. As a means of simplifying the what-if scenario and dramatizing

the impact of compliance, a single level of *L. monocytogenes* contamination, 10^6 CFU/g, was assumed for all "defective" servings. This assumption focuses the scenario on the percentage of defective servings with elevated levels of *L. monocytogenes*, i.e. the group of defective servings that is responsible for the majority of listeriosis cases. Thus, if a serving of food was not defective i.e. in compliance, it had a level of *L. monocytogenes* at or below the specified microbiological limit based on the distribution of *L. monocytogenes* levels (Table 5) used to calculate the 100% compliance values depicted in Table 6. Conversely, if a serving of food was defective or out of compliance, it was assumed to have 10^6 CFU/g *L. monocytogenes*, or since the assumed serving size was 31.6 g, a consumed dose of 3.16×10^8 CFU. The predicted number of cases as a function of the percentage of defective servings is provided in Table 7.

As indicated in Table 6, at 100% compliance the number of predicted cases for both limits is low, with an approximate 10-fold differential between the two microbiological limits. As expected, the number of predicted cases increases with an increasing frequency of defective servings. At defect rates > 0.0001%, a 10-fold increase in the defect rate results in an approximate 10-fold increase in the number of predicted cases, regardless of the microbiological limits (i.e. 0.04 CFU/g versus 100 CFU/g). Based on the conditions and assumptions of this simple what-if scenario, the defect rate that yielded a value approximately equivalent to the baseline value of 2130 cases used in the FDA/FSIS draft risk assessment (2001) was 0.018%. This is consistent with the defect rate (0.013%) at this contamination level reported in Table 5, and the earlier observation that the dose-response relationship predicts that this group of defective servings accounts for most cases of foodborne listeriosis.

Table 7. Hypothetical "what-if" scenario demonstrating the effect that the proportion of "defective" servings has on the number of predicted cases of foodborne listeriosis.

Assumed percentage of "Defective" servings[(1)]	**Predicted number of listeriosis cases[(2)]**	
	Initial standard of 0.04 CFU/g	Initial standard of 100 CFU/g
0	0.5	5.7
0.00001	1.7	6.9
0.0001	12.3	17.4
0.001	119	124
0.01	1185	1191
0.018	2133	2133
0.1	11837	11848
1	117300	117363

NOTES: (1) For the purposes of this scenario, all defective servings were assumed to contain 10^6 CFU/g. (2) For the purposes of this scenario, an r-value of 5.85×10^{-12} was employed and a standard serving size of 31.6 g was assumed. In the case of the 100 CFU/g calculations, the defective servings were assumed to be proportionally distributed according to the number of servings within each cell concentration bin.

A more detailed consideration of compliance could be achieved by incorporation of distributions reflecting the levels of *L. monocytogenes* observed in a variety of foods. However, such a detailed consideration of compliance rates was beyond the scope of the current risk assessment. Furthermore, the simple hypothetical what-if scenario presented adequately demonstrates key concepts related to how compliance rates can strongly influence the actual risk associated with a microbiological criterion. In fact, it could be argued that the rate of compliance is a more significant risk factor than the numeric value of the criterion within the range that CCFH asked the risk assessment team to consider. The what-if scenario

also demonstrates the concept that a less stringent microbiological limit could lead to an improvement in public health if the new criterion leads to new control measures that decrease defect rates. For example, the model (Table 7) predicts that if a microbiological limit of 0.04 CFU/g with a 0.018% defect rate (2133 cases) was replaced with a 100 CFU/g limit and a 0.001% defect rate (124 cases), the predicted result based on the scenario is an approximate 95% reduction in foodborne listeriosis.

6.3 Question 2

Estimate the risk for consumers in different susceptible population groups

As discussed in the hazard characterization section of the risk assessment, listeriosis is primarily a disease of certain subpopulations with impaired or altered immune function (e.g. pregnant women and their fetuses, the elderly, individuals with chronic diseases, AIDS patients, individuals taking immunosuppressive drugs). Susceptibility varies within the broadly defined susceptible group (e.g. the risk of listeriosis appears to be less for pregnant women than for transplant recipients). It has been estimated that various subpopulations may have a 20- to 2500-fold increased risk of acquiring listeriosis (FDA/FSIS, 2001; Marchetti, 1996). The CCFH requested that the risk assessment team attempt to estimate the differences in the dose-response relations for the various subpopulations with increased susceptibility. While previous risk assessments had considered the relative susceptibility of the entire population at increased risk versus the general population, these risk assessments did not develop the type of detailed comparisons of subpopulations with increased susceptibility requested by CCFH. Thus, the current risk assessment had to develop *de novo* a means for addressing the request.

The basic approach taken to developing the requested dose-response relations was to take advantage of epidemiological estimates of the relative rates of listeriosis for different subpopulations. These "relative susceptibility" values were generated by taking the total number of listeriosis cases for a subpopulation and dividing it by the estimated number of people in the total population that have that condition. This value is then divided by a similar value for the general population. While there is a substantial uncertainty associated with these values (i.e. a relative susceptibility value is the ratio of two uncertain risk estimates) it does provide a useful estimate of the differences in the susceptibility among the different subpopulations and the role that immune status has in determining an individual's risk from *L. monocytogenes* (Table 8).

Relating the relative susceptibility values to the dose-response relations for the different subpopulations requires a means of converting these point estimates to a dose-response curve. The unique characteristics of the exponential model allowed this to be done. Being a single-parameter model, the exponential model allows the entire dose-response curve to be generated once any point of the curve is known. Thus, the r-value for an exponential dose-response curve can be estimated for a subpopulation using a relative susceptibility ratio and a reference r-value for the general population. Using the relative susceptibility value for cancer patients as an example (Table 8), the equation for the relative susceptibility is:

$$\text{Relative susceptibility} = RS = P_{cancer}/P_{healthy} = [1 - \exp(-r_{cancer}*N)] / [1 - \exp(-r_{healthy}*N)]$$

where P_{cancer} and $P_{healthy}$ denote the probability of systemic listeriosis for a cancer patient and a healthy adult, respectively, when exposed to a dose N of *L. monocytogenes*, and where r_{cancer}

and $r_{healthy}$ are the r-values of exponential dose-response relationships specific for those population sub-groups.

This equation can be rearranged to:

$$r_{cancer} = - \ln [RS * \exp(-r_{healthy}*N) - (RS -1)] / N$$

As long as the value for N, the number of *L. monocytogenes* consumed, is much smaller than the maximum assumed dose, the above relationship can be used to estimate the $r_{subpopulation}$-value. Using the above equation, the r-values for different classes of patients were estimated based on epidemiological data from France (Table 8) and the United States of America (Table 9).

Comparison of the relative susceptibility values and corresponding r-values 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 and this is reflected in a corresponding increase in the r-value of the dose-response curve. The most compromised group in the French data (transplant patients) has an r-value approximately 4 orders of magnitude greater than the reference population (i.e. individuals less than 65 years old with no other medical conditions). The relative susceptibility values for the elderly population in Tables 8 and 9 showed close agreement, 7.5 and 2.6 for the French and United States of America data, respectively. The differences reflect, in part, the different definition of the age corresponding to the category "elderly" and the reference population. The United States of America intermediate-aged population includes the patients that are separated out from the age group less-than-65-years in the French data, and the two reference populations are not expected, therefore, to have the same r-values. Nevertheless, the two tables indicate the magnitude of the impact that the impairment of the immune system by the specific conditions and disease states has on susceptibility to listeriosis.

The two outbreak r-values provide an indication on the validity of the models. The r-value for the Los Angeles outbreak in pregnant women from consumption of Hispanic cheese was very close to that estimated (Table 9). The r-value for the Finnish outbreak caused by contaminated butter among hospitalized transplant patients differed from the values based on transplant patients by 1000-fold (Table 8). This may have resulted from the smaller number of individuals exposed, the extremely compromised and highly variable immunological status of the population, a food matrix effect, or the involvement of a highly virulent strain of *L. monocytogenes*. There is a clear need in future outbreaks for exposure levels, immune status of the patients and strain characteristics to all be investigated so that these dose-response models can be further refined and validated.

Table 8. r-values (exponential dose-response model) for different susceptible populations calculated using relative susceptibility information from France. Relative susceptibilities for the different subpopulations are based on the incidence of listeriosis cases (outbreak and sporadic) in these groups in 1992.

Condition	Relative susceptibility	Calculated r-value(1)	Comparable outbreak r-value
Transplant	2 584	1.41×10^{-10}	Finland butter 3×10^{-7}
Cancer – Blood	1 364	7.37×10^{-11}	
AIDS	865	4.65×10^{-11}	
Dialysis	476	2.55×10^{-11}	
Cancer – Pulmonary	229	1.23×10^{-11}	
Cancer – Gastrointestinal and liver	211	1.13×10^{-11}	
Non-cancer liver disease	143	7.65×10^{-12}	
Cancer – Bladder and prostate	112	5.99×10^{-12}	
Cancer – Gynaecological	66	3.53×10^{-12}	
Diabetes, insulin dependent	30	1.60×10^{-12}	
Diabetes, non-insulin dependent	25	1.34×10^{-12}	
Alcoholism	18	9.60×10^{-13}	
Over 65 years old	7.5	4.01×10^{-13}	
Less than 65 years, no other condition (reference population)	1	5.34×10^{-14}	

NOTES: (1) The r-value assumed for the reference population – "Less than 65 years, no other medical condition" – was 5.34×10^{-14}, i.e. the median of the r-value calculated assuming a maximum level of 8.5 $\log_{10}$ CFU per serving.
SOURCE: Marchetti, 1996.

Table 9. Dose-response curves for different susceptible populations calculated using relative susceptibility information from the United States of America. Relative susceptibilities for the different sub-populations are based on the incidences of listeriosis cases (outbreak and sporadic) in these groups.

Condition	Relative susceptibility	Calculated r-value(1)	Comparable outbreak r-value
Perinatal	14	4.51×10^{-11}	Los Angeles cheese 3×10^{-11}
Elderly (60 years and older)	2.6	8.39×10^{-12}	
Intermediate-age population (reference population)	1	5.34×10^{-14}	

NOTES: (1) The r-value assumed for the reference population – "Intermediate-age population" – was 5.34×10^{-14}, which is the median of the r-values calculated under the assumption of a maximum level of 8.5 $\log_{10}$ CFU per serving.

SOURCE: FDA/FSIS, 2001.

6.4 Question 3

Estimate the risk from L. monocytogenes *in foods that support growth and foods that do not support growth at specific storage and shelf-life conditions.*

L. monocytogenes growth in foods is not the only determinant of risk of listeriosis. Additional factors that affect the risk associated with any food, regardless of whether it does or does not support *L. monocytogenes* growth include:

- frequency of contamination;
- level of contamination;

- frequency of consumption; and
- susceptibility of consuming population.

This question raises the possibility of alternative approaches to a simple growth/no-growth evaluation, such as a consideration of the effect on consumer risk of limiting the storage temperature and shelf-life of a product that supports the growth of *L. monocytogenes*. The risk assessment team has attempted to also consider these approaches while formulating its answer to the question.

Thus, as was discussed in response to Question No. 1 (risk from foods containing < 0.4 versus 1000 CFU/g), it is possible that a food that does not permit the growth of *L. monocytogenes* but that is frequently contaminated at moderate levels could pose a greater risk than an infrequently contaminated food, or one contaminated at low levels, but that could support the growth of *L. monocytogenes.* Also, as noted previously, it is clear that an increase in the *total* numbers of *L. monocytogenes* in a food (whether through growth or increased frequency of contamination) will lead to increased consumer risk because, for *L. monocytogenes*, the dose-response model used indicates that public health risk is proportional to the total number of *L. monocytogenes* in the food when consumed. Furthermore, as bacterial growth is exponential, the risk might be expected to increase exponentially with storage time.

Three approaches for answering this question are provided:

(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, in part, to evaluate the effect of growth on risk, and

(iii) comparison of what-if scenarios for the foods evaluated that do support *L. monocytogenes* growth if they did not support *L. monocytogenes* growth

Each of the evidential approaches is discussed below.

6.4.1 Growth rates in foods

L. monocytogenes is able to grow in many RTE foods, even if stored under appropriate refrigeration conditions. Factors affecting the growth of *L. monocytogenes* in foods are discussed in detail in Sections 3.5 and 4.4 of the *L. monocytogenes* risk assessment Technical Report (FAO/WHO, 2004). These include product formulation, storage time and temperature, and interactions with other microorganisms present in the product. In vacuum-packed foods, lactic acid bacteria can reach stationary phase without product spoilage. This can slow, or even prevent, the subsequent growth of *L. monocytogenes*. Table 10 presents representative generation times for different foods as a function of product type and storage temperature. For every three generations of growth, there is approximately a 10-fold increase in bacterial population. As discussed in Question No. 1 and assuming the same strain(s), a 10-fold increase in the levels of *L. monocytogenes* ingested produces a corresponding 10-fold increase in risk to humans (Figure 2). Thus, the risk from a food that supports the growth of *L. monocytogenes* increases with increasing storage time. However, the degree that the risk increases is dependent on the extent of growth in the food, which, in turn, is largely a function of *L. monocytogenes'* growth rate in the food and the duration and conditions of storage.

L. monocytogenes has been reported to grow in foods at temperatures as low as 0°C, water activities as low as 0.91-0.93 and pH as low as 4.2. Combinations of sub-optimal

levels reduce growth rate and can prevent growth at less extreme conditions than any of these factors acting alone. This principle, often referred to as hurdle technology or combination treatment, is exploited in food processing to prevent or limit the growth of bacteria in RTE foods.

Table 10. Representative generation times (hours) and growth potential of *Listeria monocytogenes* at different temperatures and shelf lives at 5°C in various RTE foods.

Temperature (°C)	Generation time (hours)			
	Milk	Vacuum-packed cold-smoked fish	Vacuum-packed processed meats	Sliced vegetables
5[(1)]	27.6	46.6	29.6	111
(95% confidence interval)	(14–226)	(20–infinite)	(14–infinite)	(28–infinite)
5[(2)]	25–30	40–49	16–48	–
10[(2)]	5–7	8–11	7–10	–
25[(2)]	0.7–1.0	1.2–1.7	1- 1.6	–
	Growth potential[(3)]			
5	~2–3	~4–5	~8–9	~0.3
	Advisory shelf-life (weeks)			
5	1–2	4–6	6–8	1

NOTES: (1) Values based on data collated in FDA/FSIS, 2001.
(2) Representative predictions and ranges from several published predictive models developed for growth rate of *L. monocytogenes.* No predictions were possible for vegetables because none of the published models were developed, or validated, for use with sliced vegetables.
(3) Log increase ignoring lag phase or suppression of growth by lactic acid bacteria.

The potential extent of growth varies among different foods, being dependent on the pathogen's growth rate in a specific food, which is a function of the product's composition and storage conditions, and on the shelf-life of the product. From Table 10 it is evident that the growth of *L. monocytogenes* within the normal shelf-life of products could be substantial. For example, fresh cut vegetables have a relatively short shelf-life and do not support as rapid growth of *L. monocytogenes* as some other foods, such as milk or deli-meats. Thus, it would be expected that the extent of growth in fresh cut vegetables would not be as great as those other foods, resulting in a lower risk for given initial contamination rates and levels.

The example of the effect of storage time and temperature on the growth of *L. monocytogenes* and the subsequent risk of listeriosis can be considered a "worst-case scenario" in that it only considers the effect of temperature on generation times. Additional factors that act to delay the initiation of growth of *L. monocytogenes* (e.g. consideration of the lag phase), reduce the rate of growth (e.g. modified atmosphere packaging), or suppress the maximum level reached by *L. monocytogenes* (e.g. growth of lactic acid bacteria) would decrease the extent of growth within a specified period of a product's shelf-life, with a corresponding decrease in risk. The actual calculation of risk would also have to consider that different servings would be consumed at different times within the total shelf-life of the product, i.e. only a small fraction of a product is typically consumed close to the end of its declared shelf-life.

6.4.2 Comparison of four foods

The four foods evaluated in the risk assessment (i.e. milk, ice cream, cold-smoked fish, and fermented meat products) were selected, in part, to compare the effect of various product characteristics on growth. This included specific consideration of the ability of foods to

support growth. Thus, milk and ice cream were compared because they have similar compositions, servings sizes, frequencies of consumption, and rates and extents of initial contamination. However, milk supports *L. monocytogenes* growth while ice cream does not. Similarly, cold-smoked fish and fermented meat products have similar rates of initial contamination, serving sizes, and frequencies of consumption, but, because of different compositions, the former supports the growth of *L. monocytogenes* while the latter does not.

Comparisons of the predicted for risk per million serving values (Table 3) between milk and ice cream, and between cold-smoked fish and fermented meat products, indicate that the ability of a product to support growth within its shelf-life can increase substantially the risk of that product being a vehicle for foodborne listeriosis. Thus, the predicted risk per million servings of milk was approximately 100-fold greater than that for ice cream, and the risk for smoked fish was approximately 10 000-fold greater than the corresponding risk for fermented meat products.

6.4.3 What-if scenarios

One of the useful features of a quantitative risk assessment is that the underlying mathematical models can be modified to allow various what-if scenarios to be conducted to evaluate the likely impact of different risk management options. Accordingly, a limited number of what-if scenarios were evaluated for milk and cold-smoked seafood, the two foods considered in the risk assessment that supported the growth of *L. monocytogenes*. The results of these analyses were then compared to the predicted baseline risks (Table 3) to determine the impact of the intervention.

6.4.3.1 Milk

The initial assessment of risk associated with recontaminated pasteurized milk considered the likely growth of *L. monocytogenes* during the shelf-life of the product (see Section 4.3 of the Technical Report (FAO/WHO, 2004)) using Canadian consumption characteristics as an example. To help answer CCFH Question No. 3, the model was re-executed after being modified such that growth was no longer considered. The results of the two calculations were then compared to estimate the effect of growth on risk (Table 11).

The results suggest that an approximately 1000-fold increase in risk can be attributed to the predicted growth of *L. monocytogenes* in pasteurized milk by either measure of risk, i.e. risk per 1 million meals, or risk per 100 000 population. The uncertainty measures associated with the comparison suggested that the predicted increase in risk attributable to growth could be as little as 100-fold, or as much as > 10 000-fold.

Several what-if scenarios were calculated for milk to illustrate the interactions of the various factors in determining the risks (Table 12). In one scenario, if all milk were consumed immediately after purchase at retail, the risks per serving and cases per population in both susceptible and healthy populations would decrease approximately 1000-fold. In contrast, if the contamination levels of milk were truncated at 100 CFU/g at retail, but with growth still allowed, the incidence of listeriosis is predicted to be reduced only by about two-thirds. Two scenarios examined the impact of storage temperatures and times. When the temperature distribution was shifted so the median increased from 3.4 to 6.2°C, the mean number of illnesses increased over 10-fold for both populations. When the storage time distribution was shifted from a median of 5.3 days to 6.7 days, the mean rate of illnesses increased 4.5-fold and 1.2-fold for the healthy and susceptible populations, respectively.

Table 11 Estimates of the increase in risk of listeriosis from growth during storage of pasteurized milk between purchase and consumption.

	Normal-risk population		High-risk population		Mixed population	
	Mean	(s.e.)[(1)]	Mean	(s.e.)	Mean	(s.e.)
With growth (baseline model)						
Cases per 100 000 population	1.6×10^{-2}	(5.0×10^{-4})	5.2×10^{-1}	(3.1×10^{-2})	9.1×10^{-2}	(4.7×10^{-3})
Cases per 1 000 000 servings	1.0×10^{-3}	(1.0×10^{-4})	2.2×10^{-2}	(9.0×10^{-4})	5.0×10^{-3}	(2.0×10^{-4})
Without growth						
Cases per 100 000 population	1.3×10^{-5}	(6.7×10^{-8})	3.8×10^{-4}	(1.6×10^{-6})	6.7×10^{-5}	(2.4×10^{-7})
Cases per 1 000 000 servings	5.9×10^{-7}	(3.1×10^{-9})	1.7×10^{-5}	(7.5×10^{-8})	3.6×10^{-5}	(1.4×10^{-8})
Increased risk with growth relative to that without growth (n-fold increase)						
Cases per 100 000 population	1 231		1 366		1 358	
Cases per 1 000 000 servings	1 695		1 294		139	

KEY: (1) s.e. = Standard error of the mean.

Table 12. Three "what-if" scenarios that illustrate the impact of contamination and storage on the estimated risks of listeriosis per 100 000 population and per million servings for milk under typical conditions of storage and use.

Food	Estimated mean cases of listeriosis per 100 000 people	Estimated mean cases of listeriosis per 10^6 servings
Milk baseline (from Table 10)	9.1×10^{-2}	4.6×10^{-3}
No growth	6.7×10^{-5}	
Contamination truncated at 100 CFU/g	2.8×10^{-2}	
Increase storage temperature (from 3.4 to 6.2°C)	1.2×10^{0}	
Increase storage time (from 5.3 to 6.7 days)	2.0×10^{-1}	

6.4.3.2 Smoked fish

The assumptions used with the cold-smoked fish model differ slightly from those used with the pasteurized milk example. The cold-smoked fish model also considers the effect of the growth of indigenous lactic acid bacteria in the product, which, when they grow to high numbers, suppress the growth of *L. monocytogenes* (see Section 4.5 of the *L. monocytogenes* risk assessment Technical Report (FAO/WHO, 2004). The extent of that growth suppression is not known with certainty. In the baseline model, two assumptions concerning the growth rate suppression by lactic acid bacteria were tested. In the what-if scenario the growth rate inhibition of *L. monocytogenes* by the lactic acid bacteria was set to zero(therefore not affected by the lactic acid bacteria). Table 13 compares the risk estimates when growth was modelled to occur or not, including the effect of different assumptions about the magnitude of the inhibition of *L. monocytogenes* growth rate due to the growth of lactic acid bacteria.

Table 13. Impact of the growth of *Listeria monocytogenes* during storage of cold-smoked fish between purchase and consumption on the risk of listeriosis under typical conditions of storage and use.

Growth rate inhibition due to growth of lactic acid bacteria	Cases per 1 000 000 meals		Cases per 100 000 population	
	No Growth	Growth Modelled	No Growth	Growth Modelled
80–100%	4.51×10^{-4}	4.59×10^{-1}	9.60×10^{-5}	6.57×10^{-2}
	(3.09×10^{-5})[1]	(3.29×10^{-1})	(1.07×10^{-5})	(3.78×10^{-2})
Difference[2]		1020-fold		684-fold
95%		3.82×10^{-2}		6.48×10^{-3}
		(1.96×10^{-2})		(2.26×10^{-3})
Difference[2]		85-fold		67-fold

NOTE: (1) Values in parentheses are standard deviations. (2) Increase in risk of listeriosis in the growth versus the no-growth scenarios

With either assumption concerning the effect of lactic acid bacteria on *L. monocytogenes* growth potential, growth greatly increased the risk of listeriosis. Assuming that 80 to 100% suppression occurred, it allowed more growth than the assumption of 95% growth rate suppression, a result of the faster overall growth rate after lactic acid bacteria have achieved maximum population growth. The risk per serving and cases per 100 000 population increased 700- to 1000-fold in the first assumption (80–100% growth rate suppression) and 67- to 85-fold under the latter assumption (95%) from the "no *L. monocytogenes* growth" to the baseline (growth) scenarios.

For the cold-smoked fish model, between 15 and 20% of the population were assumed to be in the "high-risk" category, but the cases attributable to the "normal" and "high-risk" categories were not explicitly estimated. Rather, as in the previous example, the predicted number of cases is a weighted mean of the normal and high-risk populations. It is known that the population at increased susceptibility of listeriosis experiences between 80 and 98% of total reported cases of listeriosis. Also, in this example, no attempt was made to differentiate consumption between these two susceptibility classes, unlike in the assessment undertaken for milk. These differences do not affect the interpretation of the results with a food, but some caution must be exercised in comparing the impact of growth of risk between the foods. However, the differences in the modelling are relatively minor and the predicted increase in risk due to growth in the two examples is roughly comparable. In the case of pasteurized milk (Table 12), the modelling also suggests that the increase in risk due to the growth of *L. monocytogenes* within the normal shelf-life of the product is between approximately 100- and 1000-fold, similar to the risk increase predicted for cold-smoked fish due to *L. monocytogenes* growth during storage.

A further what-if scenario was performed to estimate the effect on risk of reducing the shelf-life of smoked fish by 50%. This was tested by replacing the original shelf-life distribution of 1–28 days, with a most likely value of 14 days, by a shelf-life distribution of 1–14 days, with a most likely value of 7 days. The effect of this change resulted in an 80% reduction in the predicted increase in risk due to growth. The fact that the change was not greater is probably due to the effect of lactic acid bacteria, which is modelled to begin to suppress *L. monocytogenes* growth after approximately 3 weeks of storage at 5°C.

6.4.4 Summary

Three different approaches were taken to demonstrate the effect of growth of *L. monocytogenes* on the risk of listeriosis associated with RTE foods. It is apparent that the potential for growth strongly influences risk, though the extent of that increase is dependent on the characteristics of the food and the conditions and duration of refrigerated storage. However, using the examples provided in the risk assessment, the ability of these RTE foods to support the growth of *L. monocytogenes* appears to increase the risk of listeriosis on a per serving basis by 100- to 1000-fold over what the risk would have been if the foods did not support growth. While it is not possible to present a single value for the increased risk for all RTE foods, because of the different properties of the foods, the range of values here provide some insight into the magnitude of the increase in risk that may be associated with the ability of a food to support the growth of *L. monocytogenes.*

7. Key findings

- The probability of illness as a result of consuming a specified number of *L. monocytogenes* is appropriately conceptualized by the disease triangle, where the food matrix, the virulence of the strain and the susceptibility of the consumer are all important factors. Little information was found on food matrix effects for *L. monocytogenes*. Strain variation in virulence has been shown in animal studies to be large, but it is not possible at this time to determine the human virulence for any individual strain and explicitly include that in the model. However, the epidemiologically-based models used in the risk assessment implicitly consider the variation in virulence among strains. Population-based models were developed that estimate the likelihood of illness for various immunocompromised human populations after consuming specified numbers of *L. monocytogenes*. Although the maximum levels of contamination at consumption are uncertain, different models based on different values all lead to the same general findings.
- The models developed predict that nearly all cases of listeriosis result from the consumption of high numbers of the pathogen. Conversely, the models predict that the consumption of low numbers of *L. monocytogenes* has a low probability of causing illness. Old age and pregnancy increase susceptibility and thus the risk of acquiring listeriosis per exposure. Likewise, diseases and medical interventions that severely compromise the immune system greatly increase the risks. The risk of acquiring listeriosis from the consumption of contaminated food appears to be adequately described by the type of "probabilistic statement" that underlies the exponential dose-response relationship used in the risk assessment, namely, that there is a finite, albeit exceedingly small, possibility that a case could occur if an unusually susceptible consumer ingested low numbers of an unusually virulent strain.
- There is no evidence that the risk from consuming a specific number of *L. monocytogenes* varies from one country to another for the equivalent population. 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 annual number of cases per population. The former is a function of the frequency of contamination and the distribution of contamination levels within that class of food. The latter considers the number of servings of the food consumed by the population and the size of that population. A food may have a relatively high risk per serving but, if a minor component of the national diet, it may have a relatively small impact on public health as defined by the number of cases per year attributable to that food. Conversely, a food that has a relatively small risk per serving but that is consumed frequently and in large quantities may account for a greater portion of the number of cases within a population.
- Control measures that reduce the frequencies of contamination imply proportional reductions in the rates of illness, provided the proportions of high contaminations are reduced similarly.

- Control measures that prevent the occurrence of high levels of contamination at consumption would be expected to have the greatest impact on reducing the rates of listeriosis. Contamination with high numbers of *L. monocytogenes* at manufacturing and retail is rare, and foods such as ice cream and fermented meat products that do not permit growth during storage have relatively low risks per serving and low annual risks per population. In foods that permit growth during storage, particularly if stored at higher temperatures or for longer duration, the low numbers of *L. monocytogenes* at manufacture and retail may increase during storage to levels that represent substantially elevated relative risks of causing 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 reduce increase in risk due to growth of *L. monocytogenes*. Re-formulating foods so they do not support growth would be expected to reduce the occurrence of high doses and thus reduce the risk of listeriosis.

- 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 the standard is zero tolerance or 100 CFU/g. Raising a zero tolerance standard to a higher value (e.g. 1 CFU/25 g to 100/g) would be expected to result in increased incidence of listeriosis *unless* relaxing the standard led to the general adoption of control measures that significantly decreased the incidence of RTE food servings that exceeded the standard, particularly the number of servings with elevated levels of *L. monocytogenes*.

8. Limitations and caveats

No risk assessment is without its weaknesses. It is important that these are recognized, acknowledged and documented. This facilitates understanding of the risk assessment as well as its correct interpretation and use. Transparency in this area can actually help minimize the weaknesses. There are a number of limitations and caveats to this current risk assessment that the end user should be aware of so that he/she can make optimal use of the work in the appropriate manner. These are outlined below.

- 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.

9. Gaps in data

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. The uncertainty ranges about the risks per serving and number of cases in a population indicate the effect of data gaps on the estimates.

Consumption data were usually determined for nutritional purposes and lack critical information relevant to microbial quality. Contamination data were often neither recent, systematic, quantitative nor representative for different countries. In particular, the frequencies of high levels of contamination need to be better known. Additional knowledge on modelling growth would improve the estimates of the levels of *L. monocytogenes* consumed. Specific areas include the maximum levels of growth, interactions with the indigenous spoilage flora (including the lactic acid bacteria), distributions of storage times, and interactions of storage times and temperatures with spoilage.

The dose-response models are all based upon pairing population consumption patterns with epidemiological statistics. Improved investigation of outbreaks to determine the food involved, the amount of food consumed, number of *L. monocytogenes* consumed, the number of people exposed, number of people ill, the immunological status of all exposed people, and the virulence properties of the causative strain together would eventually lead to more accurate and specific dose-response models.

The dose-response models used in the current risk assessment should be applicable to all countries. Conversely, the exposure assessments are unique to each country and depend upon specific data on the factors that affect that population's exposure. At the present time, the amount of data available varies widely from one country to another, i.e. from marginal to adequate. No country has an "excess" of data.

10. Recommendations for future risk assessments

This risk assessment reflects the current state of knowledge about the contamination of foods with *L. monocytogenes* and rates of listeriosis. Implementation of systematic surveys to determine the handling, consumption and contamination of foods would improve future risk assessments. Research to further the understanding of microbial growth dynamics would increase the ability to estimate final levels of contamination. More complete investigation of outbreaks and determination of the virulence characteristics of *L. monocytogenes* will make the dose-response relationships more accurate and precise.

This risk assessment did not attempt to evaluate the factors that lead to the contamination of a food at retail. Additional product pathway exposure assessments for selected foods would provide additional understanding of how these foods become contaminated and the factors that have the greatest impact on preventing or eliminating that contamination. Creating valid product pathway assessments would then permit testing the impact on the incidences of listeriosis of various mitigations or postulated effects of regulatory changes.

The critical factor in evaluating the risk from a food is the frequency distribution of the levels of contamination when that food is consumed. Estimating the actual effect of a proposed regulatory programme on this distribution is highly uncertain, yet determining the resulting change in the distribution is fundamental to reducing the occurrence of listeriosis.

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Listeria monocytogenes is widely dispersed in the environment and foods, and is capable of growing even at refrigeration temperatures. Foodborne listeriosis, although relatively rare, is a clinically serious disease with a high case-fatality rate that largely affects specific higher-risk segments of the population. Cases of listeriosis appear to be predominately associated with ready-to-eat products. FAO and WHO have undertaken a risk assessment to address the risk of listeriosis associated with such foods and to answer specific risk management questions posed by the Codex Committee on Food Hygiene (CCFH). This volume provides a summary of that risk assessment.

The interpretative summary includes an overview of the risk assessment with a particular focus on information that would be relevant to risk managers faced with addressing problems posed by this pathogen in ready-to-eat foods. It includes answers to the specific risk management questions posed by the CCFH and outlines the issues to be considered when implementing control measures, including the establishment of microbiological criteria.

This volume and others in this *Microbiological Risk Assessment Series* contain information that is useful to both risk assessors and risk managers, the Codex Alimentarius Commission, governments and food regulatory agencies, industries and other people or institutions with an interest in the area of *Listeria monocytogenes*, its impact on public health and food trade, and the use of microbiological risk assessment in developing control strategies.

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