Quantitative Assessment of Relative Risk to Public Health from Foodborne
Listeria monocytogenes **Among Selected Categories of Ready-to-Eat Foods**

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SUMMARY OF PUBLIC COMMENTS AND FDA/FSIS REVISIONS TO RISK ASSESSMENT

A notice of availability of a draft risk assessment on the relationship between foodborne *Listeria monocytogenes* and human health, and a proposed risk management action plan was published in the Federal Register of January 19, 2001 (66 FR 5515) by the Food and Drug Administration (FDA), in cooperation with the Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA), and the Centers for Disease Control and Prevention (CDC). As part of a peer evaluation of the draft risk assessment, FDA/FSIS requested comments on the technical aspects of the draft risk assessment in the following areas: (1) the assumptions made; (2) the modeling techniques; (3) the data used; and (4) the transparency of the document. Comments were solicited for a 60-day period, ending March 20, 2001. Extensions were granted to comment on the risk assessment, extending the comment period to July 18, 2001.

We received 20 submissions of public comments. Submissions to the docket were received from: consumer groups; industry; trade associations representing the food industry, restaurants, food processors, manufacturers, distributors, marketers; consumer groups; manufacturer of food processing equipment; expert risk assessors and modelers; food retailer; educational and scientific society; and marketer, processor and distributor of agricultural and food products. The specific comments and the corresponding FDA/FSIS action/response for each topic area are described in Appendix 2.

We wish to both acknowledge and express our appreciation to those who provided comments to us. We considered the specific public comments in preparing this revised risk assessment. On the basis of the comments received, we determined that certain modifications should be included in the revised risk assessment. These modifications include the following.

1. **Revision of the Food Categories**
   - The cheese categories have been reorganized into six categories based on moisture content.
   - The Miscellaneous Dairy Products have been split into two categories: Cultured Milk Products (includes the low pH dairy foods manufactured with lactic acid fermentation) and High Fat and Other Dairy Products (includes the remainder of the dairy products that generally support growth).
   - The frankfurter category was separated into reheated and not reheated frankfurter categories.
   - Vegetable and fruit salads with salad dressing (including cole slaw and potato salad) were moved to the Deli-type Salad food category.
   - Canned fruits and nuts were removed.
   - Pickled, dried, and processed vegetables were removed.
   - The number of food categories was increased from 20 to 23.

2. **Modifications to the Contamination Data**
   - Newly available published and unpublished contamination data (approximately 40 studies) were included.
   - Contamination data were weighted according to geographical location, year collected, and study size and an adjustment factor was used for food categories that had no new data.
   - Food category-specific generalizations were made for the shape of the *Listeria monocytogenes* concentration distributions based on enumeration studies.

3. **Modifications to the Growth Data**
   - Newly available data on growth of *Listeria monocytogenes* in various foods and post-retail storage times (frankfurters and deli meats) were included.
   - For the Deli-type Salad food category, salads were segregated into growth and non-growth salads (and included consideration of the use of preservatives in salads made for bulk distribution to retail stores).
   - For non-growth foods, the rates of inactivation were estimated from the research literature.
   - The percentage of Frankfurters frozen before consumption were excluded from the growth model.

4. **Incorporated Key New Data:**
   - American Meat Institute (AMI) consumer survey on how long (on average) deli meats and frankfurters were stored prior to consumption.
   - National Food Processors Association (NFPA)/ Joint Institute for Food Safety and Applied Nutrition (JIFSAN) retail study, detailing the frequency and prevalence of *Listeria monocytogenes* in deli meats, deli salads, bagged fresh vegetables, seafood salads, smoked seafood, soft cheeses, and Hispanic-style cheeses.
   - FDA/CFSAN study on the growth of *Listeria monocytogenes* in deli salads.
   - International Dairy Foods Association (IDFA) data on cheese and ice cream.
   - Refrigerated Foods Association study in growth of *Listeria monocytogenes* in deli salads.

5. **Dose-Response and Other Model Modifications**
   - An additional year of FoodNet data (2000) was incorporated, which slightly reduced the total number of predicted cases.
   - Separate mortality to hospitalization ratios were calculated for each sub-population.
   - A 'scaling factor' was selected to adjust each uncertainty distribution of the predicted number of cases to the FoodNet estimates. As a result the 'scaling factor' is a distribution; but the total number of predicted cases for each population is not.
   - The model was rewritten in Visual Basic for Applications to speed up the computation time required for each run of the model and to facilitate review.

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Quantitative Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods

EXECUTIVE SUMMARY

Background

The U.S. Department of Health and Human Service, Food and Drug Administration's Center for Food Safety and Applied Nutrition (DHHS/FDA/CFSAN) conducted this risk assessment in collaboration with the U.S. Department of Agriculture's Food Safety and Inspection Service (USDA/FSIS) and in consultation with the DHHS Centers for Disease Control and Prevention (CDC). The purpose of the assessment is to examine systematically the available scientific data and information to estimate the relative risks of serious illness and death associated with consumption of different types of ready-to-eat (RTE) foods that may be contaminated with *Listeria monocytogenes*. This examination of the current science and the models developed from it are among the tools that food safety regulatory agencies will consider when evaluating the effectiveness of current and future policies, programs, and regulatory practices to minimize the public health impact of this pathogen.

The Healthy People 2010 goals for national health promotion and disease prevention called on federal food safety agencies to reduce foodborne listeriosis by 50% by the end of the year 2005. Preliminary FoodNet data on the incidence of foodborne illnesses for the United States in 2001 indicated that the incidence of infection from *Listeria monocytogenes* decreased between 1996 and 2001 from 0.5 to 0.3 cases per 100,000 people per year. The level then reached a plateau. In order to reduce further the incidence to a level of 0.25 cases per 100,000 people by the end of 2005, it became evident that additional targeted measures were needed. The *Listeria monocytogenes* risk assessment was initiated as an evaluation tool in support of this goal.

Listeria monocytogenes is a bacterium that occurs widely in both agricultural (soil, plants and water) and food processing environments. Ingestion of *Listeria monocytogenes* can cause listeriosis, which can be a life-threatening human illness. In 2000, the CDC reported that of all the foodborne pathogens tracked by CDC, *Listeria monocytogenes* had the second highest case fatality rate (21%) and the highest hospitalization rate (90.5%). Serious illness almost always occurs in people considered to be at higher risk, such as the elderly and those who have a pre-existing illness that reduces the effectiveness of their immune system. Perinatal listeriosis results from foodborne exposure of the pregnant mother leading to *in utero* exposure of the fetus, resulting in fetal infection that leads to fetal death, premature birth, or neonatal illness and death. *Listeria monocytogenes* also causes listerial gastroenteritis, a syndrome typically associated with mild gastrointestinal symptoms in healthy individuals. This risk assessment focuses on the severe public health consequences.

Scope and General Approach

This risk assessment provides analyses and models that (1) estimate the potential level of exposure of three age-based population groups and the total United States population to *Listeria monocytogenes* contaminated foods for 23 food categories and (2) relate this exposure to public health consequences. The food categories consist of foods with a documented history of *Listeria monocytogenes* contamination. The models provide a means of predicting the likelihood that

severe illness or death will result from consuming foods contaminated with this pathogen. These predictions are interpreted and used to estimate the relative risks among the food categories. The foods considered in this risk assessment are ready-to-eat foods that are eaten without being cooked or reheated just prior to consumption. One food, frankfurters, may or may not be reheated prior to consumption and was considered as two separate food categories. The working assumption is that all the cases of listeriosis are attributed to the foods in 23 categories, so that the risk assessment could be 'anchored' to the United States public health statistics. However, it is recognized that additional foods or cross-contamination from raw foods before cooking to other RTE foods may also contribute to additional cases.

The published scientific literature, government food intake surveys, health statistics, epidemiological information, unpublished food product surveys acquired from state and federal public health officials and trade associations, and surveys specifically designed to augment the data available for the risk assessment are the primary sources of data used in this document. Expert advice on scientific assumptions was actively sought from leading scientists from academia, industry, and government. This included two formal reviews of the underlying model structure and assumptions by the United States National Advisory Committee on Microbiological Criteria for Foods. In addition, the risk assessment was initially published in draft form and public comments sought for six months.

While the risk assessment purposely did not look into the pathways for the manufacture of individual foods, the risk assessment model developed can be used to estimate the likely impact of control strategies by changing one or more input parameters and measuring the change in the model outputs. This process, referred to as conducting 'what-if' scenarios, can be used to explore how the components of a complex model interact. Several 'what-if' scenarios are detailed within the risk assessment to evaluate the impact of refrigerator temperature, storage times, and reduction of the number of organisms in foods at before it is sold, and reduction in the contamination levels in foods that support growth.

Results
The relative risk rankings, along with the corresponding risk estimates expressed in terms of both the predicted number of cases per serving and per annum, are provided in Summary Table 1. Both measures are important in understanding and interpreting the risks associated with foodborne listeriosis. The per serving value can be considered the inherent risk associated with the manufacturing, distribution, marketing, and use of the food category, and is reflective of the degree of *Listeria monocytogenes* control achieved. Examples of factors that influence the 'per serving' risk include the frequency of contamination, the extent of that contamination, the ability of the food category to support the growth of *Listeria monocytogenes*, the duration and temperature of refrigerated storage, and the size of the serving. The predicted relative risk per serving can be viewed as the relative risk faced by individual consumers when he/she consume a single serving of the various foods considered in this risk assessment. The 'per serving' risk is typically the value upon which risk management decisions related to a specific product are based.

Summary Table 1. Relative Risk Ranking and Predicted Median Cases of Listeriosis for the Total United States Population on a per Serving and per Annum Basis

Relative Risk Ranking	Predicted Median Cases of Listeriosis for 23 Food Categories					
	Per Serving Basis[a]			Per Annum Basis[b]		
		Food	Cases		Food	Cases
1	High Risk	Deli Meats	7.7×10^{-8}	Very High	Deli Meats	1598.7
2	High Risk	Frankfurters, not reheated	6.5×10^{-8}	High Risk	Pasteurized Fluid Milk	90.8
3	High Risk	Pâté and Meat Spreads	3.2×10^{-8}	High Risk	High Fat and Other Dairy Products	56.4
4	High Risk	Unpasteurized Fluid Milk	7.1×10^{-9}	High Risk	Frankfurters, not reheated	30.5
5	High Risk	Smoked Seafood	6.2×10^{-9}	Moderate Risk	Soft Unripened Cheese	7.7
6	High Risk	Cooked Ready-to-Eat Crustaceans	5.1×10^{-9}	Moderate Risk	Pâté and Meat Spreads	3.8
7	Moderate Risk	High Fat and Other Dairy Products	2.7×10^{-9}	Moderate Risk	Unpasteurized Fluid Milk	3.1
8	Moderate Risk	Soft Unripened Cheese	1.8×10^{-9}	Moderate Risk	Cooked Ready-to-Eat Crustaceans	2.8
9	Moderate Risk	Pasteurized Fluid Milk	1.0×10^{-9}	Moderate Risk	Smoked Seafood	1.3
10	Low Risk	Fresh Soft Cheese	1.7×10^{-10}	Low Risk	Fruits	0.9
11	Low Risk	Frankfurters, reheated	6.3×10^{-11}	Low Risk	Frankfurters, reheated	0.4
12	Low Risk	Preserved Fish	2.3×10^{-11}	Low Risk	Vegetables	0.2
13	Low Risk	Raw Seafood	2.0×10^{-11}	Low Risk	Dry/Semi-dry Fermented Sausages	<0.1
14	Low Risk	Fruits	1.9×10^{-11}	Low Risk	Fresh Soft Cheese	<0.1
15	Low Risk	Dry/Semi-dry Fermented Sausages	1.7×10^{-11}	Low Risk	Semi-soft Cheese	<0.1
16	Low Risk	Semi-soft Cheese	6.5×10^{-12}	Low Risk	Soft Ripened Cheese	<0.1
17	Low Risk	Soft Ripened Cheese	5.1×10^{-12}	Low Risk	Deli-type Salads	<0.1
18	Low Risk	Vegetables	2.8×10^{-12}	Low Risk	Raw Seafood	<0.1
19	Low Risk	Deli-type Salads	5.6×10^{-13}	Low Risk	Preserved Fish	<0.1
20	Low Risk	Ice Cream and Other Frozen Dairy Products	4.9×10^{-14}	Low Risk	Ice Cream and Other Frozen Dairy Products	<0.1
21	Low Risk	Processed Cheese	4.2×10^{-14}	Low Risk	Processed Cheese	<0.1
22	Low Risk	Cultured Milk Products	3.2×10^{-14}	Low Risk	Cultured Milk Products	<0.1
23	Low Risk	Hard Cheese	4.5×10^{-15}	Low Risk	Hard Cheese	<0.1

[a]Food categories were classified as high risk (>5 cases per billion servings), moderate risk (<5 but ≥1 case per billion servings), and low risk (<1 case per billion servings).

[b]Food categories were classified as very high risk (>100 cases per annum), high risk (>10 to 100 cases per annum), moderate risk (≥1 to 10 cases per annum), and low risk (<1 cases per annum).

The second measure, the 'per annum risk,' is the predicted number of fatal infections per year in the United States for each food category. This value takes into account the number of servings of the food category that are consumed. The predicted per annum risk of serious illnesses for each food category can be thought of as the predicted relative total public health impact for each food category. Since the 'per annum' risk is derived from the 'per serving' risk, there is generally a higher degree of uncertainty associated with the former. The predicted per serving and per annum relative risks are used to develop risk rankings to compare the various food categories. In addition to presenting the 'most likely' relative risk rankings for the different population groups and food categories, the risk assessment provides the inherent variability and the uncertainty associated with these rankings.

Evaluation and Interpretation
The overall interpretation of the risk assessment requires more than just a simple consideration of the relative risk rankings associated with the various food categories. First, the interpretation of the results requires an appreciation of the fact that the values being compared are the median values of distributions that may be highly skewed (i.e., not evenly distributed). The use of median values was selected as being the appropriate method for comparing the overall relative risks among the different food categories. The quantitative results must be considered in relation to the associated variability and uncertainty (i.e., the confidence intervals surrounding the median) and interpreted in the context of both the epidemiologic record and how the food categories are manufactured, marketed, and consumed. A detailed consideration of the quantitative and qualitative findings for each food category is provided in the risk assessment and its appendices.

A number of methods for objectively grouping the results were evaluated, and are discussed in detail within the risk assessment. One approach is cluster analysis. When performed at the 90% confidence level, this analysis groups the per serving rankings into four clusters and the per annum rankings into five. These clusters are used, in turn, to develop a two-dimensional matrix of per serving vs. per annum rankings of the food categories (Summary Figure 1). In this approach, the 'per serving' clusters are arrayed against the 'per annum' clusters. The matrix is then used to depict five risk designations: Very High, High, Moderate, Low, and Very Low.

The risk characterization combines the exposure and dose-response models to predict the relative risk of illness attributable to each food category. While the risk characterization must be interpreted in light of both the inherent variability and uncertainty associated with the extent of contamination of ready-to-eat foods with *Listeria monocytogenes* and the ability of the microorganism to cause disease, the results provide a means of comparing the relative risks among the different food categories and population groups considered in the assessment and should prove to be a useful tool in focusing control strategies and ultimately improving public health through effective risk management. As described above, cluster analysis techniques are employed as a means of discussing the food categories within a risk analysis framework. The food categories are divided into five overall risk designations (see Summary Figure 1), which are likely to require different approaches to controlling foodborne listeriosis.

Decreased Risk per Annum

	Clusters A and B	Clusters C and D	Cluster E	
	Very High Risk (Clusters 1-A, 1-B) Deli Meats Frankfurters (not reheated)	**High Risk (Clusters 1-C, 1-D)** Pâté and Meat Spreads Unpasteurized Fluid Milk Smoked Seafood	**Moderate Risk (Cluster 1-E)** No food categories	**Cluster 1**
	High Risk (Clusters 2-A, 2-B) High Fat and Other Dairy Products Pasteurized Fluid Milk Soft Unripened Cheese	**Moderate Risk (Clusters 2-C, 2-D)** Cooked RTE Crustaceans	**Moderate Risk (Cluster 2-E)** No food categories	**Cluster 2**
	Moderate Risk (Clusters 3-A, 3-B) No food categories	**Moderate Risk (Clusters 3-C, 3-D)** Deli-type Salads Dry/Semi-dry Fermented Sausages Frankfurters (reheated) Fresh Soft Cheese Fruits Semi-soft Cheese Soft Ripened Cheese Vegetables	**Low Risk (Cluster 3-E)** Preserved Fish Raw Seafood	**Cluster 3**
	Moderate Risk (Clusters 4-A, 4-B) No food categories	**Low Risk (Clusters 4-C, 4-D)** No food categories	**Very Low Risk (Cluster 4-E)** Cultured Milk Products Hard Cheese Ice Cream and Other Frozen Dairy Products Processed Cheese	**Cluster 4**

Decreased Risk per Serving (vertical axis label, left side)

Summary Figure 1. Two-Dimensional Matrix of Food Categories Based on Cluster Analysis of Predicted per Serving and per Annum Relative Rankings

[The matrix was formed by the interception of the four per serving clusters vs. the per annum clusters A and B, C and D, and E. For example, Cluster 3-E (Low Risk) refers to the food categories that are in both Cluster level 3 for the risk per serving and Cluster level E for the risk per annum.]

Risk Designation Very High. This designation includes two food categories, Deli Meats and Frankfurters, Not Reheated. These are food categories that have high predicted relative risk rankings on both a per serving and per annum basis, reflecting the fact that they have relatively high rates of contamination, support the relatively rapid growth of *Listeria monocytogenes* under

refrigerated storage, are stored for extended periods, and are consumed extensively. These products have also been directly linked to outbreaks of listeriosis. This risk designation is one that is consistent with the need for immediate attention in relation to the national goal for reducing the incidence of foodborne listeriosis. Likely activities include the development of new control strategies and/or consumer education programs suitable for these products.

Risk Designation High. This designation includes six food categories, High Fat and Other Dairy Products, Pasteurized Fluid Milk, Pâté and Meat Spreads, Soft Unripened Cheeses, Smoked Seafood, and Unpasteurized Fluid Milk. These food categories all have in common the ability to support the growth of *Listeria monocytogenes* during extended refrigerated storage. However, the foods within this risk designation appear to fall into two distinct groups based on their rates of contamination and frequencies of consumption.

- Pâté and Meat Spreads, Smoked Seafood, and Unpasteurized Fluid Milk have relatively high rates of contamination and thus high predicted per serving relative risks. However, these products are generally consumed only occasionally in small quantities and/or are eaten by a relatively small portion of the population, which lowers the per annum risk. All three products have been associated with outbreaks or sporadic cases, at least internationally.

  These foods appear to be priority candidates for new control measures (i.e., Smoked Seafood, Pâté and Meat Spreads) or continued avoidance (i.e., Unpasteurized Fluid Milk).

- High Fat and Other Dairy Products, Pasteurized Fluid Milk, and Soft Unripened Cheeses have low rates of contamination and corresponding relatively low predicted per serving relative risks. However, these products are consumed often by a large percentage of the population, resulting in elevated predicted per annum relative risks. In general, the predicted per annum risk is not matched with an equivalent United States epidemiologic record. However, the low frequency of recontamination of individual servings of these products in combination with their broad consumption makes it likely that these products are primarily associated with sporadic cases and normal case control studies would be unlikely to lead to the identification of an association between these products and cases of listeriosis.

  These products (High Fat and Other Dairy Products, Pasteurized Fluid Milk, and Soft Unripened Cheeses) appear to be priority candidates for advanced epidemiologic and scientific investigations to either confirm the predictions of the risk assessment or identify the factors not captured by the current models that would reduce the predicted relative risk.

Risk Designation Moderate. This risk designation includes nine food categories (Cooked Ready-to-Eat Crustaceans, Deli Salads, Fermented Sausages, Frankfurters-Reheated, Fresh Soft Cheese, Fruits, Semi-soft Cheese, Soft Ripened Cheese, and Vegetables) that encompass a range of contamination rates and consumption profiles. A number of these foods include effective bactericidal treatments in their manufacture or preparation (e.g., Cooked Ready-to-Eat Crustaceans, Frankfurters-Reheated, Semi-soft Cheese) or commonly employ conditions or compounds that inhibit the growth of *Listeria monocytogenes* (e.g., Deli Salads, Dry/Semi-dry Fermented Sausages). The risks associated with these products appear to be primarily associated with product recontamination, which in turn, is dependent on continued, vigilant application of proven control measures.

Risk Designation Low. This risk designation includes two food categories, Preserved Fish and Raw Seafood. Both products have moderate contamination rates but include conditions (e.g., acidification) or consumption characteristics (e.g., short shelf-life) that limit *Listeria monocytogenes* growth and thus limit predicted per serving risks. The products are generally consumed in small quantities by a small portion of the population on an infrequent basis, which results in low predicted per annum relative risks. Exposure data for these products are limited so there is substantial uncertainty in the findings. However, the current results predict that these products, when manufactured consistent with current good manufacturing practices, are not likely to be a major source of foodborne listeriosis.

Risk Designation Very Low. This risk designation includes four food categories, Cultured Milk Products, Hard Cheese, Ice Cream and Other Frozen Dairy Products, and Processed Cheese. These products all have in common the characteristics of being subjected to a bactericidal treatment, having very low contamination rates, and possessing an inherent characteristic that either inactivates *Listeria monocytogenes* (e.g., Cultured Milk Products, Hard Cheese) or prevents its growth (e.g., Ice Cream and Other Frozen Dairy Products, Processed Cheese). This results in a very low predicted per serving relative risks. The predicted per annum relative risks are also low despite the fact that these products are among the more commonly consumed ready-to-eat products considered by the risk assessment. The results of the risk assessment predict that unless there was a gross error in their manufacture, these products are highly unlikely to be a significant source of foodborne listeriosis.

Conclusions
The following conclusions are provided as an integration of the results derived from the models, the evaluation of the variability and uncertainty underlying the results, and the impact that the various qualitative factors identified in the hazard identification, exposure assessment, and hazard characterization have on the interpretation of the risk assessment.

- The risk assessment reinforces past epidemiological conclusions that foodborne listeriosis is a moderately rare although severe disease. United States consumers are exposed to low to moderate levels of *Listeria monocytogenes* on a regular basis.

- The risk assessment supports the findings of epidemiological investigations of both sporadic illness and outbreaks of listeriosis that certain foods are more likely to be vehicles for *Listeria monocytogenes*.

- Three dose-response models were developed that relate the exposure to different levels of *Listeria monocytogenes* in three age-based subpopulations [i.e., perinatal (fetuses and newborns), elderly, and intermediate-age] with the predicted number of fatalities. These models were used to describe the relationship between levels of *Listeria monocytogenes* ingested and the incidence of listeriosis. The dose of *Listeria monocytogenes* necessary to cause listeriosis depends greatly upon the immune status of the individual.

  1. Susceptible subpopulations (such as the elderly and perinatal) are more likely to contract listeriosis than the general population.

2. Within the intermediate-age subpopulation group, almost all cases of listeriosis are associated with specific subpopulation groups with increased susceptibility (e.g., individuals with chronic illnesses, individuals taking immunosuppressive medication).

3. The strong association of foodborne listeriosis with specific population groups suggests that strategies targeted to these susceptible population groups, i.e., perinatal (pregnant women), elderly, and susceptible individuals within the intermediate-age group, would result in the greatest reduction in the public health impact of this pathogen.

- The dose-response models developed for this risk assessment considered, for the first time, the range of virulence observed among different isolates of *Listeria monocytogenes*. The dose-response curves suggest that the relative risk of contracting listeriosis from low dose exposures could be less than previously estimated.

- The exposure models and the accompanying 'what-if' scenarios identify five broad factors that affect consumer exposure to *Listeria monocytogenes* at the time of food consumption.

  1. Amounts and frequency of consumption of a ready-to-eat food

  2. Frequency and levels of *Listeria monocytogenes* in a ready-to-eat food

  3. Potential of the food to support growth of *Listeria monocytogenes* during refrigerated storage

  4. Refrigerated storage temperature

  5. Duration of refrigerated storage before consumption

Any of these factors can affect potential exposure to *Listeria monocytogenes* from a food category. These factors are 'additive' in the sense that foods where multiple factors favor high levels of *Listeria monocytogenes* at the time of consumption are typically more likely to be riskier than foods where a single factor is high. These factors also suggest several broad control strategies that could reduce the risk of foodborne listeriosis such as reformulation of products to reduce their ability to support the growth of *Listeria monocytogenes* or encouraging consumers to keep refrigerator temperatures at or below 40 ºF and reduce refrigerated storage times. For example, the 'what-if' scenarios using Deli Meats predicts that consumer education and other strategies aimed at maintaining home refrigerator temperatures at 40 ºF could substantially reduce the risks associated with this food category. Combining this with pre-retail treatments that decrease the contamination levels in Deli Meats would be expected to reduce the risk even further.

This risk assessment significantly advances our ability to describe our current state of knowledge about this important foodborne pathogen, while simultaneously providing a framework for integrating and evaluating the impact of new scientific knowledge on public health enhancement.

ABBREVIATIONS AND ACRONYMS

Abbreviation/ Acronym	Definition
ARS:	USDA's Agricultural Research Service
CDC:	Centers for Disease Control and Prevention
CFSAN:	FDA's Center for Food Safety and Applied Nutrition
CFU:	Colony forming unit
CSFII:	USDA's Continuing Survey of Food Intakes by Individuals
EGR:	Exponential Growth Rate
FDA:	US DHHS's Food and Drug Administration
FSIS:	USDA's Food Safety and Inspection Service
GMP:	Good Manufacturing Practice
GSD:	Geometric Standard Deviation
HACCP:	Hazard Analysis Critical Control Point
IP:	Intraperitoneal
LD_{50}:	The 50 % Lethal Dose (See Glossary)
LLO:	Listeriolysin O (see Glossary)
NACMCF:	National Advisory Committee on Microbiological Criteria for Foods
NAS:	National Academy of Sciences
NFS:	Not further specified; a term used by CSFII
NHANES III:	Third National Health and Nutrition Examination Survey
PFGE:	Pulsed Field Gel Electrophoresis
RAC:	The interagency Risk Assessment Consortium
RTE:	Ready-to-Eat
SSOP:	Sanitation Standard Operating Procedure
UHT:	Ultra high temperature
US DHHS:	United States Department of Health and Human Services
USDA:	United States Department of Agriculture
WHO:	World Health Organization

GLOSSARY

Term

Antibody Titer: A measure of the activity of an antibody solution.

Antibody: A protein capable of specifically reacting with a particular antigen.

Antigen: A substance capable of eliciting the formation of an antibody.

Asymptomatic: Without symptoms, or not exhibiting symptoms.

Attack Rate: The numbers of people at risk who develop a disease out of the total number of people at risk. The attack rate is useful in comparing the risk of disease in groups with different exposures.

Colony Forming Unit: A cell or cluster of two or more attached sister cells capable of multiplying to form a macroscopic colony of cells.

Cumulative Distribution: A representation of a distribution where the values are arranged in ascending or descending order.

Distribution: A series of values or a mathematical equation describing a series of values.

Dose: The amount or number of a pathogen that is ingested or interacts with an organism (host).

Dose-response Assessment: The determination of the relationship between the magnitude of exposure and the magnitude and/or frequency of adverse effects.

Elderly: United States population 60 years of age and older.

Empirical Distribution: A series of observed values or data.

Exposure Assessment: A component of a risk assessment that characterizes the source and magnitude of human exposure to the pathogen.

Fetus: The term used to refer to an unborn child from 16 weeks after fertilization to birth.

Foodborne Pathogen: A microorganism (bacteria, virus, protozoa) that is capable of causing disease and is transmitted by food.

Food Code: A number representing a food in the food consumption surveys; each food has its own food code.

Food Matrix: The food environment that a pathogen is in. It includes the food's fat levels, acidity, salt level and other characteristics of the food that affect the pathogen's ability to cause disease.

FoodNet: Foodborne Diseases Active Surveillance Network. A surveillance system led by the Centers for Disease Control and Prevention for compiling epidemiological data on the incidences of foodborne illness (also see Appendix 4).

Frequency Distribution: A distribution describing the rate or frequency of occurrence of a value in a series or population.

Term	Definition
Gene Knock Out Model:	An animal host which is specifically used because it has a known genetic defect or gene disruption in order to determine the role of the missing gene in a biological process such as resistance to infection.
Intermediate-age Subpopulation:	Total United States population excluding elderly and pregnancy-associated groups, and including susceptible populations such as cancer patients, AIDS patients, and transplant patients.
Hazard Health Effect:	A biological, chemical or physical agent in, or property of, food that may have an adverse health effect.
Hazard Identification:	The identification of known or potential health effects associated with a particular agent.
Hazard Characterization:	The qualitative or quantitative evaluation of the nature of the adverse effects associated with biological, chemical, and physical agents that may be present in food.
Incidence:	The number of new cases of a disease that occur during a specified period of time in a population at risk for developing the disease.
Infection:	When a microorganism or other pathogen becomes established in the host; this includes invasion, multiplication, and transmission.
Iteration:	A single calculation among a series of calculations (e.g. a Monte-Carlo simulation).
Intraperitoneal:	Route of introduction of an inoculum (pathogen) by a needle or syringe into the peritoneal cavity (abdomen) of the host.
Intragastrical:	The route of introducing an inoculum in which the material is injected into the stomach of the host by a tube that bypasses the mouth and esophagus. The normal route of invasion of a foodborne pathogen is through ingestion, survival in the stomach and invasion through the gastrointestinal system.
Immunosuppression:	An agent or condition that decreases a person's ability to resist infection.
LD_{50}:	The dose resulting in 50 % lethality in a population.
Listerial Gastroenteritis:	Mild, flu-like symptoms caused by *Listeria monocytogenes* infection: chills, diarrhea, headache, abdominal pain and cramps, nausea, vomiting, fatigue, and myalgia.
Listeriolysin O:	A protein produced by *Listeria monocytogenes* that disrupts red blood cells in the host.
Listeriosis:	The disease caused by infection with *Listeria monocytogenes.*

Term	Definition
Modeling (mathematical):	Attempting to predict aspects of the behavior of some system by creating an approximate (mathematical) model of it. Mathematical models contribute to the understanding of complex systems and their predicted behavior within the scope of the model.
Meat or Poultry Spreads:	A ready-to-eat product that generally is cooked and contains meat or poultry, fat, and other ingredients to result in a paste-like consistency (e.g., "Ham Spread" or "Tongue Spread"). Meat or poultry spreads differ from pâté in that the primary meat product or poultry product is liver.
Monte-Carlo Simulation:	A process for making repeated calculations with minor variations of the same mathematical equation, usually with the use of a computer. May be used to integrate variability in the predicted results for a population or the uncertainty of a predicted result. A two dimensional Monte-Carlo in simulation may be used to do both.
Neonate:	A newborn from birth to 30 days of age.
Outbreak:	The occurrence of two or more cases of similar illness resulting from the ingestion of a common food (See Sporadic).
Perinatal:	As used in this risk assessment, refers to the susceptible population that includes fetuses and neonates from 16 weeks after fertilization to 30 days after birth.
Prenatal:	As used in this risk assessment, a fetus over 16 weeks gestation.
Prevalence:	In epidemiology, the number of affected persons present in the population at a specific point in time divided by the number of persons in the population at that time.
Probability:	As used in this risk assessment, probability denotes uncertainty. The term is also sometimes used to denote frequency.
Ready-To-Eat:	Foods that may be eaten as purchased without further preparation by the consumer, particularly without additional cooking.
Relative Risk:	As used in this risk assessment, the term refers to the comparisons and rankings of the risks per serving and cases per annum of listeriosis attributed to each of 23 food categories. The food categories are ranked from 1 (highest risk) to 23 (lowest risk) based on the model predictions for the median number of cases of listeriosis. An implicate assumption is that virtually all cases of foodborne listeriosis reported by CDC can be attributed to the foods in these 23 food categories.

Term	Definition
Ribotype:	A subtype of a bacterial strain more detailed than the species or serotype level; determination of a ribotype is based on analysis of patterns formed by DNA fragments.
Risk:	The likelihood of the occurrence and the magnitude of the consequences of exposure to a hazard on human health.
Risk Analysis:	The process consisting of three components: risk assessment, risk management, and risk communication.
Risk Assessment:	The scientific evaluation of known or potential adverse health effects resulting from human exposure to hazards. The process consists of the following steps: hazard identification, exposure assessment, hazard characterization (dose-response), and risk characterization.
Risk Characterization:	Integration of hazard identification, hazard characterization and exposure assessment into an estimation of the adverse effects likely to occur in a given population, including attendant uncertainties.
Serotype:	A group of related microbes distinguished by its composition of antigens.
Serving Size:	The amount of food eaten per eating occasion. [In this risk assessment, it does not refer to the amount customarily consumed per eating occasion, as defined by FDA in the Code of Federal Regulations.]
Sporadic Case:	When a single individual becomes ill; an isolated event not documented as an outbreak.
Susceptible Population:	A group of people at increased risk for infection and illness from a pathogen, often caused by a decrease in the effectiveness of the person's immune system.
Susceptibility:	The degree that a host is vulnerable to infection; includes the ability of the host to defend itself.
T lymphocytes:	A subset of lymphocytes (white blood cells) defined by their development in the thymus gland. They are involved in most aspects of adaptive immunity including antibody production (via interaction with B-lymphocytes) and inflammation.
Uncertainty:	An expression of the lack of knowledge, usually given as a range or group of plausible alternatives.
Uncertainty Distribution:	A description of the range of plausible values for a prediction.
Variability:	A description of differences among the individual members of a series or population.
Virulence:	The capacity of a microbial pathogen to invade and/or produce illness in the host. Mediated by the presence of specific genes and their protein products that interact with the host.

Revised: Quantitative Assessment of Relative Risk to Public Health from Foodborne
***Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods**

I. INTRODUCTION

The United States DHHS Food and Drug Administration's Center for Food Safety and Applied Nutrition (FDA/CFSAN) conducted this risk assessment in collaboration with the United States Department of Agriculture's Food Safety and Inspection Service (FSIS), and in consultation with the Centers for Disease Control and Prevention (CDC). The purpose of this assessment is to systematically examine available scientific data and information in order to estimate the predicted relative risk of serious illness and death that may be associated with consumption of different types of ready-to-eat foods that may be contaminated with *Listeria monocytogenes*. This examination of current science and the models developed are among the tools that food safety regulatory agencies will use to evaluate the effectiveness of current policies, programs and regulatory practices that will minimize the public health impact of this pathogenic microorganism. This work provides a comprehensive assessment, building on and improving upon previously published assessments that related foodborne exposure to human listeriosis (Lindquist and Westöö, 2000, Buchanan *et al*., 1997; Farber *et al*., 1996; Haas *et al*., 1999; Hitchins, 1995 and 1996; and Teufel and Bendzulla, 1993).

DHHS/FDA and USDA/FSIS announced their intent to conduct a risk assessment of the public health impact of *Listeria monocytogenes* from food in the *Federal Register* (US DHHS, 1999a). At that time, the public was invited to comment on the planned assessment and submit scientific data and information for use in the assessment. The advice and recommendations of the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) were sought on the assumptions and the risk assessment model structure to be used (US DHHS, 1999b, 1999c). During the conduct of this risk assessment, FDA and FSIS solicited the technical advice and opinions of scientific experts in various disciplines. In addition, critical review of this risk assessment model and a draft document was solicited and received from members of the Interagency Risk Assessment Consortium and other government employees.

This risk assessment was preceded by the Draft Assessment of Relative Risk to Public Health from Foodborne *Listeria monocytogenes* among Selected Categories of Ready-To-Eat Foods (DHHS/USDA, 2001). In January 2001, FDA and FSIS invited comments on the draft risk assessment. These comments, additional new data, and improved modeling techniques are incorporated into this revised version. A chronology of the technical and scientific review involved in the development of this *Listeria monocytogenes* risk assessment is provided in Appendix 1. A summary of the public comments submitted in response to the January 2001 draft risk assessment and our responses to these comments is provided in Appendix 2.

An international risk assessment on *Listeria monocytogenes* is concurrently being conducted by WHO/FAO for the Codex Alimantarius, Codex Committee on Food Hygiene (WHO/FAO, 2003). This FDA/FSIS risk assessment was conducted simultaneously with but independent of the WHO/FAO effort. The latter explored the dose-response relationship in more detail but determined the risks for only four representative foods. The conclusions reached in the WHO/FAO risk assessment are compatible with those reached in this FDA/FSIS risk assessment.

This risk assessment estimates the potential levels of consumer exposure to foodborne *Listeria monocytogenes* from different types of ready-to-eat (RTE) foods (including seafood, vegetables, fruit, dairy products, and meats), and characterizes the likely impact of this exposure on public health. Included is an evaluation of the impact of foodborne *Listeria monocytogenes* on the health of three age-based subpopulations, two of which are vulnerable groups that were distinguished based on FoodNet surveillance data.

- Perinatal: This subpopulation includes fetuses and neonates from 16 weeks after fertilization to 30 days postpartum. The neonatal cases are assumed to be pregnancy-associated cases where exposure occurs *in utero* as a result of foodborne *Listeria monocytogenes* infections of the mothers during pregnancy. Manifestations of listeriosis for this subpopulation group include spontaneous abortions, stillbirths, and neonatal infections.

- Elderly: This subpopulation includes individuals who are 60 or more years of age. This group is considered to have increased susceptibility to listeriosis due, in part, to physiological changes associated with the natural aging process.

- Intermediate-Age: Because there are insufficient data to separate the remaining population into discrete subpopulations, this group includes the remaining population, both healthy individuals (with very low risk of severe illness or death from *Listeria monocytogenes*) and certain susceptible subpopulations. The subpopulations include individuals with increased susceptibility to listeriosis; such as AIDS patients or individuals taking drugs that suppress the immune systems (*e. g.*, cancer or transplant drugs). Individuals within these subpopulations account for most of the cases of listeriosis within the intermediate-age group.

In addition, the number of predicted cases of listeriosis for the total United States population was estimated on a per serving and per annum basis for each food category.

Background

A series of illness outbreaks associated with the consumption of coleslaw, pasteurized milk, and fresh soft cheese in the early 1980s led to the recognition of *Listeria monocytogenes* as a foodborne pathogen.

In 1991, the NACMCF presented its analysis of the emerging problem and its recommendations to FSIS, FDA and other United States government agencies (NACMCF, 1991). The NACMCF recommended control strategies to minimize the presence, survival, and multiplication of *Listeria monocytogenes* in foods. These control strategies included the development of an effective surveillance system for listeriosis, targeted efforts on specific foods, and the use of HACCP-based (Hazard Analysis and Critical Control Points) programs to ensure the safety of foods from processing to consumption.

Major efforts by industry and regulatory agencies during the early 1990s reduced the incidence of listeriosis by approximately 50%. However, further reductions in illness are increasingly

difficult, in part because of the unique challenges associated with controlling this pathogen. Several barriers to its control include:

- The microorganism is commonly found in the environment, including food processing, distribution, and retail environments, in foods, and in the home.
- It primarily affects a small segment of the population that has heightened susceptibility.
- It can grow slowly in many foods during refrigerated storage.
- It is more resistant than most bacteria to the conditions and treatments used to control foodborne pathogens.

Current Policies

Based on the known characteristics of this microorganism and the disease, FDA maintains a policy of "zero-tolerance" for *Listeria monocytogenes* in ready-to-eat foods (i.e., products that may be consumed without any further cooking or reheating). This means that the detection of any *Listeria monocytogenes* in either of two 25-gram samples of a food renders the food adulterated as defined by the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. 342(a)(1) (Shank *et al.*, 1996). This policy was affirmed in the 1995 United States District court decision, United States v. Union Cheese Co. (Anonymous, 1995).

FSIS's "zero-tolerance" policy applies to the detection of *Listeria monocytogenes* in ready-to-eat products. If meat or poultry products are contaminated with *Listeria monocytogenes*, the products are adulterated under the provisions of the Federal Meat Inspection Act and the Poultry Inspection Act, 21 U.S.C. 601(m) or 453 (g), respectively (Anonymous, 1994).

Other countries, including some major trading partners of the United States, have different policies for dealing with *Listeria monocytogenes* contamination. Countries such as Canada and Denmark have a "non-zero tolerance" for *Listeria monocytogenes* for some classes of foods (Health Canada, 1994). For example, in Canada, ready-to-eat (RTE) foods that have not been associated with an outbreak and do not allow any growth of *Listeria monocytogenes* during a 10-day period of refrigerated storage, may contain up to 100 *Listeria monocytogenes* organisms per

gram of food (Health Canada, 1994). Denmark has six classes of foods with various criteria for *Listeria monocytogenes*. In raw RTE foods, for example, two of five samples can contain between 10 and 100 organisms per gram, but no sample can exceed 100 organisms per gram.

There is no epidemiological evidence that demonstrates whether a zero or non-zero tolerance policy leads to a greater rate of listeriosis. Estimates of disease rates between different countries must be considered with caution because of different surveillance and reporting systems but the comparable overall rates of listeriosis for ranges from 0.1 to 11.3 cases per million persons per year in Europe, 3.4 to 4.4 cases per million people per year in the United States, and 3 cases per million per year in Australia (WHO/FAO, 2003).

Healthy People 2010 Initiative

The commitment of FDA, FSIS, and CDC to reduce foodborne listeriosis was formally reaffirmed as a national public health goal in the Healthy People 2010 initiative coordinated by the United States Department of Health and Human Services (US DHHS). The federal government established a goal of working with industry, public health, and research communities to achieve an additional 50% reduction in listeriosis by 2010. "Healthy People" is a national health promotion and disease prevention initiative that brings together national, state, and local government agencies; nonprofit, voluntary, and professional organizations; and businesses, communities, and individuals to improve the health of all Americans, eliminate disparities in health, and improve years and quality of healthy life (US DHHS, 2000).

Preliminary FoodNet data on the incidence of foodborne illnesses for the United States in 2001 indicated that the incidence of infection from *Listeria monocytogenes* decreased between 1996 and 2001 from 0.5 to 0.3 cases per 100,000 people per year. The level then reached a plateau. In order to reduce further the incidence to a level of 0.25 cases per 100,000 people by the end of 2005, it became evident that additional targeted measures were needed. The *Listeria monocytogenes* risk assessment was initiated as an evaluation tool in support of this goal.

Risk Assessment Overview

This risk assessment follows the risk assessment structure of the Joint Food and Agriculture
Organization/World Health Organization Expert Consultation on the Application of Risk
Analysis to Food Standards Issues (Joint FAO/WHO, 1995). The structure consists of four
components: (1) hazard identification, (2) exposure assessment, (3) hazard characterization, and
(4) risk characterization. Hazard identification is defined by the Joint FAO/WHO Consultation
as the identification of known or potential health effects associated with a particular biological,
chemical, or physical agent. Exposure assessment is the qualitative and/or quantitative
evaluation of the degree of intake likely to occur. Hazard characterization is the qualitative
and/or quantitative evaluation of the nature of the adverse effects associated with biological,
chemical, and physical agents that may be present in food. Finally, risk characterization is the
integration of hazard identification, hazard characterization, and exposure assessment into an
estimation of the adverse effects likely to occur in a given subpopulation, including attendant
uncertainties.

Microbiological risk assessments generally use the same conceptual framework developed for
chemical risk assessments (ICMSF, 1994). However, while there are many similarities between
chemical and microbial risk assessments, there are also differences. At this time, the major
concern with microbiological hazards is an acute illness from a single exposure, rather than
illness from a low level, chronic exposure. Even so, sequelae and other long-term effects are
beginning to be recognized for some microorganisms, but knowledge is still limited in this area.
In this microbial risk assessment, the infectious unit is a single microorganism. Low levels of
microorganisms (rather than low concentrations of a chemical substance) characterize the
frequent exposure with higher levels of exposure occurring only occasionally. While the
likelihood of disease increases with increasing numbers of pathogenic microorganisms
consumed, the potential for low levels of infectious agents to cause disease cannot be dismissed.
Another difference between microbial and chemical hazards is that the level of a microorganism
in a food can change, while chemical concentrations usually remain constant. This change in
microbial levels should be accounted for in a microbial risk assessment's model. Human
exposure levels to a pathogen in a food can rapidly increase by a million-fold within even a

relatively short period of temperature abuse. Conversely, heating food immediately before consumption can reduce pathogen levels to a negligible risk. These biological characteristics of bacteria require the inclusion of detailed modeling steps in the exposure assessment. There is usually little question as to the hazard of microbial pathogens, although the dose-response relationships may not be easily described.

Figure I-1 shows the organization of this report including the main components of each chapter such as types of data and modeling techniques described. Additional details concerning the structure and modeling techniques used in this risk assessment are provided in Appendix 3.

Hazard Identification

- ❖ Characteristics of *Listeria monocytogenes*
- ❖ Endpoints of concern: Listeriosis, Death
- ❖ Epidemiology
- ❖ Outbreaks

Exposure Assessment

Data:
- ❖ Food consumption
- ❖ Food contamination levels
- ❖ Growth rates
- ❖ Storage times
- ❖ Storage temperatures

Modeling:
- ❖ *Listeria monocytogenes* levels in food at retail
- ❖ Growth between retail and consumption
- ❖ Thermal inactivation
- ❖ *Listeria monocytogenes* in food at consumption

Hazard Characterization

Data:
- ❖ Pathogen virulence
- ❖ Host susceptibility
- ❖ FoodNet surveillance

Modeling:
- ❖ Dose-response in mice
- ❖ Adjustment factor(s)
- ❖ Dose-response curves for 3 subpopulations

Risk Characterization

- ❖ Risk per serving
- ❖ Risk per annum
- ❖ Risk rankings
- ❖ Lattitude (uncertainty) graphs

Intervention Scenarios

- ❖ Storage temperature
- ❖ Storage time
- ❖ Frequency of contamination

Figure I-1. Overview of *Listeria monocytogenes* Risk Assessment Document

II. HAZARD IDENTIFICATION

In the hazard identification, the known or potential health effects associated with *Listeria monocytogenes* are identified by establishing the general relationship between the pathogen, its presence in foods, and the adverse outcome (illness or death) associated with consumption of foods contaminated with *Listeria monocytogenes*. While the negative health impact of a hazard must be recognized for a risk assessment to be undertaken, the nature of the impact must be clearly defined, and specific endpoints, or health outcomes of interest, identified. Common endpoints for infectious agents are infection, disease (morbidity), death, and chronic sequelae (long-term after-effects). This risk assessment is concerned with the endpoints of serious illness and death.

Listeria monocytogenes

Listeria are short (0.5 μm in diameter by 1 to 2 μm long) gram positive, non-spore-forming rods. *Listeria monocytogenes* is one of six species are currently recognized within the genus (Rocourt, 1999). It can be isolated from numerous species of domestic and wild animals, as well as from soil, silage, and other environmental sources. *Listeria monocytogenes* can be classified into a number of subtypes using several methods. The most common is based upon recognition of antigens on the surface of the bacterium by specific antisera (Graves *et al*., 1999). Thirteen of these serotypes are associated with *Listeria monocytogenes* (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, 7). Some of these serotypes are also associated with other species of *Listeria* (1/2b, 4ab, 4c, 4d). The numbers and letters refer to specific combinations of bacterial antigens used for serotyping (Seeliger and Höhne, 1979). Serotyping is often used as a first step to type strains associated with human listeriosis, but it has relatively low discriminating power compared to molecular methods such as ribotyping or pulse field gel electrophoresis (PFGE). Ribotyping relies on separation and analysis of specific well-conserved DNA fragments and this method is often used in combination with serotyping to identify and trace a specific strain of *Listeria monocytogenes* associated with illness to a food source or to link seemingly unrelated illnesses. On the basis of ribotyping and PCR-restriction fragment length polymorphism of three virulence genes (hly, actA, and inlA), Wiedmann *et al*. (1997) separated *Listeria monocytogenes* into three lineages, which appear to have distinctive pathogenicities. Several reviews and books have

summarized the ecology, characteristics, presence in foods, and public health effects of *Listeria* (Farber *et al.*, 1996; Farber and Peterkin, 1991; Ryser, 1999a; Slutsker and Schuchat, 1999).

Listeriosis

Listeria monocytogenes is a well-known hazard for which there is extensive surveillance and outbreak data. Although rare when compared to many other foodborne diseases (Table II-1), listeriosis often leads to severe consequences, particularly in susceptible subpopulations. In 2000, *Listeria monocytogenes* caused higher rates of hospitalization than any other pathogen and caused over one-third of the reported deaths. Because listeriosis so often results in medical care, CDC believes that its surveillance system (FoodNet) misses only half of all cases, compared with 97% of missed cases for other pathogens (Mead *et al.*, 1999). A description of the Foodborne Diseases Active Surveillance Network (FoodNet) is provided in Appendix 4. *Listeria monocytogenes* usually causes only flu-like symptoms in healthy people. For the purposes of this risk assessment, a distinction is made between non-invasive listeriosis with mild, flu-like symptoms (referred to as listerial gastroenteritis) and invasive listeriosis that is severe and sometimes life-threatening (referred to as listeriosis in the risk assessment).

Table II-1. Incidence of Foodborne Pathogens in the United States

Pathogen	Infections (Cases per 1,000,000 population[a])
Cyclospora	0.7
Vibrio	2.1
Listeria	3.4
Yersinia	4.4
E. coli 0157:H7	21
Shigella	79
Salmonella	144
Campylobacter	157
Total Pathogens	411.6

[a] FoodNet sites include CT, MN, GA, OR, and selected counties in CA, MD, NY, TN; Total population 30.5 million. FoodNet is the Foodborne Diseases Active Surveillance Network. (CDC, 2000a)

Invasive Listeriosis

Invasive listeriosis typically has a 2 to 3 week incubation time, but can sometimes extend up to three months (Gellin and Broome, 1989). Serious conditions caused by *Listeria monocytogenes* in adults can include septicema, meningitis, enceplalitis, abortion, or stillbirth (Shelef, 1989a). Invasive diseases in nonpregenant adults can include a variety of other clinical manifestations. Endocarditis can occur in patients with underlying cardiac lesions. Cutaneous infections have been reported in persons handling animals and those exposed by accidental exposure while working in laboratories. Focal infections are rare but can include endophthalmitis, septic arthritis, osteomyelitis, pleural infection and peritonitis (Slutsker and Schuchat, 1999).

Most information on the pathogenesis of *Listeria monocytogenes* comes from studies in mice or cell biology studies using tissue culture cells (Kuhn and Goebel, 1999). When ingested, *Listeria monocytogenes* penetrates the intestinal tissue and is exposed to phagocytic cells of the immune system that function to kill microbial invaders. A portion of invading *Listeria monocytogenes* can evade the killing mechanisms, survive, and multiply within host phagocytes (macrophages). Protected within, or having escaped from these host cells, *Listeria monocytogenes* moves throughout the host via blood or lymphatic circulation to various tissues. Once in a tissue it can invade cells, multiply within them, and then use cytoskeletal acting filaments to spread to adjacent cells, without risk of exposure to humoral components of the immune system. The probability of tissue invasion depends upon the number of organisms consumed, host susceptibility, and virulence of the strain (Gellin and Broome, 1989). Most cases of listeriosis occur in fetuses or neonates and individuals with a predisposing condition that impairs the immune system (Slutsker and Schuchat, 1999).

Although *Listeria monocytogenes* is generally known to cause severe illness, there have been outbreaks in which the majority of patients only developed mild symptoms such as diarrhea, fever, headache, and myalgia (Dalton *et al.*, 1997; Salamina *et al.*, 1996; Riedo *et al.*, 1994; Aureli *et al*., 2000). The frequency of these types of outbreaks is unknown because most cases of listerial gastroenteritis are not reported to public health officials. For this reason, this risk assessment is restricted to severe cases of listeriosis.

High Risk Individuals

Two high risk (susceptible) subpopulations are considered in this risk assessment: elderly and perinatal. Persons at high risk for developing listeriosis often have deficient or immature immune systems (immunocompromised). Actual numbers of susceptible individuals are difficult to determine because these individuals belong to diverse groups including the elderly, cancer and transplant patients, and persons with immunosuppressive diseases such as AIDS (Morris and Potter, 1997). In addition, the description of an immunocompromised state is often based on qualitative or circumstantial criteria that may apply to some, but not all members of a particular group.

Susceptible subpopulations are not homogeneous with regard to susceptibility, both within and between groups. High-risk subpopulations can be separated into non-perinatal and perinatal groups. A non-pregnancy related case is a person other than a pregnant woman or her child in whom *Listeria monocytogenes* organisms are cultured from a normally sterile site. Of the non-perinatal groups, the elderly constitute the largest and most well characterized subpopulation. A case-control study revealed that of 98 cases of non-perinatal sporadic listeriosis in the United States, 98% had at least one underlying medical condition. Most (69%) of these were associated with probable immunosuppression (Schuchat *et al.,* 1992). The next largest group (33%) was associated with heart disease. Many individuals fell under more than one category. In people over the age of 60, the disease is often present with sepsis or meningitis (Schuchat *et al.*, 1991; Shelef, 1989a; Linnan *et al.*, 1988; WHO Work Group, 1988).

A perinatal infection occurs primarily as the result of transplacental transmission to the fetus following infection of the mother. The perinatal group includes fetuses or neonates from whom *Listeria monocytogenes* organisms are isolated from a normally sterile body site. Perinatal infections can occur before or after birth and outcomes include live birth of an infected neonate, stillbirth, or premature termination of pregnancy. Neonates (newborns) are defined by the American Medical Association as newborn infants from birth to one month of age. In this risk assessment, neonates are considered to be between 0-30 days of age. The term fetus is used to refer to an unborn child from 16 weeks after fertilization to birth.

Women may become infected with *Listeria monocytogenes* at any time during pregnancy, but most cases of listeriosis are reported in the third trimester (Slutsker and Schuchat, 1999). Usually three to seven days after the onset of symptoms, a woman may abort the fetus or have premature delivery (Gellin and Broome, 1989). In the first trimester, listeriosis may result in spontaneous abortion. In later stages of pregnancy, the result may be stillbirth or birth of a critically ill newborn. Listeriosis is rarely life threatening to the mother and is not known to cause increased risk in subsequent pregnancies (Skidmore, 1981; Farber and Peterkin, 1991).

Neonates may present with an early-onset or late-onset form of listeriosis. Approximately 45 to 70% of newborn cases are early-onset (Slutsker and Schuchat, 1999). Early-onset listeriosis often presents with sepsis and may progress to a syndrome known as granulomatosis infantisepticum (Gellin and Broome, 1989). This syndrome is often characterized by widely disseminated granulomas, premature birth, respiratory distress, and circulatory failure. Late-onset is defined as listeriosis in a newborn between 8 to 30 days of life. Usually late-onset neonates are born apparently healthy and at full-term. Meningitis rather than sepsis is more common in late-onset neonates (Farber, 1991a). The mothers of late-onset neonates usually have an uneventful pregnancy without illness. *Listeria monocytogenes* is rarely isolated from the mother and the source of listeriosis is often not identified in late-onset cases (Farber and Peterkin, 1991; Slutsker and Schuchat, 1999).

Non-Invasive Listeriosis (Listerial Gastroenteritis)

Gastrointestinal illness (listerial gastroenteritis) from *Listeria monocytogenes* has only recently been recognized as a distinct entity (Dalton *et al.*, 1997). Typical signs and symptoms associated with the mild form of *Listeria monocytogenes* infection are primarily those associated with gastrointestinal illness: chills, diarrhea, headache, abdominal pain and cramps, nausea, vomiting, fatigue, and myalgia. A variety of foods have been implicated as the vehicle of infection. Because symptoms are mild, there is a high potential for underreporting of listerial gastroenteritis. Data are currently unavailable through foodborne surveillance mechanisms such as FoodNet to capture the incidence of listerial gastroenteritis since routine stool cultures do not include evaluation for *Listeria monocytogenes*.

Nevertheless, outbreaks of listerial gastroenteritis have been identified. Table II-2 shows reported events where most of the cases reported mild symptoms (Heitmann *et al.*, 1997; Dalton *et al.*, 1997; Salamina *et al.*, 1996; Riedo *et al.*, 1994; Aureli *et al.*, 2000). In the vast majority of these cases, there was no evidence for invasive disease beyond the intestine. Gastrointestinal and other mild symptoms were reported in individuals with no known underlying predisposition. In two of these reports, there was evidence of very high levels of food contamination. These facts suggest that, in normal individuals, listerial gastroenteritis may be associated with exposure to high levels of *Listeria monocytogenes*. It is possible that this manifestation of *Listeria monocytogenes* infection is a different disease compared to invasive and more severe listeriosis. Because modeling in this risk assessment depends on case reporting and non-invasive gastroenteritis is not likely to be reported, listerial gastroenteritis was not considered in the risk assessment model. However, the outbreaks do provide important observations related to the exposure of populations to extremely high levels of the microorganisms without identifiable cases of invasive listeriosis.

Table II-2. Reports of Outbreaks of Listerial Gastroenteritis

Location	Year	Number of Cases	Vehicle	Reference
Northern Italy	1997	1566	Tuna/Corn Salad	Aureli *et al.*, 2000
Denmark	1996	3	Unknown	Heitmann *et al.*, 1997
United States	1994	45	Chocolate Milk	Dalton *et al.*, 1997
Northern Italy	1993	18	Rice Salad	Salamina *et al.*, 1996
United States	1989	10	Shrimp	Riedo *et al.*, 1994

Asymptomatic Carriage

The large intestine may be a reservoir for *Listeria monocytogenes* in humans. Estimates of fecal carriage in various populations of healthy adults range from <1% to 21%. It has been suggested that stress can undermine resistance in fecal carriers, and may trigger listeriosis in the carrier. Several studies have looked at fecal carriage to gain insight into listeriosis. However, it is unknown how fecal carriage relates to length of incubation or occurrence of invasive disease (Skidmore 1981; Slutsker and Schuchat, 1999; Mascola *et al.,* 1992; and Schuchat *et al.,* 1991).

Approximately 1 to 5% of normal asymptomatic carriers shed *Listeria monocytogenes* bacteria in the feces (Hof, 2001). *Listeria monocytogenes* was isolated from 2 of 100 colon biopsy

specimens from patients with colon cancer; however, neither patient exhibited signs of listeriosis (Hof, 2001).

In a retrospective study of the outbreak in 1985 that was linked to Hispanic-style fresh soft cheese, outbreak-related listeriosis patients and matched controls were asked to participate in a study of stool carriage of *Listeria monocytogenes* (Mascola *et al.*, 1992). Fecal carriage incidence was also determined for employees of the cheese plant and their household contacts. Stool specimens from 8% of those tested were positive for *Listeria monocytogenes*. The highest rate of recovery of the organism from stool samples was from employees of the cheese plant and their household contacts. It was found that the occurrence of listerial gastroenteritis or listeriosis was not associated with fecal carriage of *Listeria monocytogenes,* and was actually more common for persons with negative stool samples.

Between January 1990 and December 1991, as part of a multistate active surveillance project on sporadic listeriosis, a study was conducted to evaluate the fecal carriage of *Listeria monocytogenes* among household contacts of patients with invasive listeriosis (Schuchat *et al.*, 1993). The authors determined that the rates of carriage did not vary significantly by sex but were significantly higher in younger persons. The organism was isolated from 32% of those <30 years of age, compared to 7% from older persons. Nearly 20% of household contacts of patients with sporadic listeriosis had asymptomatic carriage of the strain associated with illness. The authors suggested that carriage of *Listeria monocytogenes* is more common in persons that have been in contact with listeriosis patients and that it was difficult to compare the fecal carriage rate in this study group to the population at large.

Epidemiological Patterns of Listeriosis: Sporadic versus Outbreak-Associated Cases

The Centers for Disease Control and Prevention (CDC) has estimated that approximately 2,500 cases of listeriosis occur annually in the United States (Mead *et al.*, 1999). The overall annual incidence of listeriosis in the United States has been estimated to range from 3.4 per million (CDC, 2000) to 4.4 per million (Tappero *et al.*, 1995). The incidence of listeriosis reported from other countries vary substantially, for example 3.5 per million persons in Bristol, England; 1.8 per million persons in England, Wales and Northern Ireland; and 6 to 7 per million persons in Denmark (Slutsker and Schuchat, 1999).

Most cases of human listeriosis occur sporadically although much of what is known about the epidemiology of the disease has been derived from outbreak-associated cases. However, it is unclear what percentage of sporadic cases may actually represent unrecognized, temporally or geographically diffuse outbreaks. Case-control studies are often used to elucidate risk factors for both outbreak-associated and sporadic cases. Investigations of outbreaks have provided much of our knowledge of the etiology of this disease organism, particularly in relation to isolation of *Listeria monocytogenes* from both the case patient and the implicated food. Investigation of sporadic cases of listeriosis often does not lead to this direct product isolate-human isolate link. Therefore, studies of sporadic cases are more likely to identify a food group, such as soft cheese, as a risk factor rather than a specific brand of soft cheese, the latter to be more likely in an outbreak investigation. Also, outbreaks of listeriosis are often associated with a processing or production failure (Slutsker and Schuchat, 1999) whereas this has been less evident among sporadic cases (Barnes *et al*., 1989).

Sporadic Listeriosis

In 1988, a microwave reheated turkey frank, consumed by an immunocompromised woman, was among the first microbiological food isolates from an RTE product associated with sporadic clinical listeriosis in the United States. Food isolates of *Listeria monocytogenes*, of the same serotype with the same electrophoretic enzyme type as the clinical isolate, were identified from both opened and unopened turkey franks from the same manufacturer (Barnes *et al*., 1989).

Likely dietary risk factors for sporadic cases of listeriosis have been identified through two case-control studies conducted by the CDC. Case-patients were identified through active surveillance conducted by CDC, and controls were selected and matched on age, geographic location, socioeconomic status, and underlying health conditions. The first case-control study of sporadic cases of listeriosis enrolled 82 patients and 239 controls from 1986 to 1987. Non-reheated frankfurters and undercooked chicken were found to have an attributable risk of 15% and 6%, respectively. These were the only foods that were statistically significantly associated with sporadic cases of listeriosis. In the subsequent and larger case-control study conducted by CDC from 1988 to 1990, 165 patients and 376 controls were enrolled in the study of sporadic listeriosis cases. This study also included a microbial assessment of patient-consumed foods.

Case-patients were significantly more likely to have consumed foods bought at a deli or to have eaten soft cheeses (Schuchat, *et al.*, 1992). Food samples were collected from 123 (75%) of patients' refrigerators and assayed for presence of *Listeria monocytogenes*. The organism was isolated from at least 1 food item in 64% of refrigerators. *Listeria monocytogenes* was found in 7.6% of ready-to-eat samples including processed meats, leftovers, cheeses, and raw vegetables. These ready-to-eat food items, as well as other food samples containing the 4b serovar of the organism, were significantly more likely to be associated with disease (Pinner *et al.*, 1992). The contamination rates, by type of food are presented in Table II-3.

The FoodNet Listeria Case-Control Study was initiated in 2000 and will be completed in 2003 (Varma, 2003). The goal of the case-control study is to further characterize established risk factors and identify other potential risk factors for *Listeria* infection. Nine FoodNet sites have enrolled cases and controls and interviewed subjects with a standardized questionnaire that explores more than 400 different dietary, behavioral, and environmental risk factors.

Table II-3. Isolation of *Listeria Monocytogenes* In Food Specimens Collected from the Refrigerators of Patients with Listeriosis

Type of Food	Number of Samples	
	Positive samples	**Total tested**
Beef	50	140
Poultry	33	108
Pork	26	95
Deli Meats	18	98
Seafood	7	57
Vegetables	72	683
Fruit	5	155
Dairy	9	533
Other[b]	6	144
Total	226	2,013

[a]Source: Pinner *et al.*, 1992.
[b]Included bread, pasta, eggs, lamb, and miscellaneous mixtures of food.

Outbreak-Associated Listeriosis

Reported outbreak-associated listeriosis cases represent a small proportion of the annual number of listeriosis cases estimated to occur in the United States (Mead *et al.*, 1999). However, data collected during outbreak investigations provide important information about both the vehicle of transmission and the mechanism by which the food contamination occurred. Published and

unpublished outbreak investigation reports for the period 1970 through 2000 were reviewed. Seventeen (32.7%) of the outbreaks occurred in the United States, with the remaining 37 outbreaks occurring outside the United States. Of the 17 domestic outbreaks, one or more contaminated food vehicles were identified in 13 (76.5%) outbreaks; in the remaining four outbreaks the source of the outbreak was not identified. In two (13.3%) outbreaks, the majority of cases were classified as having listerial gastroenteritis. Of the 37 international outbreaks, one or more vehicles were identified in 22 (59.5%) outbreaks. In all but one of the outbreaks in which no vehicle was identified, the events occurred prior to 1988. In four (10.8%) outbreaks, the majority of cases were classified as having listerial gastroenteritis.

Outbreaks in the United States. A total of 466 cases of listeriosis occurred during 12 severe listeriosis outbreaks in the United States between 1970 and 2002 (Table II-4). The mean number of cases per outbreak was 39 (median, 24.5; range 2 to 142 cases). Only two outbreaks had more than 100 associated cases, and these occurred over an extended time period. Eleven of the outbreaks involved RTE products and an outbreak of two cases involved raw eggs. Mexican-style soft cheese was the identified vehicle for the largest reported outbreak of 142 cases of which 93 (65.5%) were perinatal cases. A total of 48 perinatal and non-perinatal deaths (37.5%) were attributed to this outbreak. The second largest outbreak of 101 cases (with 21 deaths) involved two products, frankfurters and deli meats, both of which were produced by the same manufacturing establishment. During the course of the outbreak, the plant was noted to have widespread environmental disruption (with major construction being done), a known risk factor for post-kill-step recontamination of RTE products (Mead, 1999).

Among the eight outbreaks for which mortality data were available, there were 121 deaths among 466 cases (26 %) and ranged within the outbreaks from 11.1 to 44.4 %. A total of 130 (36.9%) of 352 cases occurred in a fetus or neonate (perinatal listeriosis), in nine outbreaks for which perinatal infection data were available. The serotype was reported for eight outbreaks, of which serotype 4b was responsible for seven (87.5%) outbreaks (Table II-4).

A total of four food categories were implicated in the 12 outbreaks of listeriosis listed in Table II-4. Nine outbreaks were associated with only one type of food vehicle each. A dairy product

was implicated in four outbreaks, meat was implicated in three, and one outbreak each was attributed to eggs and vegetables. The specific food vehicles included pasteurized milk, Mexican-style cheese, butter, eggs (raw), deli turkey meat, pâté, and vegetables. Considering only those outbreaks in which a single vehicle was identified, the numbers of cases by food vehicle were dairy, 309 (63.75%); meat, 103 (33.3%); vegetables, 7 (2.3%); and eggs, 2 (0.6%). More than one vehicle was implicated in three outbreaks involving a total of 157 cases. The largest outbreak involved RTE meats produced in the same processing establishment.

Table II-4. Outbreaks of Listeriosis in the United States (1970-2002) with Known Food Vehicle(s)

Year	Food Vehicle	State	Cases	Perinatal cases (% of total)	Deaths (% of total)	Serotype	Reference
1979	Raw vegetables or cheese	MA	20	0 (0)	3 (15.0)	4b	Ho, 1986
1983	Pasteurized fluid milk	MA	32	7 (21.9)	14 (43.8)	4b	Fleming, 1985
1985	Mexican-style cheese (raw milk)	CA	142	93 (65.5)	48 (33.8)	4b	Linnan, 1988
1986-1987	Ice cream, salami, brie cheese	PA	36	4 (11.1)	16 (44.4)	4b,1/2b, 1/2a	Schwartz, et al., 1989
1986-1987	Raw eggs	CA	2	Unknown	Unknown	4b	Schwartz, et al., 1988
1987	Butter	CA	11	Unknown	Unknown	Unknown	Ryser, 1999a
Not specified	Frozen vegetables	TX	7	3 (42.9)	Unknown	4b	Simpson, 1996
1998-1999	Hot dogs, deli meats	22 states	101	Unknown	21 (20.8)	4b	Mead, 1999
1999	Pâté	CT, MD, NY	11	2 (18.2)	unknown	1/2a	Carter, 2000
2000	Deli turkey meat	10 states	29	8 (27.6)	7 (24.1)	unknown	CDC, 2000b
2000-2001	Homemade Mexican-style cheese (raw milk)	NC	12	10 (83.3)	5 (41.7)	unknown	CDC, 2001
2002	Deli turkey meat, sliceable	8 North Eastern states	63	3 (4.8)	7 (11.1)	unknown	CDC, 2002b
Total			466				

Outbreaks outside the United States. A total of 1,058 listeriosis cases occurred during 18 listeriosis outbreaks outside the United States between 1970 and 2000 (Table II-5). The mean number of cases per outbreak was 59 (median, 24; range 4-355 cases). All of the reported outbreaks outside the United States in which a vehicle was identified occurred in so-called "developed" countries. Five (27.8%) outbreaks occurred in France, five (27.8%) in Oceania (Australia and New Zealand), two (11.1%) in England, and one (5.6%) each in Austria, Canada, Denmark, Finland, Sweden, and Switzerland.

Information on the number of deaths was available for 18 outbreaks. A total of 257 (24.3%) of 1,058 persons who were ill died. The number of hospitalized cases was available for five outbreaks; 91 (42.9%) of 212 cases were hospitalized. Thirteen reports contained information about the number of perinatal cases; 477 (49.1%) of 972 cases were perinatal. The serotype was reported for 15 outbreaks, of which, 9 (60.0%) were caused by serotype 4b (Table II-5).

A single food vehicle was identified in 17 outbreaks involving 1,030 cases. Dairy products were implicated in six (35.3%) outbreaks, meat products in five (29.4%) outbreaks, seafood products in four (23.5%) outbreaks, and vegetables in two (11.8%) outbreaks. The specific food items included cheese (four outbreaks), two outbreaks each for pâté, pork tongue, and smoked mussels, one outbreak each for cold-smoked trout, pasteurized cream, butter, rillettes (a RTE product made of ham cooked with fat), raw fish, cabbage, and raw vegetables. Considering only those outbreaks in which a single food vehicle was identified, the number of cases by food group were: meat, 710 (68.9%); dairy, 228 (22.1%); vegetables, 53 (5.1%); and fish, 39 (3.8%). In one outbreak in Austria in 1978, multiple food vehicles were identified during the epidemiologic investigation (unpasteurized milk, vegetables).

Examples of using outbreak information in developing dose-response curves is presented in Appendix 9 using the 1985 Mexican-style cheese outbreak and Finish butter outbreak.

Table II-5. Outbreaks of Listeriosis Outside the United States (1970-2000) with Known Food Vehicle

Year	Food Vehicle	Country	Cases	Perinatal cases (% of total)	Deaths (% of total)	Serotype	Reference
1978-1979	Vegetables (raw)	Australia	12	Unknown	0 (0)	Unknown	Le Souëf and Walters, 1981
1980	Raw seafood (finfish and mollusks)	New Zealand	22	22 (100.0)	6 (27.3)	1b	Lennon *et al.*, 1984
1981	Miscellaneous Dairy Products	England	11	Unknown	5 (45.5)	1/2a	Ryser, 1999a
1981	Vegetables (raw)	Canada	41	34 (82.9)	17 (41.5)	4b	Schlech, *et al.*, 1983
1983 - 1987	Vacherin Mont d'Or cheese	Switzer-land	122	65 (53.3)	31 (25.4)	4b	Bille, 1990; Bula *et al.*, 1995
1986	Unpasteurized milk, organic vegetables	Austria	28	24 (85.7)	5 (17.9)	Unknown	Allerberger and Guggenbichler 1989
1987-1989	Pâté and meat spreads	England	355	185 (52.1)	94 (26.5)	4b	McLaughlin *et al.,* 1991
1989 - 1990	Semi-soft Cheese (blue)	Denmark	23	Unknown	0 (0)	4b	Jensen, 1994
1990	Pâté and meat spreads	Australia	11	11 (100.0)	6 (54.5)	1/2a	Ryser, 1999a
1991	Smoked mussels	Tasmania, Australia	4	0 (0)	0 (0)	1/2a	Mitchell, 1991; Misrachi *et al.*, 1991
1992	Smoked mussels	New Zealand	4	0 (0)	0 (0)	1/2	Brett, *et al.*, 1998
1992	Pork tongue in jelly	France	280	93 (33.2)	63 (22.5)	4b	Jacquet *et al.*, 1995
1993	Rillettes	France	38	31 (81.6)	11 (28.9)	4b	Goulet, 1998
1994-1995	Smoked Seafood (finfish and mollusks)	Sweden	9	3 (33.3)	2 (22.2)	4b	Ericsson *et al.*, 1997
1995	Soft Ripened Cheese, >50% moisture (brie, camembert, feta, mozzarella)	France	33	9 (45.0)	4 (20.0)	4b	Goulet *et al.*, 1995; Jacquet *et al.*, 1995
1997	Pon l'Eveque cheese	France	14	Unknown	0 (0)	4b	Ryser, 1999a
1998-1999	Butter	Finland	25	0 (0)	6 (0)	3a	Lyytikäinen *et al.*, 2000
1999-2000	Pigs tongue in aspic	France	26	Unknown	7 (0)	Unknown	Dorozynski, 2000
Total			1058				

All outbreaks combined. Data from outbreaks from within and outside the United States were collectively summed by number of outbreaks and number of cases and each food group was ranked accordingly (Table II-6). When ranked by number of associated outbreaks, dairy

products ranked highest, followed by meat products, then seafood and finally, produce. When number of outbreak-associated cases are ranked, meat products were first and dairy products were second. Contaminated meat and dairy products were responsible for more than 90% of cases. In addition, dairy and meat products were implicated in three other outbreaks with multiple food vehicles. Serotype 4b was found in 16 (72.7%) of 22 outbreaks; 1/2a was found in four (18.0%) outbreaks (Tables II-4 and II-5).

Table II-6. A Comparative Ranking of Types of Food Vehicles by Outbreaks and Cases with Combined United States and International Outbreak Data

Type of Food Vehicle	Ranking Order by the Number of Outbreaks or Cases	
	Outbreaks	Cases
Dairy	1	2
Meat	2	1
Seafood	4	4
Produce	3	3

Dairy and RTE meat products were most often implicated in domestic and international outbreaks. The most commonly implicated dairy product was soft (fresh and mold-ripened) cheese. A variety of meat products have been involved in listeriosis outbreaks including all RTE meats, such as frankfurters, deli meat, pâté and pork tongue. These findings are similar to those from case-control studies of sporadic listeriosis, in which un-reheated frankfurters, undercooked chicken, soft cheeses and foods purchased at a deli counter were associated with listeriosis (Schwartz *et al.*, 1988; Schuchat *et al.*, 1992). "Foods purchased at a deli counter" as a food group is not specific, but a subset of case-patients identified RTE meats as the only item they had purchased at a deli counter prior to becoming ill with listeriosis. The results of this case-control study were corroborated by Pinner *et al.* (1992), who found that the foods most likely to cause listeriosis were RTE foods, foods with a high concentration of *Listeria monocytogenes*, and foods from which serotype 4b was isolated. In this analysis of outbreaks, serotype 4b was found in almost 70% of the outbreaks.

The proportion of fatal cases was similar for domestic (26%) and foreign (24%) outbreaks and agreed with other sources (Slutsker and Schuchat, 1999). A somewhat lower fatality rate has been reported (i.e., 20%) when sporadic outbreak cases were considered (Mead *et al.*, 1999).

The proportion of outbreak associated perinatal (prenatal and neonatal) cases was approximately similar (40 to 50%) between outbreaks in the United States and outside the United States. In many reports, information about the number of perinatal cases and hospitalized cases was incomplete; therefore, the proportion of perinatal cases and hospitalized cases reported are probably underestimated. For international outbreaks 42.9% of cases were reportedly hospitalized. This proportion substantially underestimates the findings reported by Mead *et al*. (1999), in which 92.2% of persons with culture confirmed listeriosis required hospitalization.

The epidemiology of listeriosis outbreaks occurring within the United States appears to be similar to outbreaks occurring outside the United States. Outbreaks appear to have disproportionately higher frequency of serotype 4b. The reported median number of cases per outbreak are 24.5 and 24, respectively; however, the means are not similar. The proportion of fatal cases (26% and 24.3%), and the food groups implicated in causing outbreaks are also similar. Therefore, it appears valid to generalize the results from international (developed countries) listeriosis outbreaks to the United States.

Outbreaks due to dairy products were most often the result of raw milk being present in a product such as soft (fresh and mold-ripened) cheese, or from post-pasteurization contamination. Dairy products were incriminated in nine outbreaks, including five due to contaminated soft (fresh and mold-ripened) cheese. Post-processing contamination of butter was blamed for an outbreak in Finland (Lyytikainen *et al*., 2000). A 1983 outbreak in Massachusetts was epidemiologically linked to pasteurized milk, suggesting that *Listeria monocytogenes* can survive the pasteurization process (Fleming *et al*., 1985); however, Ryser (1999c) has raised doubts about this conclusion, citing studies that have shown *Listeria monocytogenes* is unlikely to survive pasteurization. Schuchat and colleagues (1992) proposed that contamination of the implicated milk have occurred post-pasteurization. The source of contamination implicated in this outbreak has been frequently debated without a definitive conclusion. A Danish case-control study found unpasteurized milk to be a risk factor for sporadic listeriosis (Jensen *et al*., 1994). Additional foods associated with sporadic cases of listeriosis are discussed in earlier in this chapter in the section titled 'Sporadic Listeriosis.'

III. EXPOSURE ASSESSMENT

Exposure is a function of the quantity of a food consumed and the level of contamination in that food. While the contamination level in food at consumption is the important parameter in evaluating public health, most of the available contamination data pertain to foods sampled at retail stores. Hence, it was necessary to develop estimates of the frequency and amount of each serving of the contaminated foods likely to be consumed in the United States, as well as the *Listeria monocytogenes* levels in those foods. Limitations inherent in food consumption data and the paucity of contamination data for certain foods made certain assumptions necessary to develop the estimates. These limitations and assumptions are discussed later in this chapter.

The goal of this risk assessment was to provide information needed to focus risk management strategies among a variety of foods that could be potentially contaminated with *Listeria monocytogenes*, the purpose of the exposure assessment is to estimate the contamination and consumption of foods that have a potential for *Listeria monocytogenes* contamination. Therefore, this risk assessment modeled growth of *Listeria monocytogenes* in foods during post-retail storage and reduction of levels during home cooking or reheating of frankfurters. Growth was also modeled for some contamination data that were collected pre-retail to account for possible growth between manufacture and retail.

Foods that were included in the risk assessment were identified through a comprehensive review of the recall, microbiological and epidemiological literature. Each food was placed in one of 23 food categories. Using distributions of contamination and consumption data, estimates of exposure to *Listeria monocytogenes* in the various foods were derived. The components of the exposure assessment are provided in Figure III-1, and specific modeling details are provided in Appendix 3.

Contamination Levels At Retail (cfu/g)

- ❖ Enumeration data
- ❖ *Listeria monocytogenes* distribution in food
- ❖ % frequency data
- ❖ Adjustment for study age, region, size

- ❖ Exponential growth rate
- ❖ Storage time
- ❖ Home refrigerator temperature
- ❖ Maximum growth

Post-Retail Growth

Contamination Level at Consumption (log cfu/g)

Cooking (Frankfurters)

Serving Size

Dose at Consumption (log cfu/serving)
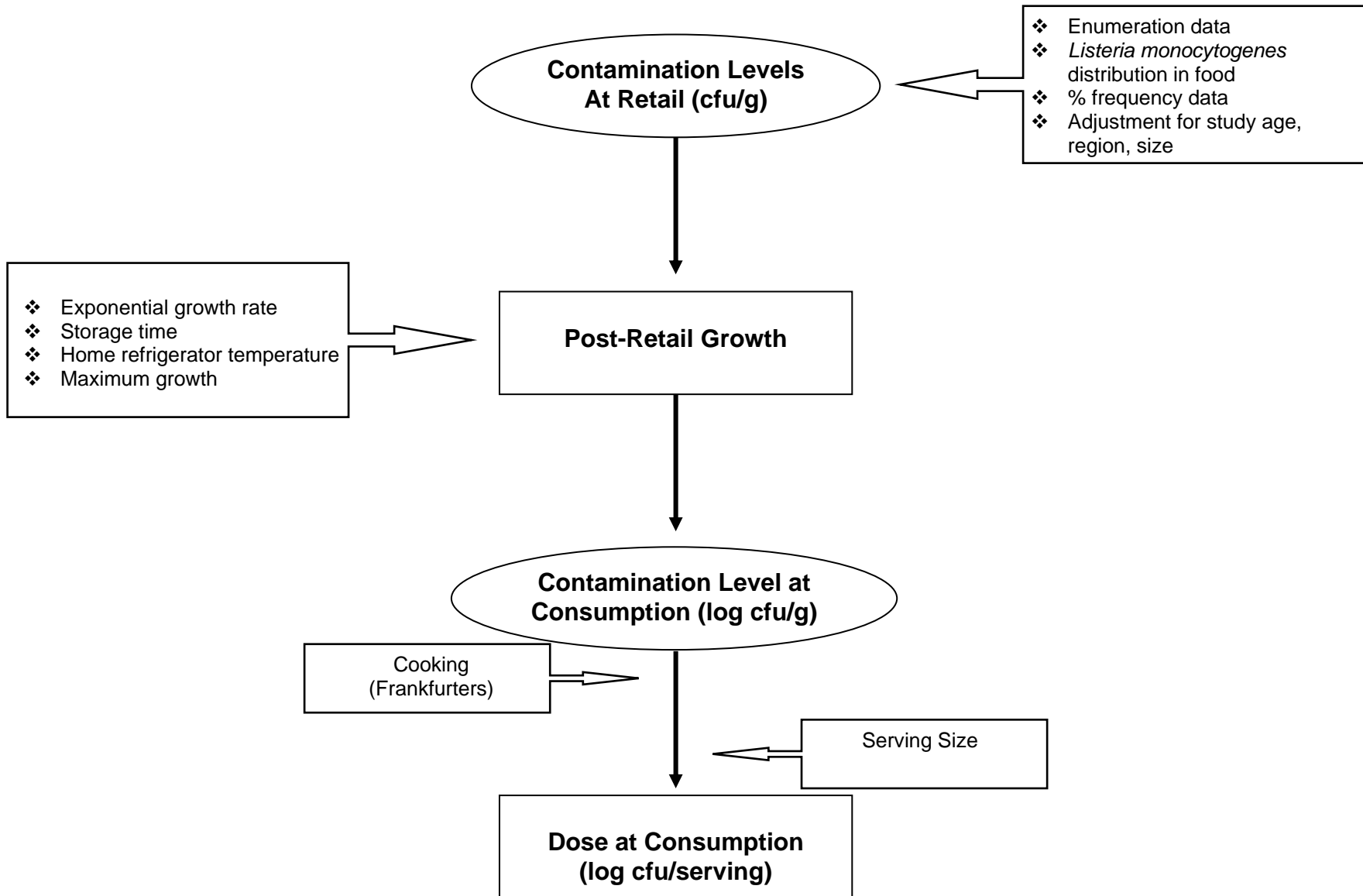
Figure III-1. Components of the Exposure Assessment Model

Food Category Identification

The first step in the exposure assessment was to consider appropriate foods to include in the risk assessment model. As the risk assessment progressed, foods and food categories were continually reevaluated and modifications were made based on new information, such as the results of growth models or new microbiological or epidemiological literature. Foods that have a significant potential for *Listeria monocytogenes* contamination were identified. They represent a subset of foods that comprise an individual's total diet. Foods that have not been linked to *Listeria monocytogenes* contamination were not included, for example, grain products (*e. g.,* bread, cookies, cakes), soft drinks, canned fruits, and cooked mixed dishes (*e. g.,* lasagna, soups). Furthermore, foods that have limited association with *Listeria monocytogenes* contamination (*e. g.,* cream-filled pastries) were not included because neither contamination level data nor appropriate data to serve as a substitute were available. It was also presumed that some foods that are cooked just prior to consumption (*e. g.,* most meats and seafoods) present a very low likelihood of containing *Listeria monocytogenes* when consumed and were not included in this risk assessment. Eggs are an example of a food category that was not included in the risk assessment, but could be a vehicle for listeriosis. Although eggs have been implicated in one outbreak with two cases (Schwartz *et al.,* 1988), *Listeria monocytogenes* has not been isolated from intact eggs and eggs products are typically cooked before consumption (Ryser and Marth, 1999).

A review of the literature was conducted to identify foods that have a significant potential for *Listeria monocytogenes* contamination. The review concentrated on the following:

- Outbreaks
- Sporadic cases, i.e. individual cases not reported as part of a documented outbreak
- Recalls and regulatory actions
- Literature related to prevalence and incidence of *Listeria monocytogenes* through analytical testing in North America (the United States and Canada)
- Literature on outbreaks, sporadic cases, and prevalence and incidence studies of *Listeria monocytogenes* in other countries

The next step in selecting foods for the risk assessment was a review of the available data on contamination and the ability of the food to support growth of *Listeria monocytogenes*. Food contamination data were compared with the available food consumption data to create food categories.

Foods that are ready-to-eat (RTE) were ultimately selected. Some RTE foods are raw and others receive some processing prior to sale. Still other RTE foods are fully cooked before sale but may be subjected to subsequent handling and storage, thereby increasing the possibility of recontamination.

The identified foods were further sorted into categories based upon food characteristics, use, and the potential for growth of *Listeria monocytogenes*. For example, Dry/Semi-dry Fermented Sausages were differentiated from other deli meats such as bologna, sliced turkey, and ham. The Cooked RTE Crustaceans food category contains peel-and-eat shrimp, steamed and boiled shrimp, and steamed crabs – foods that may be refrigerated and eaten chilled or allowed to cool after cooking, thus allowing for re-contamination and growth. The Vegetable food category includes many raw vegetables, as well as mixed vegetables such as bagged salads (without salad dressings). Similarly, the Fruits food category includes many raw and dried fruits and mixed fruits such as fruit salads (without salad dressings). In this updated risk assessment, the vegetable and fruit salads with salad dressings are included in the Deli-type Salad food category. While there is a single Deli-type Salad food category for reporting purposes, to model growth of *Listeria monocytogenes*, salads were segregated into growth and non-growth salads and considered the use of preservatives in salads made in bulk for distribution to retail stores.

In this updated risk assessment, the cheese categories have been reorganized into six categories based on moisture content. Another update to the categories included splitting the Miscellaneous Dairy Products into two categories. The Cultured Milk Products category includes the low pH dairy foods manufactured with lactic acid fermentation. Of this category, yogurt is the most frequently consumed food, followed by sour cream and buttermilk. The High Fat and Other Dairy Products category includes the remainder of the dairy products that generally support growth (including powdered products when reconstituted). Butter, cream and half and half are the most prominent foods in this category, but shakes and chocolate milk made with cocoa or syrup are also included. The frankfurter category has been divided into reheated and not

reheated frankfurters to distinguish the impact that reheating before consumption can have on the predicted risk. The number of unreheated frankfurters was represented by a triangular distribution with a minimum of 4%, most likely of 7%, and maximum of 10% of the total frankfurters consumed without reheating. These values were based on surveys conducted by USDA and American Meat Institute.

Table III-1 lists the 23 food categories that were used in this risk assessment. The food categories fall into five general groups: Seafood, Produce, Dairy, Meat, and Combination Foods. (See Appendix 5 for a detailed listing of the foods included in each food category.)

Table III-1. Food Categories Used in this Listeria monocytogenes Risk Assessment

SEAFOOD
Smoked Seafood (i.e., finfish and mollusks)
Raw Seafood (i.e., finfish and mollusks)
Preserved Fish (i.e., dried, pickled, and marinated finfish)
Cooked Ready-to-Eat Crustaceans (i.e., shrimp and crab)
PRODUCE
Vegetables (raw)
Fruits (raw and dried)
DAIRY
Fresh Soft Cheese (i.e., Queso Fresco, Queso de Creama, and Queso de Puna)
Soft Unripened Cheese, >50% moisture (i.e., cottage cheese, cream cheese, and ricotta)
Soft Ripened Cheese, >50% moisture (i.e., brie, camembert, feta, and mozzarella)
Semi-soft Cheese, 39-50% moisture (i.e., blue, brick, monterey, and muenster)
Hard Cheese, <39% moisture (i.e., cheddar, colby, and parmesan)
Processed Cheese (i.e., cheese foods, spreads, and slices)
Pasteurized Fluid Milk
Unpasteurized Fluid Milk
Ice Cream and Frozen Dairy Products
Cultured Milk Products (i.e., yogurt, sour cream and buttermilk)
High Fat and Other Dairy Products (i.e., butter, cream, other miscellaneous dairy products)
MEAT
Frankfurters (reheated)
Frankfurters (not reheated)
Dry/Semi-dry Fermented Sausages
Deli Meats (cooked, ready-to-eat)
Pâté and Meat Spreads
COMBINATION FOODS
Deli-type Salads (i.e., fruit, vegetable, meat, pasta, egg, or seafood salads with dressing)

Food Consumption Data

Data from two large-scale, nationwide food consumption surveys were used to provide estimates of exposure to *Listeria monocytogenes* via distributions of food consumption. The first survey is the Continuing Survey of Food Intakes by Individuals (CSFII 1994-96). This is the latest survey of consumers of all ages conducted by USDA's Agricultural Research Service (USDA/ARS, 1998a, 1998b). The survey consists of the following:

- Two 24-hour recalls of foods eaten during two nonconsecutive days (with the interview for the second day conducted 3 to 10 days after the interview for the first day, but not on the same day of the week).
- Sample weights for weighting the data so that they will more closely reflect consumption by the non-institutionalized United States population.
- A sample of 16,103 respondents, including:

Pregnant and/or lactating women	(n = 123)
Children under 4 years	(n = 2,284)
People 60 years and older	(n = 2,315)

- Over sampling of low income, young children, and the elderly (USDA ARS, 1998a).
- A Population Parameter of 261,897,280, appropriate for 1994-1996.

The second nationwide survey of food consumption is the Third National Health and Nutrition Examination Survey (NHANES III), which was conducted in 1988 to 1994 (US DHHS, 1998). NHANES was conducted by the National Center for Health Statistics in the Center for Disease Control and Prevention (CDC/NCHS), DHHS. The survey consists of the following:

- One 24-hour recall of foods eaten.
- Sample weights for weighting the data so that they will more closely reflect consumption by the non-institutionalized United States population.
- A sample of 30,818 respondents, including:

Pregnant and/or lactating women	(n = 399)
Children under 4 years	(n = 3,979)
People 60 years and older	(n = 3,919)

- Over sampling of young children, older persons, black persons, and Mexican Americans.

- A United States Population Parameter of 251,097,003, appropriate for 1988-1994.

Consumption data from the CSFII 94-96 survey were used for 21 of the 23 food categories. CSFII data were used preferentially because they are newer and account for up to two days of eating per respondent. Data for unpasteurized fluid milk and unreheated frankfurters were modeled based on CSFII data for pasteurized milk and all frankfurters consumed. NHANES III data were used for two food categories (Raw Seafood and Preserved Fish) for which there are fewer than 30 eating occasions (servings) in the CSFII survey.

The surveys contain consumption data for many foods and each food has an associated food code. Over 640 food codes for RTE foods were matched to one of the 23 food categories. The following information was extracted from the databases for each food category:

- Weighted descriptives (*e. g.,* mean amount eaten in grams, median amount eaten in grams, number of servings) that characterize all eating occasions in two nonconsecutive days of eating (one day for NHANES III).

- Distributions of the amount of food (in grams) eaten in all servings over two days (one day for NHANES III).

- Distributions of the amount of food (in grams) eaten in all servings, expressed as weighted percentiles.

- Weighted descriptives to describe the amount of the food (in grams) eaten per person per day, as well as the number of eaters.

- Per capita estimates of food eaten.

Several limitations of the food consumption surveys had an impact on their use for risk assessment purposes. For some foods, it was a challenge to determine consumption. Surveys listed some particular foods under several food codes, such as ham consumed alone or ham in a ham sandwich. The proportion of a particular food (such as ham) in a mixed ingredient product (such as a ham sandwich) was determined using a generic recipe provided by the survey. The gram amount of the food (ham) consumed was then calculated and added to the intake derived from other food codes for the specific food (ham). For this risk assessment, sandwiches were

broken down into individual ingredients. Specifically, for frankfurters, dry semi/dry fermented sausages, deli meats, pâté and meat spreads, and deli salads, the actual consumption of meat or deli salad product consumed alone, as well as the proportion used in sandwiches, was used. In the case of vegetable and fruit salads (in which fruits and vegetables were the major component) and deli-type salads (not included in a sandwich), however, the entire salad was used, rather than the component ingredients.

The consumption surveys do not collect information from consumers to determine whether the milk they drank was pasteurized or unpasteurized (raw). Although federal law requires milk in interstate commerce to be pasteurized, some states allow unpasteurized milk to be sold and consumed within the state. Results of a 1995 FDA/CDC survey of all 50 states, Puerto Rico, and the District of Columbia, showed that 28 states (54%) permit the sale of unpasteurized fluid milk. However, it is estimated that unpasteurized milk accounts for less than 1% of the total volume of milk sold in these states (Headrick *et al.*, 1998). Because consumption surveys did not list "drinking occasions" (servings) of unpasteurized fluid milk, the consumption of this food category was modeled by estimating it as 0.5% of the amount consumed per serving of pasteurized milk (54% x 1%). The consumption surveys did not provide any information on the storage and heating of frankfurters. Estimates for the fraction of frankfurters stored frozen before consumption and those eaten without reheating were obtained from other surveys.

Another limitation of food consumption surveys used is that some food categories have a small number of servings. Estimates based upon small sample sizes may be less statistically reliable than estimates based on larger sample sizes (USDA/ARS, 1998a). Although weighted food consumption data provide a better representation of the United States population, weighting small samples does not provide better reliability. In addition, the surveys do not provide corrections to account for underreporting and over reporting of the amount of a food eaten by consumers.

The food consumption surveys did not collect demographic information delineating consumers who are immunocompromised. Furthermore, the surveys did not measure consumption by the elderly who are living in nursing homes or other forms of assisted living outside of the home, nor did they contain a large enough sample of pregnant women to generalize consumption to all pregnant women. Thus, the available consumption data did not allow the determination of

comprehensive estimates of food consumption for each individual susceptible subpopulation. Consumption between the subpopulations was compared. Specifically, nonparametric statistical analyses were conducted to determine if there were significant differences between the distributions of the amount eaten in each serving (expressed as weighted percentiles) for the elderly and the intermediate-age population. Seventeen food categories had sufficient consumption data to permit these analyses. There were no statistically significant differences in consumption patterns for 14 of the examined 17 food categories. Thus, for the purpose of estimating the distribution of serving sizes, the food consumption data representing all eaters were used.

Note: Starting in 2002, CFSII and the dietary component of NHANES were merged into NHANES. The integrated survey will provide two 24-hour recalls of food consumption for 5,000 individuals a year and characterize "What We Eat in America."

Annual Number of Servings of Foods

In order to estimate the number of servings of the foods in each food category eaten in a year, some key data assumptions were necessary. First, it was assumed that the weighted number of servings for one (NHANES III) or two days (CSFII) of consumption of the foods in a specific food category could be extrapolated to the number of servings of those foods eaten by the population on an annual basis. Second, it was assumed that the weighted number of eaters of a food per day would represent the number of eaters of the food over 365 days. Obviously, there are some foods that individuals are more likely to eat each day (*e. g.,* vegetables, milk) and others that they eat frequently (*e. g.*, fruits, deli meats) or occasionally (*e. g.*, frankfurters, cottage cheese). Some foods are seasonal and are not available year round (*e. g.*, some fruits and vegetables), and people may not be likely to purchase more costly items (*e. g.*, shrimp, crabmeat) for regular consumption. Thus, it is important to note that when estimating the consumption of foods on an annual basis, all foods reported in food consumption surveys during a one- or two-day period are not likely to be eaten in the same frequency by the same people over an entire year. To estimate the number of annual servings for each food category, we divided the weighted number of serving consumed in two days by 2 (one-day basis) and then multiplied that value by 365 (annual basis). Table III-2 provides the annual number of servings of food consumed in the United States for each of the 23 food categories.

The annual number of servings associated with the pregnancy exposures resulting in neonatal deaths were estimated using the number of servings in the intermediate-aged group multiplied by the birth rate (1.74%) and a fractional exposure period. A triangular distribution with a minimum of 1 day, a most likely value of 7 days, and a maximum value of 30 days was used to represent the uncertainty in the exposure period. In order to estimate the number of servings in the neonatal group, the annual number of servings in the intermediate-age group was multiplied by the exposure period (triangle distribution) and divided by 365 days to estimate the number of per annum servings consumed by pregnant women. Because the perinatal exposure period is longer than neonatal (the total number of deaths includes prenatal, i.e., stillbirth, cases occurring in the last trimester), perinatal per serving death rates from listeriosis were estimated using an exposure period of 90 days (3/12 yr = 0.25) and a pregnancy rate (2.77%) rather than birth rate.

Serving Size Distributions

Empirical distributions were used to describe the serving sizes (grams of food eaten per serving) in the 23 food categories. These distributions are expressed as a series of population percentiles of the amount of food eaten per serving, weighted to reflect the consumption survey demographics. There were no uncertainties presented for these food categories because empirical distributions were used. The uncertainties associated with the serving size distributions would be relatively small, compared to other uncertainty distributions in this risk assessment for three reasons. First, even the smallest data sets used to characterize the serving size distributions are large relative to other parts of the *Listeria monocytogenes* risk model. Second, although the data may not be completely representative of the current behavior of the United States population, the data come from surveys that were explicitly designed for that purpose. Third, the variability (range) in serving sizes covers a smaller range (two logs) than many other parts of the model.

Table III-3 shows the 50th (median), 75th, 95th and 99th percentiles of the weighted distributions of serving size. For example, these percentiles for Smoked Seafood are 57, 75, 136 and 142 g/serving, respectively. This distribution indicates that half of the servings were less than 57 g and 95% of the servings were less than 136 g.

Table III-2. Estimates of the Total Number of Annual Servings of Foods Consumed in the United States by Population and Food Category

Food Category[a]	Intermediate-Age Population	Perinatal Population[b]	Elderly Population	Total Population[c]
SEAFOOD				
Smoked Seafood	1.6×10^8	1.1×10^6	4.1×10^7	2.0×10^8
Raw Seafood	1.8×10^8	1.3×10^6	5.7×10^5	1.8×10^8
Preserved Fish	8.3×10^7	5.7×10^5	2.2×10^7	1.1×10^8
Cooked Ready-to-Eat Crustaceans	4.7×10^8	3.3×10^6	8.1×10^7	5.5×10^8
PRODUCE				
Vegetables	6.8×10^{10}	4.7×10^8	1.7×10^{10}	8.5×10^{10}
Fruits	3.7×10^{10}	2.5×10^8	1.2×10^{10}	4.9×10^{10}
DAIRY				
Fresh Soft Cheese	6.9×10^7	4.8×10^5	1.3×10^6	7.1×10^7
Soft Unripened Cheese	3.4×10^9	2.3×10^7	1.0×10^9	4.4×10^9
Soft Ripened Cheese	1.7×10^9	1.2×10^7	1.8×10^8	1.9×10^9
Semi-soft Cheese	1.6×10^9	1.1×10^7	1.5×10^8	1.8×10^9
Hard Cheese	7.8×10^9	5.4×10^7	1.3×10^9	9.0×10^9
Processed Cheese	1.1×10^{10}	7.6×10^7	1.6×10^9	1.2×10^{10}
Pasteurized Fluid Milk[d]	7.2×10^{10}	5.0×10^8	1.5×10^{10}	8.7×10^{10}
Unpasteurized Fluid Milk[d]	3.6×10^8	2.5×10^6	7.5×10^7	4.4×10^8
Ice Cream and Frozen Dairy Products	1.2×10^{10}	8.2×10^7	3.1×10^9	1.5×10^{10}
Cultured Milk Products	6.1×10^9	4.2×10^7	1.2×10^9	7.2×10^9
High Fat and Other Dairy Products	1.6×10^{10}	1.1×10^8	4.3×10^9	2.1×10^{10}
MEAT				
Frankfurters, reheated[e]	5.5×10^9	3.8×10^7	5.8×10^8	6.1×10^9
Frankfurters, not reheated[e]	4.2×10^8	2.9×10^6	4.4×10^7	4.7×10^8
Dry/Semi-dry Fermented Sausages	1.5×10^9	1.1×10^7	2.5×10^8	1.8×10^9
Deli Meats	1.8×10^{10}	1.2×10^8	2.8×10^9	2.1×10^{10}
Pâté and Meat Spreads	9.7×10^7	6.7×10^5	2.1×10^7	1.2×10^8
COMBINATION FOODS				
Deli-type Salads	1.0×10^{10}	7.0×10^7	3.1×10^9	1.3×10^{10}

[a] Serving size data based on CSFII 94-96 extrapolated from two days of eating to an annual basis, except data for Raw Seafood and Preserved Fish from NHANES III were extrapolated from one day of eating. Servings denote variable amounts consumed and not a standard serving size that represents the amount customarily consumed per eating occasion.

[b] For the purposes of estimating rates of listeriosis per serving, the values for the perinatal group were calculated by adjusting the number of annual servings for the intermediate-aged group for the annual pregnancy rate: The annual pregnancy rate (2.77%) was multiplied by the number of servings for the intermediate-aged population and 0.25 (0.25 = 3/12, to estimate the number of pregnant women in the last 3 months of pregnancy).

[c] The annual number of servings for the total population was calculated by summing the values for the elderly and intermediate-aged populations. The perinatal group was not included because the servings for this population are a subset of the intermediate-aged group.

[d] Consumption of Pasteurized Fluid Milk is based on 99.5% of total milk consumption and consumption of Unpasteurized Fluid Milk is based on 0.5% of total fluid milk consumption.

[e] Consumption of not reheated frankfurters is a distribution based on an uncertainty range of 4 to 10% of the consumption of frankfurters. The value in the table is the mean of the distribution. The value for reheated frankfurters is the difference between the total frankfurters consumption and the value for not reheated frankfurters.

Table III-3. Percentiles of Serving Size Distributions for Each Food Category

Food Categories	Weighted Percentiles (grams per serving)[a]			
	50th	75th	95th	99th
Seafood				
Smoked Seafood	57	75	136	142
Raw Seafood	16	28	77	136
Preserved Fish	70	125	130	250
Cooked Ready-to-Eat Crustaceans	50	96	256	345
Produce				
Vegetables	28	55	123	220
Fruits	118	138	272	570
Dairy				
Fresh Soft Cheese	31	85	246	246
Soft Unripened Cheese	29	105	226	420
Soft Ripened Cheese	28	48	85	168
Semi-soft cheese	28	57	142	227
Hard Cheese	28	38	85	122
Processed Cheese	21	42	84	130
Pasteurized Fluid Milk	244	245	488	732
Unpasteurized Fluid Milk	244	245	488	732
Ice Cream and Frozen Dairy Products	132	186	330	454
Cultured Milk Products	114	227	245	490
High Fat and Other Dairy Products	13	30	312	510
Meats				
Frankfurters (reheated and not reheated)	57	114	171	285
Dry/Semi-dry Fermented Sausages	46	69	161	161
Deli Meats	56	75	113	196
Pâté and Meat Spreads	57	85	128	454
COMBINATION FOODS				
Deli-type Salads	97	177	301	464

[a] There are no uncertainties presented for these food categories because empirical distributions were used.

Note: Serving size denotes variable amount consumed and are not a standard serving size that represents the amount customarily consumed per eating occasion.

Food Contamination Data

Over the last fifteen years, numerous studies have been published that report on foods contaminated with *Listeria monocytogenes* in a variety of countries and locations. Contamination data included in this risk assessment were reported from the United States and other countries on six continents. Most of the studies were from the industrialized countries of North America and Western Europe. Many studies did not identify the sampling of imported foods or indicate whether imports were excluded from the study. Contaminant serotype information was not considered because the food contamination studies did not usually identify the serotypes.

Data sources included the published scientific literature, published and unpublished official government documents, and data obtained from the private sector. All data and references are available in the docket established for this risk assessment. Two types of data describing the levels of *Listeria monocytogenes* contamination in food were identified.

- Presence/absence (qualitative) data (i.e., the number of positive samples relative to the total sample collection).
- Enumeration (quantitative) data (i.e., the number of colony forming units (cfu) of *Listeria monocytogenes* that were measured from a sample). It is conventionally assumed that one cfu is equivalent to one organism.

Both qualitative and quantitative studies were used in the assessment (Table III-4; Appendix 7). Data from presence/absence studies (qualitative data) were converted to numerical data and included in the model by assigning the lowest possible contamination level that can be detected by the laboratory method. For a method that uses a 25-g sample, the lowest detectable level is 0.04 cfu/g of food. Consequently, the qualitative data could be used along with the quantitative data in the construction of the cumulative distribution curves of *Listeria monocytogenes* levels in food.

Because each food category usually includes many related types of foods, data were collected to represent all the foods in a designated food category. For example, the deli meats include, in part, ham, bologna, and sliced chicken. These deli meats have diverse microbial characteristics and there are relatively few existing studies for each of these foods. Hence, all data available on these products

were used with the assumption that the summation of the collected data represented the diverse compositional, geographic, seasonal, home vs. away-from-home, relative frequency of consumption, and other factors that affect the exposure from *Listeria monocytogenes* in these foods. Where methodologies or designations varied among multiple data sources, the original data were often regrouped or recalculated (particularly for the growth modeling work).

Table III-4. *Listeria monocytogenes* Contamination: Numbers of Qualitative and Quantitative Studies and Samples

Food Category	Number of Studies[a]				Number of Samples[b]	Percent of Positive Samples[c]
	Total	United States	Total Quant-itative	United States Quant-itative		
SEAFOOD						
Smoked Seafood	30	6	10	2	7,855	12.9
Raw Seafood	46	11	4	1	15,650	7.0
Preserved Fish	18	1	5	0	1,495	9.8
Cooked Ready-to-Eat Crustaceans	11	4	3	2	4,004	2.8
PRODUCE						
Vegetables	32	5	8	1	9,223	3.6
Fruits	4	2	0	0	254	11.8
DAIRY						
Fresh Soft Cheese	8	3	1	1	4,866	1.4
Soft Unripened Cheese	8	2	3	0	814	3.9
Soft Ripened Cheese	17	3	5	1	3,109	3.8
Semi-soft Cheese	11	3	3	1	2,615	3.1
Hard Cheese	12	2	2	0	973	1.4
Processed Cheese	4	1	1	0	325	0.9
Pasteurized Fluid Milk	30	3	3	1	12,407	0.4
Unpasteurized Fluid Milk	45	10	3	0	19,080	4.1
Ice Cream and Frozen Dairy Products	22	5	2	0	170,787	0.2
Cultured Milk Products	6	1	1	0	490	0.8
High Fat and Other Dairy Products	12	4	2	0	18,169	1.3
MEAT						
Frankfurters	9	6	2	2	3,763	4.8
Dry/Semi-dry Fermented Sausages	14	3	3	0	3,357	6.4
Deli Meats	19	4	3	1	33,824	1.9
Pâté and Meat Spreads	12	3	7	0	5,665	6.5
COMBINATION FOODS						
Deli-type Salads	16	6	5	1	17,915	3.8

[a] See Appendix 5 for the reference citation for each study.
[b] Total number of samples equals qualitative plus quantitative samples for each category.
[c] The percent of positive samples was calculated using the total positive samples in a food category. The value in the table is an unweighted percentage (i.e., does not reflect the weighting done to represent study reliability for predicting current *Listeria monocytogenes* levels in the United States).

Pairing consumption data with the appropriate contamination data was often imperfect. Dietary intake data were highly specific as to the type of food consumed (*e. g.*, smoked mussels). In contrast, the contamination data reported in the literature were often more generic (*e. g.*, samples may only be described as shellfish).

The analytical methods used in the food contamination studies to determine the presence of *Listeria monocytogenes* were generally well known and were approximately equal in sensitivity at about 1 cfu per 25 g sample (0.04 cfu/g). However, for enumeration methods of analysis, the sample size was usually less than 25 g and was not as sensitive (typically 20 to 50 cfu/g). Typically, the samples obtained for analysis were from non-composited samples of food. An exception, however, was unpasteurized fluid milk obtained from bulk tanks.

Contamination levels at consumption were modeled with the assumption that contamination distributions for a given food in the United States do not vary significantly from those in other countries, especially Western Europe and other developed countries. Similarly, it was assumed that all foods within a category have a similar pattern of contamination. Furthermore, all *Listeria monocytogenes* food isolates were accepted as having the potential to cause human illness. No differences in ability to grow or other characteristics between food and clinical isolates were assumed. As will be discussed later, the impact of these assumptions was considered in the uncertainty associated with relative risk determinations.

The available data on *Listeria monocytogenes* levels had some limitations that affected the distributions for levels of *Listeria monocytogenes* in foods. First, there are relatively few data points above the limit of detection (0.04 cfu/g). This is because the occurrence of detectable levels of *Listeria monocytogenes* in food is rare and because most surveys of the occurrence of *Listeria monocytogenes* in food did not quantify the levels in positive samples. Second, some of the data are not from the United States and this data may not always be representative of food and processing procedures in the United States. To create an estimate of the current United States distribution, the data sets were weighted by the number of samples in the data set, likelihood of the food in that country to be imported to the United States food supply, and the recency of the data. Third, there was a wide degree of variation between studies in the

occurrence of high levels of *Listeria monocytogenes*. The extent to which this variation reflects true variation in a particular food, is not known.

Many of the studies found in the published literature were conducted in the late 1980s and early 1990s. The extent that improved sanitation and other control measures implemented by the food industry have reduced the frequency and level of contamination since 1993 (when the earlier research was conducted) is difficult to determine from published literature. It was felt that some allowance should be made for the age of data and therefore, all data were used but the more recent data were given greater weight (details below). Because some food categories had little data, which would result in a biased estimate, the overall trend in contamination for all the food categories from before 1993 to after 1998 was obtained and applied to these data sets.

The length of time a food was held at retail before it was obtained for microbial sampling was not recorded in the survey studies. It was therefore necessary to assume that foods were sampled without bias and would represent the entire range of post-production and pre-sale conditions for that food.

Growth Data

Growth of *Listeria monocytogenes* in food is a function of the storage time, storage condition, and rate of growth in specific foods. The storage times were multiplied by the rate of growth to provide an estimate of the amount of *Listeria monocytogenes* growth occurring between retail purchase of the food and its consumption. The model includes consideration of the interaction of storage time and temperature and maximum growth that specific foods support.

Storage time

Some foods are consumed on the day of purchase whereas others remain in the home refrigerator for lengthy periods of time. This is a major source of variability in the estimate of growth and ultimately, in the numbers of *Listeria monocytogenes* consumed. Except for frankfurters and deli meats, no data were found on the storage of foods in the home; therefore, storage time, including variation and uncertainty, were estimated based on the expert judgment of the risk assessment team in consideration of recommendations developed by the Food Marketing

Institute (2002) and other individuals familiar with the production and use of the various foods. It is recognized that foods may be kept beyond the recommended storage times. This risk assessment modeled estimated consumer food practices, not necessarily the recommended practices. The values were developed by consensus of the risk assessment team and vetted by government subject matter experts and other scientific reviewers including those who submitted comments following the release of the draft risk assessment. The minimum, most likely and maximum storage times used to develop the distribution of storage times for the food categories are presented in Table III-5. These are skewed distributions with relatively few servings at the maximum storage time. For Smoked Seafood, as an example, over 90% of the servings are stored for less than 13 days.

Table III-5. Variation in Post-Retail Storage Times Assigned to the Food Categories

Food Categories	Storage time (days)[a]		
	Minimum	**Most Likely**	**Maximum**
SEAFOOD			
Smoked Seafood	0.5	3 to 5	15 to 30
Raw Seafood	0.5	1 to 2	10 to 20
Preserved Fish		[Not Applicable][b]	
Cooked Ready-to-Eat Crustaceans	0.5	1 to 2	10 to 20
PRODUCE			
Vegetables	0.5	3 to 4	8 to 12
Fruits	0.5	3 to 4	8 to 12
DAIRY			
Fresh Soft Cheese	0.5	1 to 5	15 to 30
Soft Unripened Cheese	0.5	6 to 10	15 to 45
Soft Ripened Cheese	0.5	6 to 10	15 to 45
Semi-Soft Cheese	0.5	6 to 10	15 to 45
Hard Cheese	0.5	6 to 10	90 to 180
Processed Cheese	0.5	6 to 10	45 to 90
Pasteurized Fluid Milk	0.5	3 to 5	10 to 15
Unpasteurized Fluid Milk	0.5	2 to 3	7 to 10
Ice Cream and Frozen Dairy Products		[Not Applicable][b]	
Cultured Milk Products	0.5	6 to 10	15 to 45
High Fat and Other Dairy Products	0.5	6 to 10	15 to 45
MEATS			
Frankfurters		[Not applicable][c]	
Dry/Semi-Dry Fermented Sausages	0.5	6 to 10	45 to 90
Deli Meats		[Not applicable][c]	
Pâté and Meat Spreads	0.5	6 to 10	15 to 45
COMBINATION FOODS			
Deli-type Salads	0.5	3 to 4	8 to 12

[a]For the food categories a BertPert distribution with these minimum, most likely and maximum parameters were used.
[b] Not applicable because this is a food category that does not support growth.
[c] Emperical data was used (see Table III-6).

Estimating duration of post-retail storage for Frankfurters and Deli Meats

Preliminary data from a study being conducted for FSIS by Georgetown University (Wachsmuth, 2000) provided information for frankfurters and deli meats used in the draft risk assessment. For frankfurters, 3 of 73 respondents gave 21 days storage and 3 gave 30 days as the maximum time. For deli meats, 2 of 81 respondents gave 21 days of storage, and 2 gave 30 days as the maximum time. FSIS also questioned people who called in to their telephone Meat and Poultry Hot Line about their frankfurter storage and cooking or reheating practices. Of 136 callers, one had kept frankfurters 90 days and one for 180 days (Wachsmuth, 2000).

In response to the need for more comprehensive information on consumer practices for frankfurters and deli meats, the American Meat Institute (AMI) commissioned a consumer survey that asked how long, on average, deli meats and frankfurters were stored before consumption (American Meat Institute, 2001). The responses are shown in Table III-6. These data were used to model storage times for frankfurters and deli meats as described in section "Modeling: Growth Between Retail and Consumption."

Table III-6. Refrigerated Storage Times for Frankfurters and Deli Meats in the Home

Average Storage Time	Distribution (Fraction) of Responders[a]	
	Pre-packaged deli meats and frankfurters	Custom sliced deli meats
1 to 3 days	0.32	0.39
4 to 7 days	0.37	0.36
8 to 10 days	0.06	0.03
11 to 14 days	0.04	0.01
15 to 21 days	0.01	0
22 to 30 days	0.01	0
31 to 60 days	0.01	0
61 days or more	0	0
Always freeze	0.03	0.01
Don't eat	0.13	0.17
Don't know/refused	0.02	0.02

[a]Source: American Meat Institute, 2001

Refrigeration Storage temperature

Data for home refrigerator temperatures were obtained from a 1999 survey conducted by Audits International (Audits International, 1999). Nine hundred thirty nine refrigerators in the United

States were included in the survey. Approximately 26% of the refrigerators exceeded 41°F (5°C) and 1.4% exceeded 50°F (10°C) (Table III-7). The refrigeration temperatures were modeled with a discrete distribution where temperature values were randomly sampled from the data provided by Audits International.

Table III-7. Frequency Distribution of Home Refrigerator Temperatures

Refrigerator Temperature (°F)	Frequency (%)
< 32	9
33 - 35	10
36 - 38	25
39 - 41	29
42 - 44	18
45 - 47	5
48 - 50	3
51 - 53	0.4
54 - 56	0.5
57 - 59	0.4
60 - 63	0.1

Total number of refrigerators in survey = 939 (Audits International, 1999)

Growth Rate

A summary of the growth rate data is presented in Table III-8 and a complete list of the literature data can be found in Appendix 8. Significant differences in composition and processes are present within many of the food categories. Within the Smoked Seafood food category, for example, there were hot and cold smoked fish, various salt levels, both aerobic and vacuum packaging, and different fish species. The modeling process used a cumulative table of the actual data points, not the means and standard deviations presented in Table III-8.

Table III-8. Mean Exponential *Listeria monocytogenes* Growth Rates and Total Number of Samples From Growth Rate Studies for Each Food Category

Food Categories	Growth Rate at 5 °C		Number of Samples[b]
	Mean (log$_{10}$ cfu/g per day)[a]	Standard Deviation	
SEAFOOD			
Smoked Seafood	0.150	0.096	27
Raw Seafood	0.152	0.126	5
Preserved Fish	**No Growth**		
Cooked Ready-to-Eat Crustaceans	0.384	0.110	3
PRODUCE			
Vegetables	0.072	0.114	26
Fruits	0.046	0.047	5
DAIRY			
Fresh Soft Cheese	0.082	0.138	10
Soft Unripened Cheese	0.090	0.286	29
Soft Ripened Cheese	- 0.013[a]	0.133	17
Semi-soft cheese	- 0.043[a]	0.032	10
Hard Cheese	- 0.053[a]	0.065	11
Processed Cheese	- 0.045[a]	0.055	6
Pasteurized Fluid Milk[c]	0.257[c]	0.105	11
Unpasteurized Fluid Milk[c]	0.257[c]	0.105	11
Ice Cream and Frozen Dairy Products	**No Growth**		
Cultured Milk Products	- 0.168[a]	0.142	5
High Fat and Other Dairy Products	0.114	0.118	6
MEATS			
Frankfurters	0.131	0.051	5
Dry/Semi-dry Fermented Sausage	- 0.016[a]	0.016	4
Deli Meats	0.282	0.196	23
Pâté and Meat Spreads	0.252	0.154	2
COMBINATION FOODS			
Deli-type Salads (growth)	0.122	0.030	2
Deli-type Salads (non-growth)	-0.143	0.134	19

[a] Negative values indicate a decline in population for the mean growth rate.
[b] See Appendix 8 for more details about the studies.
[c] Pasteurized and unpasteurized milk were combined for analysis of exponential growth rate of fluid milk.

Modeling: *Listeria monocytogenes* Levels in Food at Retail

The majority of the data collected on the contamination of foods only determined whether or not a sample, typically 25 g, contains *Listeria monocytogenes*. Compared to the amount of qualitative data on the presence or absence of *Listeria monocytogenes* in foods, there is relatively little recent quantitative data available. This is due to the additional laboratory effort necessary

to enumerate samples, the low frequency of detecting positive samples, the need to test a large number of samples, and regulatory requirements that do not require enumerative data. Therefore, the approach taken was to develop a generic contamination model to describe the distribution of *Listeria monocytogenes* in food.

A three-step process was used to model levels of *Listeria monocytogenes* in food at retail.

> Step 1: Characterize the distribution of *Listeria monocytogenes* across food categories using the contamination data reported in selected quantitative data sets (i.e., create a generic distribution).
> Step 2: Characterize the uncertainty distribution for the frequency of detectable contamination for each food category using prevalence data adjusted to account for study size, age, and country of origin.
> Step 3: Integrate the quantitative data from generic distributions (step 1) with the adjusted prevalence data, specific for each food category (step 2).

The general approach was to assume that the contaminated samples are detectable contaminations arising from a continuous log normal distribution of contamination. The minimum detectable level from presence/absence tests is typically 1 organism in 25 g or 0.04 organisms per gram. A low percentage of samples has contamination at or above this level and the remainder has non-detectable levels (i.e., <0.04 organisms/g). There may be no detectable *Listeria monocytogenes* in a specific sample (a 25.0 g package), but if 1000 packages from that lot are analyzed *Listeria monocytogenes* might be found. The average contamination could be one organism in 1000 packages (or a level of 0.00004 organisms per gram), far below the detectable level of 0.04 organisms/g. Therefore, what is observable with the presence/absence and quantitative tests is only the upper tail of the distribution. As shown in Figure III-2, the model fits a curve to the log cfu/g data and the mean and standard deviation are calculated. This curve represents a food category with approximately 10% of the samples positive for *Listeria monocytogenes*. It also shows that 3.1% of the samples have more than 100 cfu/g.
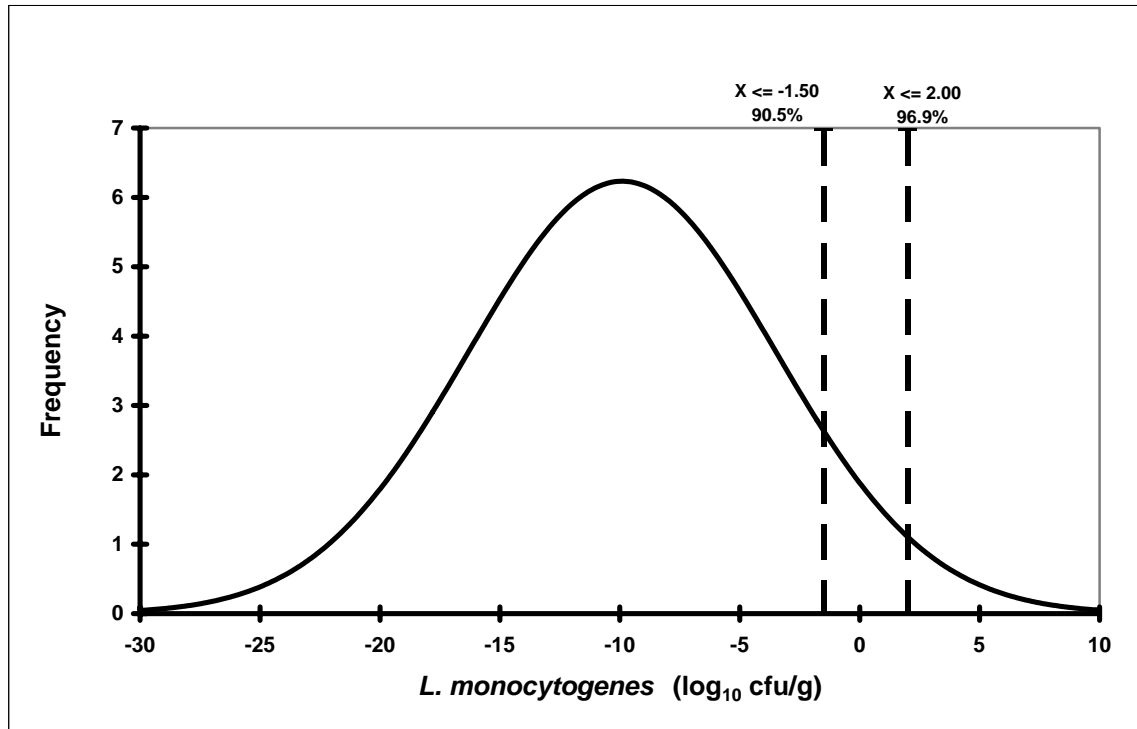
Figure III-2. Example of the Contamination Curve for a Typical Food Category Showing Frequencies of Detectable and Nondetectable Samples

Studies with enumerated samples were selected and fitted to a normal distribution. The standard deviations from each of these studies were used to estimate the uncertainty in the distribution. The presence/absence data for each food category were then used to create a frequency distribution of contamination at the 0.04 cfu/g level. A normal curve with the appropriate standard deviation was then fit to the presence/absence data by "sliding" the mean until the percentage of positive samples corresponded to the presence/absence data. A normal curve for the log cfu/g was chosen because studies enumerating spoilage flora that are at sufficiently high levels to observe the curve showed that this distribution was appropriate (Kilsby and Pugh, 1981; Gill *et al.*, 1996).

Step 1: Characterize the distribution of *Listeria monocytogenes* across food categories

Seventeen studies were selected for quantitative analysis (Table III-9). All of these studies had at least ten samples with enumerated values. The levels of *Listeria monocytogenes* in the

samples were transformed to log scale and the data for each study were fit using a normal distribution (Figure III-3). The mean level of *Listeria monocytogenes* (log cfu/g) and the standard deviation of the contamination data sets were calculated. This process was repeated for the 17 studies with adequate enumeration data.
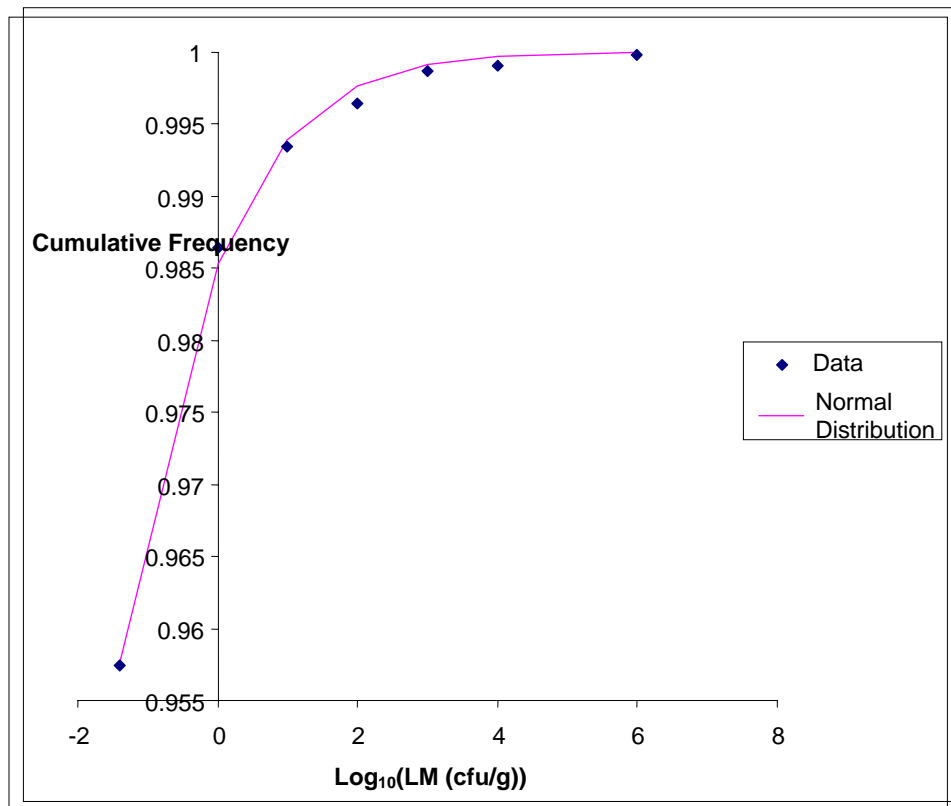
Figure III-3. A Lognormal Distribution for *Listeria monocytogenes* in Smoked Seafood

The standard deviations and mean levels of *Listeria monocytogenes* (log cfu/g) are summarized in Table III-9**.** The standard deviations of the distribution for each study ranged from 1.1 to 10.7 although most were less than 5.0.

Table III-9. Selected Studies Used to Characterize the Distribution of *Listeria monocytogenes* in Food at Retail

Study Reference[a]	Food Category	Number of Samples		Calculated Mean Level LM[a] (log cfu/g)	Estimated Standard Deviation[b]
		Total Tested	Total Positive		
Rawles, 1995	Cooked RTE Crustaceans	126	10	-9.9	6.4
NFPA, 2002	Deli Meat (CA)	4600	28	-12.2	4.3
NFPA, 2002	Deli Meat (MD)	4599	54	-7.7	2.8
WNYJWG, 1991	Deli-type Salad	149	21	-12.5	10.7
NFPA, 2002	Deli-type Salad (CA)	5504	126	-4.2	1.4
NFPA, 2002	Deli-type Salad (MD)	5606	191	-4.3	1.6
Hayes, *et al*., 1992	Frankfurter	40	12	-1.9	1.1
Morris and Ribeiro, 1991	Pâté	73	37	-1.2	4.0
Morris and Ribeiro, 1992	Pâté	216	75	-2.9	3.9
Jørgensen and Huss, 1998	Preserved Fish	91	23	-4.6	5.3
NFPA, 2002	Semi-soft Cheese	1623	23	-5.6	1.9
Cortesi, *et al*., 1997	Smoked Seafood	165	32	-4.4	3.5
Jørgensen and Huss, 1998	Smoked Seafood	420	163	-2.1	2.8
Dominguez *et al*., 2001	Smoked Seafood	170	38	-4.8	4.6
NFPA, 2002	Smoked Seafood	2687	114	-6.7	3.1
Loncarevic *et al*., 1995	Soft Ripened Cheese	31	13	-2.0	3.9
NFPA, 2002	Vegetables	2963	22	-8.9	3.1

[a]NFPA = National Food Processors Association; WNYJWG = West and North Yorkshire Joint Working Group; LM = *Listeria monocytogenes*.
[b]Standard Deviation of the log data.

These standard deviations were used to characterize the variation and uncertainty of the distribution of *Listeria monocytogenes* concentration in the food categories. The ranges of standard deviations used are given in Table III-10. A default range of 2 to 5 standard deviations was used for all food categories unless additional information was available to refine the uncertainty. Refined standard deviation ranges were used for four food categories (smoked seafood, pâté and meat spreads, deli meats, and deli-type salads) based on information as described in Table III-10. For example, the range of standard deviations assigned to Smoked

Seafood is narrower than the default range based on consideration of the standard deviations from four enumeration studies for this food category.

Table III-10. Standard Deviation Ranges for Each Food Category

Food Category	Standard Deviation Range	Comment
Default	2 to 5	This range was used as a default for all food categories (except Smoked Seafood, Pâté and Meat Spreads, Deli Meats, and Deli-type Salads) for which there was little or no empirical basis for estimating a distribution.
Smoked Seafood	2.8 to 4.6	This range encompasses the range for the four enumeration studies of smoked seafood samples.
Pâté and Meat Spreads	3.8 to 4.8	The standard deviation values for these products fit in a relatively narrow range and were generally higher than for other food categories.
Deli Meats	3.8 to 4.8	The standard deviation values for these meat products fit in a relatively narrow range and were generally higher than for other food categories
Deli-type Salads	1.5 to 2.5	The standard deviations for Deli Salads from the 2002 NFPA study were low (1.4; 1.6) in samples collected from both California and Maryland. A much higher value (10.7) was indicated by West Yorkshire study conducted 20 years ago in the U.K. Since the latter study is probably less representative of the current United States food supply, it was acknowledged by slightly raising the maximum range indicated by the NFPA study.

Step 2: Characterize the Uncertainty Distribution

The set of presence/absence studies for each food category was used to generate a discrete uncertainty distribution (a histogram) for the frequency of detectable contamination. First, the presence/absence data were used to generate a single estimate of the fraction of positive samples (i.e., a rate-concentration estimate) for each study. The concentration level was equal to the detection limit of the analysis (typically 0.04 cfu/g; based on 1 organism per 25 g sample). Next, the individual studies were adjusted (weighted) to account for sample size, geographic region of food origin, and date of collection. In addition, some data sets were obtained by sampling at the manufacturer instead of at retail. These data sets were adjusted to allow for growth between

manufacture and retail. With this adjustment the data collected at manufacture would then have the same percentage of positive samples but they were assigned higher cfu/g values.

Adjust for sample size, geographic location, and study date

The relevance of a particular contamination data set to represent current United States retail foods for the purposes of this risk assessment was a difficult judgment. If abundant, quantitative, recent and United States data were available, only this data would be used in the risk assessment. However, for most food categories these data were not available. Therefore, all data sources were used and weights were assigned to each data set so that the more relevant sets were given greater importance in this risk assessment. These weights were obtained from a panel comprised of government subject matter experts (Carrington and Dennis, 2001).

The individual studies were weighted by sample size, geographic region, and study date as follows in Equation 1.

$$\text{Study Weight} = n * gw * dw \qquad\qquad \text{Equation [1]}$$

Where:
   n is the total number of samples in the study. A larger study would provide a better estimate of the percentage of positive samples than a small study.
   gw is the geographic weight. A value of 1 was used unless the study was conducted in a region and food category for which there is little or no contribution (importation) to the United States food supply, in which case a value of 0.3 was used.
   dw is the weight for the date of the study. Evidence exists that improved sanitation and HACCP programs have reduced the contamination of foods since the recognition of the public health problem from *Listeria monocytogenes* in the 1980's. A value of 1 was used for studies published within the past three years, a value of 0.7 was used for studies published between 1993 and 1999, while a value of 0.4 was used for studies published before 1993.

The width of the probability interval assigned to each study was proportional its relative weight as shown in Equation 2.

$$\text{Study Probability} = \text{Study Weight} / \text{Total Weight} \qquad\qquad \text{Equation [2]}$$

where Total Weight is the sum of all the Study Weights for the food category.

Adjustment of older data for food categories without large recent studies

About half of the food categories had large studies that were conducted within the past three years. As a result of the weighting scheme used to weight the studies, these recent studies usually received at least half the probability interval, dominating the analysis. Ten food categories had only older studies and those studies tended to have higher prevalence rates. The higher prevalence ranges may result from higher actual contamination levels or non-representative sampling. In either case, the data may tend to overestimate current *Listeria monocytogenes* concentrations, thereby biasing these categories compared to categories with recent data. To represent the uncertainty of this bias, the impact of large new studies on prevalence of *Listeria monocytogenes* was evaluated (Table III-11). Ratios were calculated by dividing the weighted pooled prevalence of 1999 and earlier data (percentage positive samples) by the weighted pooled prevalence of data for all years. A ratio less than 1 indicates that the prevalence of contaminated samples is currently higher than in the past. The reduction ratio values were used to adjust the food categories for which recent, large studies were not available. Specifically, the set of values in Table III-11 were used as an uncertainty distribution to reduce the number of positive values from older studies in categories without newer data. The food categories adjusted with the ratios to account for the lack of newer data include: Preserved Fish, Cooked RTE Crustaceans, Fruits, Hard Cheese, Processed Cheese, and Cultured Milk Products.

Table III-11. Prevalence Reduction Ratios for *Listeria monocytogenes* Using Study Age

Food Category	Prevalence Reduction Ratio[a]
High Fat and Other Dairy Products	0.9
Raw Seafood	1.0
Fluid Milk, Unpasteurized	1.0
Soft Ripened Cheese	1.8
Semi-soft Cheese	1.8
Vegetables	2.1
Deli-type Salads	2.3
Fluid Milk, pasteurized	2.6
Deli Meats	3.4
Fresh Soft Cheese	8.7
Frankfurters	9.7
Ice Cream and Frozen Dairy Products	31.3

[a]Prevalence reduction ratio = percentage of positive samples from data collected prior to 1999 divided by the total data set for each food category.

Adjustment for growth between production and retail for samples taken at manufacturing/ production

Some studies collected samples at manufacturing/ production prior to the point of retail (see Appendix 7). Since growth can be anticipated between production and purchase, the prevalence of positive samples for those data sets from sampling at manufacture were adjusted with estimates derived from the growth models (see section, "Modeling: Exponential Growth Rates").

The temperature ranges and storage times for the food categories are presented in Table III-12. These values were estimated as likely to be encountered between manufacture and retail. Because the distributions are narrow, rectangular distributions were used for storage time and for the temperature range. The median value from the growth models were used to adjust the contamination level but not the frequency of the presence/absence data. If, for example, the estimated growth was 0.5 logs prior to retail, a study with 5% positive at 0.04 cfu/g (-1.394 log) at manufacture would become 5% positive at 0.13 cfu/g (-0.884 log) at retail [0.5 log + -1.394 log = -0.894 log]. The contamination level was therefore increased from 0.04 cfu/g to 0.13 cfu/g to account for the possible growth of *Listeria monocytogenes* in food between production and retail.

Table III-12. Estimated Storage Temperature and Duration Between Manufacture and Retail and Predicted Median Growth

Food Category	Temperature Range[a] (°C)	Storage Time[a, b] (days)		Median Growth [c] (log cfu)
		Minimum	Maximum	
SEAFOOD				
Smoked Seafood	1 to 5	10	30	1.08
Raw Seafood	1 to 5	1	3	0.11
Preserved Fish		**Not applicable[b]**		
Cooked RTE Crustaceans	1 to 5	1	3	0.28
PRODUCE				
Vegetables	1 to 5	1	10	0.10
Fruits		**Not applicable[b]**		
DAIRY				
Fresh Soft Cheese		**Not applicable[b]**		
Soft Unripened Cheese		**Not applicable[b]**		
Soft Ripened Cheese	1 to 5	10	30	0.04
Semi-Soft Cheese		**Not applicable[b]**		
Hard Cheese	1 to 5	10	45	-0.94
Processed Cheese		**Not applicable[b]**		
Pasteurized Fluid Milk	1 to 5	1	3	0.20
Unpasteurized Fluid Milk		**Not applicable[b]**		
Ice Cream and Frozen Dairy Products		**Not applicable[b]**		
Cultured Milk Products		**Not applicable[b]**		
High Fat and Other Dairy Products	1 to 5	3	10	0.24
MEATS				
Frankfurters	1 to 5	10	30	1.03
Dry/ Semi-dry Fermented Sausage		**Not applicable[b]**		
Deli Meats	1 to 5	10	30	1.86
Pâté and Meat Spreads	1 to 5	1	7	0.34
COMBINATION FOODS				
Deli-type Salads		**Not applicable[b]**		

[a] Rectangular distributions were used for both the temperature range and storage times.
[b] Not applicable because none of the samples were collected at manufacture so growth between manufacture and retail was not calculated for these food categories.
[c] Median growth (log cfu) is calculated by multiplying the storage times and the exponential growth rates (see Section "Modeling: Growth Between Retail and Consumption").

Step 3: Integration of Prevalence Data and Quantitative Analysis

Frequency distributions for *Listeria monocytogenes* concentration for each food category were generated by integrating the standard deviation estimates with the rate estimates for detectable *Listeria monocytogenes*. This was accomplished with a 300 iteration simulation in which pairs of values were randomly selected from a uniform distribution of the standard deviations (Table III-10) and the weighted collection of the presence/absence data sets for each food category (including those at 0.04 cfu/g at retail and those adjusted for pre-retail growth). For each of the 300 pairs of values, a mean of the log cfu/g value was calculated (using the Excel Goal Seek procedure) to find the geometric mean that matches the cumulative frequency of positive samples at the detection limit of the assay (0.04 cfu/g or the adjusted value) with the selected standard deviation. Therefore, for each food category, 300 contamination curves were generated. The average frequency for each contamination level was determined to create the variability of contamination levels. The standard deviation of the frequencies for each contamination level became the uncertainty of the distribution for the contamination data.

Example of the Modeling for *Listeria monocytogenes* in Food at Retail Using Smoked Seafood

Step 1. Characterize the distribution of *Listeria monocytogenes* across food categories
Data from NFPA (2002) for Smoked Seafood is used to illustrate this step. As shown in Figure III-3, at the 0.04 cfu/g (-1.4 on log scale) contamination value, 0.958 (95.8%) of the samples (2573/2687) contain less than or equal to that contamination level. Sixty-seven more samples had levels < 0.1 cfu/g and eleven samples were contaminated at less than or equal to 1 cfu/g (0.0 on log scale). Therefore the fraction of negative samples is 0.986 [(2573 + 67 + 11)/2687]. This procedure is repeated for the samples that had higher levels of contamination. A normal curve was fitted to the data points by least-squares and the mean and standard deviation were estimated as –6.7 and 3.1, respectively. This process was repeated for the 17 selected enumeration studies and the resulting means and standard deviations are summarized in Table III-9.

Step 2. Characterize the uncertainty distribution for the frequency of detectable contamination
- Adjust for sample size, geographic location, and study date. The study weight and study probability are calculated as described by Equations 1 and 2 using the total number of samples in the study (n), the geographic weight (gw), and the weight for the date of the

study (dw). These values are shown for Smoked Seafood in Table III-13. For example for the Aguado *et al.*, 2001 study, the study weight is 52 (52 x 1 x 1) and the study probability is 0.009 (52/6034.7).

- Adjustment of older data for food categories without large recent studies. This step is not applicable for smoked seafood as recent large studies were available. However an adjustment was made using the range of prevelance ratios given in Table III-11 for Preserved Fish, Cooked RTE Crustaceans, Fruits, Hard Cheese, Processed Cheese, and Cultured Milk Products.

Adjustment for growth between production and retail for samples taken at manufacturing. In Table III-13 the 'collection' column indicates which studies were collected at manufacturing/ product and at retail. For the studies collected prior to retail, the level of *Listeria monocytogenes* was increased to account for anticipated growth between manufacturing and retail. From Table III-12, the mean exponential growth for smoked seafood of 0.15 logs/day at 5°C was multiplied by a uniform distribution (minimum of 1 day, most frequent of 10 days, and maximum of 30 days of storage) and the median of this resulting distribution was 1.08 logs. The fraction of positive samples (0.04 cfu/g or -1.4 log cfu/g) at manufacture was increased to a fraction of positive samples with a value of 0.48 cfu/g (-0.32 log cfu/g) at retail (-1.4 log + 1.08 log = -0.32 log cfu/g). In Step 3 described below, the procedure for the fitting of the contamination distribution the fraction of positive samples remained the same but the contamination level was now represented by a value of –0.32 log cfu/g for these studies.

Table III-13. Prevalence Studies of *Listeria monocytogenes* in Smoked Seafood

Study Reference	n[a]	# neg[b]	gw[c]	dw[d]	Collection[e]	Study Weight[f]	Cumulative Probability[g]	LM% negative[h]
Aguado *et al*., 2001	52	36	1	1	R	52	0.009	0.69
Baek *et al*., 2000	68	65	1	1	R	68	0.020	0.96
Cortesi *et al*., 1997	165	133	1	0.7	R	115.5	0.039	0.81
Dauphin *et al*., 2001	36	20	1	1	R	36	0.045	0.56
Dillon *et al*., 1994	258	246	1	0.7	R	180.6	0.075	0.95
Dominguez *et al*., 2001	170	132	1	1	R	170	0.103	0.78
Eklund *et al*., 1995	61	13	1	0.7	P	42.7	0.110	0.21
Ericsson *et al*., 1997	9	6	1	0.7	R	6.3	0.111	0.67
Farber, 1991b	32	22	1	0.4	P	12.8	0.113	0.69
Garland, 1995	285	284	1	0.7	P	199.5	0.146	1.00
NFPA, 2002	2687	2573	1	1	R	2687	0.592	0.96
Guyer and Jemmi, 1990	64	60	1	0.4	P	25.6	0.596	0.94
Hartemink and Georgsson, 1991	31	30	1	0.4	R	12.4	0.598	0.97
Heinitz and Johnson, 1998	1080	929	1	0.7	P	432	0.669	0.86
Hudson *et al*., 1992	26	13	1	0.4	R	10.4	0.671	0.50
Inoue *et al*., 2000	92	87	1	1	R	92	0.686	0.95
Jemmi, 1990	820	732	1	0.4	R	328	0.741	0.89
Jørgensen and Huss, 1998	420	257	1	0.7	R	294	0.790	0.61
Maija *et al*., 2001	232	222	1	1	R	232	0.828	0.96
Miettinen, *et al*., 2001	25	22	1	1	R	25	0.832	0.88
Ng and Seah, 1995	2	1	1	0.7	R	1.4	0.832	0.50
Norton *et al*., 2000	38	32	1	1	P	38	0.839	0.84
Norton *et al*., 2001	96	85	1	1	P	96	0.855	0.89
Oregon State Dept of Agriculture, 2001	168	167	1	1	R	168	0.882	0.99
Scoglio *et al*., 2000	21	18	1	1	R	21	0.886	0.86
Teufel and Bendzulla, 1993	380	353	1	0.4	R	152	0.911	0.93
Vogel *et al*., 2001a	324	231	1	1	P	324	0.965	0.71
Vogel *et al*., 2001b	200	65	1	1	P	200	0.998	0.33
Yamazak *et al*., 2000	13	10	1	1	R	13	1.000	0.77
TOTAL						6034.7		

[a] n = total number of samples in the study

[b] # neg= total number of non-detectable samples in the study (i.e., <0.04 cfu/g)

[c] gw= geographic weight. A value of 1 was used unless the study was conducted in a region and food category for which there is little or no contribution (importation) to the United States food supply, in which case a value of 0.3 was used.

[d] dw= weight for the date of the study. A value of 1 was used for studies published within the past three years; a value of 0.7 was used for studies published between 1993 and 1999; and a value of 0.4 was used for studies published before 1993.

[e] Collecction. R= sample collected at retail; and P = sample collected at production/ manufacturing

[f] Study weight = n x gw x dw

[g] Cumulative probability.

[h] LM% negative = percentage of *Listeria monocytogenes* below the method of detection (i.e., <0.04 cfu/g)

Step 3: Integration of Prevalence Data and Quantitative Analysis

The *Listeria monocytogenes* concentration model for Smoked Seafood is presented in Figure III-4. The model estimates are compared to the prevalence studies and the enumeration data. The median (50th percentile), lower (5th percentile) and upper (95th percentiles) bounds reflect the *Listeria monocytogenes* concentration model (i.e., the set of Lognormal disitribution parameter values). Each data point in the "Prevalence Studies" data set represents an individual study (weighted for sample size and other study characteristics as described in Step 2). The data points in the "Enumeration Studies" data set are pooled from four different studies as noted in Table III-5. The prevelance studies at the –0.32 log cfu/g level represent the studies collected at manufacturing/ production and were adjusted for potential growth between production and retail.
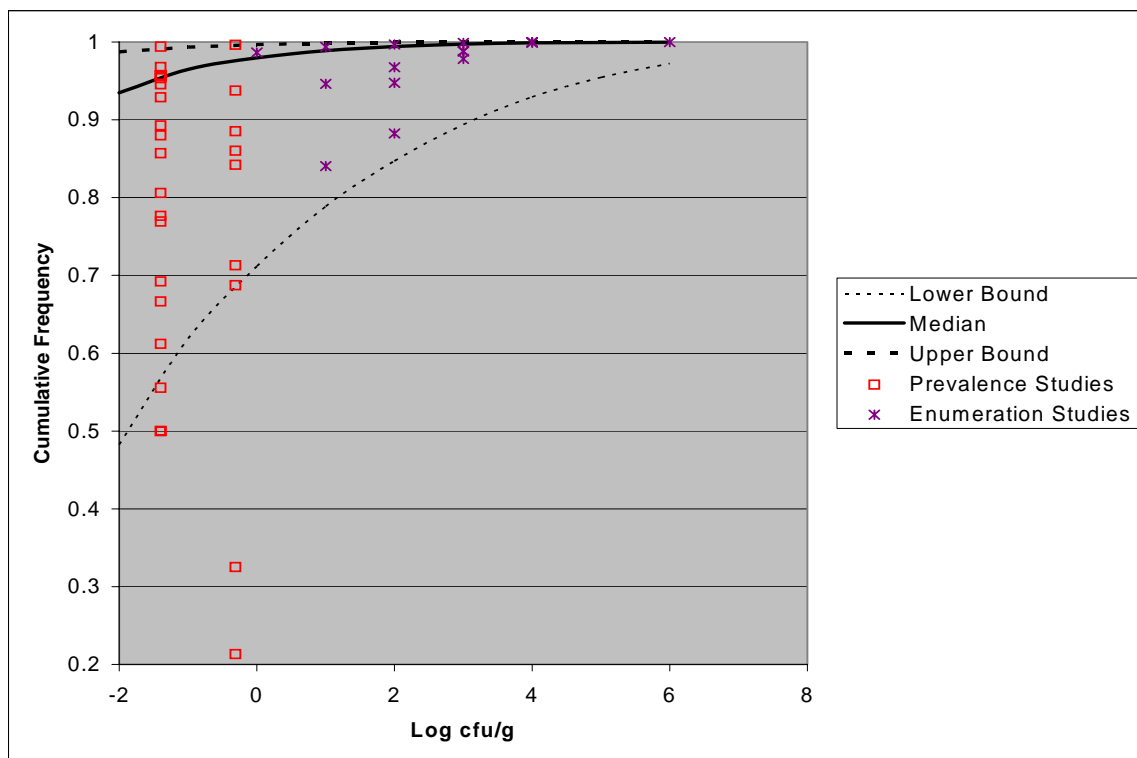
Figure III-4. Modeled Contamination Data for Smoked Seafood Food Category

Results: Modeled Contamination at Retail

Table III-14 shows the modeled distributions for *Listeria monocytogenes* contamination for the 23 food categories at retail. The first column of data in Table III-14 provides the median percentage of servings with less than one organism per serving, this estimate is not the same as undetectable values (<0.04 cfu/g) because different foods have different serving sizes. The predicted median of the servings having less than one organism of *Listeria monocytogenes* per serving ranged from 91.3 to 99.9% for the various food categories. In other words, less than 0.1 to 8.7% of the servings had one or more *Listeria monocytogenes* per serving, depending on the food category. The 5th and 95th percentiles provide information to estimate the uncertainty distributions for each of these median values. Although some servings of all food categories are likely to be contaminated at the retail level, servings of certain food categories (*e. g.,* Smoked Seafood, Raw Seafood, Deli Meats, Dry/Semi-Dry Fermented Sausages, and Deli Salads) were the most likely to be contaminated. Other columns in Table III-14 provide the percentage of servings with higher levels of contamination. Most frequently, the food categories are contaminated with 1 to 1000 cfu/serving. The calculations in the risk assessment model used 0.5 log intervals (referred to as bins) instead of the 3 log intervals shown in Table III-14.

The bar chart in Figure III-5 provides a graphic depiction of the modeled distributions. Most of the servings for each food category are in the <1 cfu/serving level (back row of bars). As the level of contamination per serving rises (moving into the front rows of bars), the fraction of servings decreases markedly for most of the food categories.

Thus, for the Smoked Seafood category, the fraction of servings at <1, 1 to 10^3, 10^3 to 10^6, 10^6 to 10^9, and >10^9 cfu/serving are about 93.6, 5.8, 0.8, 0.1, and 0.0% of servings, respectively. The sum of the fractions of servings for a food category do not necessarily equal 100% because of rounding and because adding medians is not mathematically correct.

Table III-14. Modeled Percentage Distribution of Food Servings Contaminated with *Listeria monocytogenes* at Retail

Food Category	Median Percentage of Servings Contaminated at Different Levels									
	<1 cfu/serving		1 - 1000 cfu/serving		$10^3 - 10^6$ cfu/serving		$10^6 - 10^9$ cfu/serving		> 10^9 cfu/serving	
	Median	Percentiles[a]	Median	Percentiles[a]	Median	Percentiles[a]	Median	Percentiles[a]	Median	Percentiles[a]
Seafood										
Smoked Seafood	93.6	(51.6, 98.7)	5.8	(0.9, 28.5)	0.8	(0.1, 12.8)	0.1	(<0.1, 5.9)	<0.1	(<0.1, 0.1)
Raw Seafood	91.3	(87.2, 98.6)	7.6	(1.3, 11.4)	0.8	(0.1, 1.7)	<0.1	(<0.1, 0.3)	<0.1	(<0.1, <0.1)
Preserved Fish	94.5	(70.8, 99.8)	4.8	(0.2, 20.4)	0.4	(<0.1, 4.1)	<0.1	(<0.1, 0.8)	<0.1	(<0.1, <0.1)
Cooked Ready-to-Eat Crustaceans	96.0	(93.9, 97.0)	3.6	(2.7, 6.0)	0.3	(0.1, 0.6)	<0.1	(<0.1, 0.1)	<0.1	(<0.1, <0.1)
Produce										
Vegetables	98.9	(98.7, 99.0)	1.1	(0.9, 1.3)	0.1	(<0.1, 0.1)	<0.1	(<0.1, <0.1)	<0.1	(<0.1, <0.1)
Fruits	97.3	(70.4, 99.8)	2.5	(0.2, 22.0)	0.1	(<0.1, 6.8)	<0.1	(<0.1, 1.3)	<0.1	(<0.1, <0.1)
Dairy										
Fresh Soft Cheese	99.5	(95.1, 99.7)	0.5	(0.3, 4.8)	<0.1	(<0.1, 0.5)	<0.1	(<0.1, 0.1)	<0.1	(<0.1, <0.1)
Soft Unripened Cheese,	98.0	(90.0, 99.9)	2.0	(0.1, 8.6)	0.2	(<0.1, 3.3)	<0.1	(<0.1, 0.7)	<0.1	(<0.1, <0.1)
Soft Ripened Cheese	98.5	(83.4, 99.9)	1.4	(0.1, 13.4)	0.1	(<0.1, 2.9)	<0.1	(<0.1, 0.4)	<0.1	(<0.1, <0.1)
Semi-soft Cheese	98.0	(90.8, 98.6)	1.8	(1.2, 7.2)	0.1	(<0.1, 1.5)	<0.1	(<0.1, 0.2)	<0.1	(<0.1, <0.1)
Hard Cheese	99.9	(97.8, 100.0)	0.1	(<0.1, 2.0)	<0.1	(<0.1, 0.2)	<0.1	(<0.1,< 0.1)	<0.1	(<0.1, <0.1)
Processed Cheese	99.1	(97.5, 99.9)	0.8	(0.1, 2.4)	<0.1	(<0.1, 0.2)	<0.1	(<0.1, <0.1)	<0.1	(<0.1, <0.1)
Pasteurized Fluid Milk	99.7	(97.8, 99.9)	0.3	(0.1, 2.0)	<0.1	(<0.1, 0.1)	<0.1	(<0.1, <0.1)	<0.1	(<0.1, <0.1)
Unpasteurized Fluid Milk	96.1	(90.0, 100.0)	3.3	(<0.1, 8.5)	0.3	(<0.1, 4.0)	<0.1	(<0.1, 0.9)	<0.1	(<0.1, 0.1)
Ice Cream and Frozen Dairy Products	99.6	(99.3, 99.8)	0.4	(0.2, 0.6)	<0.1	(<0.1, <0.1)	<0.1	(<0.1, <0.1)	<0.1	(<0.1, <0.1)
Cultured Milk Products	99.4	(94.0, 99.9)	0.6	(0.1, 5.5)	<0.1	(<0.1, 0.5)	<0.1	(<0.1, 0.1)	<0.1	(<0.1, <0.1)
High Fat and Other Dairy Products	98.9	(98.3, 99.1)	1.1	(0.7, 1.7)	0.1	(<0.1, 0.2)	<0.1	(<0.1, <0.1)	<0.1	(<0.1, <0.1)
Meats										
Frankfurters (reheated)	94.5	(88.5, 95.5)	4.8	(3.6, 9.4)	0.7	(0.7, 2.0)	0.1	(0.1, 0.5)	<0.1	(<0.1, <0.1)
Frankfurter (not reheated)	94.5	(88.5, 95.5)	4.8	(3.6, 9.4)	0.7	(0.7, 2.0)	0.1	(0.1, 0.5)	0.1	(<0.1, <0.1)
Dry/Semi-dry Fermented Sausages	93.6	(77.7, 97.6)	5.4	(2.1, 19.7)	0.5	(<0.1, 4.1)	<0.1	(<0.1, 1.1)	<0.1	(<0.1, <0.1)
Deli Meats	92.5	(87.8, 99.3)	6.3	(0.7,11.1)	1.0	(<0.1, 1.3)	<0.1	(<0.1, 0.2)	<0.1	(<0.1, <0.1)
Pâté and Meat Spreads	96.2	(79.7, 98.0)	3.3	(1.8, 14.9)	0.5	(0.2, 4.5)	0.1	(<0.1, 1.2)	<0.1	(<0.1, <0.1)
Combination Foods										
Deli-type Salads	92.2	(86.5, 97.7)	7.6	(2.3, 13.3)	0.1	(<0.1, 0.4)	<0.1	(<0.1, <0.1)	<0.1	(<0.1, <0.1)

[a] The 5 [th] and 95th percentiles uncertainty levels, respectively.
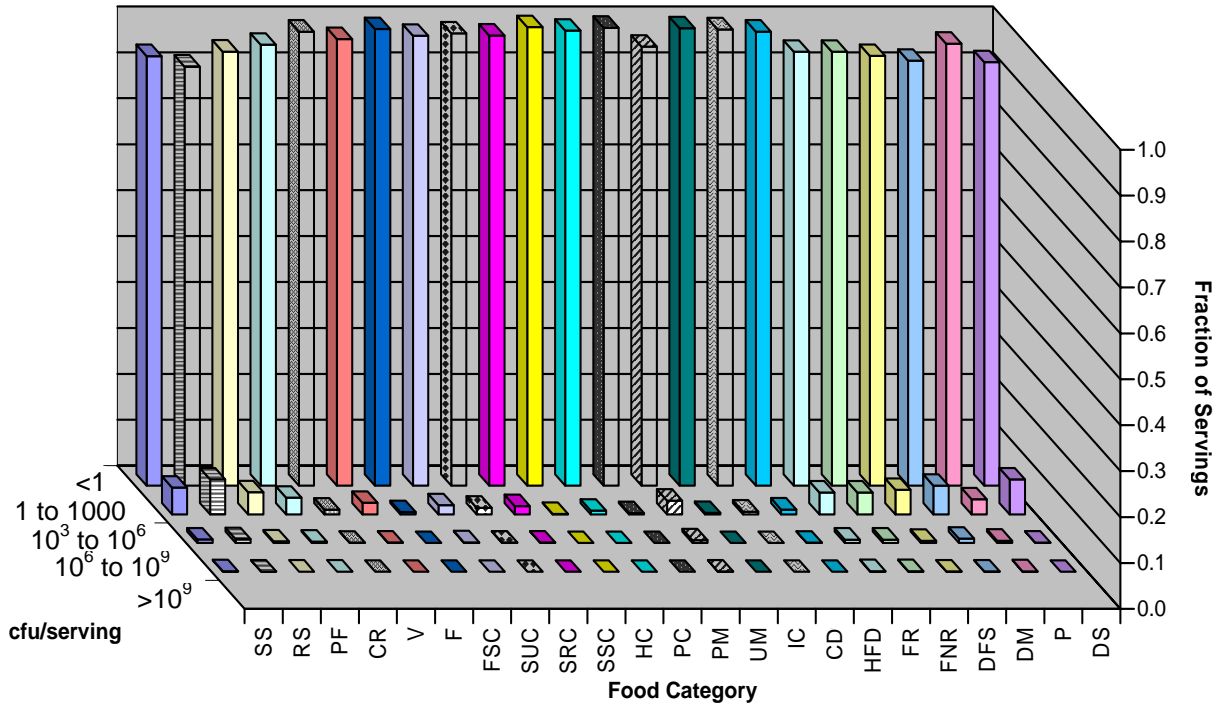
LEGEND	
SS = Smoked Seafood	PC = Processed Cheese
RS = Raw Seafood	PM = Pasteurized Fluid Milk
PF = Preserved Fish	UM = Unpasteurized Fluid Milk
CR = Cooked Ready-To-Eat Crustaceans	IC = Ice Cream and Frozen Dairy Products
V = Vegetables	CD = Cultured Milk Products
F = Fruits	HFD High Fat and Other Dairy Products
FSC = Fresh Soft Cheese	FR = Frankfurters (reheated)
SUC = Soft Unripened Cheese	FNR = Frankfurters (not reheated)
SRC = Soft Ripened Cheese	DFS = Dry/Semi-Dry Fermented Sausages
SSC = Semi-soft Cheese	DM = Deli Meats
HC = Hard Cheese	P = Pâté and Meat Spreads
	DS = Deli Salads

Figure III-5. Modeled Distribution of *Listeria monocytogenes* Contamination Levels in Food Servings at Time of Retail

Modeling: Growth Between Retail and Consumption

Most of the contamination data used in this risk assessment were from samples collected at retail. Because *Listeria monocytogenes* can grow slowly at refrigeration temperatures, a growth module was incorporated into the exposure assessment to account for the potential growth of the organism in the food during storage in the home, prior to consumption. The growth model provides an estimate of the numbers of *Listeria monocytogenes* in the food at the time of consumption.

The growth model included the initial level of *Listeria monocytogenes* in the foods at retail where the food is purchased, the storage temperature in the home refrigerator, the exponential growth rate of *Listeria monocytogenes* in a food stored at a specific temperature, the storage time in the home and the maximum growth (stationary phase). Inoculated food studies, where growth of *Listeria monocytogenes* inoculated into a food was measured, showed that maximum growth at low refrigeration temperatures (<5°C) was often less than growth in the same foods at higher temperatures. It was also concluded that refrigeration temperature and storage time are not independent factors. High storage temperatures and long storage times would not be likely to occur because this combination would lead to obvious spoilage and the food would not be consumed. The output from the growth model was a frequency distribution of the log cfu/g for each food category at the time of consumption.

Exponential Growth Rates

The square root model for exponential growth rate (EGR) was chosen because of its simplicity and general acceptance as indicated by the documented use in the microbiology literature (Ratkowsky *et al.*, 1982). A straight line results when the square root of the EGR is graphed for different growth temperatures. The equation for the model is:

$$\sqrt{EGR} = a(T - T_0) \hspace{5cm} \text{Equation [3]}$$

where **EGR** is the exponential growth rate ($\log_{10}$ cfu/day), **T** is the growth temperature (°C), **T$_0$** is the extrapolated minimum notational growth temperature (°C), and **a** is the slope parameter for *Listeria monocytogenes* in the specific food. T$_0$ values were estimated from four sources (Alavi *et al.*, 1999; Duh and Schaffner, 1993; USDA, 1997 Pathogen Modeling Program; Wijtzes *et al.*, 1993) and an average of these values (-1.18°C) was used in the model.

Different storage temperatures were used in the studies from the published literature that reported growth of *Listeria monocytogenes* in various foods. Therefore, using the data from these studies, equivalent EGRs ($\log_{10}$ cfu/day) at 5°C were calculated. The equation, presented as Equation 4, is a ratio and rearrangement of Equation 3. The slope factor (a) is a constant and cancels out in the equation.

$$\frac{EGR_s}{EGR_{lit}} = \left[\frac{a(T_s + 1.18)}{a(T_{lit} + 1.18)}\right]^2 = \left[\frac{6.18}{(T_{lit} + 1.18)}\right]^2 \qquad \text{Equation [4]}$$

where:

EGR$_5$ is the converted growth rate at 5°C,

EGR$_{lit}$ is the growth rate from the inoculated pack study,

T$_5$ is set to 5°C to standardize the EGRs, and

T$_{lit}$ is the temperature used in the literature.

If a category had five or more data points, variation was modeled by fitting statistical distributions to the resulting values (using the software program ParamFit). Paramfit employs ten different distribution models: Beta, Cauchy, Exponential, Gamma, Logistic, Lognormal, Normal, Rectangular (Uniform), Triangular, and Weibull. There is no theoretical support for any one distribution to be more appropriate than any other distribution. Therefore, the range of values generated by each of the ten statistical distributions reflects the uncertainty.

The 10 distribution models are used to construct a probability tree for the predictive model. Within an iterative simulation, the frequency of use of each model is allocated according to its relative model weight which is calculated as follows:

$$\text{Model Weight} = (((1 + n / Pn)^{O}) \times ((1 - \text{gof})^{H}) \qquad \text{Equation [5]}$$

where
n = number of observations
Pn = number of model parameters
gof = Goodness-of-Fit
O = an arbitrary constant to describe parameter penalty, a value of 19 was used
H = An arbitrary constant to modify and provide a better fit, a value of 141 was used

ParamFit uses least residual squares for the predicted percentiles as the optimization criteria. The ratio of the sum of residual squares to the sum of total squares for the predicted percentile is used as a goodness-of-fit statistic. This approach fits the middle of the distribution, so that outliers have less impact on the shape of the distribution.

In some food categories (such as Dry/Semi-dry fermented sausages and Deli-type Salads), the *Listeria monocytogenes* levels decline at a slow rate. The rate of decline was modeled with the same square root model (Equation 3) as for growth with a negative slope (**a**) and a negative EGR. Negative EGR values from the literature were combined with positive data to create one distribution, which was fitted to the growth models as explained earlier. The rate of decline was adjusted for temperature, after being converted to a positive value, by the same ratio method of Equation 4. Increasing the storage temperature above 5°C increases the rate of decline and conversely temperature decreases below 5°C decrease the rate of decline. This approach agrees with the USDA Pathogen Modeling Program (USDA, 1997), which predicts faster rates of decline at higher storage temperatures. This relatively simple approach to modeling growth versus decline (survival) sufficiently accounted for the relatively slow rates of declines encountered in this risk assessment.

If all of the growth values were positive, the data were fit with all ten distributions and the four with the highest weights were used in the probability tree. If some of the growth values were negative (reflecting a possible decline in *Listeria monocytogenes* numbers), then the data were

only fit with the Beta, Cauchy, Normal, Triangular, and Rectangular distributions as these are the only distributions of the ten that will accept negative values. Of these five distributions those with the three highest weights were used.

Several of the food categories had only two or three data points. Under this circumstance, probability trees were constructed with equiprobable rectangular or normal distribution. The maximum and minimum values were used as the parameters for the rectangular distribution. A standard algebraic formula was used to calculate the mean and standard deviation of the normal distribution.

Details on the variations and uncertainties used in the risk assessment for each food category are provided in Appendix 5. A value of zero for the EGR at all refrigeration temperatures is assigned to food categories that did not support growth (such as ice cream) and in which the pathogen levels remained stable over an extended period.

As an example, data from the Smoked Seafood food category (see Appendix 5) will be used to illustrate how the exponential growth rate of *Listeria monocytogenes* was calculated. Briefly, the data sets of EGR values at 5 °C are placed in order of ascending magnitude. Figure A5.1.2 (see Appendix 5) titled 'Cumulative Distribution for the Exponential Reference Growth Rate (EGR) at 5 °C,' is a cumulative frequency graph where the x-axis is the EGR in $\log_{10}$ cfu/day and the y-axis is the fraction of data points from the literature with that value or lower (values are from Appendix 8). Different statistical distributions are fitted to the cumulative frequency distribution with the residual sums of squares for each frequency distribution used to weight the distributions. The probability column from Table A5.1.6 (see Appendix 5) indicates the weights for the four best-fitting distributions. In this example, the Lognormal and Gamma distributions have 40 and 31% of the weight, respectively. The Beta and triangular distributions had poorer fits and carried relatively little weight (16 and 13%, respectively). The probability of each growth model dictates the frequency of selection of each distribution for use in each uncertainty iteration during a Monte Carlo simulation (Cassin, *et al*., 1998; Vose, 1998). The variation predominantly reflects the shape(s) of the most heavily weighted statistical distribution.

Post-Retail Storage Times

The distribution of storage times were multiplied by the EGR to provide an estimate of the amount of *Listeria monocytogenes* growth occurring between retail purchase of the food and its consumption. Some foods are consumed on the day of purchase whereas others remain in the home refrigerator for lengthy periods of time. This is a major source of variability in the estimate of growth and ultimately, in the numbers of *Listeria monocytogenes* consumed. The variation in storage time was described using a modified BetaPert distribution (Figure III-6). A BetaPert distribution is defined by minimum, most likely, and maximum values. The distribution was modified by increasing the weight for the central value from 4 to 7. This modification reduced the frequency of values in the extended tails. The storage times were not used in the modeling for foods where *Listeria monocytogenes* does not grow. The uncertainty was described using a $\pm$20% uniform distribution for the most frequent value and a $\pm$50% uniform distribution for the maximum value, with a 100% correlation between the two distributions.
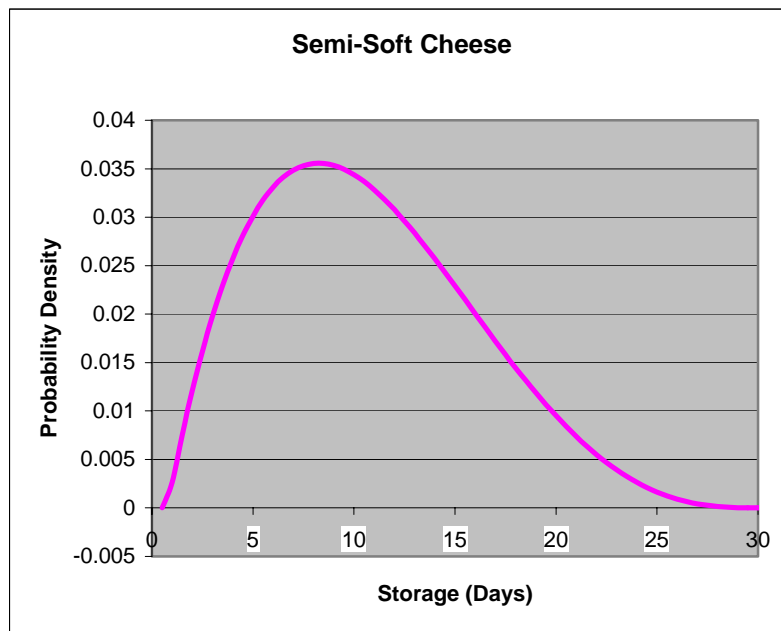
Figure III-6. Example of a Modified BetaPert Distribution

Frankfurters and Deli Meats

The survey sponsored by the American Meat Institute (AMI, 2001) asked for the average time consumers keep frankfurters and deli meats in the refrigerator. For example, 4% of the survey responders indicated that they stored frankfurters for an average of 11 to 14 days (Table III-6). This means that those responders consumed some individual servings of frankfurters after shorter storage times and others were kept longer than 14 days. While this is helpful information, what was needed for the model was the likely distribution of storage times for individual servings of frankfurters and deli meats. Thus, AMI data estimates inter-household variation. To get information on intra-household variation, consumers could be asked how long they stored the product the last time it was consumed. In order to introduce intra-household variation to the AMI data set, a log normal distribution was applied to the empirical AMI data points. The magnitude of the intra household variation, expressed as the Geometric Standard Deviation (GSD), ranged from 0.4 to 0.6 to be consistent with the data from the USDA/FSIS hotline study. The USDA hotline study asked for the 'last storage time' (Wachsmuth, 2000).

Figure III-7 shows a comparison of the USDA/FSIS hotline data (used in the draft risk assessment) and the AMI survey (indicated in the figure as 'individual average' data). The uncertainty bounds (GSD 0.2 to 0.6) are also shown in Figure III-7.
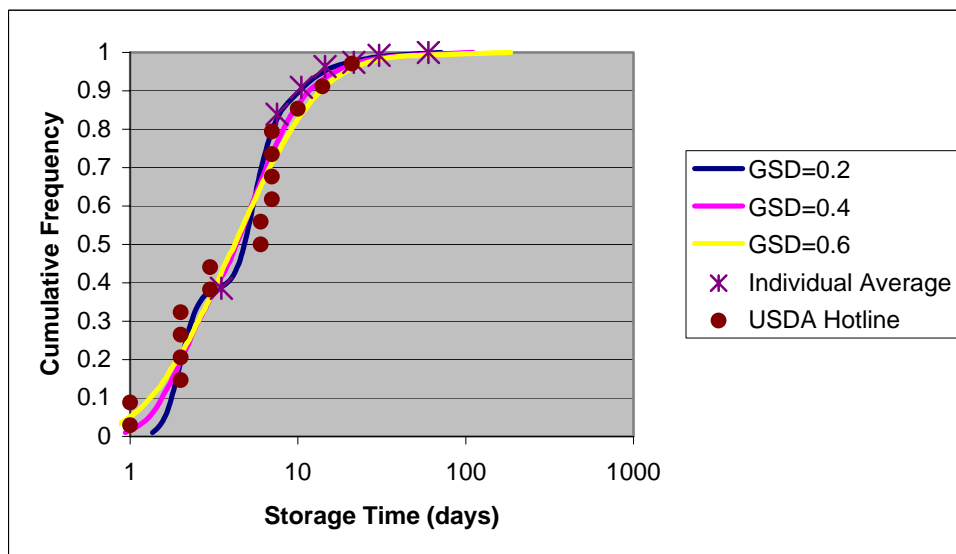
Figure III-7. Storage Time Distribution for Frankfurters and Deli Meats

Deli-type Salads

The data and assumptions behind growth estimates in deli salads were reexamined after the 2001 draft risk assessment. Data provided by Johnson (1993) and studies conducted in FDA's laboratories (Eblen, 2002a) showed that *Listeria monocytogenes* populations decline during the refrigerated storage of most deli foods. This is a consequence of processor-made salads having sufficient acidity and other preservatives to prevent growth. Locally- or store-made salads may not have these ingredients. The FDA studies indicated that growth only occurred in the shrimp and crab seafood salads. With the assistance of industry production data (Mitchell, 2001) the split between store-made and processor-made salads was estimated to be 15:85. It was also estimated that shrimp and crab salad are less than 10% of the total salad sales. Therefore, a triangular distribution of (1, 1.5, 3) was used to represent the fraction of deli salads that support growth and the uncertainty in that estimate. The growth rate at 5°C averaged 0.122 logs/day in the salads that supported growth and the declining rate averaged 0.143 logs/day in the majority of salads that did not support growth.

Modeling: Interaction of Storage Times and Temperatures

Increases in the levels of *Listeria monocytogenes* were calculated as the product of the EGR (which is dependent on the refrigeration temperature) and storage time. The Monte Carlo simulation program randomly selects different values from each calculated distribution. Both temperature and time distributions are concentrated toward the center of their ranges, 4°C and 8 days, respectively for Smoked Seafood. As a result, the most frequent estimates of growth would reflect these conditions. The simulation process would also select, at a lower frequency, the combination of low refrigeration temperatures and short storage times. Such combinations would result in relatively little growth. Similarly, the process could select high refrigeration temperatures and long storage times, 10°C and 45 days, which would result in extensive growth. However, this combination of temperatures and times would likely result in the food showing obvious spoilage and hence would not be consumed. Modeling the refrigeration temperature and storage time distributions as independent distributions was not believed to be appropriate. Therefore, the uncertainties in the mode and maximum storage times were negatively correlated

to the temperature. For example, for Smoked Seafood, this means the mode ranged from 6 to 10 days. When refrigeration temperature was 10°C, the time was 6 days and when the temperature was 0°C the time was 10 days. The maximum storage time similarly ranged from 15 to 45 days for 10°C and 0°C storage, respectively. This means that at higher temperatures the distribution for storage times is much compressed relative to the distribution at lower temperatures.

Maximum Growth Levels

Growth is the product of the EGR (at a specific temperature) and the storage time. For each iteration of the Monte Carlo simulation, the logarithm of growth estimated during storage was added to a contamination level at retail. No lag phase was calculated; it was assumed that the *Listeria monocytogenes* cells were already in the food and adjusted to the food's environment during the period before retail purchase. The only change made from retail to storage was to a new refrigerator temperature. This relatively small change would take several hours for a packaged food and the cells would effectively adjust as the temperature changes.

The populations for the stationary phases of *Listeria monocytogenes* in foods were obtained from the published literature and were used to establish limits for the maximum calculated growth levels for each food category (Appendix 8). If the calculated level for *Listeria monocytogenes* exceeded the maximum level, the maximum value was used. The literature also indicated that the maximum growth level is dependent upon the storage temperature. However, there were only a few studies in the literature that provided for the growth in a food to the stationary phase at more than one temperature to permit accurate estimation of this behavior.

Duffes *et al.* (1999) showed maximum levels (cfu/g) in smoked salmon to be less than 10^5 at 4°C and $10^{8.1}$ at 8°C. Pelroy *et al.* (1994a) found maximum levels in smoked salmon to be 10^5 and $10^{6.5}$ at 5 and 10°C, respectively. Maximum populations were reported in cream as 10^7 and $10^{7.5}$ at 4 and 8°C, respectively (Rosenow and Marth, 1987); in butter it was reported as $10^{5.5}$ and 10^6 at 4 to 6 and 13°C, respectively (Olsen *et al*, 1988); and in lettuce it was reported as 10^5 to $10^{5.5}$ and $10^{6.5}$ to $10^{7.5}$ at 5 and 10°C, respectively (Beuchat and Brackett, 1990b). In addition to direct

comparisons, a collection of individual growth studies also showed this tendency to grow to higher population levels at higher temperatures.

The maximum growth levels (cfu/g) used were applied across all food categories with 10^5, $10^{6.5}$ and 10^8 used as maximums for temperatures of <5, 5 to 7 and >7°C, respectively. For milk, sufficient data were found in the literature for growth levels of 10^7, $10^{7.5}$ and 10^8, to use as the maximums for the three temperatures, respectively. A uniform range of one logarithm was used to represent the uncertainty for each of the maximum growth levels. The calculated growth levels were added to the retail contamination levels during each iteration of the model, and these new levels of *Listeria monocytogenes* contamination in food were compared to the maximum growth level. If the calculated growth level exceeded the maximum growth level in any iteration, the amount of growth was reduced to the maximum growth level.

Modeling: Thermal Inactivation

Frankfurters have been implicated in outbreaks of listeriosis although they are generally reheated before serving. Because they are precooked during manufacturing, frankfurters are considered to be a RTE food. Reheating will kill *Listeria monocytogenes* in food. Frankfurters are usually, but not always, reheated before consumption. Therefore, a thermal inactivation step was included in the model for frankfurters. The frequency of insufficient reheating and the extent of inactivation of *Listeria monocytogenes* when not properly reheated were estimated in this step of the model.

No data describing the prevalence or extent of under-reheating of frankfurters has been published. However, the Georgetown survey (n=90) found approximately 1% of the respondents did not reheat their frankfurters (Wachsmuth, 2000). In an FSIS Hotline survey, 14% of the respondents indicated that at least one household member has eaten frankfurters directly from the package (Wachsmuth, 2000).

Some frankfurters are frozen by the consumer when they are brought home from the retail store. Information on the proportion of frozen frankfurters from the AMI survey and FDA Food Safety survey (Lando, 2003) led the risk assessment team to assign a uniform distribution from 3.0 to 8.7 % to represent this proportion and its uncertainty. To the frozen portion of frankfurters, the growth of *Listeria monocytogenes* would be set to zero, that is, the bacteria don't grow or die during the frozen storage. The time of storage would be irrelevant. It was assumed that all of the frozen frankfurters would be reheated before consumption. Therefore, the distribution of *Listeria monocytogenes* inactivation used for part of the non-frozen frankfurters was applied to all of the frozen frankfurters.

The final distribution of *Listeria monocytogenes* consumed per serving in reheated frankfurters is the summation of the respective proportions of the frankfurters stored frozen and reheated and the frankfurters stored refrigerated and reheated. The number of cases per annum was calculated from the total number of frankfurter servings and the proportion of the total in these two groups. The distribution of *Listeria monocytogenes* consumed per serving in non-reheated frankfurters represents the remaining proportion, represented by a triangular distribution of (4, 7, 10) percent of the non-frozen frankfurter servings (uncertainty distribution).

It was recognized that frankfurters are reheated in boiling water and microwave ovens more frequently than grilling, and that frankfurters are more likely to be contaminated on the surface than the interior. The Georgetown survey showed that 20% of the frankfurters were microwaved; the percentage of all responses for the FSIS Hotline was 19.4% with 4.7% microwaved less than 1 minute (Wachsmuth, 2000). In a preliminary experiment conducted by FDA/CFSAN, the heating of frankfurters by microwave ovens was measured with low (600 W) and high (1,100 W) powered microwave ovens (Buchanan, 2000). Four types were tested, including chicken frankfurters, low salt frankfurters, and two different size diameter frankfurters. Using various combinations of the two microwave power settings and four types of frankfurters, it was shown that the surface temperature increased faster than the center temperature. Heating for 25 seconds in the high power oven (1,100 W) and 40 seconds in the lower power oven (600 W) raised the surface temperature to at least 59 °C and, in some cases, raised the surface temperatures to over 70 °C. There is no information on what combinations of heating times and

temperatures are actually realized by consumers, but this preliminary experiment suggests that microwave heating is likely to be sufficient to cause substantial inactivation of any *Listeria monocytogenes* that might be present.

Inadequate data were found with which to directly model thermal inactivation in the frankfurters that receive some heating by microwaving, boiling, frying, grilling, broiling or other means. Therefore, data from inactivation of *E. coli* O157:H7 in hamburgers were adapted (Juneja *et al.,* 1997). These authors determined that survival of *E. coli* O157:H7 after cooking to maximum temperatures ranging from 54 to 77ºC (129 to 171 ºF) may be estimated by this equation:

$$\log_{10} \text{survivors} = 20.53 - 0.12 \text{ T} \qquad \qquad \text{Equation [6]}$$

The maximum cooking temperature to calculate the decrease (T) is in degrees Fahrenheit. Because the initial contamination was 6.6 logs, the equation can be rearranged to calculate the decrease in contamination and applied to any initial level of contamination. The temperature was also converted into degrees Celsius:

$$\log_{10} \text{reduction} = 0.216 \text{ (T - 46.4)} \qquad \qquad \text{Equation [7]}$$

A standard deviation of 0.5 logs was used to represent the uncertainty in the estimated reduction. This value reflects the sampling error from a similar experiment with *E. coli* O157:H7 (Jackson *et al*., 1996) where a 4.1 $\log_{10}$ reduction was observed after cooking to 68.3ºC.

Reductions in *Listeria monocytogenes* levels were calculated by estimating a distribution of cooking temperatures with a triangular distribution having a minimum of 54 ºC, most frequent temperature in the range of 69 to 73 ºC, and a maximum of 77 ºC. The four-degree range for the most frequent temperature represents uncertainty in the cooking temperature distribution. Table III-15 contains the resulting cumulative distribution in log reductions for the frankfurters that were given some reheating. The remainder had no reduction in *Listeria monocytogenes* after the growth modeling.

Table III-15. Cumulative Distribution of the Reduction (log$_{10}$) of
Listeria monocytogenes **in Reheated Frankfurters**

Percentile	Median Reduction, log$_{10}$ cfu/g [a]
1st	0.00 (0.00, 0.00)
5th	2.09 (1.90, 2.29)
10th	2.63 (2.52, 2.77)
25th	3.50 (3.38, 3.62)
50th	4.49 (4.32, 4.63)
75th	5.30 (5.13, 5.45)
90th	5.89 (5.78, 6.01)
95th	6.18 (6.05, 6.29)
99th	6.68 (6.57, 6.77)

[a] Values in parentheses are the 5th and 95th uncertainty levels.

Results: Modeled *Listeria monocytogenes* Levels in Food at Consumption

The estimated levels of *Listeria monocytogenes* at consumption are presented on Table III-16.
This table has the same format as the table for *Listeria monocytogenes* contamination at retail
(Table III-5), and may be directly compared to it to observe the shift in levels of *Listeria
monocytogenes* after storage and/or heating. The median percentage of servings that fall within
designated ranges of *Listeria monocytogenes* levels per serving are presented. The actual
simulation modeling was at narrower levels (every logarithm and half-logarithm cfu/serving).
The 5 and 95% values for the distributions for *Listeria monocytogenes* levels in each food are
also given. These distributions indicate the uncertainty in the value for each median. The
distribution observed with the values across a row gives the variation in *Listeria monocytogenes*
levels expected for each food category. Because these medians are from skewed uncertainty
distributions and because of rounding errors, a row may not sum to exactly 1.00.

As shown previously with the retail contamination estimates, every food category has some
fraction of servings with at least 1 cfu/serving. The food categories range from 0.1% in hard
cheeses to 8.7% in raw seafood. The column in Table III-16 showing 10^6 to 10^9 *Listeria
monocytogenes* per serving is the level where the occurrence of listeriosis would be expected to
be most likely. Smoked Seafood, Cooked RTE Crustaceans, Frankfurters not reheated, Deli
Meats, and Pâté and Meat Spreads categories comprise a group of foods estimated to have the
greatest likelihood of containing 10^6 to 10^9 *Listeria monocytogenes* per serving. These levels are

illustrated in Figure III-8. The row in the rear represents the fraction of servings with <1.0 cfu *Listeria monocytogenes*. All of the food categories have more than 90% of their servings in this contamination range. The rows have increasing levels of contamination toward the front of the figure.

Comparing corresponding values in Tables III-14 and III-16 shows the predicted effect of storage on the levels of *Listeria monocytogenes* at consumption. Cooked RTE Crustaceans, Frankfurters (not reheated), Deli Meats, and Pâté and Meat Spreads have some of the most dramatic changes. For example, at retail, 1.0% of Deli Meat servings would be in the 10^3 to 10^6 cfu/serving group. This increases to 1.6% at the time of consumption. In addition, the reduction in *Listeria monocytogenes* from reheating frankfurters is evident by comparing the <1, 1-1000 and 10^3 to 10^6 cfu/serving groups in Table III-16. The levels of *Listeria monocytogenes* in foods that do not permit growth, such as ice cream, do not show a change in comparing the values in Table III-14 (at retail levels) and Table III-16 (at consumption levels).

Table III-16. Modeled Percentage Distribution of Food Servings Contaminated with *Listeria monocytogenes* at Time of Consumption

Food Category	Median Percentage of Servings Contaminated at Different Levels[a]									
	<1 cfu/serving		1 - 1000 cfu/serving		$10^3 - 10^6$ cfu/serving		$10^6 - 10^9$ cfu/serving		> 10^9 cfu/serving	
	Median	Percentiles[a]	Median	Percentiles[a]	Median	Percentiles[a]	Median	Percentiles[a]	Median	Percentiles[a]
Seafood										
Smoked Seafood	93.6	(51.6, 98.7)	5.3	(0.8, 24.6)	1.2	(0.2, 15.0)	0.2	(<0.1, 8.2)	<0.1	(<0.1, 0.5)
Raw Seafood	91.3	(87.3, 98.6)	7.2	(1.2, 10.8)	1.2	(0.1, 2.2)	<0.1	(<0.1, 0.2)	<0.1	(<0.1, <0.1)
Preserved Fish	94.5	(70.8, 99.8)	4.8	(0.2, 20.4)	0.4	(<0.1, 4.1)	<0.1	(<0.1, 0.8)	<0.1	(<0.1, <0.1)
Cooked Ready-to-Eat Crustaceans	96.0	(93.9, 97.0)	3.2	(2.5, 5.5)	0.7	(0.4, 1.0)	0.1	(<0.1, 0.2)	<0.1	(<0.1, <0.1)
Produce										
Vegetables	98.9	(98.7, 99.0)	1.0	(0.9, 1.3)	0.1	(<0.1, 0.1)	<0.1	(<0.1, <0.1)	<0.1	(<0.1, <0.1)
Fruits	97.3	(70.4, 99.8)	2.5	(0.2, 21.4)	0.2	(<0.1, 7.5)	<0.1	(<0.1, 1.4)	<0.1	(<0.1, <0.1)
Dairy										
Fresh Soft Cheese	99.5	(95.2, 99.7)	0.5	(0.3, 4.5)	<0.1	(<0.1, 0.7)	<0.1	(<0.1, 0.1)	<0.1	(<0.1, <0.1)
Soft Unripened Cheese	98.1	(90.1, 99.9)	1.8	(0.1, 7.5)	0.2	(<0.1, 3.7)	<0.1	(<0.1, 1.0)	<0.1	(<0.1, <0.1)
Soft Ripened Cheese	98.6	(84.0, 99.9)	1.3	(0.1, 12.8)	0.1	(<0.1, 3.0)	<0.1	(<0.1, 0.4)	<0.1	(<0.1, <0.1)
Semi-soft Cheese	98.2	(91.4, 98.8)	1.7	(1.1, 6.9)	0.1	(<0.1, 1.3)	<0.1	(<0.1, 0.2)	<0.1	(<0.1, <0.1)
Hard Cheese	99.9	(98.3, 100.0)	0.1	(<0.1, 1.6)	<0.1	(<0.1, 0.2)	<0.1	(<0.1, <0.1)	<0.1	(<0.1, <0.1)
Processed Cheese	99.2	(97.8, 99.9)	0.7	(0.1, 2.1)	<0.1	(<0.1, 0.2)	<0.1	(<0.1, <0.1)	<0.1	(<0.1, <0.1)
Pasteurized Fluid Milk	99.7	(97.8, 99.9)	0.2	(0.1, 1.8)	<0.1	(<0.1, 0.4)	<0.1	(<0.1, <0.1)	<0.1	(<0.1, <0.1)
Unpasteurized Fluid Milk	95.6	(90.0, 99.6)	3.0	(0.4, 7.6)	0.6	(<0.1, 5.1)	<0.1	(<0.1, 1.3)	<0.1	(<0.1, 0.2)
Ice Cream/Frozen Dairy Products	99.6	(99.3, 99.8)	0.4	(0.2, 0.6)	<0.1	(<0.1, <0.1)	<0.1	(<0.1, <0.1)	<0.1	(<0.1, <0.1)
Cultured Milk Products	99.6	(95.8, 99.9)	0.4	(0.1, 3.8)	<0.1	(<0.1, 0.3)	<0.1	(<0.1, <0.1)	<0.1	(<0.1, <0.1)
High Fat and Other Dairy Products	98.9	(98.3, 99.2)	0.9	(0.6, 1.5)	0.2	(0.1, 0.4)	<0.1	(<0.1, 0.1)	<0.1	(<0.1, <0.1)
Meats										
Frankfurters (reheated)	98.9	(97.3, 99.1)	0.8	(0.7, 2.1)	0.2	(0.2, 0.5)	0.1	(<0.1, 0.1)	<0.1	(<0.1, <0.1)
Frankfurters (not reheated)	94.5	(88.5, 95.5)	4.2	(3.1, 8.1)	1.0	(1.0, 2.5)	0.3	(0.2, 0.8)	0.1	(0.1, 0.3)
Dry/Semi-dry Fermented Sausages	93.6	(77.7, 97.6)	5.4	(2.1, 19.7)	0.5	(<0.1, 4.1)	<0.1	(<0.1, 1.1)	<0.1	(<0.1, <0.1)
Deli Meats	92.5	(87.8, 99.3)	4.8	(0.5, 8.6)	1.6	(0.1, 2.4)	0.5	(<0.1, 0.7)	0.3	(<0.1, 0.6)
Pâté and Meat Spreads	96.3	(79.8, 98.0)	2.2	(1.2, 8.6)	1.3	(0.6, 7.8)	0.4	(0.2, 3.8)	0.1	(<0.1, 0.6)
Combination Foods										
Deli-type Salads	93.5	(88.7, 98.2)	6.4	(1.8, 11.1)	0.1	(<0.1, 0.3)	<0.1	(<0.1, <0.1)	<0.1	(<0.1, <0.1)

[a] The 5th and 95th percentiles uncertainty levels, respectively.
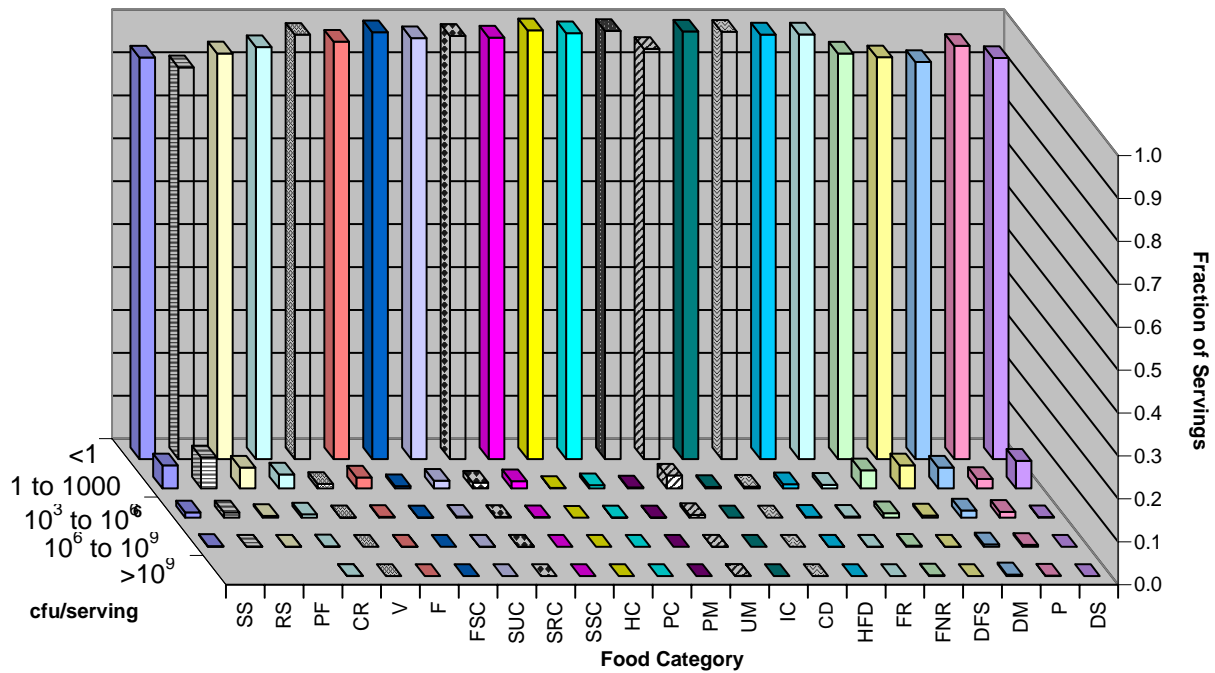
LEGEND	
SS = Smoked Seafood	PM = Pasteurized Fluid Milk
RS = Raw Seafood	UM = Unpasteurized Fluid Milk
PF = Preserved Fish	IC = Ice Cream and Frozen Dairy Products
CR = Cooked Ready-To-Eat Crustaceans	CD= Cultured Milk Products
V = Vegetables	HFD High Fat and Other Dairy Products
F = Fruits	FR = Frankfurters (reheated)
FSC = Fresh Soft Cheese	FNR= Frankfurters (not reheated)
SUC = Soft Unripened Cheese	DFS = Dry/Semi-Dry Fermented Sausages
SRC = Soft Ripened Cheese	DM = Deli Meats
SSC = Semi-soft Cheese	P = Pâté and Meat Spreads
HC = Hard Cheese	DS = Deli-type Salads
PC = Processed Cheese	

Figure III-8. Three Dimensional Graph of the Modeled Distribution of *Listeria monocytogenes* Levels of Contamination at the Time of Consumption for the Food Categories

An approximation of the overall frequency of consumption of *Listeria monocytogenes* by the United States population can be made by multiplying the fraction of servings in each food category-dose bin (Table III-16) by the annual number of servings in each food category (Table III-2). The numbers of servings are then summed for each dose for all of the food categories. Table III-17 shows that most of the servings have less than one *Listeria monocytogenes* and the number of contaminated servings decreases with increasing levels of contamination. If the number of contaminated servings is divided by the United States population (2.6×10^8), an approximation of how frequently the "average person" would encounter these levels of *Listeria monocytogenes* each year can be calculated. This "average" person would consume a serving with 10^3 to 10^6 microorganisms 2.4 times per year, 10^6 to 10^9 microorganisms once every two years and more than 10^9 microorganisms once every three years.

Table III-17. Number of Servings of Food per Year Containing Various Levels of *Listeria monocytogenes*

Listeria monocytogenes Levels in Food (per serving)	Number of Servings (per year in the United States)	Number of Servings (per person per year)
0	3.3×10^{11}	1300
1 to 1000	4.9×10^9	19
1×10^3 to 1×10^6	6.2×10^8	2.4
1×10^6 to 1×10^9	1.3×10^8	0.5
$> 1 \times 10^9$	7.3×10^7	0.3

IV. HAZARD CHARACTERIZATION

Hazard characterization describes the adverse effects of a particular substance, organism, or other entity. The relationship between the exposure level (dose) and frequency of illness or other adverse effect (response) is estimated and the severity of the health effects is also evaluated, often by considering multiple biological endpoints (e.g., infection, morbidity, fatalities, sequelae). In the case of *Listeria monocytogenes*, the overall incidence of illness, its severity, and the differential risk to immunocompromised subpopulations are well characterized (see section titled "II: Hazard Identification"). In contrast, the relationship between the amount of *Listeria monocytogenes* consumed (the dose) and the likelihood or severity of illness resulting from that dose (the response) is not well understood. This part of the *Listeria monocytogenes* risk assessment focuses on characterization of the dose-response relationship.

Three factors, often called the disease triangle, affect the dose-response relationship: the environment (in this case, the food matrix), the pathogen (virulence characteristics or factors), and the host (susceptibility or immune status factors). Data may be obtained from humans (outbreaks, case reports, case-controlled studies, volunteer feeding trials), animals (mice, rats, primates, and other species), or *in vitro* (e.g., tissue culture) studies. For this risk assessment, surveillance data were used to describe the magnitude and the incidence of severe disease. This human data from surveillance studies was combined with data from surrogate studies using animals to establish the dose-response relationship for the subpopulations.

Based upon the available information and the objectives of this risk assessment, the total population was separated into three groups: the elderly (60 years and older), pregnancy related cases (perinatal), and the remaining population (designated the intermediate–aged). Perinatal deaths result from foodborne infection of a pregnant woman that is transmitted to the fetus before birth. Neonatal death rates from surveillance data were adjusted to include prenatal infections that resulted in very early termination of pregnancy (i.e., miscarriages). Distinct disease surveillance data on prenatal deaths were not consistently reported and had to be estimated based on neonatal death rates. The intermediate-aged group contains both individuals

with fully competent immune systems and individuals with decreased immune function that are at greater risk of listeriosis.

In this revised FDA/FSIS risk assessment, adjustment ('dose-response scaling') factors were used to account for the variability of the many parameters (e.g., host susceptibility and *Listeria monocytogenes* strain virulence) that influence the relationship between the level of the dose and the severity of illness. For example, variability in the effect of host susceptibility on the level of a lethal dose was determined using mortality data from animal studies that compare normal mice with those having various forms of immune suppression. Animal studies were also used to characterize the range of *Listeria monocytogenes* strain virulences.

The WHO/FAO Risk Assessment of *Listeria monocytogenes* in Ready-To-Eat Foods (WHO/FAO, 2002) contains estimations for the risk of listeriosis for individuals with a range of medical conditions. This degree of detail was not undertaken in the current risk assessment since it would not improve the primary objective of this revised risk assessment, i.e., to compare the risk of different foods. Without food consumption information on the frequency and serving size of smoked seafood for diabetic and cancer patients, for example, it is not possible to provide additional insight from that already in the WHO/FAO document. We would also need information on the number of cases of listeriosis in the immunocompromised groups.

In the Hazard Characterization that follows, the relevant background for each component of the hazard characterization dose-response model is discussed, followed by a description of how specific related information was used for probabilistic modeling and any model outputs. The background sections describe the type of data available, including its strengths and limitations for use in this risk assessment. A diagram showing the main components of the Dose-Response model is provided in Figure IV-1.
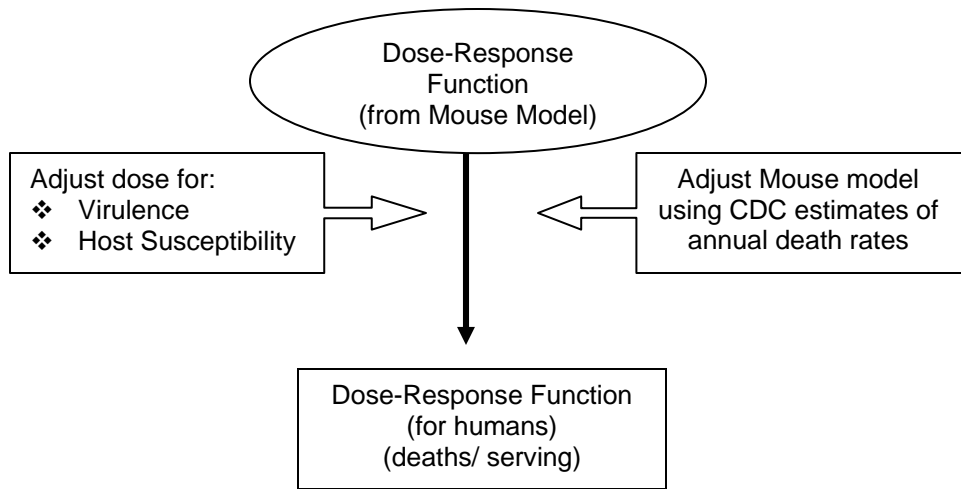
Figure IV-1. Components of the Dose-Response Model

Dose-Response Modeling

The primary variables involved in constructing dose-response models for *Listeria monocytogenes* are pathogen virulence (the ability of the pathogen to produce illness), host susceptibility (the capacity of the host to defend against the pathogen), and the effect of the food matrix (the relationship between the physico-chemical nature of *Listeria monocytogenes*-contaminated food and the fate of the organism following ingestion). Because of variability in host susceptibility and food matrix effects, there is no single infectious dose for *Listeria monocytogenes*, or any other pathogen that can be used for all individuals.

The food matrix has been theorized to affect the ability of a pathogen to survive gastric acidity or to interact with intestinal mucosa, changing the likelihood of infection. While *Listeria monocytogenes* has been found in many environments, human listeriosis has often been associated with high salt, low pH, or high fat foods (Juntilla and Brander, 1989; McLauchlin, 1996; Linnan *et al.*, 1988; Dalton *et al.*, 1997; Barnes *et al.*, 1989). While these findings are circumstantial in nature, adaptation of *Listeria monocytogenes* to acidic or high salt environments may also increase its ability to survive the stomach acid barrier or within host cells (O'Driscoll *et al.*, 1996). Similarly, high fat content in foods may protect *Listeria*

monocytogenes from gastric acid, or possibly enhance uptake and survival in host cells via interaction with cell membrane lipids (Coleman and Marks, 1998). At present, there are only limited studies in animal surrogates that assess the effects of food matrix on dose-response (Sprong *et al*., 1999), so incorporation of this parameter into the dose-response model awaits further research.

Pathogen virulence studies with different strains and serotypes of *Listeria monocytogenes* have been conducted with experimental animals (Pine *et al.*, 1990; Pine *et al.*, 1991; Stelma *et al.,* 1987). Studies have also been performed that attempt to quantify the relationship between immune function and lethal dose (Czuprynski *et al*., 1996; Czuprynski and Brown, 1986; Golnazarian *et al*., 1989). These types of studies were used to develop the relative extremes of dosages that affect lethality in laboratory animals with respect to susceptibility.

There are no human clinical trials with *Listeria monocytogenes.* Human data to anchor animal ranges (*i.e.,* relate effects observed in surrogate animals with those in humans) are limited to outbreak, case-control, and surveillance studies. Although numerous epidemiological investigations have been conducted for *Listeria monocytogenes*, the emphasis of these investigations has not been quantification of the number of organisms consumed by both ill and exposed (but not ill) subjects. However, two outbreak investigations did occur that provided quantitative data. The use of outbreak data to create a dose-response curve is described in Appendix 9.

Comparison of the FDA/FSIS Revised Dose-Response Model to Other Dose-Response Models for *Listeria monocytogenes*

Previously published risk assessments for *Listeria monocytogenes* included dose-response models (Farber *et al*., 1996; Buchanan *et al.*, 1997; Haas *et al*., 1999; Lindqvist and Westöö, 2000; WHO/FAO, 2002). These efforts share some similarities with the dose-response evaluation used in this FDA/FSIS revised risk assessment, but there are significant differences as well. In Table IV-1, several aspects of the models are compared: empirical basis for the estimates, health endpoints modeled, consideration of susceptible subpopulation, consideration of strain virulence, and models employed.

The earlier dose-response assessments each used a single mathematical model, and the model was different in each case. Farber *et al*. (1996) used a three-parameter Weibull-Gamma model, Buchanan *et al*. (1997) used a single parameter exponential model, and Haas *et al*. (1999) used a beta-Poisson model after rejecting the exponential model for lack of fit. Lindquist and Westoo (2000) used exponential and Weibull-Gamma models. The FDA/FSIS revised risk assessment used an initial battery of eight models. All the models that appeared to provide a reasonably close fit (described in Appendix 6) were used to characterize the uncertainty in the prediction arising from model selection using a probability tree.

Both Farber *et al*. (1996) and Buchanan *et al*. (1997) sought to predict cases of listeriosis, which they defined as infections serious enough to require clinical attention and generate a public health record. The endpoint modeled by Haas *et al*. (1999) was infection in mice (i.e., presence of the microorganism in the liver or spleen of mice), which does not necessarily correlate with a clinical outcome in humans (e.g., illness). The dose-response model for the revised FDA/FSIS risk assessment uses mortality as the outcome because it represents a comparable endpoint for both the human epidemiology record and experimental mouse data. The total number of listeriosis cases is estimated with a multiple for each population based on CDC epidemiological data.

The dose-response analysis by Farber *et al*. (1996) began with a presumption of the doses corresponding to illness rates of 10% and 90%. Although there may have been some empirical basis for these estimates, the basis was not specified. The dose-response model developed by Buchanan *et al*. (1997) relied on exposure and public health data collected in Germany. Haas *et al*. (1999) based their model on data collected from a study with controlled exposures of mice to *Listeria monocytogenes*. The dose-response model in the revised FDA/FSIS risk assessment uses one of the studies also employed by Haas *et al*. (1999), but also accounted for the difference in susceptibility between mice and humans using public health data collected in the United States.

Both Farber *et al*. (1996) and this revised FDA/FSIS risk assessment generate separate equations for different population groups. Farber *et al*. (1996) employed a Weibull-Gamma model with a

different set of parameters for two groups designated as susceptible and non-susceptible. The revised FDA/FSIS risk assessment includes a scaling factor that adjusts the effective dose to match the dose-response model with the surveillance data. The analysis by Buchanan *et al.* (1997) did not explicitly model susceptible subpopulations. However, the variation in host susceptibility is implicitly an integral part of the total variability represented by the equation. The dose-response model of Haas *et al.* (1999) reflected the variation of the population in the study with inbred mice in a highly controlled environment. It did not attempt to address the greater variation that might be expected in a human population.

Farber *et al.* (1996) did not specify the empirical basis of their estimate, so the extent to which strain virulence was considered is not apparent. The estimate by Haas *et al.* (1999) was based on a study with a single strain and it clearly did not address strain virulence. Although Buchanan *et al.* (1997) did not model strain variability, the variation in strain virulence was implicitly an integral part of the total variability represented by the equation because it was based upon statistics for the entire population.

The WHO/FAO (2002) risk assessment used a combination of the models from Buchanan *et al.* (1997), Lindqvist and Westöö (2000), and the draft US HHS/USDA (2001) risk assessments for its hazard characterization. The first two studies, Buchanan *et al.* (1997), Lindqvist and Westöö (2000), reported an r-value derived from the exponential dose-response curve. A third r-value was calculated from the dose-response graph reported in the draft US HHS/USDA (2001) risk assessment; this r-value was smaller than the other two. The difference in the r-values resulted from the assumption about the highest *Listeria monocytogenes* doses that would be encountered in the rare servings that were most likely to lead to illness. The draft US HHS/USDA (2001) estimated higher numbers of *Listeria monocytogenes* would be consumed resulting in a lower calculated r-value (i.e., consumption of higher cell numbers means that a cell has a lower probability of causing illness). The WHO/FAO (2002) risk assessment tested some of the consequences of this assumption, but in this comparative risk assessment, the same assumptions regarding maximum growth levels that are used to derive the dose-response model are then used to calculate the risks for the different food categories.

Table IV-1. Characteristics of This *Listeria monocytogenes* Risk Assessment (FDA/FSIS) and Previously Conducted *Listeria monocytogenes* Risk Assessments that Contain Dose-Response Models for Listeriosis

Study	Empirical Basis	Endpoint	Models Examined	Model Used	Host Susceptibility	Strain Virulence
Farber *et al.* (1996)	Subjective	Illness (including lethality)	Weibull-Gamma	Weibull-Gamma	Explicit	Unknown
Buchanan *et al.* (1997)	Epidemiology	Illness (including lethality)	Exponential	Exponential	Implicit	Implicit
Haas *et al.* (1999)	Mouse	Infection	Beta-Poisson Exponential	Beta-Poisson	Mice = Men	Not Addressed
Lindquist and Westoo, 2000	Epidemiology	Illness	Exponential and Weibull-Gamma	Exponential	Implicit	Implicit
FDA/FSIS draft risk assessment (US HHS/USDA, 2001)	Mouse, Epidemiology	Lethality and Infection	Multiple	Multiple	Explicit	Explicit
WHO/FAO, 2002	Epidemiology	Morbidity, Mortality	Multiple	Exponential	Explicit	Implicit
FDA/FSIS Risk Assessment (revised, current document)	Mouse, Epidemiology	Lethality and Infection	Multiple	Multiple	Explicit	Explicit

Dose-Response in Animal Surrogates

Data Collected from Animal Studies

The virulence factors of *Listeria monocytogenes* and their interaction with the host's defense systems help determine the infectious dose of listeriosis. However, because of the potential for fatal outcomes in human listeriosis, clinical studies involving human subjects have not been conducted. Experimental dose-response data are therefore derived exclusively from studies using animal and *in vitro* surrogates.

Extrapolation from animal to human infection involves the interaction of several factors related to the inherent differences between surrogates (e.g., mice) and humans. The relationship of infective dose to body mass, for example, if treated in a classic chemical toxicology approach, suggests that mouse doses may be equivalent to a 50- or 500-fold higher dose in humans, depending on age. It is not known whether this approach is directly applicable to microbial dose-response. For this reason, no explicit body weight dose adjustment factor was included.

The difference in lifetime daily exposure patterns between humans and animal surrogates is also significant. Dose-response studies in surrogates, such as mice, generally use animals that are immunologically naïve (i.e., previously unexposed) to *Listeria monocytogenes* but with normal immune systems. In humans, both food contamination data and fecal carriage studies suggest that exposure to *Listeria monocytogenes* is relatively common among humans. Most of the surveys of fecal carriage are based on point prevalence rather than cumulative exposure (Slutsker and Schuchat, 1999). Unless fecal carriage is monitored over time in the same individuals, it cannot be determined what proportions of positive isolates of *Listeria monocytogenes* represent transient passage of the organism versus asymptomatic or mildly symptomatic carrier status.

The exact relationship between fecal carriage and immunological exposure and sensitization is not clear. Prolonged exposure, such as colonization of intestinal tissues, would likely result in immune sensitization. In an outbreak involving a high infective

dose in chocolate milk, in which the major symptom was gastroenteritis, the severity of symptoms correlated with subsequent higher antibody titers against the antigen listeriolysin O (Dalton *et al.,* 1997). Another study reported that T lymphocytes that were reactive to *Listeria monocytogenes* antigens were present in the peripheral blood of 50 normal, healthy adults surveyed (Munk and Kaufmann, 1988).

This suggests that exposure and subsequent immune sensitization may commonly occur. This observation also suggests that such exposure may result in increased resistance because T lymphocytes have been shown to be an important component of resistance to *Listeria monocytogenes* in mice (Kuhn and Goebel, 1999, Unanue, 1997b). Comparison of dose-response in a normal population of mice versus a "normal" population of humans therefore results in additional uncertainty. The surrogates (mice) are uniformly immunologically naïve while the human population probably encompasses various degrees of immune sensitization resulting from an individual's response to frequent dietary exposure to *Listeria monocytogenes*.

In laboratory dose-response studies with mice, two methods of administering *Listeria monocytogenes* have been employed. One model uses oral infection of mice as a surrogate for human foodborne exposure. A great deal of additional data for mice are available from studies using the intraperitoneal (IP) infection route. Comparative studies have shown a similar dose-response for oral and IP infections in mice (Golnazarian *et al.*, 1989; Pine *et al.*, 1990). Endpoints in studies with animal surrogates are usually infection or death. Values for these endpoints are usually expressed as median infective dose (ID_{50}) and median lethal dose (LD_{50}). The infective dose in surrogate animals is determined by isolation of the organism from normally sterile sites, typically liver and spleen. It is not known whether this is directly comparable to serious illness in humans; however, this is an implicit assumption when surrogate animal data for this biological endpoint are used. The ID_{50} is influenced by the degree of sensitivity of the isolation method.

One study determined both endpoints (ID_{50} and LD_{50}) following oral dosing of inbred mice (Golnazarian *et al.*, 1989). This approach is useful for determining the relationship between these endpoints. The *Listeria monocytogenes* strain used, F5817, was a human patient isolate, serotype 4b. In this study, ID_{50} was determined by a sensitive 48-hour enrichment method, as well as by culturing directly from tissues. This tends to result in a lower ID_{50} than one determined by direct plating alone.

No dose-response studies of *Listeria monocytogenes* in animal surrogates were found that used gastrointestinal illness as an endpoint or that relied on biomarkers such as fever, neurological, or immune parameters. Therefore, the gastrointestinal endpoint of listeriosis in humans (Dalton *et al.,* 1997) was not included in the dose-response model. Development of quantitative biomarkers of exposure would be useful for establishing comparable endpoints in animals and humans. Although useful in establishing a general dose-response model for severe or lethal listeriosis, attempts to use the mouse model to establish the dose-response for neonatal listeriosis have not produced stillbirth or neonatal infection in mice. This is perhaps related to the differences between rodent and primate placental structure (Golnazarian *et al.,* 1989), and indicates a need to look for more appropriate surrogates. Recently, a primate model of oral infection has been developed (Smith et al., 2003). This model uses stillbirth following oral infection in pregnant Rhesus monkeys as an endpoint, and is currently being used to develop dose-response information. Other oral dose-response studies involving rats (Schlech *et al.*, 1993) and primates (Farber *et al.*, 1991) have also been conducted, but these systems are not as developed as the mouse system. They also lack the extensive genetic and immunological tools that are available in the mouse model.

The recent development of a transgenic mouse model expressing the human form of E-cadherin (an adhesion molecule) on the intestinal mucosa has demonstrated an increase in susceptibility following oral infection (Lecuit *et al*., 2001). This increased susceptibility is apparently based on the enhanced ability of the *Listeria monocytogenes* virulence factor, internalin A, to interact with human E-cadherin versus the normal mouse molecule. This difference is attributable to a single amino acid change in this otherwise

highly conserved molecule (i.e., the molecule is similar across a broad range of different species). If these results are replicated with other strains of *Listeria monocytogenes*, it may lead to significant improvement in the mouse model and point the way to development of other "humanized" transgenic models.

Modeling: Dose-Response in Mice

The relationship between the number of *Listeria monocytogenes* consumed and the occurrence of death (mortality) was modeled by using data obtained from mice with a single strain of *Listeria monocytogenes* (F5817) (Golnazarian *et al*., 1989). In this risk assessment, the effective dose was modified to account for strain variation, host susceptibility surveillance statistics, and differences in susceptibility of laboratory mice in a controlled environment and humans in an uncontrolled environment. Therefore, the mouse model is primarily used to establish the breadth of the range of doses that can cause illness and death. This can be seen in the shape or steepness of the dose-response curve. The animal data were not used to establish the actual doses that cause human illness, which is seen in the scale or relative position of the dose-response curve on the dose axis. As will be described below, actual doses were derived using human health statistics.

For mortality in mice (Figure IV-2), the data came from three different experiments using the same strain (F5817) with comparable results. The data were fit with six different models using the Dose Frequency curve-fitting procedure (see Appendix 6). The best five models (Probit, Exponential, Logistic, Multihit, and Gompertz-Log) were used to characterize the uncertainty in the shape of the dose-response curve. The parameters used for these models are provided in Table IV-2. The exponential model provided the best fit and received the most weight (Figure IV-2).
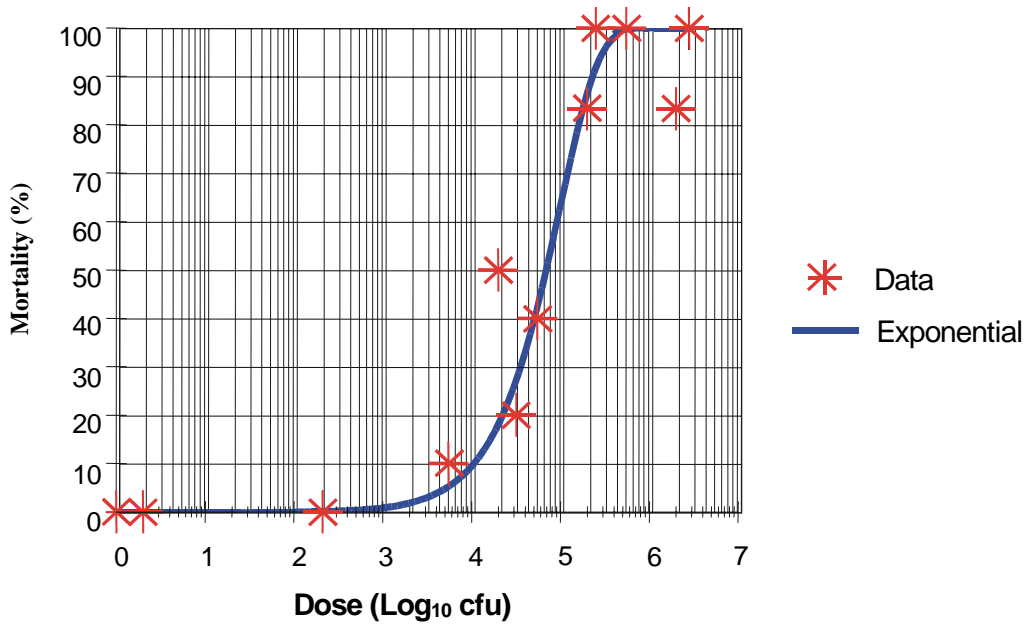
Figure IV-2. *Listeria monocytogenes* Dose vs. Mortality in Mice

Table IV-2. Parameters for the Statistical Distribution Models Used in the Probability Tree for the Mouse Dose-Frequency Relationship

Model	Parameter 1[a]	Parameter 2[a]	RSQ[b]	N[c]	CP[d]
Logistic	-14.7	1.34	0.159	2	0.14
Exponential	0.000011		0.140	2	0.50
Gompertz-Log	-10.47	0.91	0.134	2	0.68
Probit	-8.73	0.80	0.159		0.82
Multihit	0.000008	82	0.132	2	1.00

[a]See Appendix 6: Software for a description of the common names used for the parameters for these statistical distributions (models).
[b]RSQ = Residual Sum of Squares
[c]N = number of parameters
[d]CP = Cumulative Probability

Dose-Response Curves for Infection and Serious Illness

Infection in humans was not modeled in the FDA/FSIS revised risk assessment and serious illness was predicted from dose-response mortality curves. However, for illustrative purposes only, a dose-response curve for infection was developed using mouse data. The data were taken from Golnazarian *et al*. (1989), who described the results of experiments in which mice were infected by the oral route. The data were fit with six different distribution models using the Dose Frequency curve-fitting procedure.

(See Appendix 6 for information about this procedure and more details about modeling and software.) Five distribution models with the best fit (Beta-Poisson, Logistic, Gompertz-Log, and Gompertz-Power, and Gamma-Weibull) were used to characterize the uncertainty in the shape of the dose-response curve; the exponential model was discarded for lack of fit based on visual inspection. The Gompertz-Log model provided the best fit and received the most weight (Figure IV-3). The shape of the curve for infection is very shallow and rises gradually, whereas the curve for lethality (Figure IV-2) rises very sharply. Serious illness and mortality are subsets of infection that primarily correspond to the upper (higher dose) portion of the infection curve. The infection endpoint in mice was based on the detection of viable *Listeria monocytogenes* in one or more internal organs using sensitive methods that cannot be routinely applied to human infections. In human infection, it is not known how the presence of a small number of *Listeria monocytogenes* in tissues correlates with clinical illness. Therefore, because the relationship between infection in mice and the spectrum of clinical illness in humans (invasive, non-invasive, or asymptomatic) is not understood, especially at lower doses, this risk assessment used mortality rather than infection as the endpoint to model human dose-response.
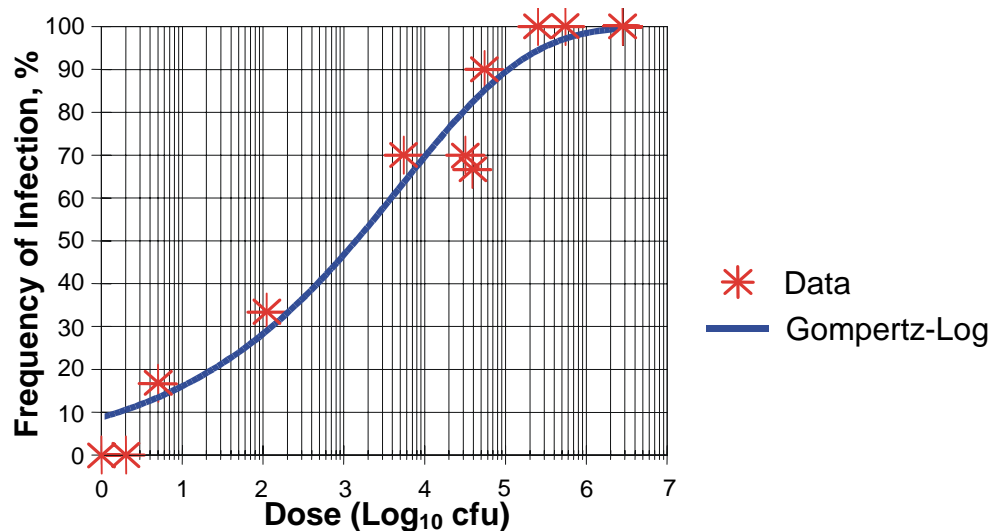
Figure IV-3. Dose vs. Frequency of Infection in Mice

Variability in Virulence

Available Data

Variation in virulence is demonstrable among *Listeria monocytogenes* strains. This variability influences the number of organisms required to produce illness and possibly the severity or manifestations of illness. From a mechanistic perspective, this problem has been extensively investigated, and a large number of virulence components of *Listeria monocytogenes* have been discovered. Studies on *Listeria monocytogenes* virulence have, of necessity, been conducted using well-characterized strains of *Listeria monocytogenes*, selected for the presence or absence of the specific virulence gene of interest. Where animal studies are involved, genetically inbred mouse strains are commonly used. While the use of tightly defined systems (clonal bacteria and genetically identical hosts) is necessary to solve the questions associated with virulence mechanisms, they are not likely to reflect the range of virulence profiles found among naturally occurring, foodborne *Listeria monocytogenes*.

There is also epidemiological evidence for variability in virulence among foodborne isolates of *Listeria monocytogenes*. Most illnesses are associated with a restricted number of serotypes, primarily 1/2a, 1/2b, and 4b. Serotype 4b occurs most frequently in outbreaks (Farber and Peterkin, 1991). In sporadic cases, the same serotypes predominate; however, the frequencies are somewhat different with 1/2a and 1/2b accounting for a higher proportion of cases than 4b (Slutsker and Schuchat, 1999). However, the frequency with which these serotypes are isolated from foods does not parallel the disease distribution. For example, while the 4b and 1/2a serotypes are most frequently associated with foodborne illness, they are not the strains most commonly isolated from foods (Pinner *et al*., 1992). In addition to serotyping, ribotyping has also been used to identify three lineages or groupings of *Listeria monocytogenes* primarily associated with large outbreaks, sporadic cases, or animal disease (Wiedman *et al.*, 1997).

With the complete sequencing of the genome of both *Listeria monocytogenes* and *L. innocua*, tools are now available to completely discover all of the relevant virulence

genes in *Listeria monocytogenes* (Glaser *et al*., 2001). Approximately 270 genes were found to be unique to *Listeria monocytogenes*, and many of these are similar structurally to already discovered virulence factors (Cabanes *et al*., 2002). This information has the potential as the basis for development of genetic tools such as microarrays to further characterize variability in virulence.

Animal surrogate studies also show a range of virulence among food isolates of *Listeria monocytogenes*. Del Corral *et al*. (1990) demonstrated a three-log LD_{50} range of virulence among 13 food isolates (all serotype 1) in immunocompromised mice following intraperitoneal inoculation. In two surveys involving multiple serotypes, Pine *et al*. (1990) and Stelma *et al*. (1987) used oral dosing with normal mice to demonstrate a range of virulence. These studies included clinical isolates, as well as strains lacking known virulence genes (*e. g*., listeriolysin O (LLO)). Major reductions in mouse lethality were seen with strains lacking LLO, but clinical strains did not prove to be consistently more virulent than food isolates with no known human disease association. Where multiple serotypes or ribotypes were compared, there was not a consistent pattern of increased virulence associated with any subtype(s) in animal (Pine *et al*., 1990, Stelma *et al*., 1987) or *in vitro* studies (Pine *et al*., 1991, Weidman *et al*., 1997). Thus, while serotype, phagetype, and ribotype data are valuable epidemiological tools for identifying and tracking outbreaks, they are not mechanistically related to virulence. The predominance of certain subtypes identified in outbreaks may not be related to the presence or absence of known virulence factors. It is possible that allelic differences in virulence genes occur that account for variability in virulence properties (Weidman *et al*., 1997), or that there are as yet unidentified virulence factors. Another consideration is the effect of pathogen adaptation to various ecological niches on the survival and virulence of certain illness-associated subtypes in foods (Boerlin and Piffaretti, 1993).

Finally, while strong circumstantial evidence exists for a predominant role of certain subtypes in human disease, there is demonstrable variation in virulence within these subtypes in animal studies and all serotypes have been associated with at least some human illness. Therefore, animal data were used to model a range of variability in

virulence among *Listeria monocytogenes* isolates, but neither animal nor human outbreak data were used to assign virulence rankings based on sub-types.

Modeling: Variability in Strain Virulence

The extent of the variation in the ability of different *Listeria monocytogenes* strains to cause human disease was based on comparisons made in mice. Specifically, the range of LD_{50} values observed in mice was also used to characterize the range of variation expected in humans. Since the strain used to establish the overall dose-response relationship was not used in any of the studies of strain variability, the model assumes that the shape of the population dose-response function is the same for all strains.

Table IV-3 describes the LD_{50} values from three studies in which *Listeria monocytogenes* was administered to healthy, immunocompetent mice by intraperitoneal injection. The data were used to develop the distributions for the range of strain virulence. Although some of the strains were obtained directly from food, most of the strains tested were clinical isolates. Since members of the latter set were identified because they resulted in disease, the set of strains represented in the sample may be biased towards strains that are more virulent. Virulence in mice ranged over seven logs; however, there were no large or obvious trends in the LD_{50} values relative to either serotype or strain source.

It is possible that the conditions under which strains are held in the laboratory can affect strain virulence. The Scott A strain, one of the clinical strains tested and found to have relatively low virulence, has been cultured for use in laboratory studies for many years. This may have allowed the accumulation of new and different mutations in the laboratory strain, which would not have occurred in the strain in nature, creating differences in virulence in the laboratory and environmental strains. Other strains may have also been altered in this way. In this instance, the effect would be to bias the set of strains represented in the sample toward strains that are less virulent.

Table IV-3. LD$_{50}$ Values for Various *Listeria monocytogenes* Strains Following Intraperitoneal Injection in Normal Mice

Strain	Serotype	Source	LD$_{50}$ (Log$_{10}$ cfu)[a]	Reference
G9599	4	clinical	2.57[a]	Pine *et al.*, 1990
G1032	4	clinical	2.69[a]	Pine *et al.*, 1990
G2618	1/2a	food	2.89[a]	Pine *et al.*, 1991
F4244	4b	clinical	3.62	Pine *et al.*, 1991
F5738	1/2a	clinical	3.67	Pine *et al.*, 1990
F6646	1/2a	clinical	4.49	Pine *et al.*, 1990
15U	4b	clinical	4.56	Pine *et al.*, 1991
F4246S	1/2a	clinical	4.57	Pine *et al.*, 1991
F7208	3a	clinical	4.61	Pine *et al.*, 1990
G2228	1/2a	clinical	4.66[a]	Pine *et al.*, 1990
F2381	4b	food	4.73	Pine *et al.*, 1991
G2261	1/2b	food	4.95[a]	Pine *et al.*, 1991
F2380	4b	food	4.96[a]	Pine *et al.*, 1990
F2392	1/2a	clinical	5.08	Pine *et al.*, 1990
NCTC 7973	1/2a	clinical	5.47[a]	Pine *et al.*, 1991
F7243	4b	clinical	5.75[a]	Pine *et al.*, 1990
F7245	4b	clinical	5.91[a]	Pine *et al.*, 1990
SLCC 5764	1/2a	clinical	6.00	Pine *et al.*, 1991
V37 CE		food	6.04	Stelma *et al.*, 1987
F7191	1b	clinical	6.23	Pine *et al.*, 1991
V7		food	6.80	Stelma *et al.*, 1987
Brie 1		food	7.28	Stelma *et al.*, 1987
Murray B		clinical	7.30	Stelma *et al.*, 1987
Scott A	4b	clinical	7.54	Stelma *et al.*, 1987
G970	1/2a	clinical	8.88	Pine *et al.*, 1991
NCTC 5101	3a	clinical	9.70	Pine *et al.*, 1991

[a] These LD$_{50}$ (50% of the lethal dose) values are averages from multiple experiments.

Table IV-4 presents the results of a study by Pine *et al.* (1990) in which *Listeria monocytogenes* was administered by intraperitoneal injection and intragastric gavage. For some strains, the intraperitoneal route was more effective (lower LD$_{50}$), and for other strains, the intragastric route was more effective. To facilitate comparison, the log$_{10}$ of the ratio of the intragastric LD$_{50}$/ intraperitoneal LD$_{50}$ was calculated. The median value for the log$_{10}$ ratios was positive, indicating that the IP values may slightly overestimate intragastric LD$_{50}$ by approximately a half log$_{10}$.

Table IV-4. Effect of Route of *Listeria monocytogenes* Administration (Intragastric vs. Intraperitoneal) on Mouse LD$_{50}$

Strain	Serotype	Source	Log$_{10}$ ratio [a] (intragastric/intraperitoneal)
F2380	4b	food	-1.81
F7243	4b	clinical	-0.75
F7245	4b	clinical	-0.47
G2228	1/2a	clinical	0.00
G2261	1/2b	food	0.00
NCTC 7973	1/2a	food	0.04
F6646	1/2a	clinical	0.21
F2380	4b	food	0.71
G9599	4	clinical	0.96
G1032	4	clinical	1.60
F5738	1/2a	clinical	1.81
G2618	1/2a	food	2.00

[a] All data from Pine *et al*., 1990. A Log$_{10}$ ratio of 0 indicates that the LD$_{50}$ by the two routes were identical. A negative number indicates a lower LD$_{50}$ (50% of the lethal dose) by the intragastric route, while a positive number indicates a greater LD$_{50}$ by the intraperitoneal route.

Data shown in Table IV-3 were modeled by fitting nine distributions with ParamFit (see Appendix 6). Figure IV-4 displays all nine distributions. The best four models (Triangular, Gramma, and Lognormal) were used to characterize the dose-response model uncertainty associated with the distribution. The parameters used for these models are provided in Table IV-5. Output from the resulting function is given in Table IV-6 and describes the extent of virulence variability in determining dose-response. Since the virulence estimated from the distribution was from intraperitoneal doses, the estimated LD$_{50}$ was increased by 0 to1 logs (uncertainty range) to produce an estimated intragastric LD$_{50}$.
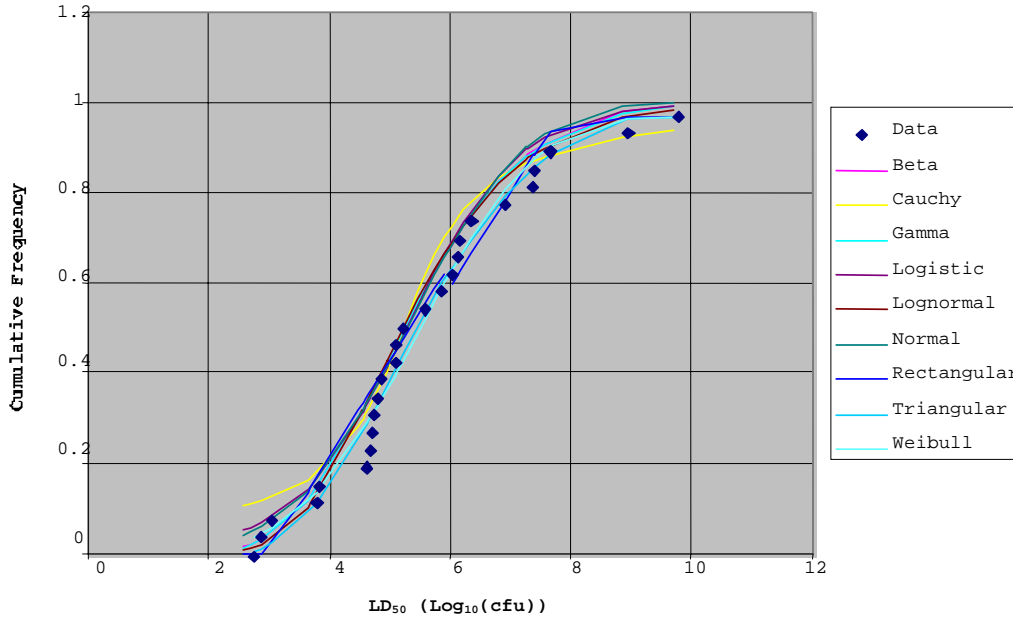
Figure IV-4. Variation (Cumulative Frequency) of *Listeria monocytogenes* Strain Virulences: Nine Distributions

Table IV-5. Parameters for the Statistical Distribution Models Used in the Probability Tree for Variation in Strain Virulence

Model	Parameter 1[a]	Parameter 2[a]	Parameter 3[a]	RSQ[b]	N[c]	CP[d]
Triangular	2.09	4.80	9.19	0.037	2	0.30
Gamma	12.0	0.440		0.037	2	0.58
Lognormal	1.65	0.289		0.038	2	0.83
Logistic	5.29	0.92		0.041	2	1.00

[a]See Appendix 6: Software for a description of the common names used for the parameters for these statistical distributions (models)
[b]RSQ = Residual Sum of Squares
[c]N = number of parameters
[d]CP = Cumulative Probability

Table IV-6. Model Output for *Listeria monocytogenes* Strain Virulence

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

[a] LD_{50} is the dose with a 50% mortality.

Host Susceptibility

Available Data

Susceptibility in Humans and Animal Surrogates

Variation in susceptibility to listeriosis among people exists. This influences the number of organisms required to produce illness and the type of illness produced. Information on susceptibility for this risk assessment was taken from epidemiology and case reports of conditions that predispose to infection, as well as studies with animal surrogates on the role of host defense components in susceptibility to *Listeria monocytogenes* infection.

Immunosuppression in Humans and Animal Surrogates

With respect to immune function, dose-response information related to susceptibility in humans must be gleaned from surveillance and other epidemiological data. Again, animals are potentially useful surrogates. The approach used was to identify biomarkers of susceptibility that reflect defects in immune mechanisms in both human populations and in animal surrogates. This approach is based on the premise that human and animal resistance mechanisms are similar. The mouse *Listeria monocytogenes* animal model was characterized with respect to the role of many specific immune defects. Host resistance mechanisms to *Listeria monocytogenes* have been studied using a variety of immune-compromised mouse models. These animal models include "gene knockout animals" in which genes for specific immune functions are disrupted. Other surrogate animal models involve depletion of cytokines or immune cells with monoclonal antibodies, and mouse strains with genetic defects related to macrophage-mediated killing of *Listeria monocytogenes* (Czuprynski and Brown, 1986; Cheers and McKenzie, 1978, Unanue, 1997a*).*

In mouse models of *Listeria* infection, certain inbred mouse strains exhibit increased susceptibility. Mouse strains C57BL10 and BL6 are relatively more resistant than Balb/c and A/J. The genetic basis of this resistance is distinct from *Nramp I* and involves2 loci on chromosomes 5 and 13, and possibly other loci as well (Kramnik and Boyartchuk, 2002). The exact mechanism is unknown, but appears to involve a defect in the ability of susceptible strains to form granulomas around foci of infection in the liver (Boyartchuk *et*

al., 2001). In addition, mapping has revealed distinct T cell epitopes recognized by Balb/c and C57BL strains (Geginat *et al.*, 2001). It is probable that similar differences exist among the genetically diverse human population.

Pregnant Women. Within some susceptible human populations, immune system defects or alterations that correlate with resistance in mouse models have been identified. In pregnancy, there is a characteristic inhibition of natural killer (NK) cell activity in the placenta (Schwartz, 1999). In the mouse, these NK cells, stimulated by Interleukin 12, are the primary source of interferon, which is a key component of resistance (Unanue, 1997a; Tripp et al, 1994). Pregnancy is also associated with development of a T-helper cell type 2 (Th-2) cytokine environment which favors the production of Interleukins 4 (IL-4) and 10 (IL-10) (Schwartz, 1999). Immune defects in the mouse, which simulate immune status alterations occurring in pregnancy impact negatively on resistance (Nakane *et al.,* 1996; Genovese *et al.,* 1999). Cytokines characteristic of a T-helper cell Type 1 (Th-1) response (*e. g.*, interferon) are critical for resistance (Unanue, 1997a, 1997b; Tripp *et al.*, 1994; Huang *et al.,* 1993). Listeriosis symptoms in pregnancy are often mild (Slutsker and Schuchat, 1999) suggesting that pregnancy may not predispose mothers to more severe illness. However, it is possible that immunosuppression as a consequence of pregnancy results in increased likelihood that even small numbers of *Listeria monocytogenes* in the circulation can colonize placental tissues, increasing the chances of fetal exposure. Because the fetus has a poorly developed immune system and is immunologically naïve with respect to *Listeria monocytogenes*, the consequences of fetal exposure are severe, often resulting in stillbirth or neonatal infection.

Elderly and Neonates. At the extremes of age, (neonates and the elderly), changes in both innate and acquired immunity have been observed. Numerous biomarkers of immune responsiveness have been measured in the elderly including decreased γ-interferon production, NK cell activity, and increased IL-4 and IL-10 production (Rink *et al.,* 1998; Mbawuike *et al.,* 1997; Di Lorenzo *et al.,* 1999). The effects on IL-4 and IL-10 are suggestive of a predominant Th-2 vs. Th-1 response. A similar imbalance, characterized by decreased interferon production and increased production of IL-10 may occur in

neonates (Lewis *et al.,* 1986; Genovese *et al.,* 1999). Thus, in the elderly and during pregnancy, as well as in neonatal immune systems, biomarkers can be documented that correlate with decreased resistance in mouse models having the same immune defect(s). Relatively few mouse studies investigate dose-response in an oral infection model in immunocompromised mice (Czuprynski *et al.,* 1996; Golnazarian *et al.,* 1989).

Cancer, Transplant, and AIDS Patients. As with pregnant women, neonates, and the elderly, there are immune defects that occur in AIDS patients, cancer patients, and organ transplant recipients. These may involve not only depletion of T-lymphocytes, but also neutropenia (depletion of neutrophils) as a result of immunosuppressive medications (Morris and Potter, 1997). Severe neutropenia would be expected to result in greatly increased susceptibility as has been demonstrated in mouse studies in which neutrophils are experimentally depleted (Czuyprynski *et al.*, 1996).

Because the experimental studies all involve highly controlled manipulation of the immune system, it is very difficult to translate their results to a highly variable, uncontrolled human population. However, because relative change in susceptibility could be determined, these compromised mouse studies were used in aggregate to set limits or bounds for a maximal degree of increased susceptibility due to immunosuppression. The validity of this approach is based upon the concept that host-resistance mechanisms targeted in animal studies are connected with human biomarkers of exposure and susceptibility. It is important to note, however, that knockout mice or treatment with monoclonal antibodies both reflect a near complete abrogation of the immune parameter in question, which is probably not the case in most humans. In addition, most of these targeted immunocompromised animal model systems have not been tried with oral infection.

Non-Immune Factors Affecting Susceptibility

While susceptibility in these groups is thought to be related primarily to impaired immune function, another physiologic parameter thought to be relevant to susceptibility is a reduced level of gastric acidity. Reduced gastric acidity (achlorhydria) may be

associated with aging or with drug treatment for gastric hyperacidity. Another factor responsible for reduction in gastric acidity in humans is infection with another bacterium, *Helicobacter pylori (*Feldman *et al*., 1999*)*. Two dose-response studies dealing with this issue involved treatment of mice or rats with the acid suppressor, Cimetidine, concurrent with oral infection with *Listeria monocytogenes*. The mouse study showed no significant effect with drug treatment (Golnazarian *et al.*, 1989), while the rat study showed increased infectivity of *Listeria monocytogenes* at the lowest dose (Schlech *et al.*, 1993). Because of the conflicting nature of these reports, and lack of additional information, no dose modification factor was included for gastric acidity.

Modeling: Host Susceptibility

Variation in host susceptibility was represented with triangular distributions that modified the effective dose for individual servings. In order to represent populations with different ranges of susceptibility, three alternative triangular distributions were applied to generate three different effective dose estimates. The distributions all had a minimum value of -1 and a median value of 0, so that the net effect of the host susceptibility adjustment was to broaden the distribution of effective doses without greatly altering the midpoint. The maximum values were 1.5, 3.0, and 4.5 $\log_{10}$ cfu for the Low, Medium, and High Variability distributions, respectively (see Table IV-7). In addition, the uncertainty in the tails of the frequency distributions were assigned uncertainty ranges using rectangular distributions, so that there was overlap in the uncertainty ranges of the three frequency distributions. A single random number was used to select the values for the tails, so that a low uncertainty percentile selects a narrow distribution, while a large uncertainty percentile results in a wide distribution.

Table IV-7. Parameters for Variability Distributions for Host Susceptibility for Listeriosis

Distribution	Minimum	Most Frequent	Maximum
Low Variability	-1 to 0	0	0 to 1.5
Medium Variability	-1 to 0	0	1 to 3
High Variability	-1 to 0	0	2.5 to 4.5

The three distributions encompass the range of susceptibility that has been observed in animal studies (see section titled 'Modeling: Dose-Response in Surrogates'). In conjunction with a population-specific dose-response scaling factor (see section titled "Dose-Response Scaling Factor"), these distributions may be used to create a unique dose-response function for a particular subpopulation. The selection of one of the three distributions for a particular population will depend on the relative homogeneity of the population being modeled. If the population is thought to be nearly as homogeneous as a population of laboratory mice, the Low Variability adjustment would be the most appropriate (one tail of the uncertainty distribution gives an overall modification of 0, implying that the population is as homogeneous as a population of laboratory mice). A population thought to include both highly susceptible and individuals displaying a normal degree of resistance, but still within the ranges documented in the animal studies would mandate the Medium Variability adjustment. Speculation that the range of susceptibility may exceed ranges in the animal studies may be expressed by using the High Variability adjustment.

Dose-response functions for specific subpopulations were developed by altering the dose-response scaling factor by 0.25 $\log_{10}$ increments so that the median estimate roughly predicted the number of annual cases estimated from surveillance data, given the number of servings consumed for each food category, and distribution estimates of effective dose in either the Low, Medium, or High Variability populations. The model output for the host susceptibility, showing the distributions for the low, medium, and high variability adjustments is provided in Table IV-8.

Table IV-8. Model Output for Variability Adjustment Factors for Host Susceptibility to Listeriosis

Percentiles	Low Variability Adjustment[a] (Log$_{10}$ cfu)	Medium Variability Adjustment[a] (Log$_{10}$ cfu)	High Variability Adjustment[a] (Log$_{10}$cfu)
1st	-0.4 (-0.8, -0.1)	-0.4 (-0.8, 0.0)	-0.4 (-0.7, 0.0)
5th	-0.3 (-0.6, 0.0)	-0.3 (-0.5, 0.0)	-0.2 (-0.4, 0.0)
10th	-0.3 (-0.5, 0.0)	-0.1 (-0.3, 0.0)	-0.1 (-0.2, 0.1)
25th	-0.1 (-0.2, 0.0)	0.1 (0.0, 0.1)	0.3 (0.2, 0.3)
Median	0.1 (0.0, 0.1)	0.4 (0.3, 0.5)	0.9 (0.7, 1.0)
75th	0.3 (0.0, 0.5)	0.9 (0.5, 1.2)	1.6 (1.3, 2.0)
90th	0.4 (0.0, 0.8)	1.3 (0.7, 1.8)	2.3 (1.8, 2.9)
95th	0.5 (0.1, 1.0)	1.5 (0.8, 2.2)	2.7 (2.0, 3.3)
99th	0.7 (0.1, 1.2)	1.8 (1.0, 2.6)	3.1 (2.3, 3.9)

[a] The median value is presented. The 5th and 95th uncertainty values are given in parenthesis.

High variability host susceptibility distributions were used for the intermediate-age and elderly subpopulations since the members of these subpopulations most probably exceed the range of physiological states characterized by the animal research. Because the susceptibilities of individuals within the elderly subpopulation or immunocompromised individuals within the intermediate-aged subpopulation may be varied, wider ranges are assigned to these groups. The neonatal dose-response functions were based on the medium variability distributions since the basis of categorization does not occur as a matter of degree. Because the adjustments were somewhat dose-response model-dependent, the adjustment is expressed as a range.

Dose-Response Scaling Factor

The relationship between dose and response (or cause and effect) is often complex and is often influenced by many different parameters. Some of these parameters (or causative factors), such as virulence variability, have quantitative data that can be incorporated into the model. However, there are a variety of host and food matrix factors that could potentially influence *Listeria monocytogenes* dose-response, but these have either not been identified or no data are available. As a result, a single additional parameter, the dose-response scaling factor, was used to account for these influences, and thus bridge the relationship between the response in humans versus surrogate animals. Without this

adjustment, the mouse dose-response model, when coupled with the exposure assessment model, greatly overestimates the incidence of lethal infections in humans from *Listeria monocytogenes*.

The dose-response curve derived from the mouse study estimates that the LD_{50} is about 4.26 logs or 20,000 cfu. The food contamination data indicate that human exposure to this number of *Listeria monocytogenes* is relatively frequent. If the mouse dose-response model were directly applicable to humans, the dose-response model would overestimate the number of human deaths due to listeriosis by a factor of over one million. This indicates that normal human beings are much less susceptible to *Listeria monocytogenes* than laboratory mice. There are a number of factors that may be responsible for the difference in susceptibility between humans and mice, any or all of which may contribute:

- Inherent differences between mice and humans: Factors, such as body mass, metabolic rate, body temperature, or gastrointestinal physiology may contribute to differences.
- Immunity: Humans are more likely to have had prior exposure to low levels of *Listeria monocytogenes* that may serve to develop immunity to challenges with larger numbers.
- Route of exposure: The *Listeria monocytogenes* dosing in the animal studies was not introduced by the dietary consumption route. The consumption of *Listeria monocytogenes* in food may reduce its ability to penetrate the intestine.
- Strain bias: The strains surveyed in mice may be more virulent than those typically encountered in food.
- Food matrix effects: The physico-chemical nature of a *Listeria monocytogenes*-contaminated food may vary depending on fat content or other factors.
- Exposure: Some fraction of the dose-response scaling factor may result from overestimate of the occurrence and growth of *Listeria monocytogenes* in the exposure assessment. This occurs because the development of a dose-response

scaling factor includes using the exposure assessment result as an estimate of dose
along with the epidemiological incidence.

Since there are no available quantitative data related to *Listeria monocytogenes* for the
factors listed above, a dose-response scaling factor (referred to as a scaling factor) was
developed to correct the mouse-derived model so that it was applicable to humans. The
size of this factor is determined by surveillance data reported to FoodNet for each of the
three subpopulations modeled in this risk assessment. Differences among subpopulations
may mainly be attributed to the first two factors listed above (i.e., inherent differences
between mice and humans, and immunity). Thus, while the shape of the dose-response
curve is initially derived from mice, the scale is determined by the human epidemiology.
The range of dose-response scaling factors for each of the three subpopulations is
provided in Table IV-9.

**Table IV-9. Model-Dependence of the *Listeria monocytogenes* Dose-Response Scaling
Factor Ranges for the Three Subpopulations**

Subpopulation	Dose-Response Scaling Factor (Log_{10} cfu)		
	Median	5th Percentile	95th Percentile
Intermediate-Age	12.8	11.1	15.9
Neonatal[a]	9.0	7.9	11.6
Elderly	11.4	10.1	14.3

[a] An adjustment to account for total perinatal deaths (prenatal and neonatal) is described in
the risk characterization section.

This single dose-response scaling factor is used to account for all of the factors listed
above, as well as any others not yet identified. In the future, it may be possible to give
specific attribution to particular influences such as the food matrix or the development of
immunity. Because the dose-response scaling factor was selected to ensure that the dose-
response model, combined with the exposure assessment, is consistent with available
public health data, new information about initial *Listeria monocytogenes* contamination
levels, growth rates, strain virulence, host susceptibility, or the annual number of reported
cases would affect the magnitude of the scaling factor. A demonstration of this effect can
be found in the hazard characterization section entitled 'Modeling: Outbreak Data."

Estimating Listeriosis Rates in Susceptible Subpopulations

FoodNet surveillance data from the CDC were used to help determine the relative susceptibility of sensitive subpopulations. Figure IV-5 shows listeriosis incidence by age using 1999 FoodNet data (CDC, 2000a) and Table IV-10 shows the number of listeriosis isolates by age and the total number of *Listeria monocytogenes* isolates per year from FoodNet from 1997 to 2000 (CDC, 1998a, 1999a, 2000a; Wong, 2000; Lay, 2001).

Mead *et al.* (1999), adjusting for underreporting, estimated that there were 2,493 cases including 499 deaths due to foodborne listeriosis using 1996-97 surveillance data and extrapolating to the 1997 total United States population. This estimate of the total foodborne illness was made by adjusting the number of reported cases to account for underreporting and estimating the proportion of illnesses specifically attributed to foodborne transmission. To calculate for underreporting (the difference between the number of reported cases and the number of cases that actually occur in the community), a multiplier of two was used based on the assumption that *Listeria monocytogenes* typically causes severe illness and one out of every two cases would come to medical attention. More information about FoodNet is available in Appendix 4.
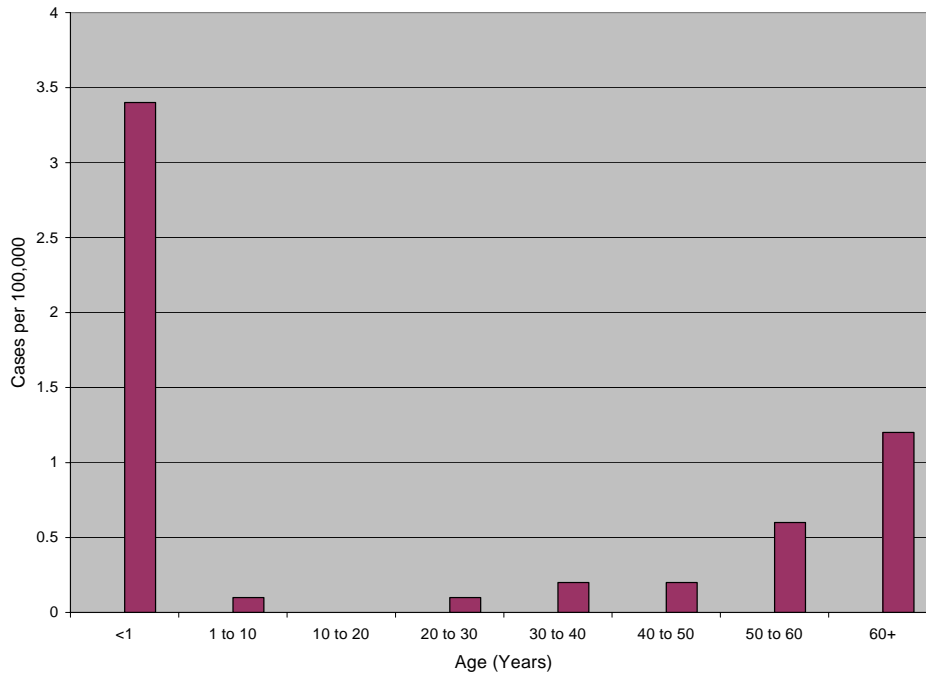
Figure IV-5. 1999 FoodNet Estimates of Listeriosis Incidence, by Age

Table IV-10. Number of *Listeria monocytogenes* Isolates by Patient Age and Year of Occurrence

Patient Age	Number of *Listeria monocytogenes* isolates			
	1997[a]	1998[b]	1999[c]	2000[d]
< 1 year old[e]	5	10	12	13
1 to 9 years old	2	1	3	2
10 to 19 years old	1	2	1	4
20 to 29 years old	3	6	5	2
30 to 39 years old	9	13	7	10
40 to 49 years old	6	6	8	8
50 to 59 years old	9	13	16	4
≥ 60 years old	42	61	48	62
Unknown age	0	0	14	0
Total	77	112	114	105

[a] CDC, 1998a (from five states).
[b] CDC, 1999a (from seven states).
[c] CDC, 2000a,d (from seven states) and Wong, 2000 (Unpublished data).
[d] Lay, 2001
[e] All of these cases were less than 30 days old.

Illness-Mortality Ratios

FoodNet data was used to estimate the numbers of serious illness relative to the number of deaths. The illness-mortality ratio was population specific (Table IV-11), and was used to estimate the number of serious illnesses (including deaths) in the Risk Characterization section. Because this conversion factor is applied after the final step in the modeling process, it affects the absolute number of listeriosis cases attributable to a given food category, but not the relative risk ranking of the food categories. The use of a conversion factor to estimate serious illness, rather than modeling illness as an endpoint is confounded by at least two recognized problems: 1) The steepness of the infectious dose-response curve in mice is much less than that for mortality so that the factor in humans may be different at various doses, and 2) if the variation in susceptibility among the three age-based groups is assumed to be different, the ratio of serious illness to mortality may also be different among these groups. Nevertheless, because the conversion factor used is based on surveillance data, it implicitly incorporates these and other uncertainties and reflects the overall relationship between serious illness and mortality across the entire dose range.

Table IV-11. Reported and National Annual Projections for Severe Listeriosis, Based of FoodNet Reports

Sub-Population	National Projected Annual[a]		FoodNet Reported 4-Year Total[b]		Illness: Mortality Ratio[c]
	Cases of Listeriosis[d]	Deaths	Cases of Listeriosis[d]	Deaths	
Neonatal	216	16[e]	38	3	12.7
Intermediate	702	67	113	10	11.3
Elderly	1159	307	194	52	3.7
TOTAL	**2078**	**390**	**345**	**65**	

[a]Adjusted cases and deaths for the total population (average of 4 years FoodNet data).
[b]Reported total cases and deaths for the FoodNet catchment areas (4 year total)
[c]The mortality: illness ratio is calculated using the reported cases and deaths in the FoodNet catchment area, i.e., deaths divided by cases.
[d] Serious cases of listeriosis requiring hospitalization.
[e] Perinatal deaths = 40. Deaths for the perinatal group are calculated by multiplying the death for neonatal by 2.5 to account for abortions and stillbirths not reported in FoodNet surveillance reports. See description of the neonatal dose-response curve below.

The estimates of cases of listeriosis and deaths shown in Table IV-11 are based on the average number of reported cases from CDC's FoodNet surveillance from 1997 to 2000. The projections are corrected for the percentage of the nation covered by FoodNet (6 to 11%) and include a factor of 2 to account for underreporting so that it is consistent with the CDC estimates.

Results: Dose-Response Curves for Three Population Groups

Intermediate-Age Dose-Response Curve

After applying the virulence distribution (Table IV-2) to the mouse dose-response mortality curve (Figure IV-2), the dose-response scaling factor is used to shift the curve towards higher doses necessary for lethality estimates similar to surveillance data. Figure IV-6 depicts the results of applying this factor to the intermediate-age subpopulation. It describes the dose required to produce death from a series of servings contaminated with different (or variable) *Listeria monocytogenes* strains. The range of values (indicated by the lower and upper bound lines) accounts for the uncertainty from three primary sources: 1) variation in the virulence of different strains; 2) uncertainty in the host susceptibility among individuals within this population; and 3) uncertainty in the exposure to *Listeria monocytogenes*.

An example of how the dose-response curve relates exposure to public health impact can be examined using Figure IV-6 as an example. By selecting a dose from the x-axis, an estimated death rate can be read off the y-axis. For example, at a dose of 1×10^{10} cfu/serving, the dose-response model predicts a median death rate of 1 in 769,231 servings. The uncertainty results in a lower bound prediction of 1 death in 40 trillion servings and an upper bound prediction of 1 in approximately 6,667 servings. Similar predictions can be made for any other dose. At higher predicted mortality rates, the number of bacteria necessary to attain that level of mortality is above the practical upper limit that would be encountered in foods. For example, doses greater than 10^9 to 10^{10} cfu/serving exceed the populations of *Listeria monocytogenes* attainable in food.
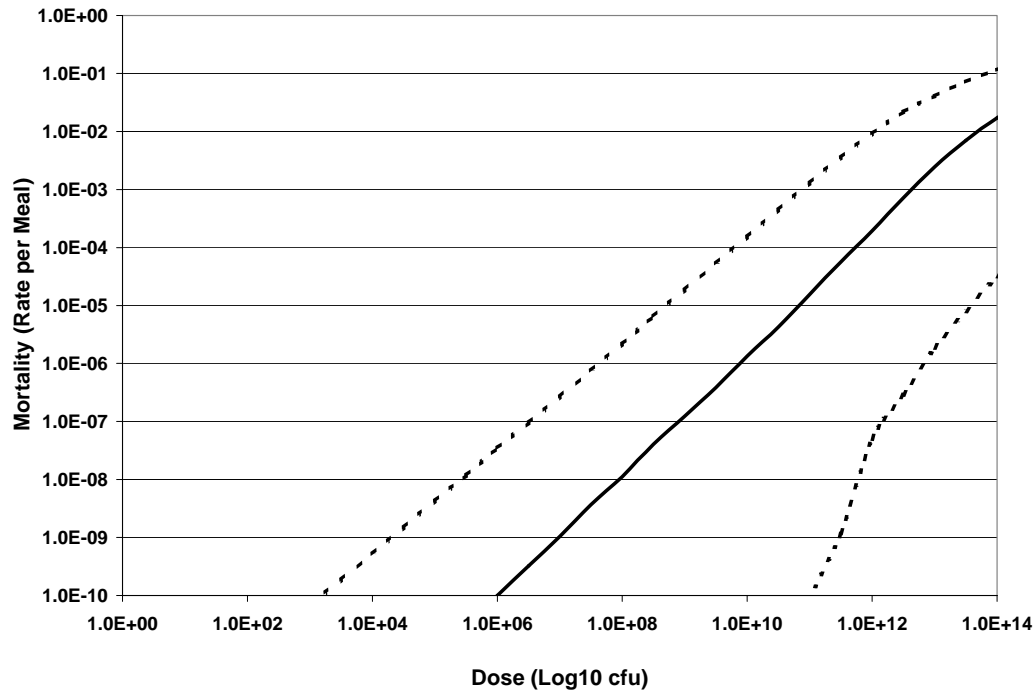
Figure IV-6. *Listeria monocytogenes* **Dose-Response for Mortality with Variable Strain Virulence for the Intermediate-Age Subpopulation**

Neonatal/Perinatal Dose-Response Curve

Figure IV-7 depicts the neonatal subpopulation dose-response curve. It describes the dose required to produce death from a series of servings, consumed maternally, that are contaminated with different (or variable) *Listeria monocytogenes* strains. The distribution (indicated by the lower and upper bound lines) accounts for the uncertainty from three primary sources: 1) variation in the virulence of different strains; 2) uncertainty in the host susceptibility among pregnant women; and 3) uncertainty in the exposure to *Listeria monocytogenes*.

By selecting a dose from the x-axis, the expected death rate can be read off the y-axis. For example, at a dose of 1 x 10^{10} cfu/serving, the dose-response model predicts a median death rate of 1 in 667 servings. However, the uncertainty introduced by the variability in virulence and in host susceptibility results in a lower bound prediction of 1 death in

303,030 servings and an upper bound prediction of 1 death in approximately 37 servings. Similar predictions can be made for any dose.
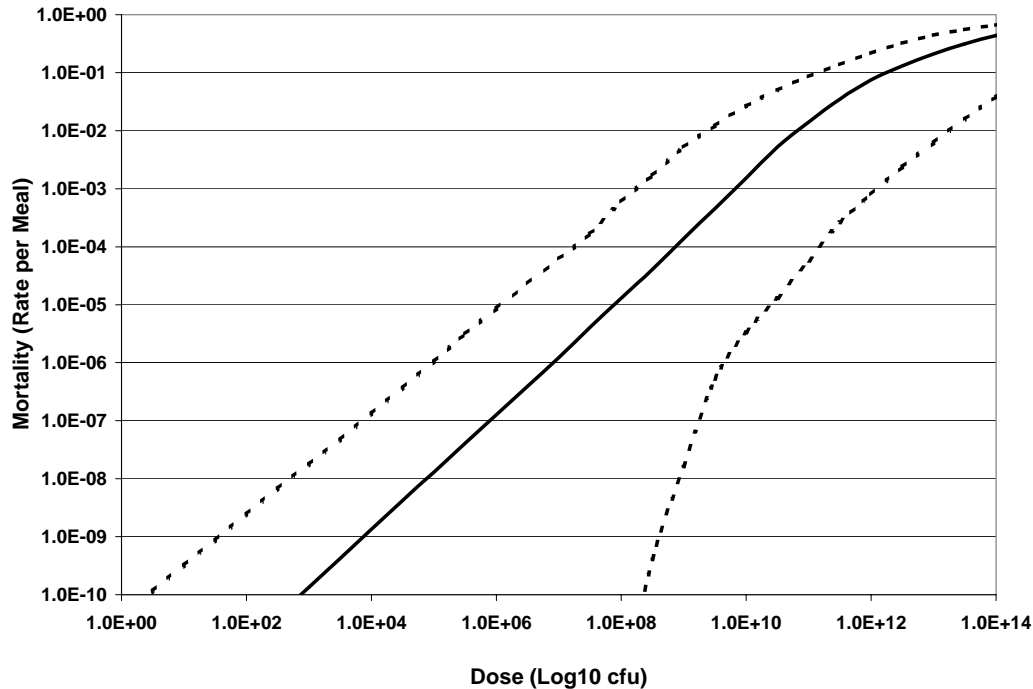
Figure IV-7. *Listeria monocytogenes* **Dose-Response for Mortality with Variable Strain Virulence for the Neonatal Subpopulation**

Data reported to FoodNet are the only national data available for estimating cases of neonatal infection and death but these data do not consistently record fetal deaths. To compensate for underreporting of death rates, data from the County of Los Angeles Department of Health Services mandatory listeriosis reporting system were used to estimate the proportion of prenatal infections that resulted in premature termination of pregnancy. These data provided detailed patient information concerning *Listeria monocytogenes* isolates from clinical laboratories indicating that the combined prenatal and neonatal deaths (perinatal deaths) were 2.5 times the neonatal deaths (Buchholz, 2000). Therefore, the number of perinatal deaths was calculated by multiplying the neonatal deaths by 2.5. [Note: The perinatal deaths include both prenatal and neonatal.] However, because non-lethal infections do not result in prenatal hospitalizations, this multiplier was not used to estimate the number of perinatal cases of listeriosis.

Elderly Dose-Response Curve

Figure IV-8 depicts the elderly subpopulation dose-response curve. It is intended to describe the dose (in colony forming units) required to produce death from a series of servings that are contaminated with different (or variable) *Listeria monocytogenes* strains. The range of values (indicated by the lower and upper bound lines) accounts for the uncertainty from three primary sources: 1) variation in the virulence of different strains; 2) uncertainty in the host susceptibility among individuals within this population; and 3) uncertainty in the exposure to *Listeria monocytogenes*.

By selecting a dose from the x-axis, the expected death rate can be read off the y-axis. For example, at a dose of 1×10^{10} cfu/serving, the dose-response model predicts a median death rate of 1 in 25,641 servings. However, the uncertainty results in a lower bound prediction of 1 death in 1.7 billion servings and an upper bound prediction of 1 death in approximately 588 servings.

Table IV-12 provides a summary of the data presented in the preceding figures for the intermediate-aged, neonatal, and elderly subpopulations. The death rate per serving is presented as the median and the upper (95th) and lower (5th) boundaries of the uncertainty. The data in Table IV-12 show a 20-fold decrease in the dose necessary to cause death from listeriosis for the elderly subpopulation compared to the intermediate-aged population. The intermediate-aged population does contain individuals with immunocompromising diseases or treatments. The neonatal population is approximately 10,000-fold more sensitive than the intermediate-aged population.
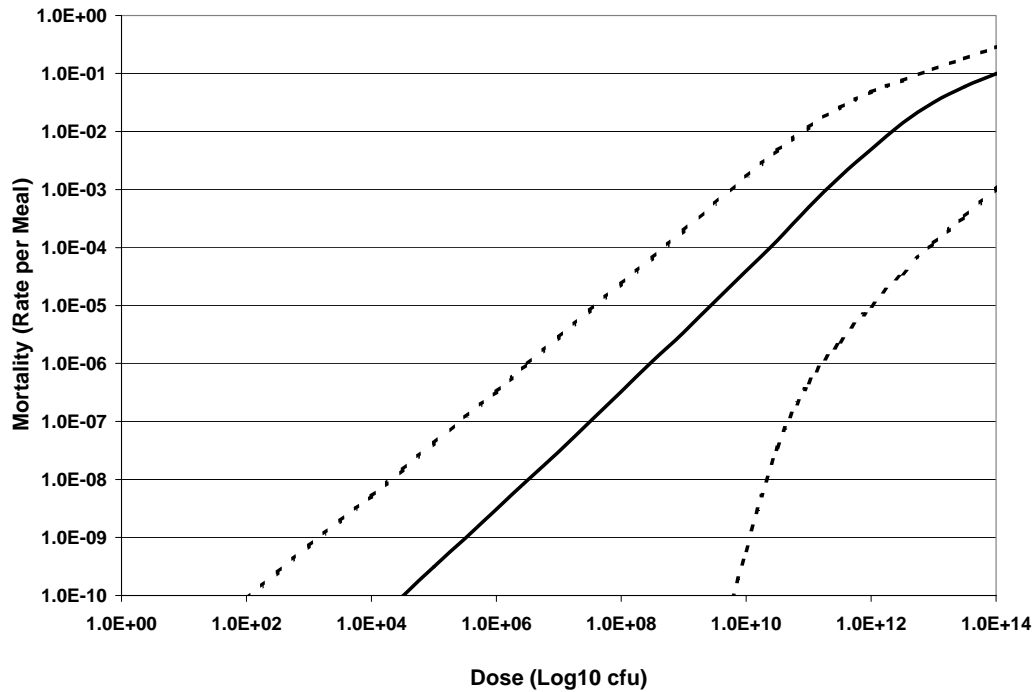
Figure IV-8. *Listeria monocytogenes* **Dose-Response for Mortality with Variable Strain Virulence for the Elderly**

Table IV-12. Dose-Response with Variable *Listeria monocytogenes* Strain Virulence for Three Age-Based Subpopulations

Dose (cfu/serving)	Median Mortality Rate per Serving[a]		
	Intermediate-Age	Neonatal[b]	Elderly
1	1.5×10^{-16} (1.2×10^{-146}, 1.9×10^{-13})	1.6×10^{-13} (1.2×10^{-99}, 4.0×10^{-11})	4.0×10^{-15} (6.3×10^{-124}, 1.8×10^{-12})
10^3	1.2×10^{-13} (5.4×10^{-92}, 6.8×10^{-11})	1.3×10^{-10} (4.3×10^{-56}, 1.7×10^{-8})	3.6×10^{-12} (2.2×10^{-74}, 7.2×10^{-10})
10^6	1.0×10^{-10} (1.9×10^{-50}, 3.5×10^{-8})	1.3×10^{-7} (1.2×10^{-25}, 8.6×10^{-6})	3.1×10^{-9} (5.7×10^{-38}, 3.3×10^{-7})
10^9	1.2×10^{-7} (6.0×10^{-22}, 1.9×10^{-5})	1.4×10^{-4} (1.6×10^{-8}, 5.1×10^{-3})	3.4×10^{-6} (1.3×10^{-14}, 1.9×10^{-4})
10^{10}	1.3×10^{-6} (2.5×10^{-15}, 1.5×10^{-4})[c]	1.5×10^{-3} (3.3×10^{-6}, 2.7×10^{-2})	3.9×10^{-5} (6.0×10^{-10}, 1.7×10^{-3})
10^{12}	1.9×10^{-4} (4.9×10^{-8}, 9.2×10^{-3})	7.4×10^{-2} (7.8×10^{-4}, 2.2×10^{-1})	4.9×10^{-3} (9.8×10^{-6}, 4.8×10^{-2})

[a] The 5th and 95th percentiles from the uncertainty are in parenthesis.
[b] An adjustment to account for total perinatal deaths (prenatal and neonatal) is in the risk characterization section.
[c] The median mortality rate per serving of 1.3×10^{-6} for the intermediate-age subpopulation at the 10^{10} cfu/serving dose level, corresponds to 1 death in approximately 769,231 servings ($1/1.3 \times 10^{-6}$).

Dose-Response for an Epidemic with an Unknown Strain

Figure IV-9 represents the dose-response relationship for an epidemic with a single strain
of unknown virulence. This simulation treated the strain virulence as a source of
uncertainty, rather than as a source of variability that contributed to the rate. This is
because a single strain has a single virulence rate (therefore, no variation); however, it is
not known what that the actual rate is (therefore, there is uncertainty). As a result the
slope is somewhat steeper and the uncertainty bounds wider (i.e., compared to
Figure IV-7).
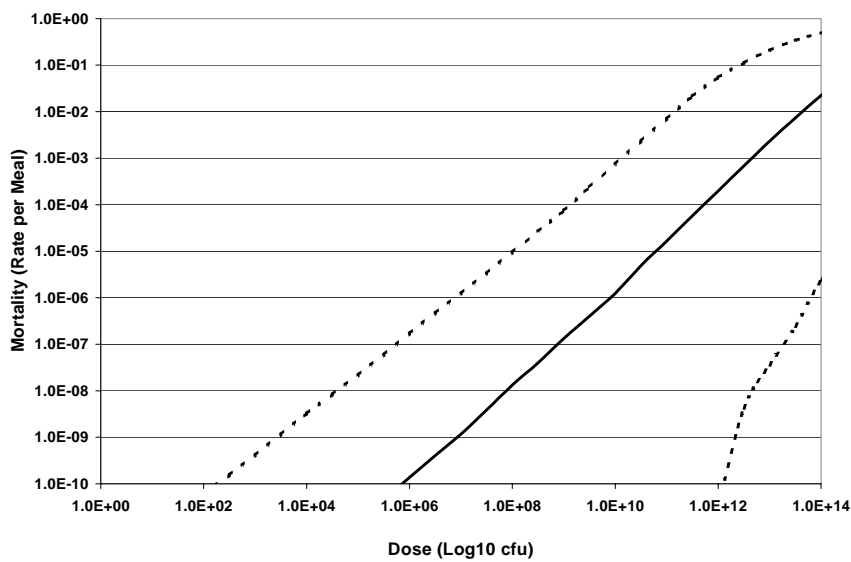
**Figure IV-9. Dose Frequency Function for Elderly Population with a Single
Strain of Unknown Virulence**

V. RISK CHARACTERIZATION

Risk characterization integrates information and data acquired during the hazard identification, hazard characterization, and exposure assessment into an estimate of the adverse effects likely to occur in a given population. In this risk assessment, the risk characterization links the probability of exposure to *Listeria monocytogenes* from consumption of foods with the adverse health outcomes. The primary focus is on the prediction of the relative probability of contracting listeriosis from consumption of a single serving of food in one of the 23 food categories. Additional predictions also consider the extent of annual consumption of the various foods and the predicted contribution of each of the individual food categories to the number of listeriosis cases nationally.

This risk assessment is based on contaminated foods at the retail level. The risk characterization of the overall burden of listeriosis on public health includes both sporadic (i.e., illnesses not associated with a documented outbreak) and outbreak illnesses. Illnesses attributed to documented outbreaks are a small proportion of the total estimated annual cases of listeriosis. At this time it is not possible to separate the risk attributable to specific foods to sporadic and outbreak cases. Outbreaks frequently represent a breakdown in food production, manufacturing, or distribution systems instituted to prevent *Listeria monocytogenes* contamination. Assessing the likelihood that these systems will fail requires detailed information about the manufacture of individual foods that is beyond the scope of this assessment. However, an important benefit of conducting a risk assessment is the identification of knowledge and data gaps. Continuing research is needed to facilitate future *Listeria monocytogenes* risk assessment work (see Appendix 11: Reseach Needs).

Simulation Modeling

The model is comprised of two major components—exposure and dose-response. These models are integrated for the risk characterization simulations as shown in Figure V-1.
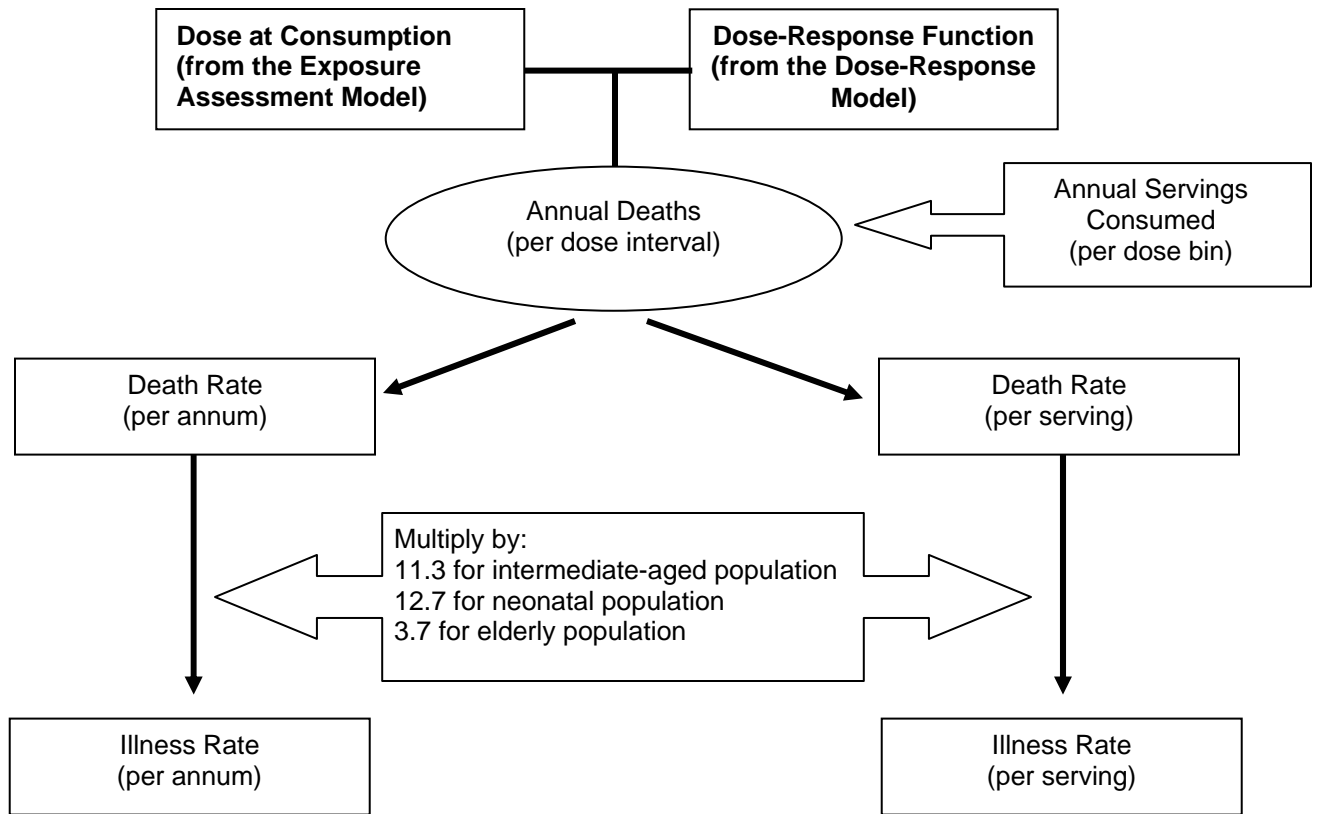
Figure V-1. Components of the Risk Characterization Model

A separate exposure simulation was constructed for each food category. Results from all the food categories were then carried forward to the dose-response simulations, where a separate simulation was constructed for each of the three subpopulation groups. Details of the various modeling steps are provided in Appendix 3.

The exposure assessment modeled the effect of various factors (e.g., frequency and extent of contamination at retail, consumption patterns, the growth potential of *Listeria monocytogenes* in foods, length of refrigerated storage, and refrigeration temperatures) that might affect levels of *Listeria monocytogenes* contamination in a food at the time of consumption. For the exposure assessment, a two-dimensional Monte-Carlo simulation (100,000 variability and 300 uncertainty iterations; total of 30,000,000 iterations) was

used to integrate the components of the exposure model for each of the food categories. The result of each exposure simulation is the fraction of servings that occur at designated dose levels (broken out into half-log$_{10}$ intervals), which are referred to as dose bins. The conversion to dose bins was necessary in order to integrate the exposure simulation, (which evaluated the exposure from individual servings) with the dose-response model (which predicted the number of cases at a population level). The exposure simulations produce 300 distributions (sets of dose bins) of predicted doses for each food category.

The dose-response simulation was carried out in several steps. First, the two-dimensional Monte-Carlo simulation (100,000 variability and 300 uncertainty iterations) was used to integrate the variability and uncertainty of the strain-virulence and host susceptibility functions for each of the subpopulations to provide dose-adjustment factors. The variability dimension for these combined dose-adjustment factors were then grouped into half-log$_{10}$ bins, which ranged from -5 to +10 logs. Second, a one-dimensional (4,000 uncertainty iterations) dose-response simulation was run for each food category by selecting one of the 300 sets of dose bins from the exposure assessment.

These two sets of distributions (exposure dose bins for each food category and dose-response scaling factors for each subpopulation) consist of a relatively small set of finite values and were combined algebraically by adding the arrays. Although some resolution was lost through the creation of the bins for the distribution, avoidance of the use of random numbers provides greater precision at the tails of the summed distribution. In order to calculate the annual rates of cases of listeriosis, the number of deaths per year were multiplied by factors of 11.3 for intermediate-aged population, 12.7 for neonatal, and 3.7 for the elderly population. To calculate the number of perinatal deaths per year, the neonatal death estimate was multiplied by a factor of 2.5. The 2.5 is the approximate ratio of perinatal (106) to neonatal (41) deaths from the County of Los Angeles Department of Health Services (Buchholz, 2000).

The dose-response scaling factor was adjusted so that the sum of the dose-response function (derived from the mouse model) times the exposure assessment doses equaled

the CDC estimates for the annual number of cases of listeriosis. This procedure anchors the overall predicted incidence of listeriosis with the actual incidence of listeriosis. An implicit assumption is that the foods encompassed by the 23 food categories account for all cases of foodborne listeriosis.

The medians of the 4,000 iterations of predicted deaths (per serving) for each food category and each subpopulation were reported. These predictions were ranked from highest to lowest. Because of the variability incorporated into the model (i.e., from differences in consumption of the foods in each categories, pathogen virulence, host susceptibility, and inherent uncertainty), the predicted relative ranking of food categories changes with each of the 4,000 iterations (in some cases significantly). To illustrate the degree of uncertainty associated with the relative risk ranking, the results of each set of the 4,000 iterations was ranked and compared. To this end, the ranking of each food category from 1st to 23rd was determined for each set of the 4,000 uncertainty iterations. The number of times each food category was observed to be ranked at each specific position was determined. These data were compiled and presented graphically (see the latitude graphs, Figures V-4a to V-26b in the section below titled "Summaries of the Food Categories").

For a more detailed explanation of two-dimensional Monte-Carlo and the dose-binning process, see Appendix 3.

Results

The results of this risk assessment, the predicted relative risks of listeriosis associated with each food category, are presented first as an initial overview followed by a more detailed consideration of the individual food categories. The individual food category discussions further interpret the meaning and significance of the analyses in relation to the goal of the risk assessment, as well as discuss factors contributing to the variability and uncertainty associated with the predictions.

A significant difference between the FDA/FSIS risk assessment (the 2001 draft and this revised version) approach and prior attempts to evaluate the risks associated with ready-to-eat foods is the complexity of factors considered in the hazard characterization (Lindquist and Westöö, 2000, Buchanan *et al*., 1997; Farber *et al*., 1996; Haas *et al*., 1999; Hitchins, 1995 and 1996; and Teufel and Bendzulla, 1993). In addition to establishing a general dose-response relation, models were developed for three distinct age-based subpopulations and for assessing the full range of virulence potential that is likely to occur among *Listeria monocytogenes* isolates. It also emphasizes the fact that most exposures to *Listeria monocytogenes* seldom lead to listeriosis, even among highly susceptible segments of the population.

Medians (the value with 50% of the values above and 50% of the values below) are used to represent the "expected" (central tendency) of the estimated risk values. We used medians rather than means because the distributions have long "tails" (high uncertainty and skewed distributions). Medians are less influenced by these extreme values in the distribution but still allow us to represent the central tendency of the distribution with a single value. For other purposes, such as summing the food categories or additional calculations, the mean values are provided in Appendix 10.

Risk Per Serving

A key value used to assess the predicted relative risk among the 23 food categories is the "per serving" likelihood that consumption of a food will lead to listeriosis. This can be viewed as the risk that individual consumers face when they eat a serving of a food. The risk assessment results indicate that listeriosis could potentially be caused by foods in any of the food categories; that is, no food category is risk-free because almost any food could become contaminated with *Listeria monocytogenes*. It is equally apparent that there are substantial differences in risk among the different food categories.

As anticipated from the review of the scientific literature that was conducted in conjunction with this risk assessment, five factors have a large influence on the results of the exposure assessment and thus, the characterization of the predicted relative risk. These factors include the following.

- Frequency and extent of *Listeria monocytogenes* in the food

- Amounts and frequency of food consumption

- Potential for growth of *Listeria monocytogenes* in food during refrigerated storage

- Duration of refrigerated storage before consumption

- Temperature at which the food was held during refrigerated storage

Any of these factors alone affects the potential contamination level at consumption. Those food categories in which one or more of these factors produce a greater risk of exposure to higher levels of *Listeria monocytogenes* contamination are more likely to increase consumers' risk of listeriosis. Examination of the food categories shows that certain factors may have a larger role in driving the predictions of higher risk. Food categories that contained foods that have a high growth potential, based on moderate or high growth rates, coupled with moderate or long storage times, were often the categories that had higher predicted relative risk values. These results have to be interpreted being cognizant of the fact that data on actual consumer storage practices were generally not available, so storage times were estimated based on expert judgment and USDA recommended practices. It is likely that the actual consumer storage times of food are longer than USDA recommendations.

As previously indicated in the description of the exposure assessment, other assumptions related to factors that could affect the frequency or extent of contamination could have a significant impact on the predicted relative risk per serving associated with individual food categories. These, in turn, could affect the predicted relative risk rankings of other food categories. For example, during manufacturing frankfurters are fully cooked to temperatures that are lethal for *Listeria monocytogenes.* However, subsequent recontamination prior to packaging may occur followed by growth of the pathogen. Although frankfurters are usually reheated prior to consumption, a portion of the population consumes them without reheating. To estimate the proportion of frankfurters consumed unreheated, a triangular distribution was used with a minimum of 4%, most

likely of 7% and maximum of 10%. The impact of these types of assumptions on the predicted relative risk is considered in the discussion of the individual food categories.

<u>Predicted Cases of Listeriosis per Serving</u>. The results are summarized in Table V-1 as the median number of cases of listeriosis per serving for each of the three age-based subpopulations and the total United States population. Figure V-2 also shows the differential in median predicted risk per serving (the median values on a log scale are represented in the graph as a box) for the total United States population. The figure illustrates the point that elimination of *Listeria monocytogenes* from any single food will not eliminate foodborne listeriosis; control of listeriosis will require consideration of a variety of foods. However, some foods represent a substantially greater risk per serving and are likely to warrant additional attention from industry and regulators.

In addition to the median values, the 5th and 95th percentile values were also calculated for each of the subpopulations and the total United States population (Table V-1). These lower and upper bound values provide a method of estimating the uncertainty associated with the predictions. Figure V-2 shows these lower and upper bounds for the total United States population. In order to more easily present the data in a graph, the cases of listeriosis for each of the food categories is presented in Figure V-2 on a log scale. It is apparent that for some foods, the range covered was substantial. This was largely the result of exposure distributions where either a small percentage of the foods were predicted to have elevated levels of the pathogen or a high degree of uncertainty had to be assumed due to limitations in available data. The predicted relative risk values must be evaluated in relation to observed variability and uncertainty when using them to determine the best course of action for each of the different food categories. This interpretation of the results is discussed in greater depth for each of the individual food categories later in this chapter.

Table V-1. Estimated Number of Cases of Listeriosis per Serving for Each Food Category and Subpopulation

Food Category	Number of Cases of Listeriosis per Serving[a]											
	Intermediate-Age [b]			Elderly			Perinatal[c]			Total		
	Percentiles			Percentiles			Percentiles			Percentiles		
	Median	5th	95th	Median	5th	95th	Median	5th	95th	Median	5th	95th
SEAFOOD												
Smoked Seafood	2.1×10^{-9}	8.8×10^{-11}	1.2×10^{-7}	1.9×10^{-8}	9.7×10^{-10}	1.0×10^{-6}	8.4×10^{-7}	4.3×10^{-8}	4.6×10^{-5}	6.2×10^{-9}	3.0×10^{-10}	3.3×10^{-7}
Raw Seafood	1.3×10^{-11}	1.1×10^{-17}	2.9×10^{-10}	1.3×10^{-10}	1.7×10^{-14}	2.9×10^{-9}	6.7×10^{-9}	7.4×10^{-12}	2.0×10^{-7}	2.0×10^{-11}	7.4×10^{-15}	4.6×10^{-10}
Preserved Fish	6.4×10^{-12}	5.5×10^{-20}	2.6×10^{-9}	6.7×10^{-11}	3.9×10^{-17}	2.2×10^{-8}	4.1×10^{-9}	2.1×10^{-14}	9.9×10^{-7}	2.3×10^{-11}	6.9×10^{-17}	7.5×10^{-9}
Cooked RTE Crustaceans	2.2×10^{-9}	2.5×10^{-10}	2.1×10^{-8}	1.9×10^{-8}	2.4×10^{-9}	1.6×10^{-7}	7.4×10^{-7}	9.7×10^{-8}	6.1×10^{-6}	5.1×10^{-9}	6.5×10^{-10}	4.6×10^{-8}
PRODUCE												
Vegetables	8.4×10^{-13}	1.5×10^{-19}	6.3×10^{-11}	8.2×10^{-12}	3.7×10^{-16}	5.7×10^{-10}	4.8×10^{-10}	1.4×10^{-13}	3.1×10^{-8}	2.8×10^{-12}	2.8×10^{-16}	1.9×10^{-10}
Fruits	5.0×10^{-12}	6.0×10^{-20}	9.6×10^{-9}	5.1×10^{-11}	5.3×10^{-17}	5.7×10^{-8}	2.8×10^{-9}	1.3×10^{-14}	3.0×10^{-6}	1.9×10^{-11}	4.5×10^{-17}	2.3×10^{-8}
DAIRY												
Fresh Soft Cheese	1.2×10^{-10}	4.6×10^{-13}	2.1×10^{-9}	1.0×10^{-9}	5.0×10^{-12}	1.7×10^{-8}	4.2×10^{-8}	2.6×10^{-10}	7.0×10^{-7}	1.7×10^{-10}	8.0×10^{-13}	2.9×10^{-9}
Soft Unripened Cheese	5.8×10^{-10}	8.4×10^{-14}	1.6×10^{-8}	4.9×10^{-9}	7.2×10^{-13}	1.2×10^{-7}	2.0×10^{-7}	4.8×10^{-11}	5.3×10^{-6}	1.8×10^{-9}	2.8×10^{-13}	4.4×10^{-8}
Soft Ripened Cheese	2.1×10^{-12}	1.8×10^{-21}	1.3×10^{-9}	2.2×10^{-11}	3.3×10^{-18}	1.1×10^{-8}	1.3×10^{-9}	3.5×10^{-15}	5.2×10^{-7}	5.1×10^{-12}	7.9×10^{-18}	2.6×10^{-9}
Semi-soft Cheese	2.9×10^{-12}	9.3×10^{-17}	2.9×10^{-10}	3.0×10^{-11}	5.5×10^{-15}	2.7×10^{-9}	1.6×10^{-9}	9.2×10^{-13}	1.4×10^{-7}	6.5×10^{-12}	2.5×10^{-15}	5.8×10^{-10}
Hard Cheese	3.4×10^{-15}	5.3×10^{-47}	1.9×10^{-12}	9.2×10^{-15}	5.8×10^{-39}	1.9×10^{-11}	8.1×10^{-13}	3.4×10^{-32}	1.3×10^{-9}	4.5×10^{-15}	2.5×10^{-35}	5.5×10^{-12}
Processed Cheese	1.4×10^{-14}	3.2×10^{-30}	2.3×10^{-12}	9.3×10^{-14}	8.8×10^{-25}	2.2×10^{-11}	6.7×10^{-12}	6.6×10^{-20}	1.4×10^{-9}	4.2×10^{-14}	5.4×10^{-23}	6.0×10^{-12}
Pasteurized Fluid Milk	4.4×10^{-10}	2.8×10^{-11}	5.7×10^{-9}	3.4×10^{-9}	2.5×10^{-10}	3.9×10^{-8}	1.5×10^{-7}	1.2×10^{-8}	1.7×10^{-6}	1.0×10^{-9}	7.5×10^{-11}	1.3×10^{-8}
Unpasteurized Fluid Milk	2.9×10^{-9}	3.5×10^{-11}	6.8×10^{-8}	2.2×10^{-8}	3.4×10^{-10}	5.1×10^{-7}	9.9×10^{-7}	1.7×10^{-8}	2.3×10^{-5}	7.1×10^{-9}	9.7×10^{-11}	1.6×10^{-7}
Ice Cream/Frozen Dairy Products	1.3×10^{-14}	2.7×10^{-35}	1.8×10^{-12}	9.2×10^{-14}	1.4×10^{-28}	1.9×10^{-11}	6.5×10^{-12}	2.7×10^{-23}	1.3×10^{-9}	4.9×10^{-14}	1.7×10^{-26}	6.3×10^{-12}
Cultured Milk Products	9.5×10^{-15}	2.4×10^{-40}	1.7×10^{-11}	5.6×10^{-14}	6.5×10^{-33}	1.7×10^{-10}	4.7×10^{-12}	5.1×10^{-26}	9.9×10^{-9}	3.2×10^{-14}	3.3×10^{-29}	4.9×10^{-11}
High Fat and Other Dairy Products	1.0×10^{-9}	1.0×10^{-10}	8.2×10^{-9}	8.3×10^{-9}	8.9×10^{-10}	5.7×10^{-8}	3.2×10^{-7}	3.7×10^{-8}	2.0×10^{-6}	2.7×10^{-9}	2.9×10^{-10}	1.9×10^{-8}
MEATS												
Frankfurters (reheated)	2.7×10^{-11}	4.2×10^{-15}	3.4×10^{-10}	2.7×10^{-10}	8.6×10^{-13}	3.4×10^{-9}	1.6×10^{-8}	2.1×10^{-10}	2.6×10^{-7}	6.3×10^{-11}	2.7×10^{-13}	8.0×10^{-10}
Frankfurters (not reheated)	3.3×10^{-8}	3.1×10^{-9}	2.8×10^{-7}	2.9×10^{-7}	3.2×10^{-8}	2.3×10^{-6}	1.1×10^{-5}	1.3×10^{-6}	8.3×10^{-5}	6.5×10^{-8}	7.1×10^{-9}	5.2×10^{-7}
Dry/Semi-Dry Fermented Sausages	6.0×10^{-12}	6.8×10^{-20}	2.7×10^{-9}	6.2×10^{-11}	2.0×10^{-16}	2.4×10^{-8}	3.7×10^{-9}	5.1×10^{-14}	1.1×10^{-6}	1.7×10^{-11}	1.5×10^{-16}	6.3×10^{-9}
Deli Meats	3.3×10^{-8}	6.8×10^{-9}	4.1×10^{-8}	3.0×10^{-7}	5.8×10^{-8}	3.9×10^{-7}	1.2×10^{-5}	3.2×10^{-6}	1.4×10^{-5}	7.7×10^{-8}	1.7×10^{-8}	9.9×10^{-8}
Pâté and Meat Spreads	1.2×10^{-8}	1.0×10^{-9}	1.4×10^{-7}	1.1×10^{-7}	1.1×10^{-8}	1.1×10^{-6}	4.5×10^{-6}	4.7×10^{-7}	4.5×10^{-5}	3.2×10^{-8}	3.1×10^{-9}	3.3×10^{-7}
COMBINATION FOODS												
Deli-type Salads	1.7×10^{-13}	1.8×10^{-31}	1.3×10^{-10}	1.4×10^{-12}	3.3×10^{-25}	1.2×10^{-9}	8.8×10^{-11}	9.3×10^{-20}	5.5×10^{-8}	5.6×10^{-13}	8.0×10^{-23}	4.1×10^{-10}

[a]This table provides estimates of the rate of listeriosis per serving and the confidence intervals about that estimate. For example, for the perinatal group in the Smoked Seafood category, the risk assessment estimates that there is only a 5% probability that the rate of listeriosis is less than 4.3×10^{-8} and a 95% probability that it is less than 4.6×10^{-5} (or a 5% probability that it is greater). The median risk estimate has a 50% probability of being greater or smaller than the rate of listeriosis. [b]The Intermediate-age population includes susceptible populations not captured in the other groups, such as cancer, AIDS, and transplant patients, for whom there are insufficient data to consider as a separate population. [c]The Perinatal population is a susceptible population that includes fetuses and neonates. Exposure occurs *in utero* from contaminated food eaten by the pregnant woman. The predicted cases are predominately neonatal, therefore to estimate the perinatal cases presented in this table, an exposure period of 10 days was used. The value of 10 approximately corresponds to the mean of the triangle distribution (1, 7, 30) used in the simulation.
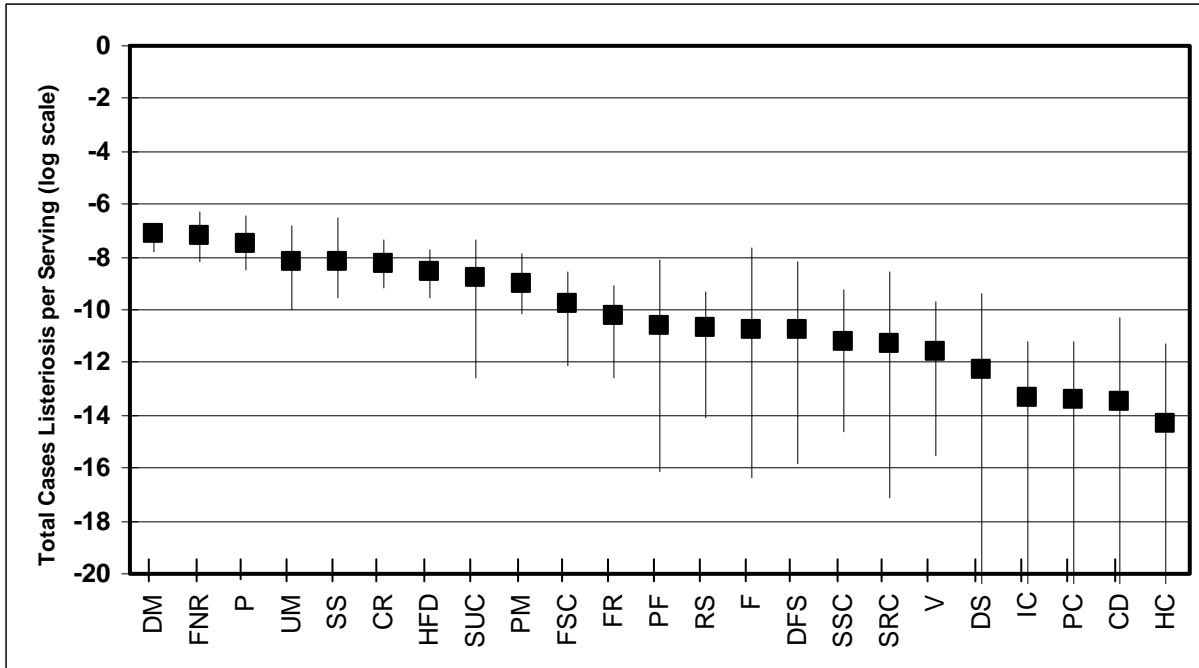
Figure V-2. Predicted Cases of Listeriosis (log scale) Associated with Food Categories for the Total United States Population on a per Serving Basis

[The box indicates the median predicted number of cases of listeriosis (log scale) and the bar indicates the lower and upper bounds (i.e., the 5th and 95th percentiles). The y-axis values are presented on a log scale. For example a log of –6 is equivalent to 1 in a million.]

DM = Deli meats; FNR = Frankfurters (not reheated); P= Pâté and Meat Spreads; UM= Unpasteurized Fluid Milk; SS= Smoked Seafood; CR = Cooked Ready-To-Eat Crustaceans; HFD = High Fat and Other Dairy Products; SUC = Soft Unripened Cheese; PM = Pasteurized Fluid Milk; FSC = Fresh Soft Cheese; FR = Frankfurters (reheated); PF = Preserved Fish; RS = Raw Seafood; F = Fruits; DFS= Dry/Semi-dry Fermented Sausages; SSC = Semi-soft Cheese; SRC = Soft Ripened Cheese; V = Vegetables; DS = Deli-type Salads; IC= Ice Cream and Frozen Dairy Products; PC = Processed Cheese; CD = Cultured Milk Products; HC = Hard Cheese.

Predicted Risk Ranking. The predicted median values for the cases of listeriosis on a per serving basis were used to develop predicted relative risk ranks. The median predicted relative risk ranking among the different food categories is summarized for the three subpopulations and the total United States population in Table V-2. It is apparent that the predicted relative risk rankings of the food categories are similar for the three subpopulations, but not identical.

The uncertainty associated with the risk ranking is described in the latitude ranking graphs that are presented as part of the discussion of each of the individual food categories (see Figures V-4a to V-26b). It is important to note that in a number of instances there are only minor differences separating the rankings of various food categories.

Although the number of iterations in the ranking process was very high (4,000), analysis of variance techniques were used to provide an indication of the statistical certainty of the rankings. Nonparametric analysis of variance technique (i.e. Kruskal-Wallis Test), followed by a multiple comparison procedure, was used to evaluate the differences in the median rankings of risk per serving for the total United States population. The analyses were performed using NCSS (NCSS, 2001) to determine which of the median rankings were not significantly different based on the number of simulation samples (iterations) and an alpha level of 0.05 for the family-wide error rate with respect to all pairwise comparisons of the 23 food categories. The results are shown in Table V-2.

Table V-2. Predicted Relative Risk Rankings for Listeriosis Among Food Categories for Three Age-Based Subpopulations and the United States Total Population Using Median Estimates of Predicted Relative Risks for Listeriosis on a per Serving Basis

| Food Categories[a] | Subpopulation | | | |
	Intermediate Age[b]	Elderly[b]	Perinatal[b]	Total [b, c]
SEAFOOD				
Smoked Seafood	6	5	5	5b
Raw Seafood	12	12	12	13d
Preserved Fish	13	13	13	12d,e
Cooked Ready-to-Eat Crustaceans	5	6	6	6b
PRODUCE				
Vegetables	18	18	18	18
Fruits	15	15	15	14e
DAIRY				
Fresh Soft Cheese	10	10	10	10
Soft Unripened Cheese, >50% moisture	8	8	8	8c
Soft Ripened Cheese, >50% moisture	17	17	17	17f
Semi-soft Cheese, 39-50% moisture	16	16	16	16f
Hard Cheese, <39% moisture	23	23	23	23
Processed Cheese	20	20	20	21g
Pasteurized Fluid Milk	9	9	9	9c
Unpasteurized Fluid Milk	4	4	4	4b
Ice Cream and Frozen Dairy Products	21	21	21	20g
Cultured Milk Products	22	22	22	22g
High Fat and Other Dairy Products	7	7	7	7
MEATS				
Frankfurters, reheated	11	11	11	11
Frankfurters, not reheated	2	2	2	2a
Dry/Semi-Dry Fermented Sausages	14	14	14	15d
Deli Meats	1	1	1	1a
Pâté and Meat Spreads	3	3	3	3
COMBINATION FOODS				
Deli-type Salads	19	19	19	19

[a] Food categories are grouped by type of food but are not in any particular order.

[b] A ranking of 1 indicates the food category with the greatest predicted relative risk per serving of causing listeriosis and a ranking of 23 indicates the lowest predicted relative risk of causing listeriosis.

[c] Ranks with the same letter are not significantly different based on the Bonferroni Multiple Comparison Test (alpha = 0.05).

Risk per Annum

A full picture of listeriosis risk requires consideration of the number of servings consumed, as well as the risk per serving. These data were considered for each of the food categories and used to calculate the predicted cases of listeriosis on a per annum basis. If the "risk per serving" is considered the predicted relative risk faced by each consumer, then the "risk per annum" is a measure of the predicted relative risk faced by the country. The risk per annum is greatly affected by the number of servings per year. Thus, a food that has a relatively high risk on a per serving basis but is seldom consumed may have a relatively low per annum risk. Conversely, a food with a relatively low risk on a per serving basis that is consumed extensively is likely to have a higher risk on a per annum basis. Table III-2 shows the wide range in number of annual servings among the food categories. The per annum relative risks inherently have a greater degree of uncertainty than the corresponding per serving relative risk because of the additional uncertainty associated with the number of annual servings. Another factor that affects predicted relative risk on a per annum basis is the size of the subpopulations, in proportion to the total population. They are substantially different, i.e., perinatal, elderly, and intermediate-age groups, represent approximately 2%, 13%, and 85% of the total population, respectively.

The results were generated in a manner similar to that described above for the predicted relative risk per serving. Table V-3 provides the predicted median number of cases of listeriosis on a per annum basis for each of the age-based populations. The upper and lower bounds (5th and 95th percentile values) are also provided in Table V-3 to show the range of variability and uncertainty of the estimates. The range in the predicted number of cases of listeriosis is depicted in Figure V-3 for the total United States population.

The predicted relative risk ranking is presented in Table V-4. The uncertainty associated with the ranking is also described using individual latitude ranking graphs based on the rankings for the total United States population (see Figures V-4a to V-26b). These graphs are provided in the discussions of individual food categories. It is important to note that the differences among several of the food categories were very small, so differences between adjacent or closely

occurring ranks must only be considered in conjunction with the estimates of uncertainty which are provided as part of the discussion of the individual food categories.

In most instances, the food categories that had high predicted relative risk rankings on a per serving basis also had a high predicted relative risk ranking on a per annum basis. However, there were instances where foods with lower risk per serving rankings had higher risk per annum values and vice versa. For example, Pâté and Meat Spreads had a higher predicted relative risk on a per serving basis than on a per annum basis. This reflects the fact that foods in this category are eaten relatively infrequently and in relatively small amounts. Conversely, Vegetables and Pasteurized Fluid Milk are products where a predicted low or moderate per serving relative risk was elevated on a per annum basis. In these examples, this appears to be a function of two factors. The first is the variability in the data sets available on a worldwide basis (see discussion of individual foods in the section titled "Overview and Discussion of Food Categories"). A wide degree of variability increases the number of predicted exposure values in the "tails" of the distribution. To a large extent, it is these extremes of the distributions that determine the per annum risk. The second is that the numbers of servings consumed annually for Vegetables and Pasteurized Fluid Milk are several orders of magnitude higher than other food categories. Again, this strongly influences the per annum predicted relative ranking for these foods. With both of these food categories, the results of the risk assessment must be interpreted in relation to the uncertainty estimates. The best interpretation may be the need to assure continued vigilance. However, these data do demonstrate how a risk assessment can provide a means of systematically examining risks from different vantage points. The results clearly point out that a relatively low predicted relative risk per serving associated with foods that are consumed extensively (such as Pasteurized Fluid Milk or Vegetables) could lead to a potentially greater impact on the relative risk of listeriosis per annum.

Table V-3. Estimated Number of Cases of Listeriosis per Annum for Each Food Category and Subpopulation

Food Category	Intermediate-Age [b]			Elderly			Perinatal [c]			Total		
	Median	5th	95th	Median	5th	95th	Median	5th	95th	Median	5th	95th
SEAFOOD												
Smoked Seafood	0.3	<0.1	19.4	0.8	<0.1	43.2	0.1	<0.1	5.8	1.3	0.1	68.1
Raw Seafood	<0.1	<0.1	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Preserved Fish	<0.1	<0.1	0.2	<0.1	<0.1	0.5	<0.1	<0.1	0.1	<0.1	<0.1	0.8
Cooked Ready-to-Eat Crustaceans	1.0	0.1	10.0	1.5	0.2	13.2	0.3	<0.1	2.2	2.8	0.4	25.7
PRODUCE												
Vegetables	0.1	<0.1	4.3	0.1	<0.1	9.7	<0.1	<0.1	1.6	0.2	<0.1	15.7
Fruits	0.2	<0.1	351.4	0.6	<0.1	680.4	0.1	<0.1	85.4	0.9	<0.1	1127.7
DAIRY												
Fresh Soft Cheese	<0.1	<0.1	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.2
Soft Unripened Cheese	2.0	<0.1	52.3	5.1	<0.1	128.8	0.5	<0.1	13.6	7.7	<0.1	193.5
Soft Ripened Cheese	<0.1	<0.1	2.1	<0.1	<0.1	2.1	<0.1	<0.1	0.7	<0.1	<0.1	4.9
Semi-soft Cheese	<0.1	<0.1	0.5	<0.1	<0.1	0.4	<0.1	<0.1	0.2	<0.1	<0.1	1.1
Hard Cheese	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Processed Cheese	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.1
Pasteurized Fluid Milk	31.4	2.0	410.1	49.8	3.7	584.4	8.0	0.7	95.8	90.8	6.5	1084.6
Unpasteurized Fluid Milk	1.1	<0.1	24.7	1.7	<0.1	38.3	0.3	<0.1	6.5	3.1	<0.1	69.2
Ice Cream/Frozen Dairy Products	<0.1	<0.1	<0.1	<0.1	<0.1	0.1	0.0	<0.1	<0.1	<0.1	<0.1	0.1
Cultured Milk Products	<0.1	<0.1	0.1	<0.1	<0.1	0.2	0.0	<0.1	<0.1	<0.1	<0.1	0.4
High Fat and Other Dairy Products	17.0	1.7	135.0	35.1	3.8	241.3	4.0	0.5	25.3	56.4	6.0	398.9
MEATS												
Frankfurters (reheated)	0.1	<0.1	1.9	0.2	<0.1	2.0	0.1	<0.1	1.1	0.4	<0.1	4.9
Frankfurters (not reheated)	13.8	1.3	119.4	13.0	1.4	103.0	3.6	0.4	26.9	30.5	3.3	245.4
Dry/Semi-Dry Fermented Sausages	<0.1	<0.1	4.1	<0.1	<0.1	6.0	<0.1	<0.1	1.3	<0.1	<0.1	11.2
Deli Meats	589.1	120.6	736.4	849.6	164.6	1106.2	161.2	44.5	197.0	1598.7	341.2	2038.2
Pâté and Meat Spreads	1.2	0.1	13.2	2.2	0.2	23.5	0.3	<0.1	3.4	3.7	0.4	39.5
COMBINATION FOODS												
Deli-type Salads	<0.1	<0.1	1.3	<0.1	<0.1	3.8	<0.1	<0.1	0.4	<0.1	<0.1	5.4

[a]This table provides estimates of the rate of listeriosis per annum and the confidence intervals about that estimate. [b]The Intermediate-age group includes susceptible populations not captured in other groups, such as cancer, AIDS, and transplant patients, for whom there are insufficient data to consider as a separate population. [c] The Perinatal population is a susceptible population that includes fetuses and neonates. Exposure occurs most often in utero from contaminated food eaten by the pregnant woman.
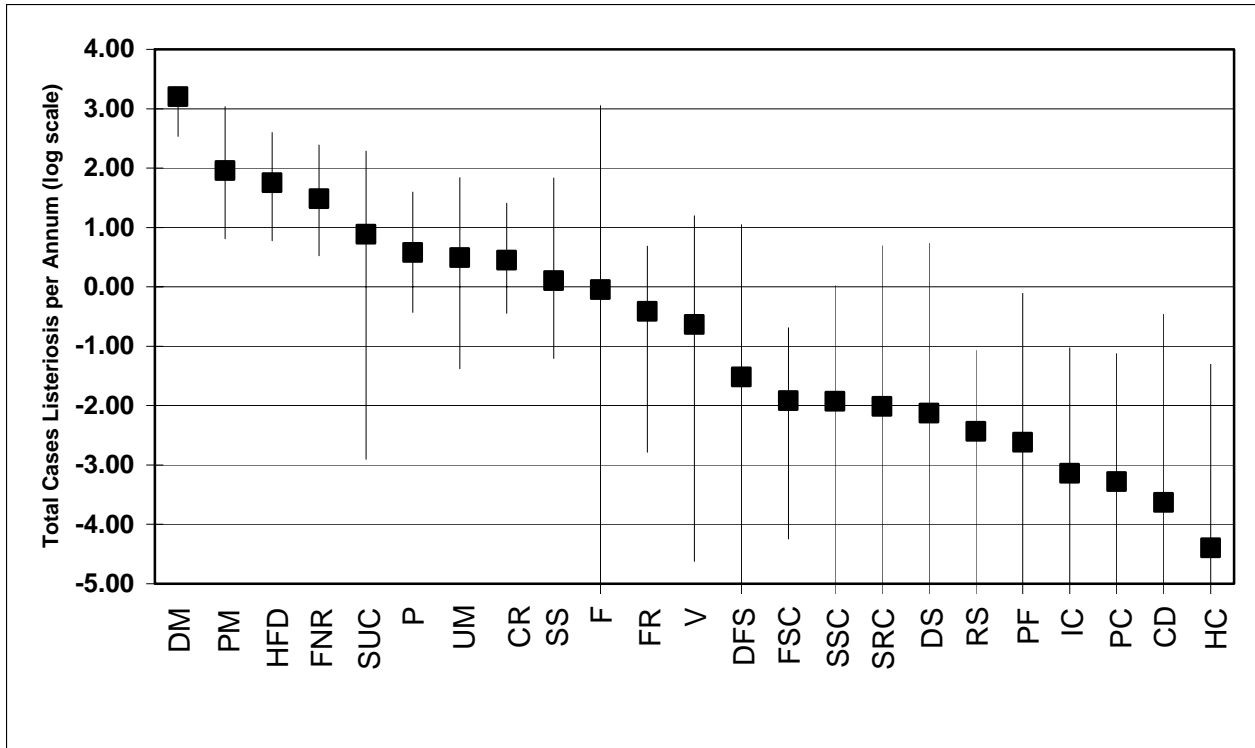
Figure V-3. Predicted Cases of Listeriosis (log scale) Associated with Food Categories for the Total United States Population on a per Annum Basis

[The box indicates the median predicted number of cases of listeriosis (log scale) and the bar indicates the lower and upper bounds (i.e., the 5th and 95th percentiles. The y-axis values are presented on a log scale. For example a log of –3 is equivalent to 1 in a thousand.]

DM = Deli meats; PM = Pasteurized Fluid Milk; HFD = High Fat and Other Dairy Products; FNR = Frankfurters (not reheated); SUC = Soft Unripened Cheese; P= Pâté and Meat Spreads; CR = Cooked Ready-To-Eat Crustaceans; UM= Unpasteurized Fluid Milk; SS= Smoked Seafood; F = Fruits; FR = Frankfurters (reheated); V = Vegetables; DFS= Dry/Semi-dry Fermented Sausages; FSC = Fresh Soft Cheese; SSC = Semi-soft Cheese; SRC = Soft Ripened Cheese; DS = Deli-type Salads; RS = Raw Seafood; PF = Preserved Fish; IC= Ice Cream and Frozen Dairy Products; PC = Processed Cheese; CD = Cultured Milk Products; HC = Hard Cheese.

Table V-4. Predicted Relative Risk Rankings for Listeriosis Among Food Categories for Three Age-Based Subpopulations and the United States Total Population Using Median Estimates of Relative Predicted Risks for Listeriosis on a per Annum Basis

Food Categories[a]	Subpopulation			
	Intermediate Age[b]	Elderly[b]	Perinatal[b]	Total [b, c]
SEAFOOD				
Smoked Seafood	9	9	9	9
Raw Seafood	17	21	17	18g
Preserved Fish	19	17	19	19g
Cooked Ready-to-Eat Crustaceans	8	8	8	8b,d,e
PRODUCE				
Vegetables	12	12	12	12
Fruits	10	10	10	10
DAIRY				
Fresh Soft Cheese	14	18	14	14f
Soft Unripened Cheese, >50% moisture	5	5	5	5b,c
Soft Ripened Cheese, >50% moisture	16	16	16	16f
Semi-soft Cheese, 39-50% moisture	15	15	15	15f
Hard Cheese, <39% moisture	23	23	23	23
Processed Cheese	20	20	21	21h
Pasteurized Fluid Milk	2	2	2	2a
Unpasteurized Fluid Milk	7	7	7	7d,e
Ice Cream and Frozen Dairy Products	21	19	20	20h
Cultured Milk Products	22	22	22	22h
High Fat and Other Dairy Products	3	3	3	3a
MEATS				
Frankfurters, reheated	11	11	11	11
Frankfurters, not reheated	4	4	4	4
Dry/Semi-Dry Fermented Sausages	13	13	13	13
Deli Meats	1	1	1	1
Pâté and Meat Spreads	6	6	6	6b,c,d
COMBINATION FOODS				
Deli-type Salads	18	14	18	17f

[a] Food categories are grouped by type of food but are not in any particular order.

[b] A ranking of 1 indicates the food category with the greatest predicted relative risk of causing listeriosis and a ranking of 23 indicates the lowest predicted relative risk of causing listeriosis.

[c] Ranks with the same letter are not significantly different based on the Bonferroni Multiple Comparison Test (alpha=0.05).

Overview and Discussion of Food Categories

Because *Listeria monocytogenes* is ubiquitous in foods and the food-processing environment, a large number of foods needed to be considered in this risk assessment. In order to have a practicable number of food groupings, 23 categories were formed from the more than 640 ready-to-eat foods in the consumption surveys. These categories are sometimes broadly defined to include several distinct but similar classes of food, while in other instances they are quite small and specific. The foods included in this risk assessment are primarily organized into categories based on primary origin of the foods (e.g., seafood, vegetable, dairy, meat), composition and processing (moisture content, raw vs. cooked, pH, salt level), contamination with *Listeria monocytogenes,* and association with listeriosis. Although generally similar, some characteristics of foods within a single category may vary. For example, within a single food category, consumption may be greater for one food, contamination higher in another, and average rate of growth in a third food. In the future, if further investigations of an individual food category or a particular food within a category are conducted, the model developed in the current risk assessment could be modified to provide a more detailed analysis.

Consumption

Consumption estimates on a per serving basis were determined, as well as the amount of food eaten per person per day. Data indicate that, for the one or two days of the consumption surveys, there were 1.8×10^9 servings consumed of the foods identified in the 23 categories. Extrapolated to an annual basis, there were 3.4×10^{11} servings consumed in a year. The vast majority (96.3% or 2.5×10^8 individuals) of the population reported eating the foods included in this risk assessment. There were a relatively low number of eaters for some of the food categories (e.g.*,* Smoked Seafood, Fresh Soft Cheese, Pâté and Meat Spreads), while other food categories are consumed widely and often (e.g.*,* Pasteurized Milk, Vegetables). Consumption information for each food category is included in the discussion below.

Contamination

Contamination levels at retail ranged from less than 0.04 cfu/g to more than 10^6 cfu/g in the food data considered in this analysis. The highest levels reported for specifically identified food products were in the range of 10^5 to 10^6 cfu/g, although the results of laboratory investigations

indicate that contamination levels greater than 10^6 cfu/g can occur. Studies that were limited to the determination of presence or absence were assigned a contamination value commensurate with the lowest limit of detection possible: 0.04 cfu/g. The highest frequency of contaminated samples was 12.9 % (Smoked Seafood). All food categories demonstrated some contamination, with a range of positive samples from 0.2 % to 12.9 % (see Table III-4). The frequency of occurrence of contaminated samples was lower at higher contamination levels. The contamination studies used in this study were published over a period of seventeen years (1985-2002). Because there was a major effort worldwide to control foodborne listeriosis, the incidence of contamination was evaluated for differences in data published pre-1993, 1993 to 1998, and post-1998. To estimate the current variation in contamination, studies were weighted by number of samples, country, and date of publication as explained in Chapter II: Exposure Assessment. Food categories with no recent data were adjusted by a factor relative to the other food categories.

Growth of *Listeria monocytogenes*

To predict possible growth between retail sampling and consumption, a growth model was created, based on growth rates from studies of various foods inoculated with *Listeria monocytogenes* under laboratory conditions. These studies were conducted at a number of temperatures. The reported growth rates were adjusted to give the equivalent growth rate at 5°C. Within each food category, the adjusted Exponential Growth Rate (EGR) from individual studies was used to develop a distribution of growth rate values. As previously mentioned, little data were available that adequately described the distribution of storage times (except for frankfurters and deli meats). Therefore, a modified BetaPert distribution was created for each food category, with minimum, most likely and maximum times (days) to account for the variation in storage times. The minimum time for all food categories (0.5 day) represents food consumed within 24 hours of purchase. For each specific food category, the most likely and maximum values were given an uncertainty range. For frankfurters and deli meats, an empirical data set was used (AMI, 2001). For each iteration of the growth simulation, the model selected a refrigeration storage temperature (that varied from 1 to 11°C) and calculated the EGR (log_{10} cfu/day) at that temperature. The EGR was multiplied by the storage time to estimate growth from retail to consumption and the estimated growth was added to the initial number of *Listeria*

monocytogenes to calculate the total *Listeria monocytogenes.* The projected growth was limited by temperature-dependent maximum growth values (stationary phase). The maximum growth was greater at higher storage temperatures than at lower temperatures. In addition, the model contained a negative correlation between storage temperature and storage times. This minimized combinations of long storage times and high temperatures that would most likely result in detectable spoilage from other microorganisms and disposal of the food rather than consumption.

Summaries of the Food Categories

Because the risk assessment model is based on many parameters and an extensive amount of both qualitative and quantitative data, it can be difficult to determine the impact of each of the factors considered. Accordingly, sets of qualitative descriptors were developed to aid in the discussion and comparison of these parameters in the food categories. The criteria used to characterize data among food categories as low/moderate/high or short/moderate/long for each parameter are presented in Table V-5a. Table V-5b provides a characterization of each of the parameters for each food category. See Appendices 5, 7 and 8 for the supporting data.

An overview of each of the 23 food categories is provided in this chapter including information for each food category on cases of listeriosis, consumption, contamination, and growth of *Listeria monocytogenes*, and a summary of the designated parameter levels based on the criteria listed in Table V-5a. In addition, the latitude graphs (Figures V-4a to V-26b) show the uncertainty associated with the predicted relative risk rankings on both a per serving and per annum basis for each food category. These graphs show how frequently a food category ranked 1st, 2nd , and so on to 23rd. A food category that primarily ranked 1st or 2nd should be considered a higher risk than a food category that primarily ranked 22nd or 23rd. The distribution of rankings shown for a food category is an indication of the certainty of its ranking. The narrower the range, the greater is the certainty associated with the relative risk ranking.

As an initial means of categorizing the results of the risk assessment in order to relate them to the characteristics of the different food categories, the relative predicted risk on a per serving basis was classified as high, moderate, or low. The following criteria was used: high = >5 predicted cases of listeriosis per billion servings; moderate = <5 but ≥ 1 predicted case per billion servings,

and low = <1 predicted case per billion servings. Based on these criteria, five of the foods were considered to be high risk, four were in the moderate risk group, and the remaining foods fell into the low risk per serving category (Table V-6). The number of predicted cases per annum in the United States for the total population was classified as low (less than 1 case per annum), moderate (>1 to 10 cases per annum), high (>10 to 100 cases), and very high (>100 cases). Based on these criteria, one food category was considered very high, three food categories were considered to cause a high number of cases and five food categories a moderate number of cases, with the remaining considered low. Additional means of grouping the results are considered later in the document (see the cluster analysis in Chapter VII).

Table V-5a. Criteria Used to Designate Parameter Ranges for *Listeria monocytogenes* Among the Food Categories

Parameter	Designated Parameter Level		
	Low/Short	Moderate	High/Long
Number of Annual Servings	$\leq 1 \times 10^9$	$> 1 \times 10^9$ to $< 1 \times 10^{10}$	$\geq 1 \times 10^{10}$
Median Amount Consumed per Serving (g)	≤ 40 g	> 40 g to < 90 g	≥ 90 g
Contamination Frequency (%)	$\leq 2\%$	$> 2\%$ to $< 5\%$	$\geq 5\%$
Contamination at Retail—Predicted Servings at 10^3 to 10^6 cfu (%)	$\leq 0.1\%$	$>0.1\%$ to $< 0.6\%$	$\geq 0.6\%$
Exponential Growth Rate at 5 °C ($\log_{10}$ cfu/day)	≤ 0.1	> 0.1 to < 0.2	≥ 0.2
Most Likely Storage Time (days)	≤ 2 days	> 2 to 5 days	≥ 6 to 10 days

Table V-5b. Summary of Data Used to Model *Listeria monocytogenes* Exposure for Each Food Relative to Other Food Categories

Food Category	Number of Annual Servings	Median Amount Consumed	Contamination Frequency	Contamination Level at Retail	Growth Rate During Storage	Storage Time
SEAFOOD						
Smoked Seafood	Low	Moderate	High	High	Moderate	Moderate
Raw Seafood	Low	Low	High	High	Moderate	Short
Preserved Fish	Low	Moderate	High	Moderate	—[a]	—[a]
Cooked Ready-to-Eat Crustaceans	Low	Moderate	Moderate	Moderate	High	Short
PRODUCE						
Vegetables	High	Low	Moderate	Low	Low	Moderate
Fruits	High	High	High	Low	Low	Moderate
DAIRY						
Fresh soft cheese	Low	Low	Low	Low	Low	Moderate
Soft Unripened Cheese, >50% moisture	Moderate	Low	Moderate	Moderate	Low[b]	Long
Soft Ripened Cheese, >50% moisture	Moderate	Low	Moderate	Low	Low[b]	Long
Semi-soft cheese, 39-50% moisture	Moderate	Low	Moderate	Low	Low[b]	Long
Hard Cheese, <39% moisture	Moderate	Low	Low	Low	Low[b]	Long
Processed Cheese	High	Low	Low	Low	Low[b]	Long
Pasteurized Fluid Milk	High	High	Low	Low	High	Moderate
Unpasteurized Fluid Milk	Low	High	Moderate	Moderate	High	Moderate
Ice Cream and Frozen Dairy Products	High	High	Low	Low	—[a]	—[a]
Cultured Milk Products	Moderate	High	Low	Low	Low[b]	Long
High Fat and Other Dairy Products	High	Low	Low	Low	Moderate	Long
MEATS						
Frankfurters, reheated	Moderate	Moderate	Moderate	High	Moderate	Moderate
Frankfurters, not reheated	Low	Moderate	Moderate	High	Moderate	Moderate
Dry/Semi-Dry Fermented Sausages	Moderate	Moderate	High	Moderate	Low[b]	Long
Deli Meats	High	Moderate	Moderate	High	High	Long
Pâté and Meat Spreads	Low	Moderate	High	Moderate	High	Long
COMBINATION FOODS						
Deli-type Salads	High	High	Moderate	Low	Low[c]	Moderate

[a] A non-growth food category; growth rates and storage times are not applicable. [b] Includes probabilities that *Listeria monocytogenes* numbers will decline during storage. [c]Overall *Listeria monocytogenes* declines in deli salads, but it can grow at a moderate rate in a small fraction of salads.

Table V-6. Relative Risk Ranking and Predicted Median Cases of Listeriosis for the Total United States Population on a per Serving and Per Annum Basis

Relative Risk Ranking	Predicted Median Cases of Listeriosis for 23 Food Categories					
	Per Serving Basis[a]			Per Annum Basis[b]		
		Food	Cases		Food	Cases
1	High Risk	Deli Meats	7.7×10^{-8}	Very High	Deli Meats	1598.7
2		Frankfurters, not reheated	6.5×10^{-8}	High Risk	Pasteurized Fluid Milk	90.8
3		Pâté and Meat Spreads	3.2×10^{-8}		High Fat and Other Dairy Products	56.4
4		Unpasteurized Fluid Milk	7.1×10^{-9}		Frankfurters, not reheated	30.5
5		Smoked Seafood	6.2×10^{-9}	Moderate Risk	Soft Unripened Cheese	7.7
6		Cooked Ready-to-Eat Crustaceans	5.1×10^{-9}		Pâté and Meat Spreads	3.8
7	Moderate Risk	High Fat and Other Dairy Products	2.7×10^{-9}		Unpasteurized Fluid Milk	3.1
8		Soft Unripened Cheese	1.8×10^{-9}		Cooked Ready-to-Eat Crustaceans	2.8
9		Pasteurized Fluid Milk	1.0×10^{-9}		Smoked Seafood	1.3
10	Low Risk	Fresh Soft Cheese	1.7×10^{-10}	Low Risk	Fruits	0.9
11		Frankfurters, reheated	6.3×10^{-11}		Frankfurters, reheated	0.4
12		Preserved Fish	2.3×10^{-11}		Vegetables	0.2
13		Raw Seafood	2.0×10^{-11}		Dry/Semi-dry Fermented Sausages	<0.1
14		Fruits	1.9×10^{-11}		Fresh Soft Cheese	<0.1
15		Dry/Semi-dry Fermented Sausages	1.7×10^{-11}		Semi-Soft Cheese	<0.1
16		Semi-soft Cheese	6.5×10^{-12}		Soft Ripened Cheese	<0.1
17		Soft Ripened Cheese	5.1×10^{-12}		Deli-type Salads	<0.1
18		Vegetables	2.8×10^{-12}		Raw Seafood	<0.1
19		Deli-type Salads	5.6×10^{-13}		Preserved Fish	<0.1
20		Ice Cream and Other Frozen Dairy Products	4.9×10^{-14}		Ice Cream and Other Frozen Dairy Products	<0.1
21		Processed Cheese	4.2×10^{-14}		Processed Cheese	<0.1
22		Cultured Milk Products	3.2×10^{-14}		Cultured Milk Products	<0.1
23		Hard Cheese	4.5×10^{-15}		Hard Cheese	<0.1

[a]Food categories were classified as high risk (>5 cases per billion servings), moderate risk (<5 but >1 case per billion servings), and low risk (<1 case per billion servings).

[b]Food categories were classified as very high risk (>100 cases per annum), high risk (>10 to 100 cases per annum), moderate risk (>1 to 10 cases per annum), and low risk (≤1 cases per annum).

Food Category: Smoked Seafood

The foods in the Smoked Seafood category had a high predicted relative risk of causing listeriosis on a per serving basis. This reflects the fact that Smoked Seafood has a high frequency of contamination; high levels of contamination at retail, supports a moderate rate of growth; and is often stored for moderate lengths of time (and occasionally long periods of time). This is offset somewhat by the moderate serving sizes and the low number of servings associated with this food category. These combine to make Smoked Seafood a moderate contributor to the total number of predicted cases of listeriosis per year.

The predicted relative risk per serving for Smoked Seafood is consistent with various smoked seafoods having been associated with listeriosis. Smoked mussels have been linked to outbreaks of listeriosis in Australia and New Zealand, cold smoked rainbow trout to an outbreak in Sweden, smoked salmon to sporadic cases in Australia, and smoked cod roe to sporadic cases in Denmark (Ryser, 1999a; Brett *et al.*, 1998; Ericsson *et al.*, 1997). Contaminated retail packages are regularly identified by regulatory surveillance programs (Ryser and Marth, 1999a). However, the small volume of most production lots and a low number of servings consumed means that outbreaks are unlikely from a contaminated product; sporadic cases would be expected to be the typical consequences of *Listeria monocytogenes* in this food category.

Foods included in this category from the consumption databases are smoked salmon, trout, herring, oysters, and other smoked fish not identified as to species. Both hot and cold smoked products are included in this category, in part because the consumption databases do not distinguish between these two processes. The predicted median amount consumed per serving for this category is 57.0 g (approximately 2 ounces), and the annual total number of servings in the United States is only 2.0×10^8 (i.e., less than 1 serving per person per annum, on average).

Data from 30 smoked seafood studies provided the contamination data used for this category. Only six of these studies were conducted in the United States. Quantitative data were available in 10 studies. The contamination database included samples from both hot and cold smoking, but the process or the species was not always specified. Salmon was the most frequent product tested but other finfish and mussels were represented. The smoking process for this category,

when specified, was usually cold smoking. The impact of different smoking methods on contamination is not known, but available literature suggests that inactivation resulting from hot smoking is often lost due to recontamination. Cold smoking has no significant effect on *Listeria monocytogenes*. The percentage of retail samples with detectable contamination was high, about 13% overall. In a few cases, the observed level of *Listeria monocytogenes* in the enumerated samples was very high. For example, the NFPA (2002) study (Gombas et al., 2003) collected 2,686 samples at retail and found 113 positive for *Listeria monocytogenes*. Two of these samples were between 10^5 and 10^6 cfu/g.

The growth rate data for this category came from 10 studies containing a total of 25 individual growth rates for hot- and cold-smoked salmon, trout, and cod. The average exponential growth rate adjusted to 5°C was a moderate 0.15 logs/day. Home storage times tend to be moderate in most instances but occasionally samples are stored for lengthy periods. The most likely and maximum storage times used were 3 to 5 days and 15 to 30 days, respectively. The estimated number of *Listeria monocytogenes* consumed per serving was high. The median estimate was 6.7% of servings exceeded 1 x 10^3 cfu/serving and 0.2% of the servings exceeded 1x10^6 cfu/serving.

The predicted median number of cases of listeriosis per serving for Smoked Seafood was 6.2x10^{-9}. This corresponds to a relative risk ranking of fifth for the Smoked Seafood category for the total United States population. The range for the per serving ranking distribution for Smoked Seafood is clustered in the higher ranks, with a normal distribution with a single mode (Figure V-4a). The level of uncertainty was typical of that observed with most food category rankings. The predicted median per annum relative risk rankings were ninth for the total United States population. The median predicted number of cases per annum of 1.3 for the total United States population was moderate. The relative ranking distribution for the per annum value (Figure V-4b) was shifted slightly to the lower risk ranks, reflecting the lower number of servings per year of foods in this category. Although the uncertainty for the cases per annum was greater than for the per serving value, the uncertainty associated with the per annum value was still typical for those observed with most food categories.
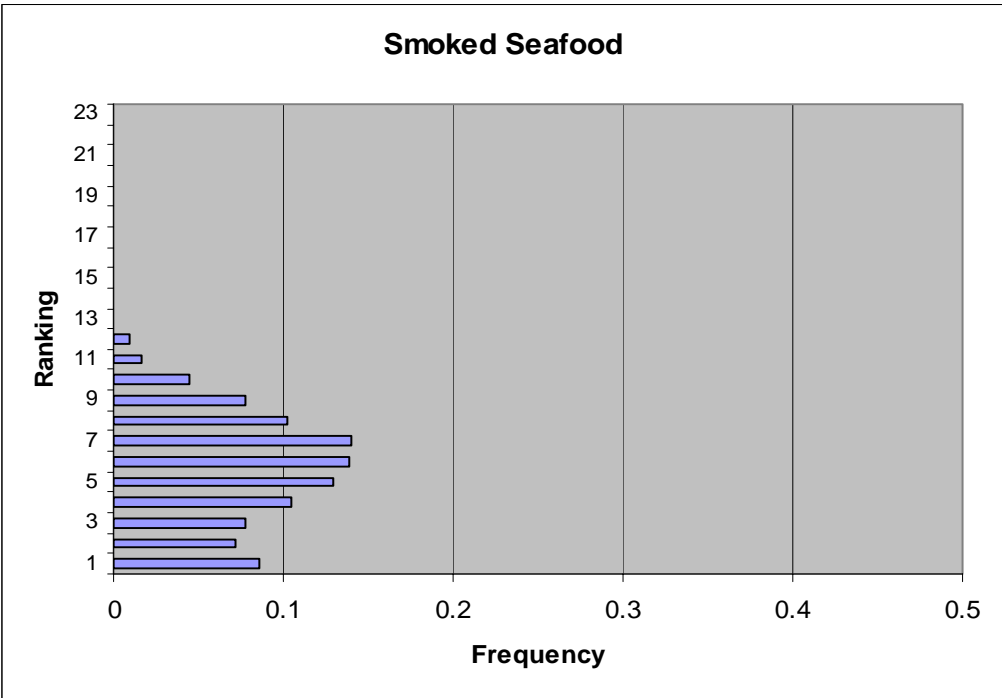
Figure V-4a. Rankings of Total Predicted Listeriosis Cases per Serving for Smoked Seafood
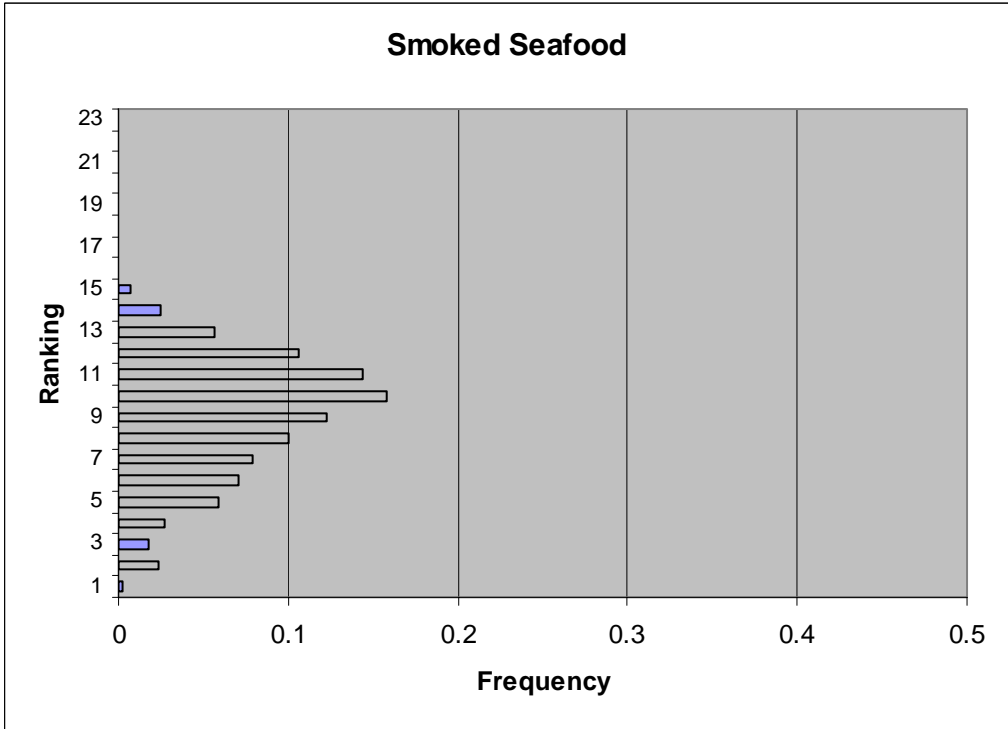

Figure V-4b. Rankings of Total Predicted Listeriosis Cases per Annum for Smoked Seafood

Food Category: Raw Seafood

Raw Seafood has a low predicted relative risk per serving of causing listeriosis in the United States. The foods in this category generally were characterized by a low annual number of servings, a low percent of the population consuming the food, and small serving sizes. However, the contamination levels at retail were high and *Listeria* can grow in these foods at moderate rates. As perishable foods, storage times are typically short which effectively limits the growth and the numbers of organisms likely to be consumed. This combination of factors made the predicted estimates of exposure and illness low. Though the Raw Seafood category has a low predicted relative risk of causing listeriosis in the United States, products in this category have been linked to an outbreak in New Zealand and to a sporadic case in Italy (Farber and Peterkin, 1991).

This category is fairly heterogeneous. Foods for which there were consumption data were flounder, pompano, tuna, sturgeon roe, squid, oysters, and sushi. The median amount consumed per serving is 16.0 g (approximately 0.5 ounce), and the annual total number of servings is low at 1.8×10^8.

Forty-six contamination studies (including 11 from the United States) analyzed over 15,500 samples of uncooked seafood and seafood products, primarily to determine the presence or absence of *Listeria monocytogenes*. Four studies provided quantitative data.

Contamination data were mainly for fresh or frozen whole animals, but products such as cakes, fingers, minces, sushi, and unspecified fish parts are also included. These can be categorized as finfish and non-finfish. Finfish, when specified, included butterfish, catfish, red snapper, trout, and tuna. Both wild caught and aquaculture-reared fish were included. Non-finfish included shellfish and crustaceans. Among the specified foods were lobster, squid, langostino, oyster, shrimp, mussel, clams, and scallops. The percentage of samples with detectable contamination was high (7.0%). Pathogen levels were predicted to be in the high range for the percentage of servings with 10^3 to 10^6 cfu at retail.

Six papers provided *Listeria monocytogenes* growth rates in these foods. Individual foods were trout, catfish, shrimp, and oysters. The growth rates averaged 0.15 logs per day at 5°C. Storage times were relatively short for these foods; the most likely storage time was 1 to 2 days, and the maximum time was 10 to 20 days.

The predicted median risk per serving for the Raw Seafood category was 2.0×10^{-11} and ranked 13th for the total United States population. The range for the per serving ranking distribution (Figure V-5a) is relatively narrow and concentrated in the lower risk ranks. This indicates that there is little uncertainty associated with the predicted per serving relative risk ranking for the Raw Seafood category. The predicted median per annum relative risk ranking was 18th for the total United States population. The range for the per annum ranking distribution (Figure V-5b) was narrow, indicating that there is also little uncertainty associated with the per annum predicted relative risk for Raw Seafood. This decrease in the per annum ranking compared to the per serving ranking is consistent with the small number of servings consumed per year.
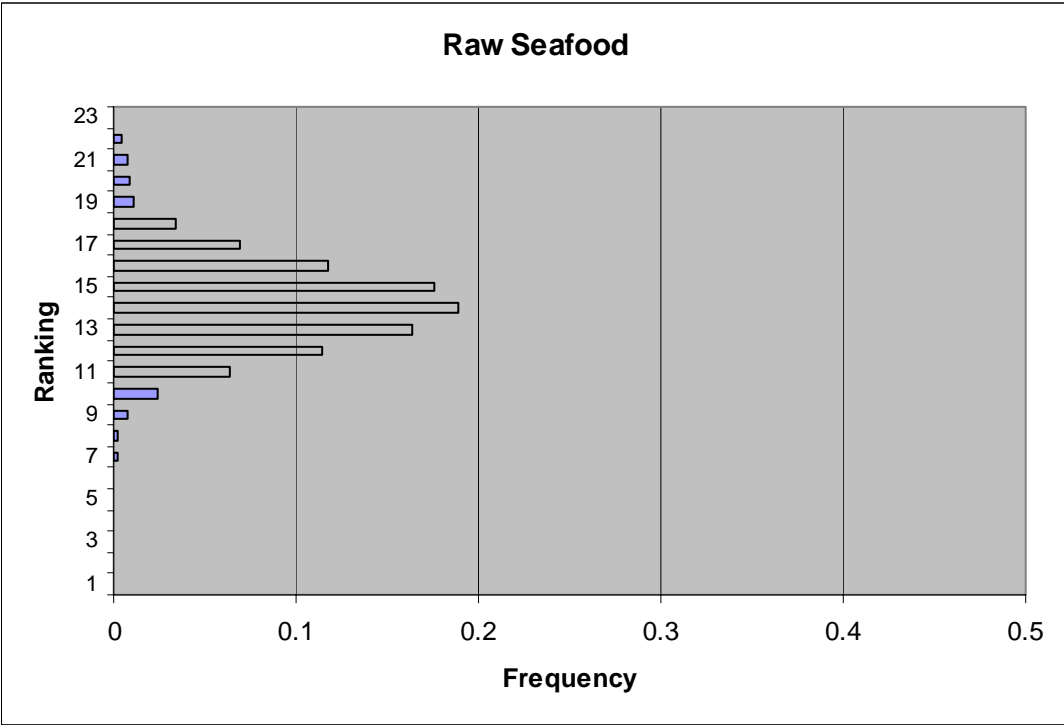
Figure V-5a. Rankings of Total Predicted Listeriosis Cases per Serving for Raw Seafood
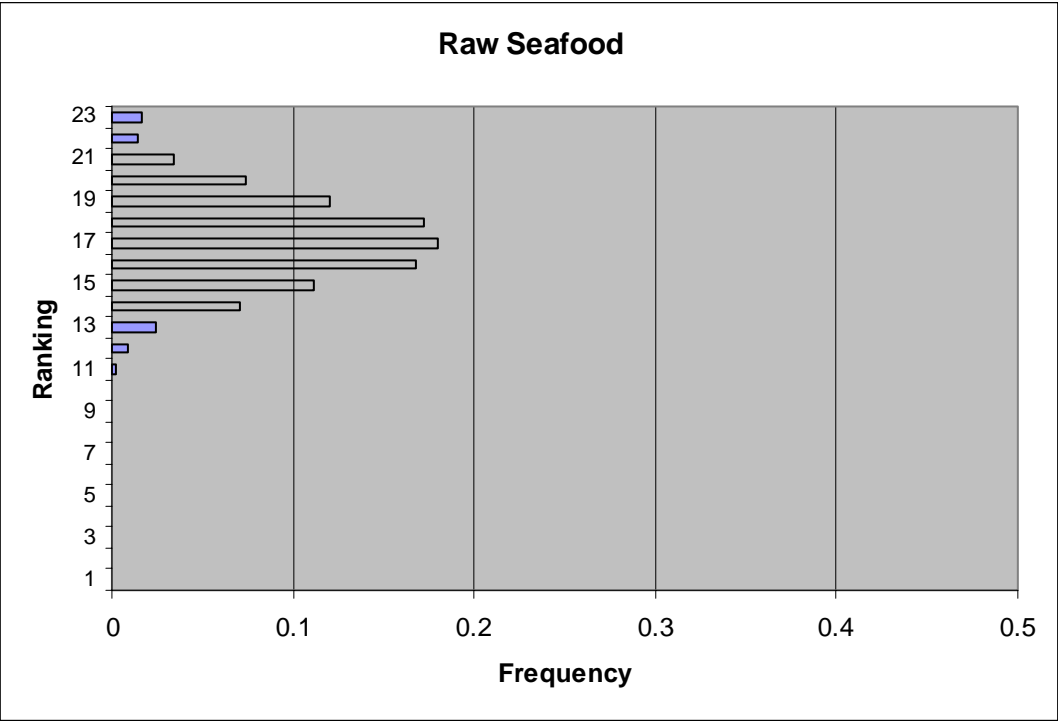

Figure V-5b. Rankings of Total Predicted Listeriosis Cases per Annum for Raw Seafood

Food Category: Preserved Fish

Preserved Fish, including pickled, marinated, or dried products, had a low predicted relative risk of causing listeriosis on a per serving basis and a low predicted contribution to the total number of cases on a per annum basis. The foods in this category had a low annual number of servings and a low percent of the population consuming the food, but had moderate serving sizes, high frequency of contamination, and moderate contamination levels at retail. Growth was not modeled for this category, since preserved fish do not support growth. Typically, the inability of a food category to support the growth of *Listeria monocytogenes* results in a low per serving relative risk. However, in this instance the lack of growth appears to be offset by the frequency of contamination at retail. Moderate level contamination likely occurs because foods in the Preserved Fish category are often prepared using traditional techniques, which require long processing times and occasionally may not meet stringent sanitary standards. This creates the potential for substantial growth of *Listeria monocytogenes* during initial production steps (e.g., brining) before the product equilibrates to the salt and pH levels that are the basis of preservation. Gravad rainbow trout has been linked to an outbreak of listeriosis in Sweden (Ericsson *et. al.*, 1997).

The Preserved Fish category includes consumption data for pickled or marinated fish, such as ceviche and pickled herring, dried and salted cod, and non-specified dried fish. The median amount consumed per serving for this category is 70 g (approximately 2.5 ounces), and the annual total number of servings is 1.1×10^8.

Contamination data for this food category was from 18 studies. Haddock, gravad trout, ceviche, and unspecified finfish that were pickled, smoked, dried, salted, or preserved were included. Of these studies only one was from the United States. Five studies contained quantitative data. The percentage of samples with detectable contamination was 9.8%, higher than for Raw Seafood, but just slightly less than Smoked Seafood. The predicted percentage of servings contaminated with 10^3 to 10^6 cfu at retail was moderate.

Because these products do not allow growth of *Listeria monocytogenes,* storage times are not a factor in the levels of *Listeria monocytogenes* present at the time of consumption. Although not

a factor, storage times were also believed to be somewhat shorter than those for Smoked Seafood. The high salt and acidity present in the final products prevent growth of *Listeria monocytogenes*. However, the microorganism is known to survive these conditions, particularly if held at refrigeration temperatures.

The predicted median risk per serving for the Preserved Fish category was 2.3×10^{-11}, which corresponds to a relative risk rankings of twelfth for the total United States population. The range for the per serving relative ranking distribution is relatively broad (Figure V-6a) with a bimodal distribution. The wide spread indicates a high degree of uncertainty which likely is due to a combination of the limited quantitative data and broad variability in conditions under which these products are produced. The bimodal distribution may indicate that there are differences among different foods within this food category, and may require that the category be subdivided if additional data become available in the future in order to achieve a more accurate measure of the relative risks associated with the different foods. The predicted median per annum relative risk ranking was low, at less than one case per annum and ranked nineteenth for the total United States population. The range for the per annum ranking distribution was also a bimodal distribution (Figure V-6b), again indicating a substantial degree of uncertainty or variability. Overall this food category is not predicted to make a substantial contribution to the cases of listeriosis in the United States, however, the uncertainty in the risk per serving indicates that it may be a concern for the small population that consumes these products.
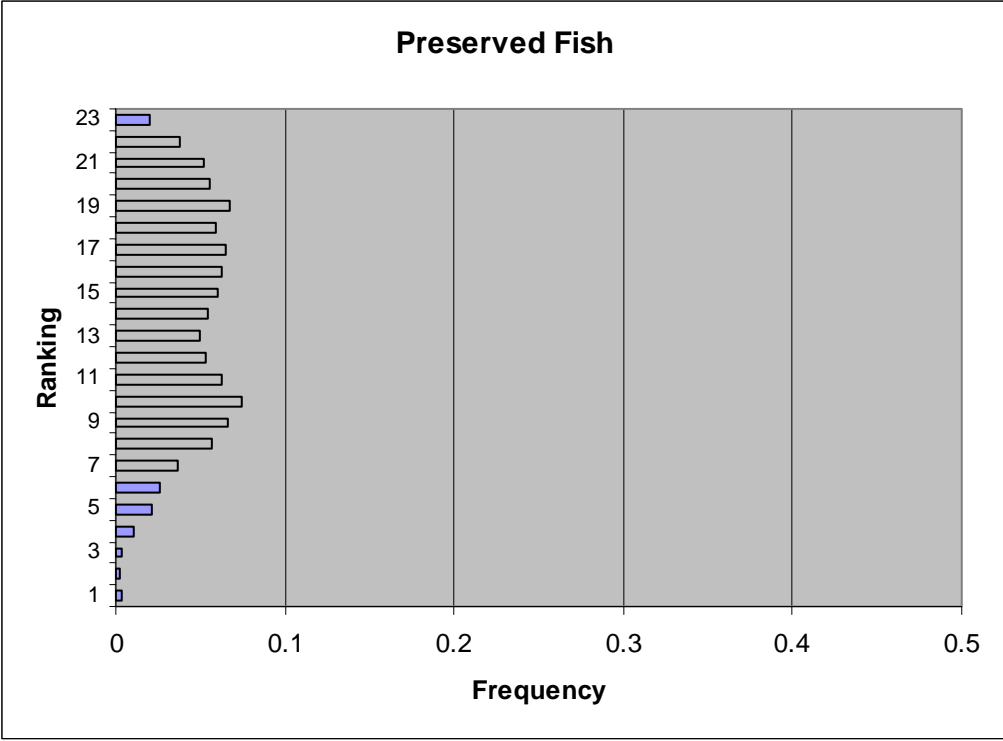
Figure V-6a. Rankings of Total Predicted Listeriosis Cases per Serving for Preserved Fish
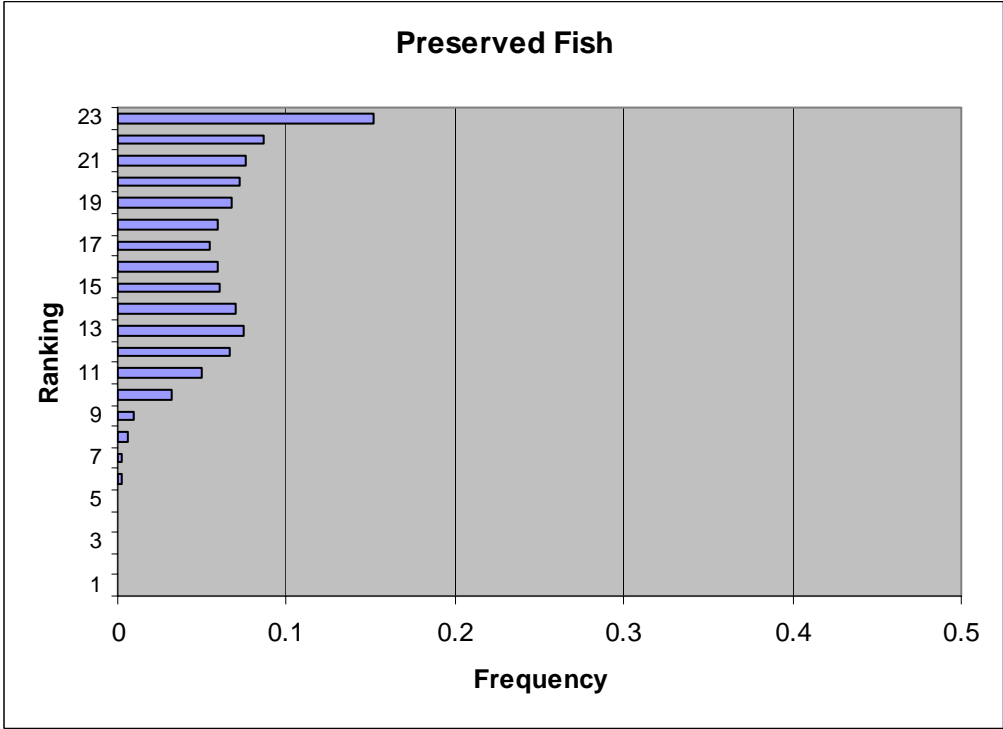

Figure V-6b. Rankings of Total Predicted Listeriosis Cases per Annum for Preserved Fish

Food Category: Cooked Ready-to-Eat Crustaceans

Cooked Ready-to-Eat (RTE) Crustaceans (crab and shrimp) had a high predicted relative risk of causing listeriosis in the United States on a per serving basis. The foods in this category generally were consumed at a low frequency and with moderate serving sizes. The relatively high growth rate of *Listeria monocytogenes* in these foods, one of the usual factors that drives listeriosis risk in food, was offset by relatively short storage times. It would be expected that the cooking step in the preparation of these foods would eliminate *Listeria monocytogenes*. However, foods in this category may often be stored refrigerated after cooking, allowing for recontamination and growth.

Imitation crabmeat has been linked to an outbreak of listeriosis in Canada and shrimp was epidemiologically linked to an outbreak in the United States. (Ryser, 1999a; Riedo *et al.*, 1994). The FDA has also monitored recalls for cooked shrimp and crab.

The Cooked RTE Crustaceans category includes consumption data for steamed, hard shell crab; steamed or boiled shrimp; and cocktail shrimp. The median serving size for this category was 50 g (approximately 1.8 ounces), and the annual total number of servings was 5.5×10^8.

Eleven contamination studies provided data mainly from cooked crab and shrimp. Four studies were for product in the United States. Two studies, both from the United States, provided quantitative data. The percentage of contaminated samples was moderate at 2.8%. A small number of samples with high contamination levels (greater than 10^3 cfu/g) have been reported. The predicted percentage of servings with 10^3 to 10^6 cfu/serving at retail was moderate. Only three papers were found that reported growth rates for pasteurized crab and for cooked shrimp and lobster. This category had the fastest reported growth rates of any food category, averaging 0.38 logs/day at 5° C**.** Storage times were estimated to be relatively short; the most likely storage time was only 1 to 2 days, and the maximum time was 10 to 20 days.

The predicted median risk per serving for the Cooked RTE Crustaceans category of 5.1×10^{-9} corresponded to a relative risk ranking of sixth for the total United States population. The range

for the per serving ranking distribution for Cooked RTE Crustaceans (Figure V-7a) is narrow and concentrated in the lower risk rankings (i.e., a higher risk food). This indicates that there is little uncertainty associated with the predicted per serving relative risk for the Cooked RTE Crustaceans category. The predicted median per annum risk is approximately three cases of listeriosis per annum and a relative risk ranking of eighth for the total United States population. The range for the per annum ranking distribution is narrow and generally normally distributed (Figure V-7b), suggesting relatively little variability or uncertainty in the extent to which this food category is consumed.
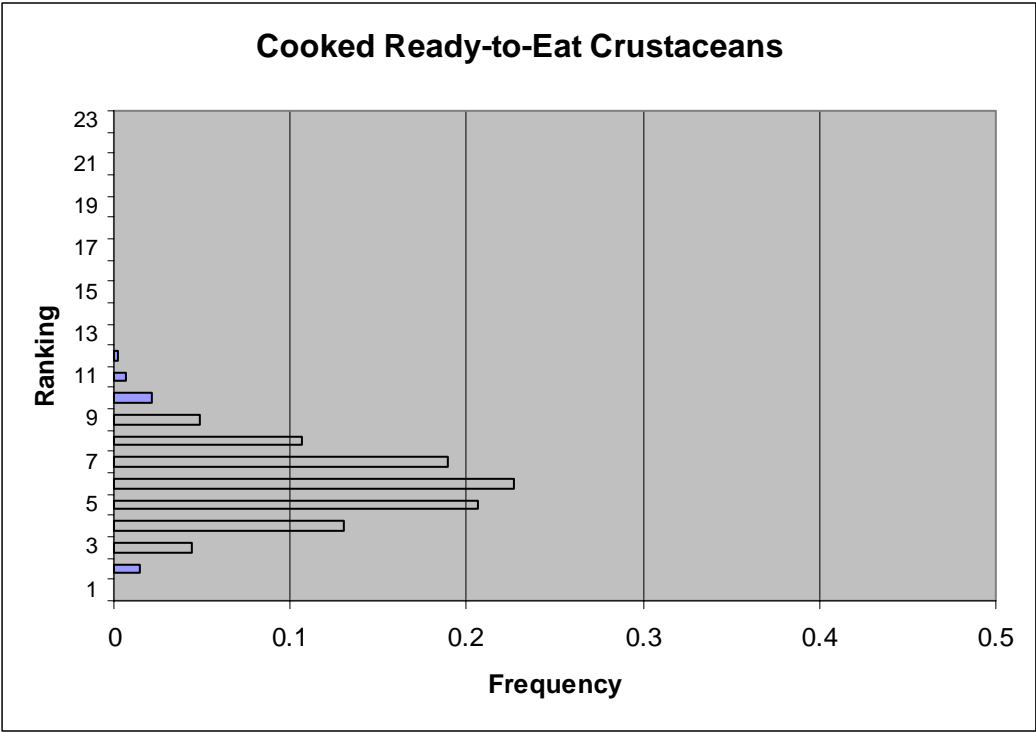
Figure V-7a. **Rankings of Total Predicted Listeriosis Cases per Serving for Cooked Ready-to-Eat Crustaceans**
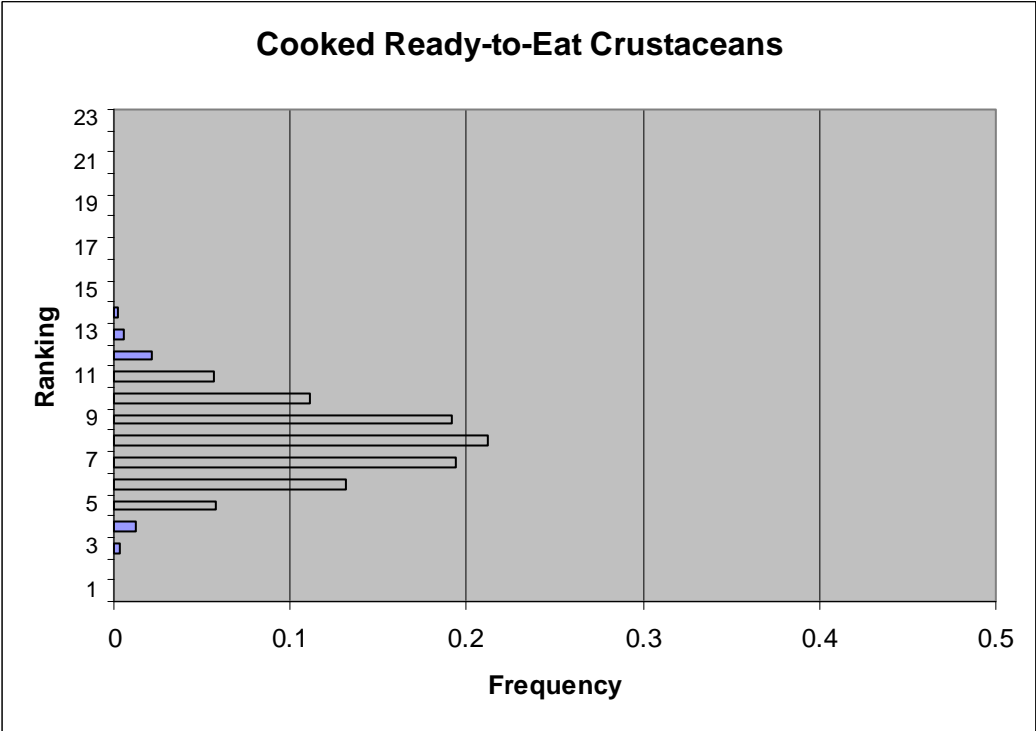
Figure V-7b. **Rankings of Total Predicted Listeriosis Cases per Annum for Cooked Ready-to-Eat Crustaceans**

Food Category: Vegetables

Foods in the Vegetables category had a low predicted relative risk of causing listeriosis in the United States on a per serving basis. The Vegetables category is difficult to characterize because it encompasses a diverse set of products that are typically consumed without cooking. The annual number of servings of Vegetables is high, while the median serving size, contamination level, and growth rate are low. The storage time and the contamination frequency are moderate.

Both raw and processed vegetables have been implicated in outbreaks. Raw vegetables have been linked to outbreaks of listeriosis in Austria and Western Australia; frozen broccoli, cauliflower, celery, tomatoes, and lettuce in the United States (Ryser, 1999a; Simpson, 1996; Riedo *et al.*, 1994; Farber and Peterkin, 1991; Allerberger and Guggenbichler, 1989). In addition, raw vegetables have been linked to sporadic cases in Australia, the U.K. (English lettuce, vegetable rennet), and Finland (salted mushrooms) (Ryser, 1999a; Farber and Peterkin, 1991).

Foods included in the Vegetables category are raw as well as mixed vegetable salads that contain raw vegetables but not salad dressing. In addition to vegetables typically consumed raw (e.g., spinach, carrots, tomatoes, celery, lettuce, onions), this category includes less frequently consumed vegetables such as artichokes, sprouts, and raw seaweed. However, salads such as cole slaw and potato salads are included in the Deli-type Salads food category because of the creamy dressing base and frequent handling in the retail deli. The median amount consumed per serving for this category is 28 g (i.e., ~ 1 ounce), and the annual total number of servings is 8.5×10^{10}. The low median serving size most likely reflects the consumption patterns associated with the wide span of vegetable types included in the analysis, though certain vegetables may be eaten in substantially larger amounts (e.g., tomatoes).

Thirty-two contamination studies were found that examined individual raw vegetables or mixed vegetables (without dressing). Of these studies, five were from the United States and eight contained quantitative data. The vegetables analyzed included raw bean sprouts, broccoli, cabbage, carrot, celery, cilantro, cress, cucumber, fennel, legumes, lettuce, mushrooms, parsley,

green peppers, onions, radish, scallion, tomato, and watercress. The NPFA (2002) survey collected 2,963 samples of bagged, precut leafy salads and found 2.3% positive, with one sample containing between 10^2 and 10^3 cfu/g. Overall, the percentage of samples with detectable contamination was a moderate 3.6%. The predicted percentage of servings with high contamination levels was low.

Nine papers provided 26 estimates of growth rates for *Listeria monocytogenes* on vegetables. The vegetables included in these studies were lettuce, cabbage, broccoli, cauliflower, asparagus, tomatoes, and carrots. The average growth rate of Vegetables was slow, 0.07 logs/day at 5°C. Moderate storage times were assumed with the most likely 3 to 4 days and the maximum of 8 to 12 days.

The predicted median risk per serving for the Vegetables category was 2.8×10^{-12} and the relative risk ranking was eighteenth for the total United States population. The range for per serving distribution for Vegetables (Figure V-8a) is similar to what was observed with most food categories and clustered in the lower risk rankings. This indicates that there is relatively little uncertainty associated with the predicted per serving relative risk for the Vegetables category. The predicted median per annum risk was less than one case and the corresponding relative risk ranking was twelfth for the total population. The per annum ranking distribution (Figure V-8b) had a relatively broad range, indicating substantial uncertainty. The distribution was shifted to the higher risk ranks compared to the per serving distribution. These results presumably reflect the large number of servings of Vegetables consumed, as well as the variability in the products encompassed in this highly diverse category. The broad range suggests that this food category and its ranking could benefit from additional investigations and the possible subdivision of the food category into several smaller groupings.

Vegetables

Figure V-8a. Rankings of Total Predicted Listeriosis Cases per Serving for Vegetables
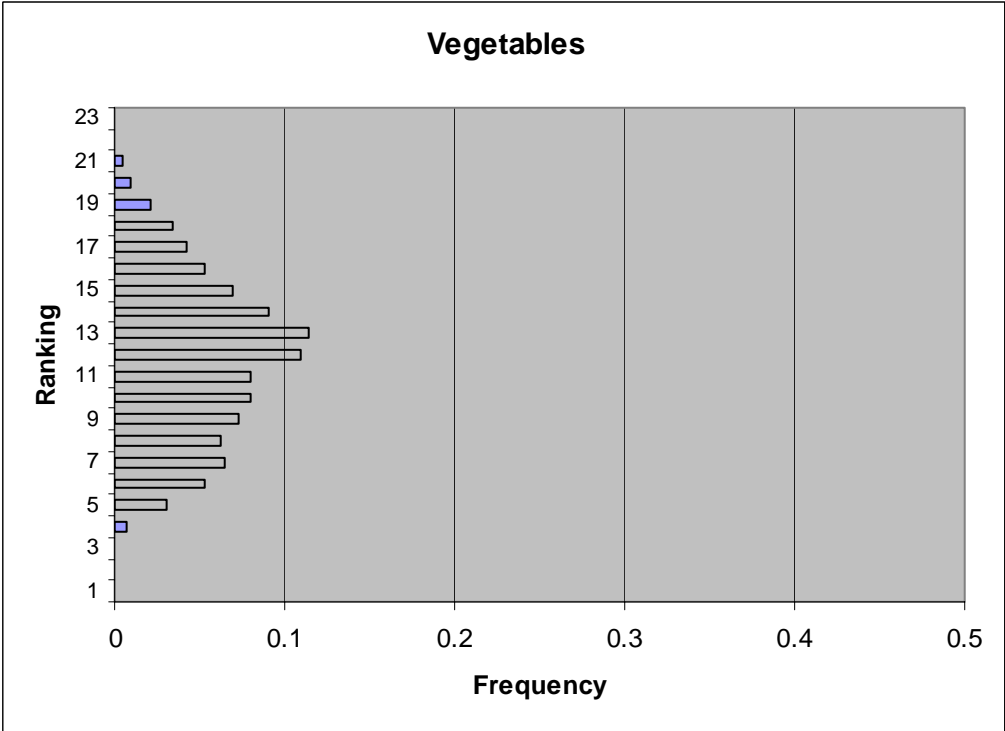
Vegetables

Figure V-8b. Rankings of Total Predicted Listeriosis Cases per Annum for Vegetables

Food Category: Fruits

Foods in the Fruits category had a low predicted relative risk of causing listeriosis on a per serving basis. Fruits have not been linked to outbreaks or sporadic cases of listeriosis, and this might explain why there is little contamination data in the published literature available for this category. The annual number of servings, median serving size and contamination frequency of Fruits are high. These factors lead to a high risk for fruits on a per annum basis even though the growth of *Listeria monocytogenes* during storage would be low. The high level of uncertainty indicates a need for more information and data for this food category. This is a diverse food category that includes acidic fruits (such as pineapples) and pH neutral fruits (such as cantaloupes).

The Fruits category includes consumption data for many types of raw and dried fruits, as well as fruit salads (with fruits as the main ingredient without salad dressing). This category is simplified from the 2001 draft risk assessment in that fruit salads containing salad dressing were moved to the Deli-type Salad food category. The median amount consumed per serving for this category is 118 g (i.e., slightly over 4 ounces), and the annual total number of servings is 4.9×10^{10}.

Only four contamination studies, two of which were from the United States were available. None of these studies included quantitative data. Fruits specified in these studies included apples, blueberries, cantaloupes, pears, pineapples, and fruit products. The percentage of samples with detectable contamination was 11.8%, a high contamination frequency. The contamination levels were estimated from the presence/absence data assuming the standard deviation of the frequencies of contamination levels. The high frequency of contamination would indicate that high levels of contamination could also occur.

Two studies (orange juice and fresh apple slices) were found that characterized the rate of *Listeria monocytogenes* growth in fruits. When the pH was less than 4.8, *Listeria monocytogenes* did not grow. At pH 5.0, growth was slow, at 0.05 logs/day. Moderate storage

times were assigned for this category, with a most likely time of 3 to 4 and a maximum time of 8 to 12 days.

The predicted median risk per serving for the Fruits category was 1.9×10^{-11} which corresponds to a relative risk ranking of fourteenth for the total United States population. The range for the ranking distribution for Fruits (Figure V-9a) is broad. The predicted median risk per annum is approximately 1 case per year and the relative risk ranking is tenth for the total United States population (Figure V-9b). This increase in relative risk compared to the per serving value reflects the large number of servings consumed annually. The range for the ranking distribution was broad indicating substantial uncertainty in the predicted relative risk ranking. This likely reflects the limited data available, the diversity of the products that fall within this food category, and the variability in the frequency and extent of contamination rates among the data that were evaluated. The bimodal nature of the distribution suggests that the food category may need to be subdivided when additional data become available. Overall, the Fruits category is a broad category with varied consumption and contamination, and few data were available to characterize this category. Thus, there is a high degree of uncertainty associated with this category.
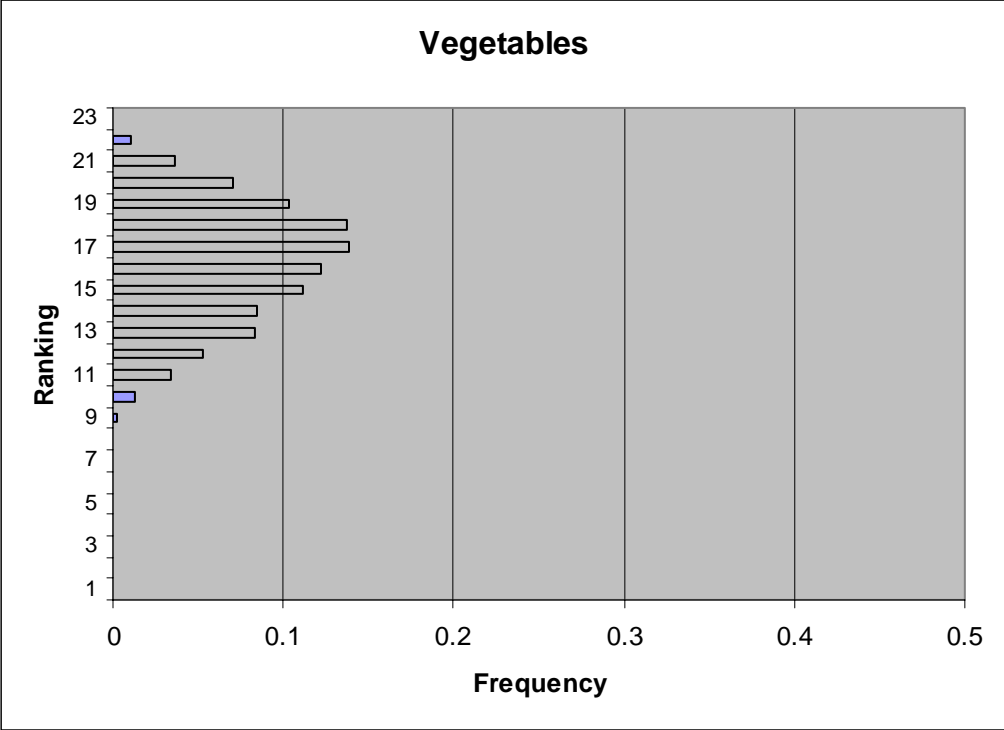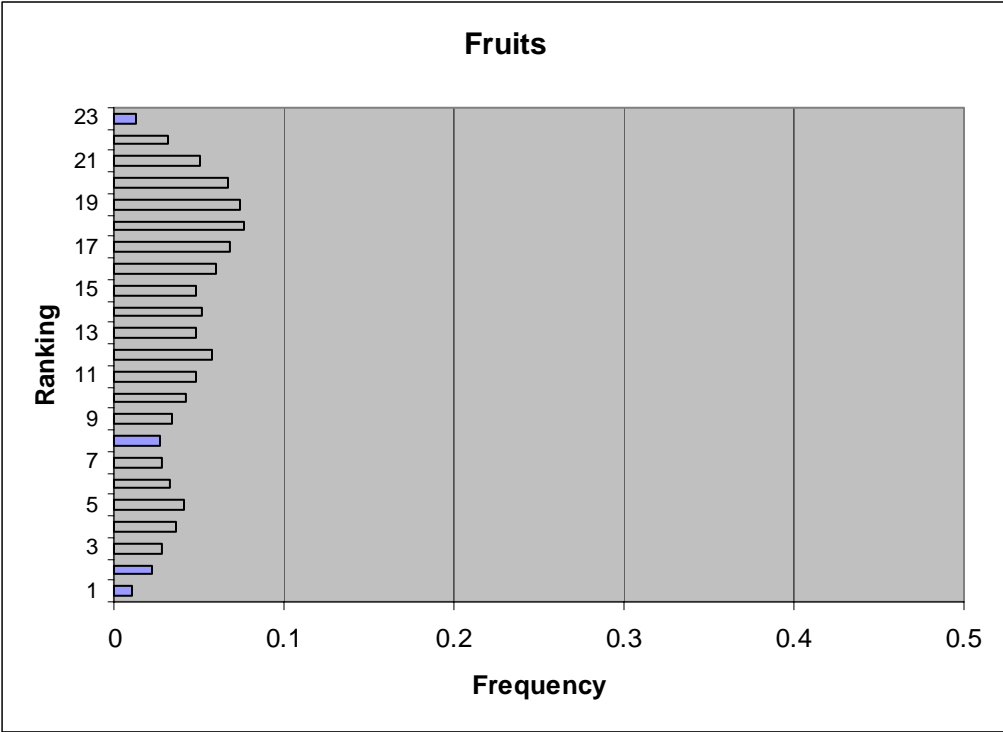
Figure V-9a. Rankings of Total Predicted Listeriosis Cases per Serving for Fruits
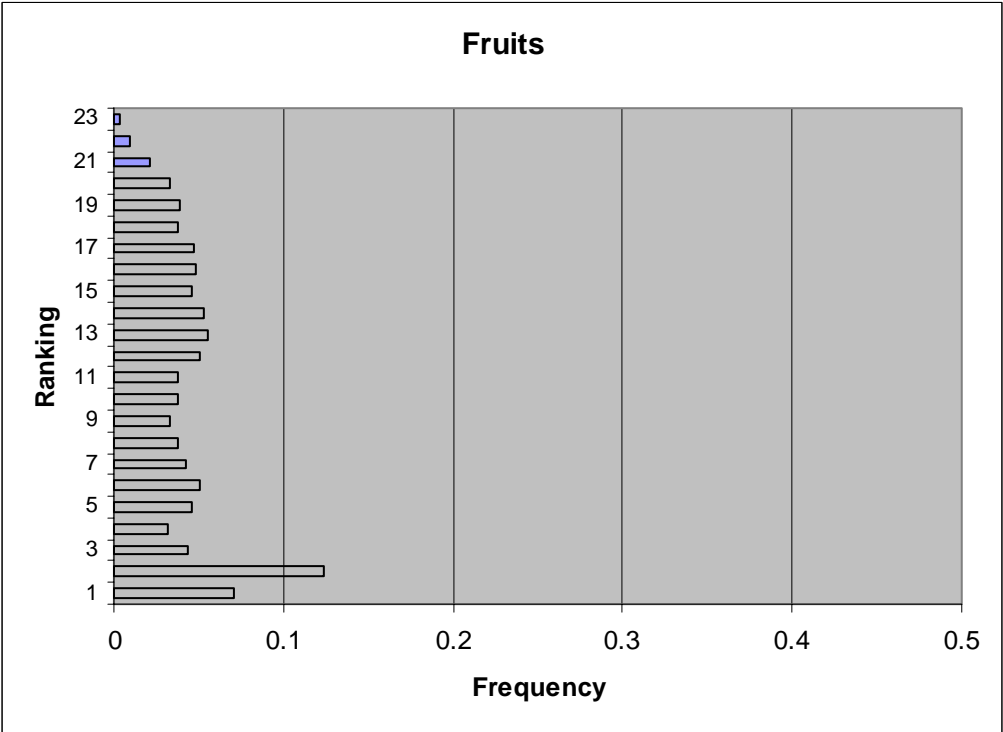
Figure V-9b. Rankings of Total Predicted Listeriosis Cases per Annum for Fruits

Food Category: Fresh Soft Cheese

Fresh Soft Cheese had a low predicted relative risk of causing listeriosis on a per serving basis. These cheeses are high moisture (>50%) fresh cheeses consumed shortly after manufacture. This category includes traditional Hispanic-style soft cheese (sometimes made from raw, unpasteurized fluid milk) such as panela, Queso de Crema, Queso Fresco,and Queso de Puna. The 2001 draft risk assessment included Queso Chihuahia and Queso Asadero attributed to this category, but these cheeses were moved from this category because they are not fresh, high moisture cheeses. The contamination level at retail, contamination frequency, growth rate during storage, and the annual number of servings are all low.

Fresh Soft Cheese (suspected to be made from unpasteurized milk) has been linked to both outbreaks and sporadic cases of listeriosis in the United States (Ryser, 1999a; Linnan *et al.*, 1988; CDC, 2001), including an outbreak in Los Angeles in 1985 and one in North Carolina in 2001. The 1985 outbreak in Los Angeles was the incident that convincingly established *Listeria moncytogenes* as an important serious foodborne pathogen. In 2000/2001, an outbreak in the Carolinas associated with homemade cheese made from unpasterurized milk resulted in 12 cases of serious listeroisis.

Consumption data was only available for one type of Fresh Soft Cheese, Queso fresco. The median amount consumed per serving for this category is 31 g (just over 1 ounce), and the annual number of servings is 7.1×10^7. Data are not available to estimate the proportion of Fresh Soft Cheese that is consumed in the United States made from unpasteurized milk; however, since the initial outbreak there has been a concerted effort to reduce the consumption of soft fresh cheeses made from unpasteurized milk. Fresh soft cheese made from unpasteurized milk does not meet FDA standards for interstate commerce.

Data from eight contamination studies were used to model the frequency of contamination for the Fresh Soft Cheese category. Cheeses in these studies were described as Hispanic-style, Queso Fresco, panela, requesoy, and fresh cow and goat milk cheeses. The most recent study was the NFPA (2002) survey and the contamination levels found in this study were much lower than those previously observed. In that study, 5 contaminated samples out of 2,936 total samples

were positive, all at a level of less than 100 cfu/g. The samples from the NFPA study were collected in retail stores and were most likely made from pasteurized milk. Products made outside the retail system (including those made from unpasteurized milk) were not reflected in the NFPA survey. A 'what if' scenerio test was conducted to allow a comparison of the expected estimate of the risk per serving for fresh soft cheese made from pasteurized vs. raw, unpasteurized milk (see below).

Only one growth rate study with these cheeses was available. That study reported a low growth rate of 0.082 logs/day when adjusted to 5° C. The assumed storage times for Fresh Soft Cheese were 1 to 5 days and 15 to 30 days for most likely and maximum times, respectively.

The median risk per serving for the Fresh Soft Cheese category of 1.7×10^{-10} corresponds to a relative predicted risk ranking of tenth for the total United States population. The range for the predicted per serving risk ranking distribution for Fresh Soft Cheese (Figure V-10a) is relatively narrow and concentrated in the middle of the risk rankings. This indicates that there is little uncertainty associated with the per serving predicted relative risk for the Fresh Soft Cheese category. The predicted median per annum risk was less than one case per year and the relative risk ranking was fourteenth for the total United States population. The range for the per annum ranking distribution is concentrated in the higher risk rankings (Figure V-10b) indicating a lower risk. The breadth of the range indicates that there was somewhat more uncertainty associated with the per annum predicted relative risk ranking for the Fresh Soft Cheese category. This is likely associated with variability in the number of servings and the serving sizes.

An area of uncertainty associated with this food category that is not captured in this risk assessment is the consumption of "homemade" soft cheeses made from raw, unpasteurized milk. Raw milk soft cheeses are not produced and marketed through typical commercial means and have in the past been illegally brought into the United States. Data on such cheeses are not captured in the contamination data base used to develop this risk assessment. However, we recognize that a substantial portion of soft cheeses consumed in the United States may be made from unpasteurized milk.

Scenario Testing: Fresh Soft Cheese Made From Contaminated Unpasteurized Milk

Unlike the 2001 draft risk assessment, the revised risk assessment indicates that the risk from Fresh Soft Cheese is low. This change is largely attributable to the inclusion of additional new data indicating a very low prevalence rate in this food category. However, in the past there has been a strong epidemiological correlation between Hispanic-style fresh soft cheese (Queso Fresco) and listeriosis. A likely explanation for this discrepancy is that the data collected for this category is not representative of the cheese linked to the disease (i.e., fresh soft cheese made from raw, unpasteurized milk). In particular, although most commercial sources of fresh soft cheese are manufactured from pasteurized milk, some sources of queso fresco are made from raw milk. Many of these sources appear to be restricted to specific local areas and have not had the benefit of FDA oversight.

To characterize the risk from highly contaminated queso fresco an exposure model was constructed using the same analog as in the 2001 draft risk assessment – soft unripened cheese made from raw milk (Loncarevik, *et al*., 1995), where 50% of the samples tested were positive. The tested 'high prevalence' scenario increased the predicted risk on a per serving basis approximately 40-fold for the perinatal and elderly subpopulations. (For additional details, see Chapter VI 'What-If' Scenarios.)

Figure V-10a. Rankings of Total Predicted Listeriosis Cases per Serving for Fresh Soft Cheese

Figure V-10b. Rankings of Total Predicted Listeriosis Cases per Annum for Fresh Soft Cheese

Food Category: Soft Unripened Cheese

The Soft Unripened Cheese category has a moderate predicted relative risk of causing listeriosis on a per serving basis. The cheeses in this category have moderate frequency and levels of contamination and can have a long storage time. However, they support only a low rate of growth. Serving sizes are typically low, whereas the annual number of servings and contamination levels at retail are moderate. There was a sporadic listeriosis case in the United States linked to the consumption of a highly contaminated ricotta cheese (Ryser, 1999a). There are no reported cases of listeriosis associated with consumption of cottage and cream cheese, but there have been FDA recalls of cream cheese products.

The category represents high moisture (>50%), white curd varieties such as cottage cheese, baker's cream, and American-type Neufchatel cheese. Milk to be manufactured into soft unripened cheese is coagulated through the production of acid by the starter culture (or by direct acidification of milk) rather then by addition of a coagulant. Unlike fresh soft cheese, the refrigerated shelf-life is typically up to 60 days.

Consumption data available were available for cottage, cream, and ricotta cheeses. The median amount consumed per serving for this category is 29 g (about 1 ounce), and the annual total number of servings is 4.4×10^9.

There were eight studies with contamination data for these cheeses, with two from the United States. Three quantitative studies provided quantitative data. Cheeses in the contamination database included Anari, Halloumi, farmer, gournay, Quark, and cottage cheese. Of the 32 positive samples, four samples contained over 500 cfu/g and four samples over 10^6 cfu/g. The percentage of positive samples was 3.9%.

Twenty-nine data sets provided data on the growth or survival of *Listeria monocytogenes* in these cheeses. Nine of these studies showed a decline in levels over time. The research literature indicates that growth or decline of *Listeria monocytogenes* in these low salt cheeses is largely dependent upon pH. For example, ricotta cheese (pH=5.9 to 6.1) permitted rapid growth,

whereas declines were observed in some cream cheeses (pH=4.8). The growth rates were standardized to 5 °C and a distribution fitted to the data to allow growth or decline (i.e., negative growth) in proportion to the available data. The average growth rate was 0.09 logs/day. Storage times were relative long, with the most likely 6 to 10 days and the maximum 15 to 45 days.

The median risk per serving for the Soft Unripened Cheese category of 1.8×10^{-9} corresponds to a relative predicted risk ranking of eighth for the total United States population. The range for the predicted per serving risk rankings for Soft Unripened Cheese (Figure V-11a) is bimodal but concentrated in the higher risk rankings. This indicates some uncertainty associated with the per serving predicted relative risk for this category. The median per annum risk was predicted as approximately 8 cases per year and the relative risk ranking was fifth for the total United States population. The range for the per annum ranking distribution is concentrated in the lower risk rankings, which corresponds to a higher risk (Figure V-11b). However, the broad ranges in uncertainty likely result from the differences of the products in this food category to support growth or cause a decline in levels of *Listeria monocytogenes*. Based on these results, this food category could benefit from subdivision.
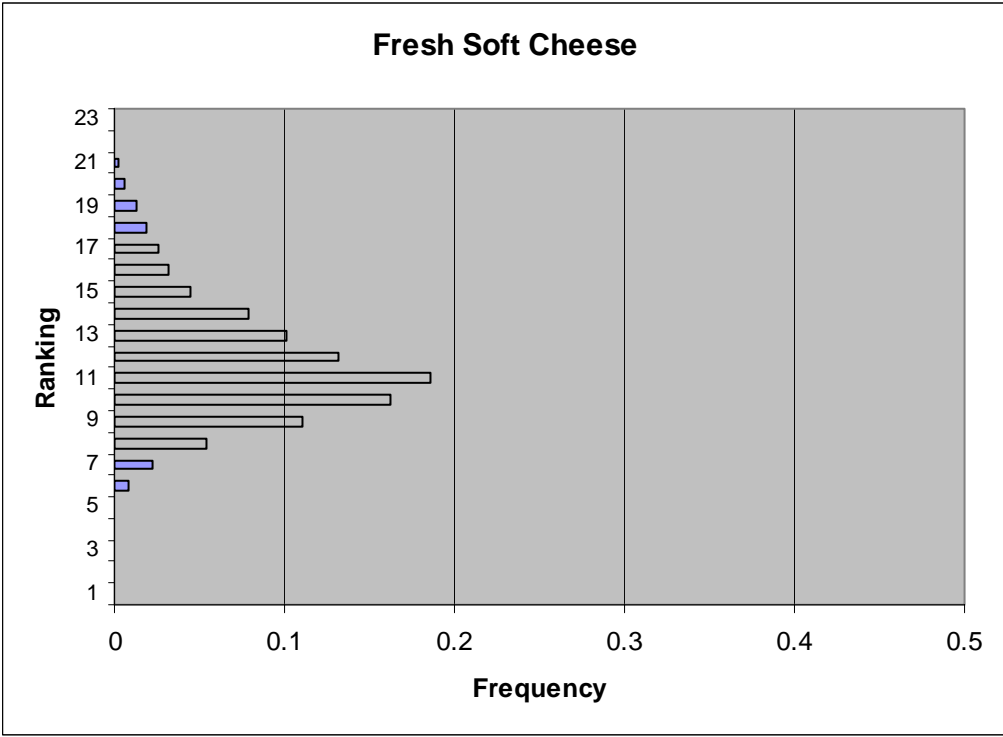
Figure V-11a. Rankings of Total Predicted Listeriosis Cases per Serving for Soft Unripened Cheese

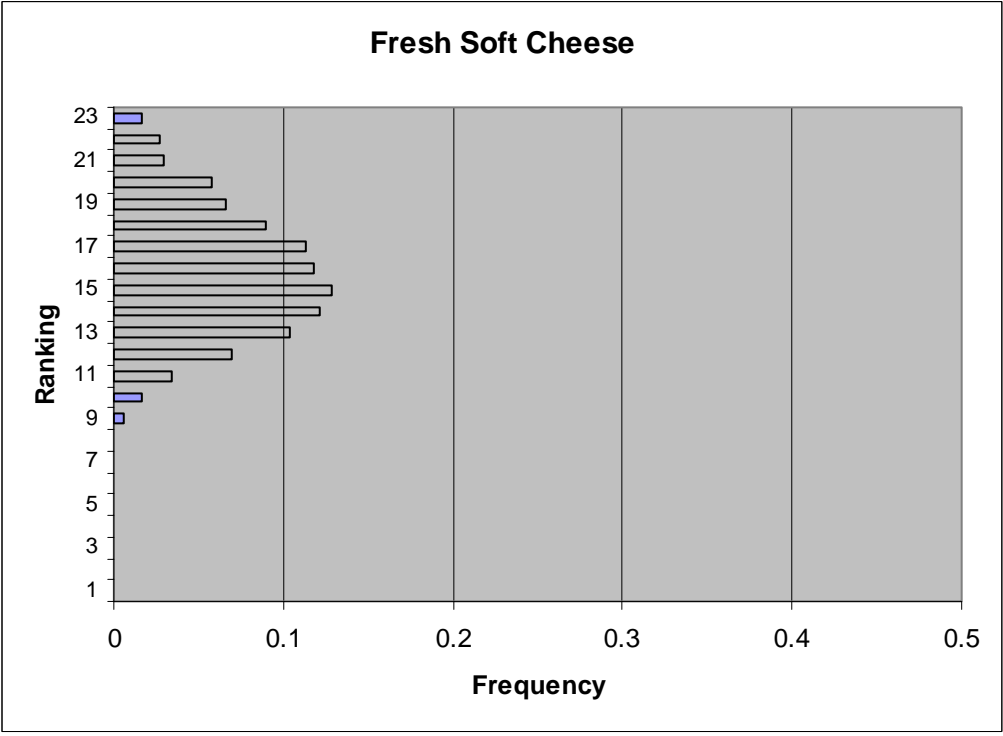**Figure V-11b. Rankings of Total Predicted Listeriosis Cases per Annum for Soft Unripened Cheese**

Food Category: Soft Ripened Cheese

The cheeses in the Soft Ripened Cheese food category had a low predicted relative risk of causing listeriosis in the United States on a per serving basis. This food category includes high moisture (>50%), ripened cheeses such as mold surface-ripened cheeses (Brie, Camembert), pickled (white brined) cheeses, feta, and soft Italian-style cheeses (mozzarella). There are a moderate number of annual servings and small serving sizes. Growth rates were low but, contamination frequencies and levels at retail were moderate and storage times were long. Soft Ripened Cheeses including mold-ripened cheeses have been linked to outbreaks of listeriosis in Denmark, France and Switzerland and linked to sporadic cases in Belgium, Canada, and the U.K (Ryser, 1999a; Riedo *et al.*, 1994; Art and Andre, 1991; Farber and Peterkin, 1991). There have not been any confirmed reports of sporadic cases or outbreaks associated with these cheeses in the United States.

The median amount consumed per serving for this category is 28 g (~1 ounce) and the annual number of servings is 1.9×10^9. Data are not available on the proportion of United States or imported cheese that is made from unpasteurized fluid milk. Market data indicate that the United States imports approximately 50% of the Camembert and Brie Cheese and 20% of the feta cheese sold in the United States (National Cheese Institute, 1998).

Contamination data was obtained for 17 studies with three being from the United States. Five studies provided quantitative data. Brie, Camembert, Feta, and Taleggio are some of the cheeses represented in the contamination data. Of the 17 studies, 6 contained quantitative contamination data. In the 2001 NFPA study, two samples were positive for *Listeria monocytogenes* with levels less than 10 cfu/g. The frequency of contamination was 3.8%.

Listeria monocytogenes populations were reported in the research literature to both increase and decrease in these cheeses. Of 17 studies, 7 showed declines, one no change, and 9 indicated growth. Therefore, the growth rate distribution used with this food category (–0.013 logs/day) included both growth and decline, with the 'average' response being a slow rate of decline. Storage times for this food category were long, with a maximum of 15 to 45 days.

The median risk per serving for the Soft Ripened Cheese category of $5.1\text{x}10^{-12}$ corresponds to a relative predicted risk ranking of seventeenth for the total United States population. The range for the predicted per serving risk ranking distribution for this category (Figure V-12a) is broad but concentrated in the higher risk rankings (low predicted risk). This indicates substantial uncertainty associated with the per serving predicted relative risk for this category resulting from the ability of some of these cheeses to support growth of *Listeria monocytogenes* and other cheeses to cause a decline. The median per annum risk was predicted as less than one case of listeriosis per year and the relative risk ranking was sixteenth for the total United States population. With the wide range for the per serving rankings, the resulting range for the per annum ranking distribution is quite broad (Figure V-12b) indicating high uncertainty associated with the per annum predicted relative risk ranking.
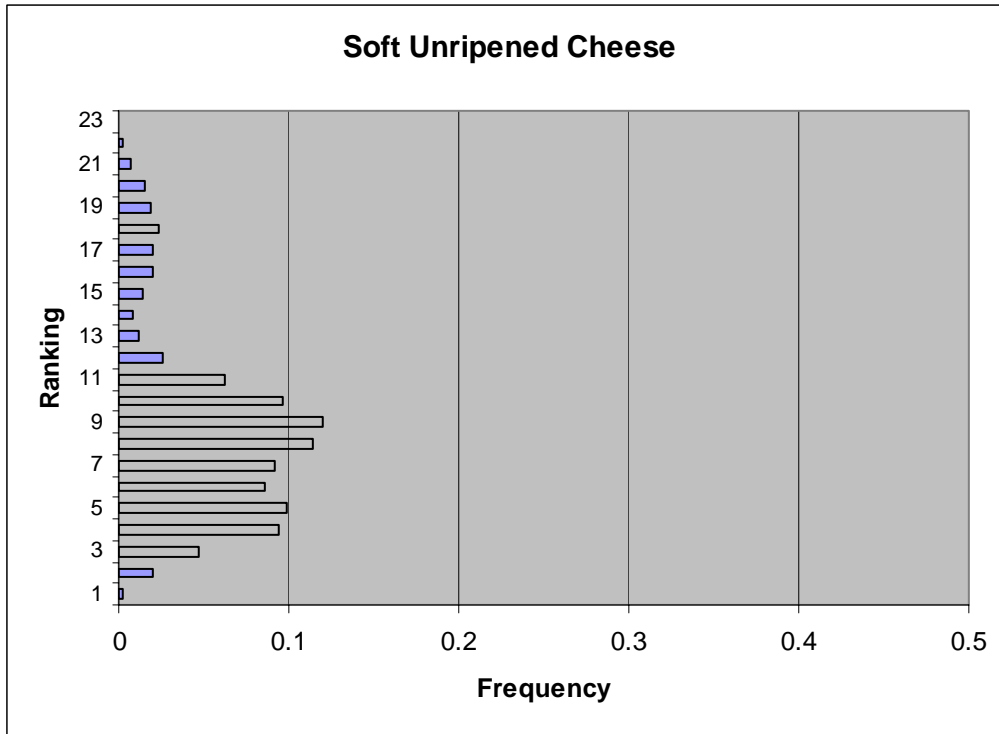
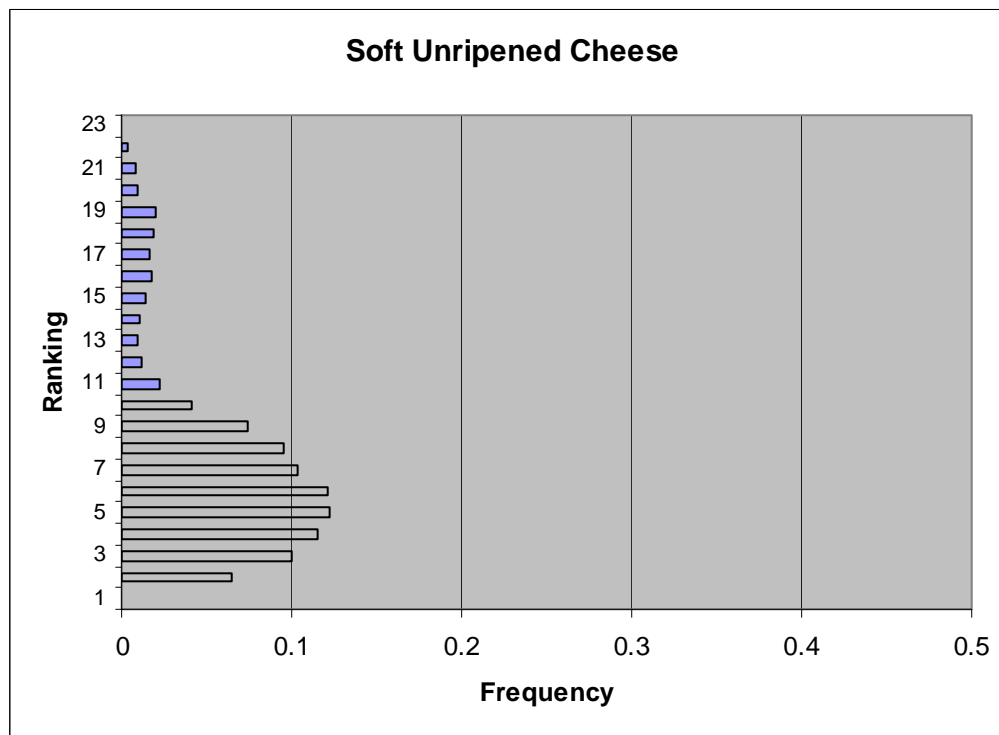**Figure V-12a. Rankings of Total Predicted Listeriosis Cases per Serving for Soft Ripened Cheese**

Figure V-12b. Rankings of Total Predicted Listeriosis Cases per Annum for Soft Ripened Cheese

Food Category: Semi-soft Cheese

The Semi-soft Cheese food category has a low predicted relative risk of causing listeriosis on a per serving basis. Semi-soft Cheese has a moisture content that ranges between 39% and 50%. The cheeses in this food category include blue, brick, Edam, Gouda, havarti, Limburger, Monterrey jack, Muenster, and provolone. The serving sizes are small, the annual number of servings, and contamination frequency are moderate, and the levels at retail are low. Although the storage times are long, the growth rates are low. Blue cheese has been linked to an outbreak of listeriosis in Denmark (Jensen *et al.*, 1994) and Monterrey jack cheese made from raw milk to a sporadic case in the United States (Ryser, 1999a). FDA has monitored recalls of several semi-soft cheeses because of the presence of *Listeria monocytogenes*.

The median amount consumed per serving for this category is 28 g (1 ounce), and the annual number of servings is 1.8×10^9. Data are not available to describe the proportion of United States or imported cheese that is made from unpasteurized fluid milk. Market data indicate that the United States imports approximately 20% of the blue cheese (including Gorgonzola) sold in the United States (National Cheese Institute, 1998).

There were eleven studies with contamination data, including three from the United States. Three studies provided quantitative data. The average frequency of contamination from these studies was 3.1%. The recent NFPA survey (NFPA, 2002) collected 1,623 samples of semi-soft cheeses, of which 23 were positive. The highest contamination observed was less than 100 cfu/g.

Semi-Soft Cheeses do not generally permit growth of *Listeria monocytogenes*. Of the 10 data sets found in the literature, levels declined in eight studies and the mean exponential growth rate was -0.043 logs/day at 5 °C. The storage times were long with a maximum of 15 to 45 days.

The median risk per serving for the Semi-soft Cheese category of 6.5×10^{-12} corresponds to a relative predicted risk ranking of sixteenth for the total United States population. The range for the predicted per serving risk ranking distributions for this category (Figure V-13a) is relatively

narrow and concentrated in the higher risk rankings (low predicted risk). This indicates relatively little uncertainty associated with the per serving predicted relative risk for this category. The median per annum risk was predicted as less than one case of listeriosis per year and the relative risk ranking was fifteenth for the total United States population. As with the range for the per serving rankings, the range for the per annum ranking distribution is similar to that typical of most food categories (Figure V-13b) indicating relatively little uncertainty associated with the per annum predicted relative risk ranking.
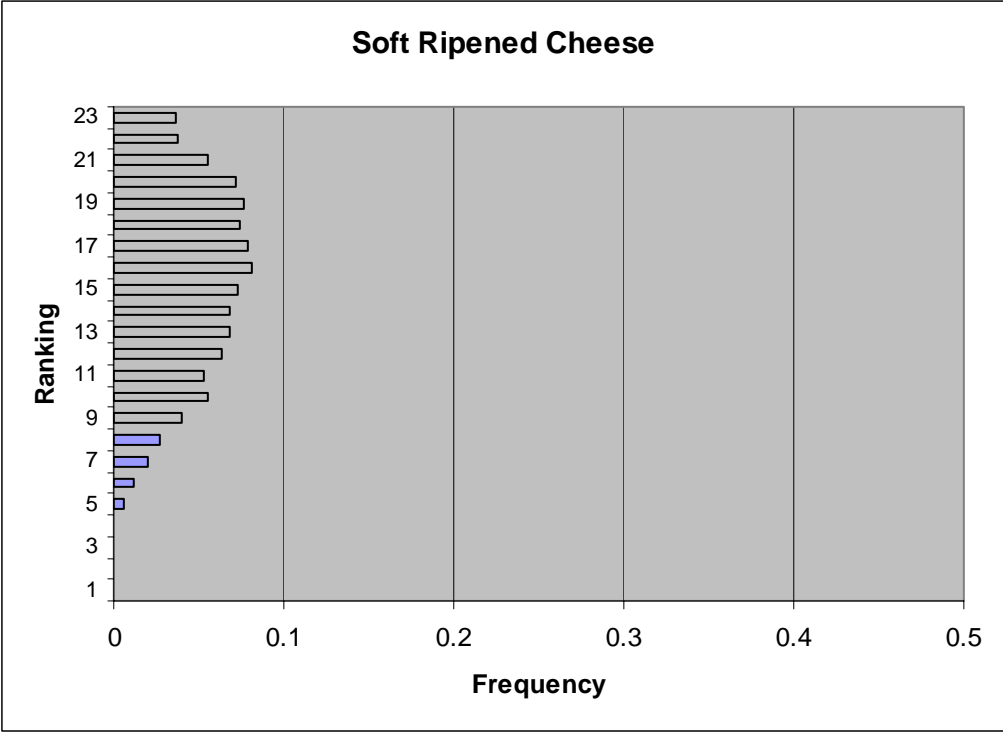
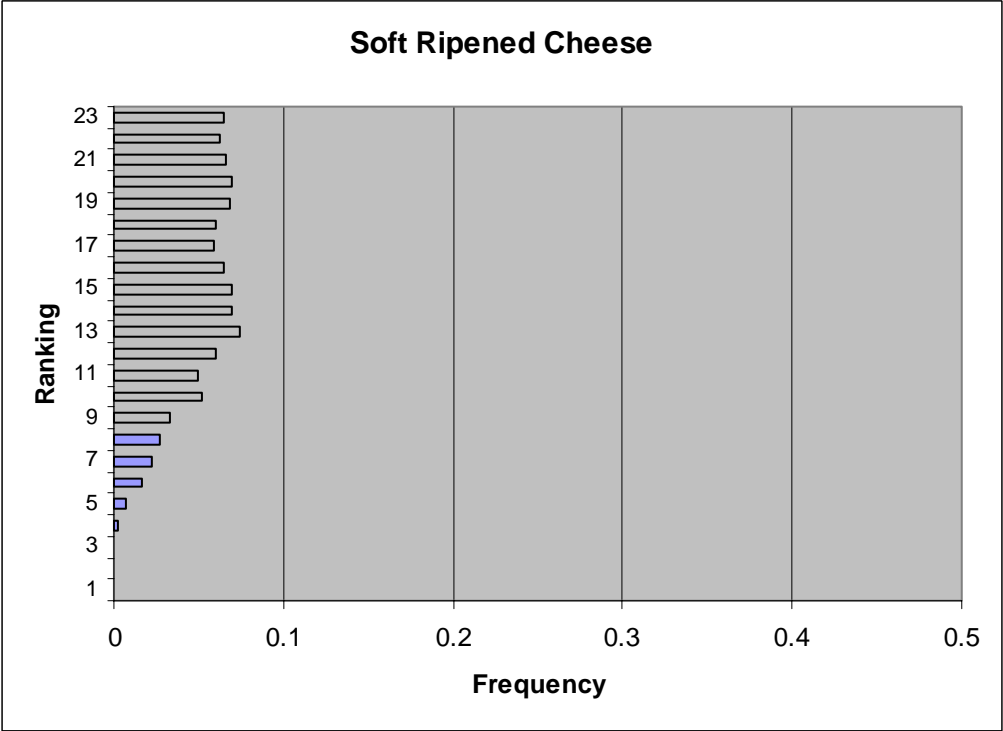**Figure V-13a. Rankings of Total Predicted Listeriosis Cases per Serving for Semi-soft Cheese**

Figure V-13b. Rankings of Total Predicted Listeriosis Cases per Annum for Semi-soft Cheese

Food Category: Hard Cheese

The Hard Cheese food category had a low predicted relative risk of causing listeriosis on a per serving basis. The low relative risk can be attributed to the small amount consumed, low contamination level at retail, and little, if any, growth during storage, despite the long storage times, and a moderate annual number of servings. Hard Cheeses have less than 39% moisture and include cheddar, Emmemtaler, Gruyere, parmesan, Queso Chihuahua, romano, silton, and Swiss. These types of cheeses typically have a high salt content, which limits the growth of *Listeria monocytogenes*. There are no recognized outbreaks or illnesses traced to Hard Cheese.

This cheese category includes consumption data for a variety of cheese types, including Swiss, cheddar, and parmesan. The median amount consumed per serving for this category is 28 g (~1 ounce) and the annual number of servings is 9.0×10^9.

Twelve studies, including two from the United States, provided contamination data for this category. Two studies provided quantitative data. Some of the data were collected from 2000 and later but the majority of the data was collected before 1993. The quantititive data were from the U.K, in 1990 and 1991. The frequency of contamination was only 1.4%, a low rate.

Seven studies provided data on the growth and survival of *Listeria monocytogenes* in hard cheeses. Of the 11 data points available, 10 indicated declines in *Listeria monocytogenes* populations, with an average of –0.053 logs/day at 5 °C. Storage times for this category of cheese were longer than other cheese categories. The most likely storage time was 6 to 10 days and the maximum was 90 to 180 days.

The median per serving and per annum predicted relative risk ranking for the Hard Cheese category were both last (23rd) for the total United States population. The range of the per serving ranking distribution (Figure V-14a) was moderately narrow and strongly concentrated in the higher rankings (low risk). The per annum ranking distribution (Figure V-14b) is similar to the per serving distribution. This indicated that there was little uncertainty with the predicted rankings.
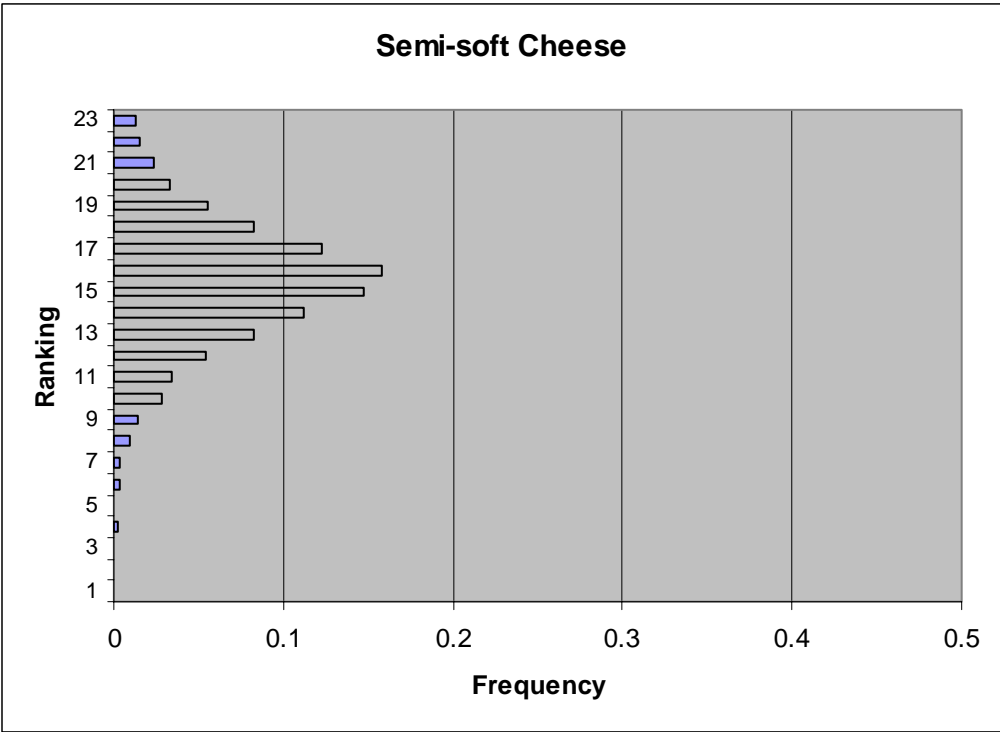
Figure V-14a. Rankings of Total Predicted Listeriosis Cases per Serving for Hard Cheese
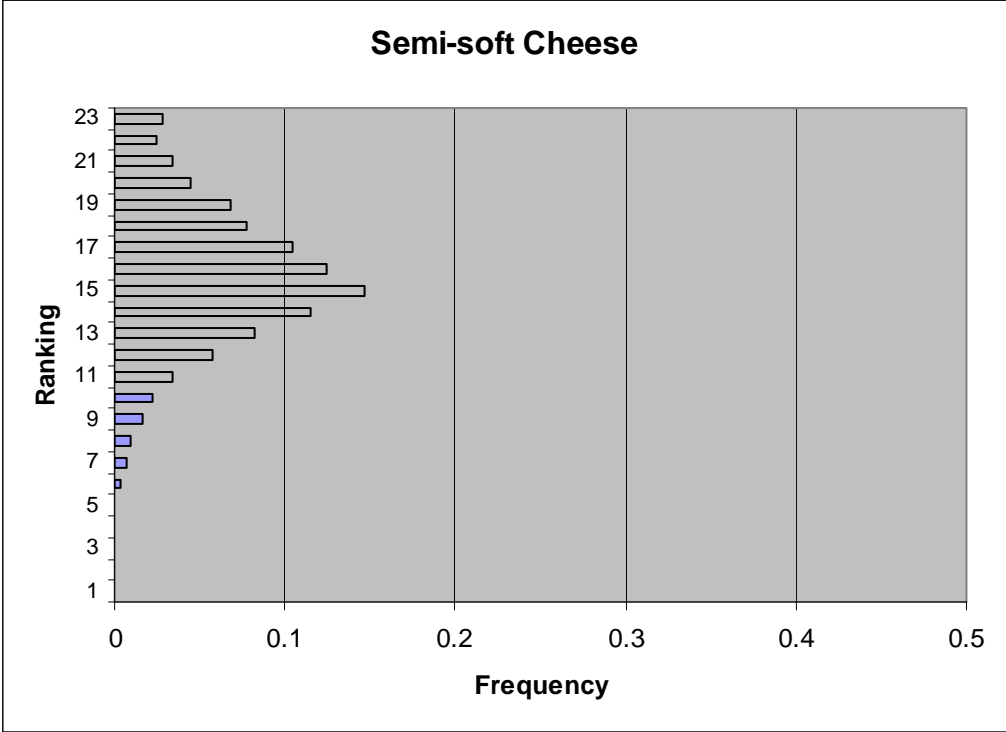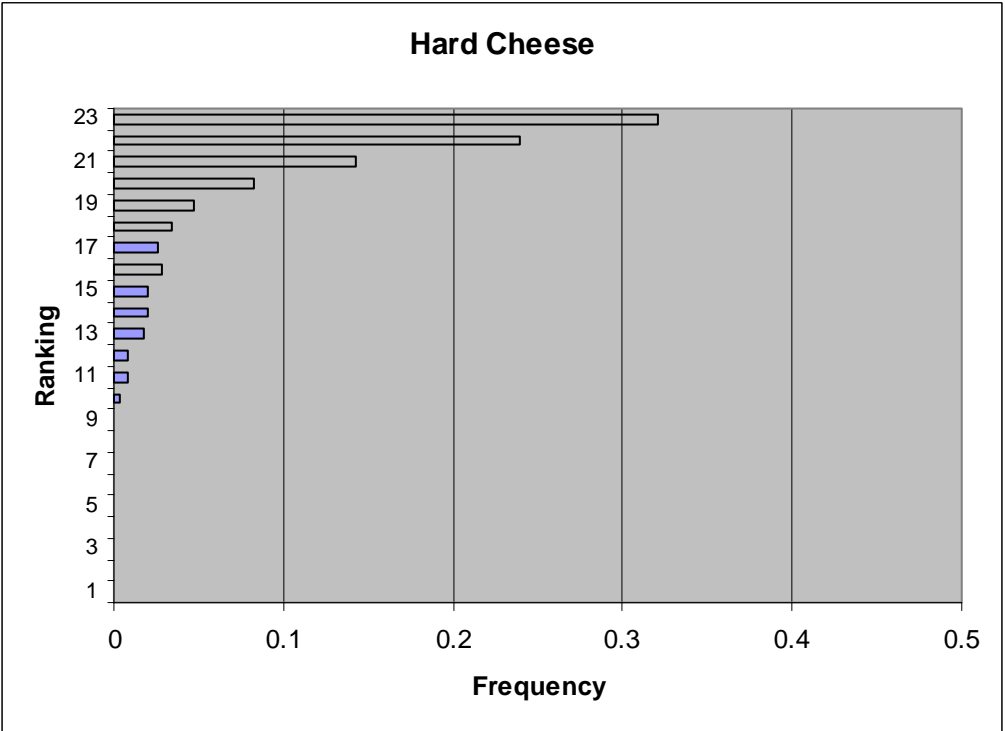

Figure V-14b. Rankings of Total Predicted Listeriosis Cases per Annum for Hard Cheese

Food Category: Processed Cheese

The Processed Cheese category had a low predicted relative risk of causing listeriosis on a per serving basis. This category has a high annual number of servings and a long storage time, but a low growth rate during storage and low contamination frequency. The median amount consumed per serving is 21 g (about 0.75 ounce), and the annual total number of servings is $1.2x10^{10}$.

Processed cheeses are made with natural cheese, dairy ingredients, and emulsifying salts. These cooked (pasteurized) and packaged cheeses include cheese food, cheese spreads, cheese sauces, and cheese slices. Processed cheeses from this category have not been linked to outbreaks or sporadic cases of listeriosis, but FDA has monitored recalls of cheese foods and cheese spreads because of the presence of *Listeria monocytogenes*.

There were four contamination studies available for this category, of which one was from the United States. A total of 325 samples were analyzed with only 0.9% found to contain *Listeria monocytogenes*. In two of these studies, the three positive samples were enumerated with the highest level being less than 100 cfu/g. In two recent studies, the 49 collected samples were negative for *Listeria monocytogenes*. The predicted percentage of servings with 10^3 to 10^6 cfu at retail was low.

Six data points for the survival of *Listeria monocytogenes* in Processed Cheese were found in the literature. All showed decreasing numbers during storage. Overall, a survival rate of -0.045 logs/day at 5°C was used. Storage times were long for this category; the assumed most likely time was 6 to 10 days and the maximum time was 45 to 90 days.

The median risk per serving for the Processed Cheese category of $4.2x10^{-14}$ corresponds to a relative predicted risk ranking of twenty-one for the total United States population. The predicted median per annum relative risk rankings was also twenty-first for the total United States population, with less than one case per year predicted for this food category. Both ranking distributions for Processed Cheese (Figures V-15a and V-15b) are moderately wide and

concentrated in the higher rankings (i.e., a lower risk). The degree of uncertainty was slightly greater for the per annum rankings. Overall, there was a moderate degree of uncertainty in both the predicted per serving and per annum predicted relative risk rankings for the Processed Cheese category. This reflects the fact that there was only limited data that were available for this food category.
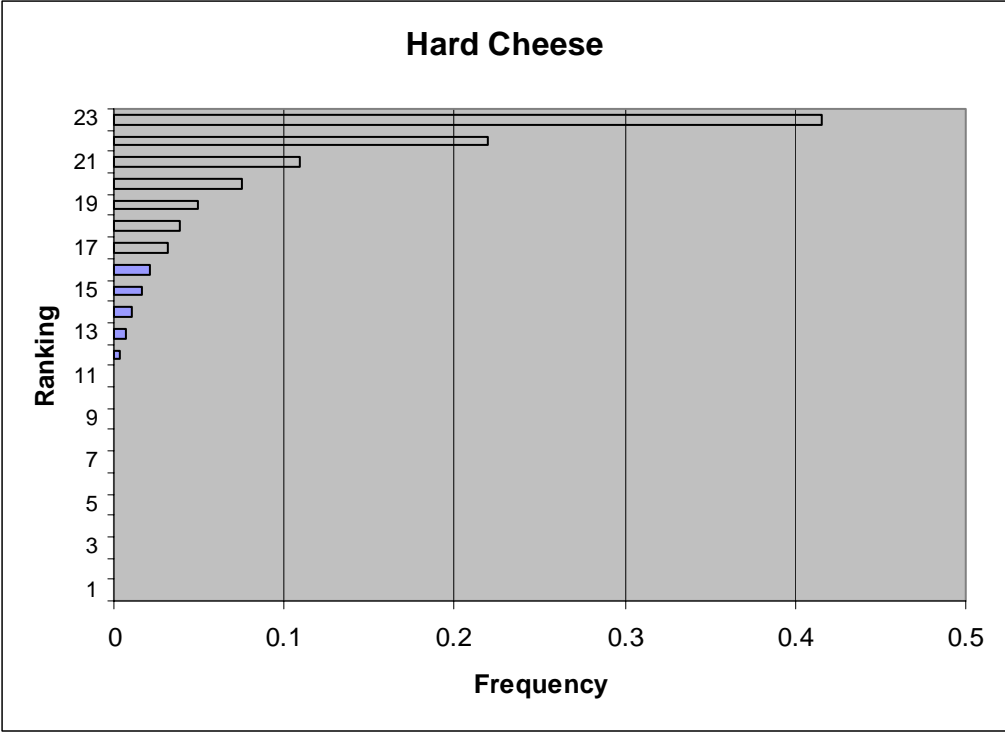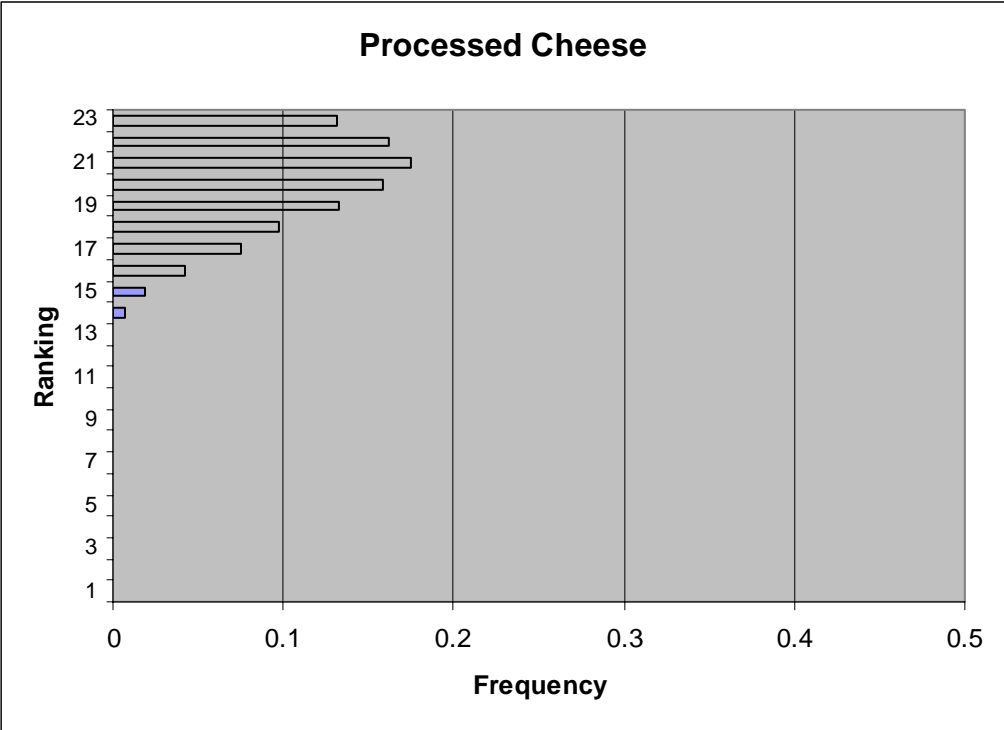
Figure V-15a. Rankings of Total Predicted Listeriosis Cases per Serving for Processed Cheese
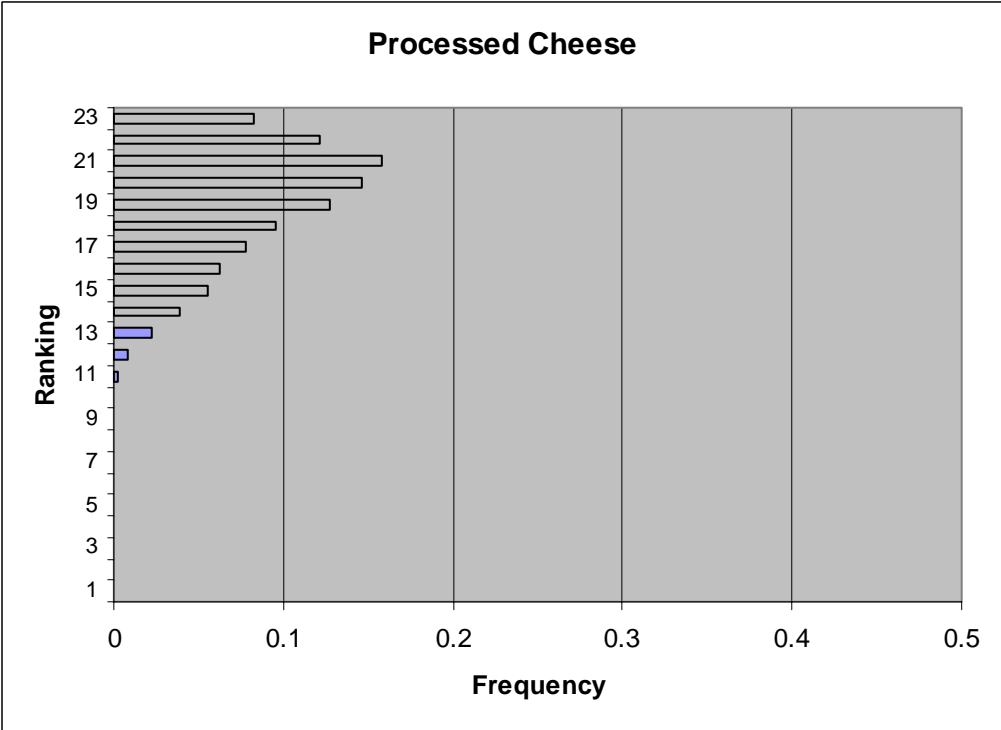
Figure V-15b. Rankings of Total Predicted Listeriosis Cases per Annum for Processed Cheese

Food Category: Pasteurized Fluid Milk

Pasteurized Fluid Milk had a moderate predicted relative risk of causing listeriosis on a per serving basis. The Pasteurized fluid milk category includes cow and goat milk, chocolate milk, other flavored milk, and malted milk. This is the most commonly consumed food category. Products in this category are eaten 4 to 100 times more often than foods in most other categories. Powdered milk and other dairy products that are reconstituted by the food preparer and milk shakes were included in the High Fat and Other Dairy Products food category.

Contamination frequency at retail for this category is low (average of 0.4%) due to pathogen inactivation during pasteurization. However, this is offset somewhat by the large serving sizes associated with this product and high potential for growth of *Listeria monocytogenes* in the product during storage. The median amount consumed per serving is 244 g (approximately 8 ounces), which is substantially larger than the serving sizes of most other foods considered in this risk assessment. The frequency of serving (8.7×10^{10}) is also the highest among the food categories.

It is generally assumed that contamination of Pasteurized Fluid Milk is the result of post-pasteurization recontamination, since normal pasteurization will effectively eliminate the microorganism. One of the most likely sites is during filling which would lead to the occasional recontamination of individual cartons. Accordingly, control of recontamination is likely to be a key factor in further risk reduction. Experimental studies have demonstrated that pasteurized milk will support growth of *Listeria monocytogenes* to high levels at refrigeration temperatures within the normal shelf-life of the food.

An outbreak of listeriosis has been associated with post-pasteurization recontamination of pasteurized chocolate milk (Dalton, *et al.*, 1997). A second outbreak was epidemiologically linked to pasteurized whole or 2% milk; however, this could not be confirmed by laboratory analyses (Ryser, 1999a; Fleming *et al.*, 1985). Such outbreaks likely represent a significant loss of control whereas the sporadic recontamination of individual contains would be likely to be

expected to produce sporadic cases. Sporadic cases would be difficult to identify using traditional case control studies due to the high rate of consumption of this food. Over 12,400 samples from 30 studies were available to provide data on the frequency of detectable contamination. Most of these studies were from samples collected outside of the United States. Approximately 0.03% of the milk consumed in the United States is imported (Frye and IDFA, 2000a). Two reports (Kozak *et al.*, 1996; Frye and IDFA, 2000b) of surveys conducted in the United States and one survey from Canada were available to estimate the frequency of contamination in North America. The overall frequency of contamination was low at 0.4%. The survey conducted in the United States by the International Dairy Foods Association (Frye and IDFA, 2000b) observed only one positive sample in 4,552 collected samples and the level in that sample was below quantitation (<1 cfu/g). The other studies with enumeration data (conducted in Germany and the U.K.) analyzed a total of 1,559 samples of which only 4 were positive.

Five laboratory investigations of the growth rate of *Listeria monocytogenes* in pasteurized, unpasteurized, Ultra High Temperature (UHT), skim, and chocolate milks were found. The mean exponential growth rate was 0.26 logs/day, a relatively rapid rate of growth compared with other food categories. In contrast to the other food categories, the literature indicated that milk supported higher maximum levels of *Listeria monocytogenes* and that the storage temperatures did not affect as much the maximum growth potential in fluid milk. The storage intervals used in the model for storage ranged from 0.5 to 15 days, with 3 to 5 days as the most likely storage time.

The median per serving predicted relative risk rankings for the Pasteurized Fluid Milk category were ninth for the total United States population. The range for per serving ranking distribution for Pasteurized Fluid Milk was moderately broad. The distribution of rankings was normally distributed, and similar to that observed with other food categories (Figure V-16a). Thus, the predicted per serving relative risk ranking was considered to have a moderate degree of uncertainty. The number of servings predicted to contain 10^3 to 10^6 cfu/g after refrigerated storage is low. Furthermore, the number of servings associated with the limited quantitative data

required the use of a broad distribution for post storage contamination levels. This, in turn, may lead to an overestimation of the relative risk associated with this product.

The median per annum predicted risk was approximately 91 cases per year which corresponds to a relative risk ranking for Pasteurized Fluid Milk of second for the total United States population. The increase in the predicted per annum relative risk ranking compared to the per serving ranking reflects the frequency of consumption. Pasteurized milk is the most extensively consumed food category both in terms of frequency of consumption and serving sizes. These factors, in combination with the uncertainty associated with the lack of quantitative data for the levels of *Listeria monocytogenes* in contaminated pasteurized milk results in a small percentage of contaminated servings being assigned a high level of contamination. These few, highly contaminated servings predicted by the model drive the risk estimates.

While the per annum ranking distribution is relatively narrow (Figure V-16b), it is strongly influenced by the highly uncertainty values in the "tails" of the broad distributions that had to be incorporated into the models. Definitive interpretation of the per annum risk and its ranking will have to await the acquisition of additional quantitative data and possibly more sophisticated epidemiologic investigations that could shed more light on the differences between the epidemiologic record and the risk predicted by the current model.
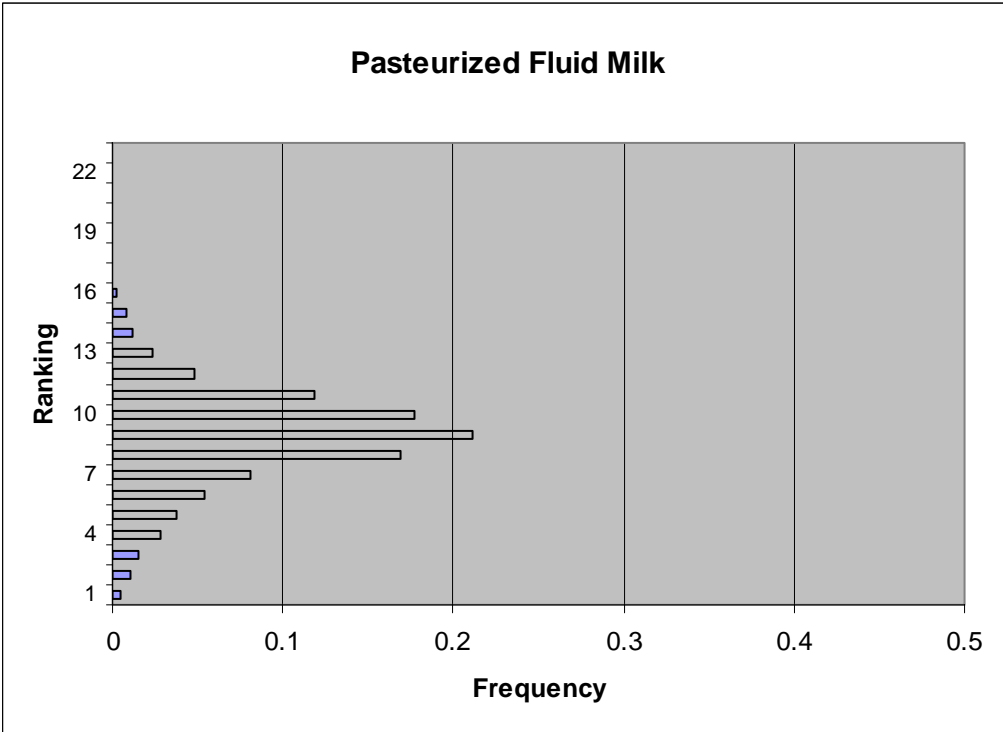
Figure V-16a. Rankings of Total Predicted Listeriosis Cases per Serving for Pasteurized Fluid Milk
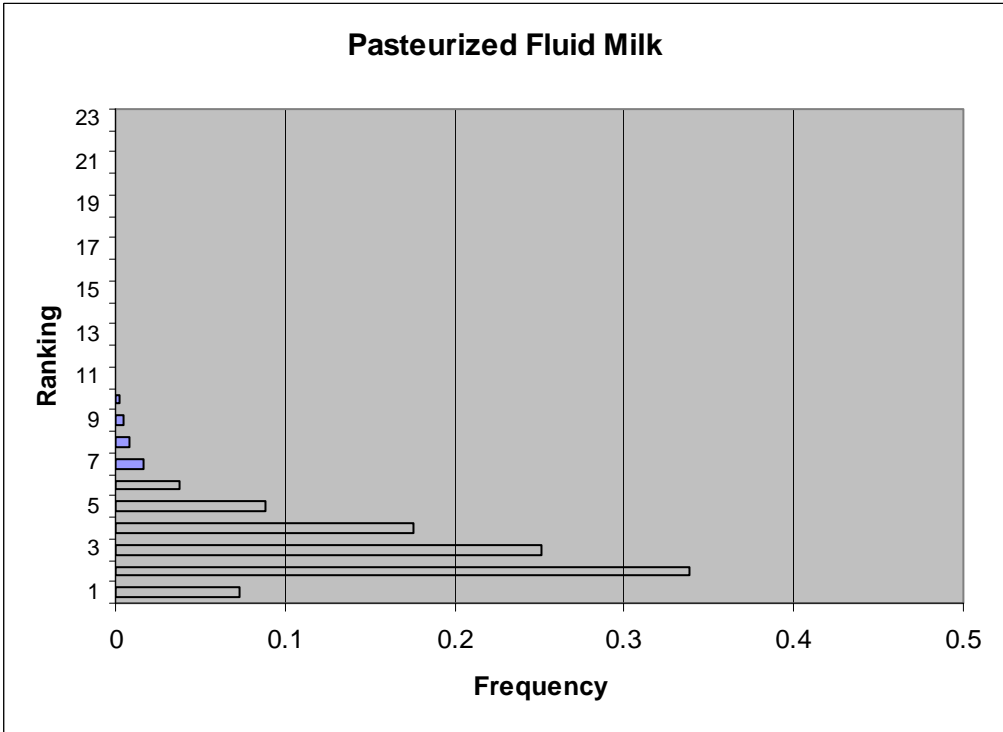
Figure V-16b. Rankings of Total Predicted Listeriosis Cases per Annum for Pasteurized Fluid Milk

Food Category: Unpasteurized Fluid Milk

Unpasteurized Fluid Milk had a high predicted relative risk of causing listeriosis on a per serving basis. Although consumption of unpasteurized fluid milk is infrequent, relatively large serving sizes and a moderate frequency of contamination, coupled with a significant (high) potential for growth during its refrigerated shelf-life affect the relative risk for this category. The annual number of servings consumed of Unpasteurized Fluid Milk was estimated to be low, 4.4×10^{8} servings (0.5% of pasteurized fluid milk). The median serving size (244 g, or approximately 8 ounces) was assumed to be the same as for pasteurized fluid milk.

Although federal law requires milk in interstate commerce to be pasteurized, some states allow milk consumed within the state to be sold and drunk as unpasteurized milk. Results of a 1995 FDA/CDC survey of all 50 states, Puerto Rico, and the District of Columbia showed that 28 states (54%) permit the sale of unpasteurized milk. In the states where the sale of unpasteurized milk is legal, the estimated volume of unpasteurized milk sold, as a percentage of total milk sold, was less than 1% by volume (or weight) (Headrick *et al.*, 1998). Several studies have shown that *Listeria monocytogenes* is present in 1 to 6% of unpasteurized milk samples on a worldwide basis. There has been an outbreak linked to unpasteurized milk in Austria and a sporadic case of listeriosis was linked to unpasteurized milk in Denmark (Jensen *et al.*, 1994; Allerberger and Guggenbichler, 1989). The use of unpasteurized milk to manufacture other dairy products has also been linked to outbreaks and sporadic cases of listeriosis.

There were 45 contamination studies, including 10 from the United States. Three studies (all non-United States) provided quantitative data. Almost all of the samples were cow's milk but a small portion was goat or other non-bovine milk. The contamination frequency was moderate at 4.1%. The three recent studies from the United States found a contamination frequency of 1.6%, i.e., 20 positive samples out of 1,263 total samples (Abou-Eleinin *et al*., 2000; Frye and IDFA, 2000b; and Oregon Dept of Agriculture, 2001).

In general, the initial frequency of contamination is greater in unpasteurized milk than in pasteurized milk, 4.1% vs. 0.4%, respectively. Although the prevalence of low level

contamination is much higher in unpasteurized milk than for pasteurized milk, the calculated relative risk per serving is only slightly higher. This appears to be due to two factors. The first is that higher contamination rates are offset somewhat by the shorter storage time assumed for unpasteurized milk. The storage times used in the analysis were 0.5 to 10 days with a most likely time of 2 to 3 days. Because of the presence of a more extensive spoilage microflora, the product tends to be held for a shorter time period than pasteurized milk. The second factor that influenced the predicted per serving relative risk associated with Unpasteurized Fluid Milk is the small degree of variability in the frequency and levels of contamination reported in a large number of studies. This availability of substantially more quantitative data led to a substantially narrower range of contamination values and eliminated the distribution "tails" that increased the uncertainty discussed in the preceding section on Pasteurized Fluid Milk. This emphasizes the impact that the degree of uncertainty has on the calculation of risk.

The predicted percentage of servings contaminated with 10^6 to 10^9 cfu/serving at retail was low. Unpasteurized Fluid Milk would be characterized by frequent contamination at low levels. This is in contrast to Pasteurized Fluid Milk, which would have infrequently contaminated cartons. Because unpasteurized milk does not receive any treatment that would reduce *Listeria monocytogenes* levels, several of the studies used were of bulk tank milk instead of milk in retail containers. The extent to which this might affect the estimated exposure is unclear. Higher median levels of contamination with *Listeria monocytogenes* might be expected in unpasteurized milk; however, the limited data do not support this.

It has been hypothesized that competition from more numerous spoilage microorganisms present in Unpasteurized Fluid Milk may slow the growth rate of *Listeria monocytogenes* and also reduce the maximum growth. However, no data were available to allow this to be factored into the risk assessment. There were two growth studies using unpasteurized fluid milk. They did not indicate any clear difference in growth rates compared to pasteurized fluid milk. Therefore, the growth characteristics of the Pasteurized Fluid Milk category were assumed for Unpasteurized Fluid Milk. As indicated previously, while storage times for unpasteurized milk were moderate, the values used were shorter than those for pasteurized milk. If this assumption

is not correct, this would lead to a degree of understating the relative risk due to the food category.

The median per serving predicted relative risk rankings for the Unpasteurized Fluid Milk category was fourth for the total United States population (Figure V-17a). The range of the ranking distribution for Unpasteurized Fluid Milk was similar to those observed with the other food categories (Figures V-17a and V-17b) and tended to the lower relative risk rankings (i.e., higher risk). This indicates that there was moderate uncertainty associated with the relative ranking for the Unpasteurized Fluid Milk category. This uncertainty is likely due to the variability in the frequencies and extents of contamination among the different studies. The median per annum predicted relative risk ranking was seventh for the total United States population (Figure V-17b). This decrease in predicted relative risk in comparison to the per serving values reflects the relatively few servings consumed annually. The distribution of per annum rankings was moderately broad and nearly normally distributed, indicating a moderate but typical degree of uncertainty associated with the predicted per annum risk ranking.
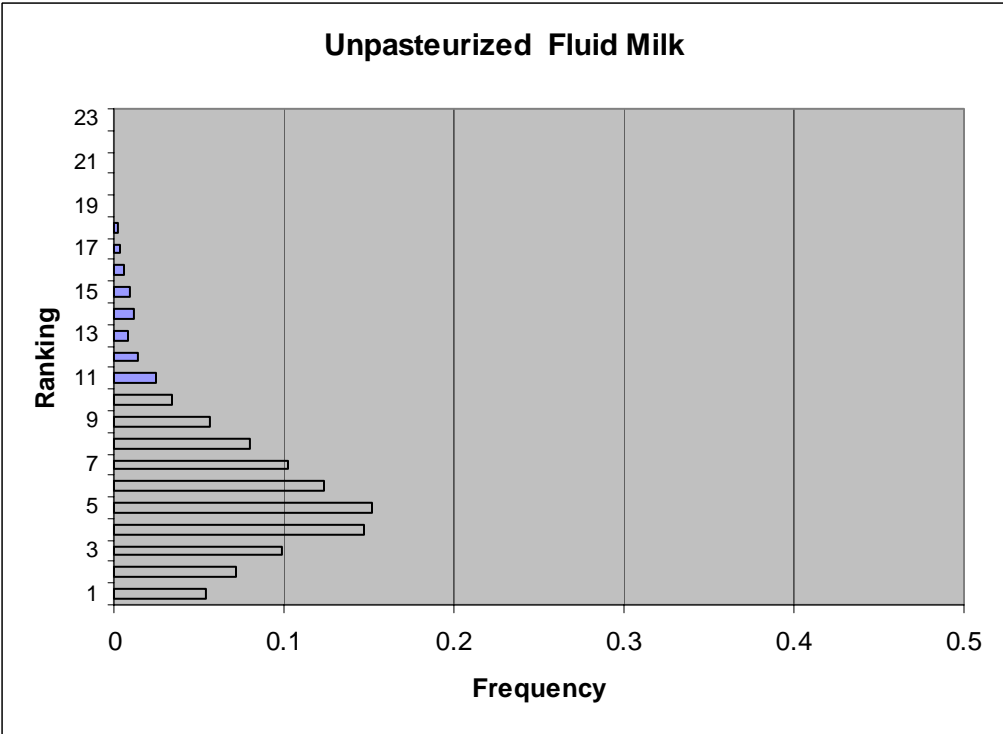
Figure V-17a. Rankings of Total Predicted Listeriosis Cases per Serving for Unpasteurized Fluid Milk
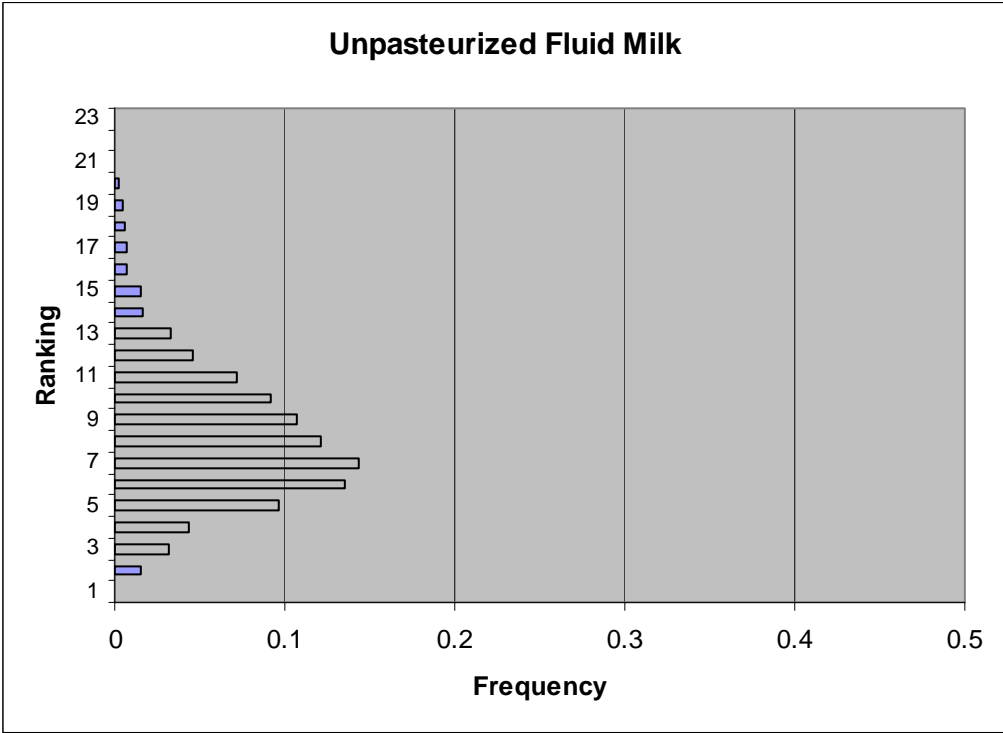
Figure V-17b. Rankings of Total Predicted Listeriosis Cases per Annum for Unpasteurized Fluid Milk

Food Category: Ice Cream and Other Frozen Dairy Products

Ice Cream and Frozen Dairy Products had a low predicted relative risk of listeriosis on both a per serving and per annum basis. While ice cream and frozen dairy products are consumed frequently and the median serving size is large, contamination frequency is low and is usually at low levels. Growth is not supported at freezer temperatures. The only association between listeriosis and ice cream or other frozen dairy products was a sporadic case in Belgium, which was linked to commercially prepared ice cream made from contaminated cream (Ryser, 1999a). Like Pasteurized Fluid Milk, contamination of Ice Cream and Other Frozen Dairy Products appears to be largely the result of occasional post-pasteurization recontamination of individual cartons, which would be more consistent with sporadic cases that outbreaks.

Consumption data included many types of ice cream and frozen dairy products. The median amount consumed per serving for this category is 132 g (approximately 4.7 ounces) and the annual number of servings consumed is 1.5×10^{10}.

Twenty-two studies provided contamination data. Five were conducted in the United States and two studies (none from the United States) provided quantitative data. Tested products included ice cream, frozen yogurt, ice milk, ice cream mix, and novelty ice cream products. The percentage of positive samples was low (0.12%). A recent, large quantitative study from Germany (Hartun, 2001) observed only two positive from a total of 1,696 samples and both of these samples contained less than 100 cfu/g of *Listeria monocytogenes*.

Although *Listeria monocytogenes* cannot grow at freezer temperatures, it is able to survive. If temperature abuse occurs that permits changes in the texture of these products (i.e., warming and refreezing), the product does not become warm enough to permit *Listeria monocytogenes* growth. More drastic temperature abuse, of the kind that would allow growth, results in an inedible product. The levels of *Listeria monocytogenes* found in the retail surveys of ice cream and frozen dairy products would not increase prior to consumption.

The predicted median per serving and per annum relative risk rankings for the Ice Cream and Frozen Dairy Products category were both twentieth for the total United States population. The

ranges for both predicted ranking distributions for ice cream and frozen dairy products are clustered in the high rankings (low risk) (Figures V-18a and V-18b). The uncertainty was similar to that observed with other food categories. The extensive database available and the characteristics of the food category provide significant confidence in the relative rankings for the Ice Cream and Other Frozen Dairy Products category.

Figure V-18a. Rankings of Total Predicted Listeriosis Cases per Serving for Ice Cream and Frozen Dairy Products
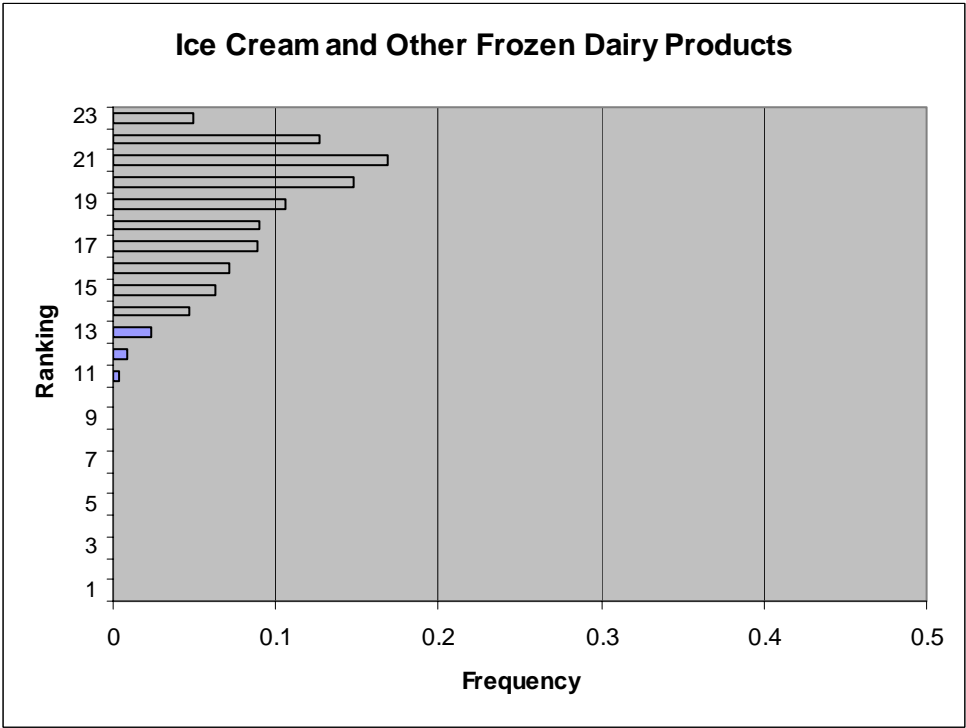
Figure V-18b. Rankings of Total Predicted Listeriosis Cases per Annum for Ice Cream and Frozen Dairy Products

Food Category: Cultured Milk Products

Cultured Milk Products had a low predicted relative risk of listeriosis on both a per serving and per annum basis. The Cultured Milk Products category had a relatively low contamination frequency and levels of contamination. Because of the breadth of the category, there were a moderate number of servings annually, high amounts consumed, and the proportion of the population eating products from this category was high.

The Cultured Milk Products category includes low pH dairy foods manufactured with lactic acid fermentation. Of these foods, yogurt is the most frequently consumed food. Others include buttermilk and sour cream. These products had previously been grouped with High Fat Dairy Products (referred to as the Miscellaneous Dairy Products) in the 2001 draft risk assessment. In this revised risk assessment, the cultured milk products and high fat milk products have been separated into two food categories based on product characteristics. No illnesses have been linked to Cultured Dairy Products.

Consumption data for Cultured Milk Products include many types of dairy products such as buttermilk, yogurt, and sour cream. The median amount consumed per serving for this category is 114 g (slightly over 4 ounces), and the annual number of servings is 7.2×10^9.

Six contamination studies were available, with the single study conducted in the United States collected only 14 samples. A 1991 study conducted in the U.K. observed four positive samples, one of which was enumerated and contained 10^3 to 10^4 cfu/g. The contamination frequency for these studies was low at 0.8%.

Inoculated pack studies showed that *Listeria monocytogenes* does not grow in these foods. Five data sets for yogurt and buttermilk were averaged and indicate an inactivation rate of -0.17 logs/day. The storage times for these products are relatively long and range from 0.5 to 45 days with the most likely storage time between 6 and 10 days.

With a low frequency of contamination and declining *Listeria monocytogenes* numbers during a potentially lengthy storage, this food category is predicted to pose a low risk per serving and a low contribution to the total cases per annum. The predicted median per serving and per annum relative risk rankings for the Cultured Milk Products were twenty-second for the total United States population. The ranges for both predicted ranking distributions are broad but clustered in the high rankings (low risk) (Figures V-19a and V-19b). There was more uncertainty for this food category than for other dairy products (such as ice cream), with more iterations having lower rankings. This is largely attributed to the limited data available.
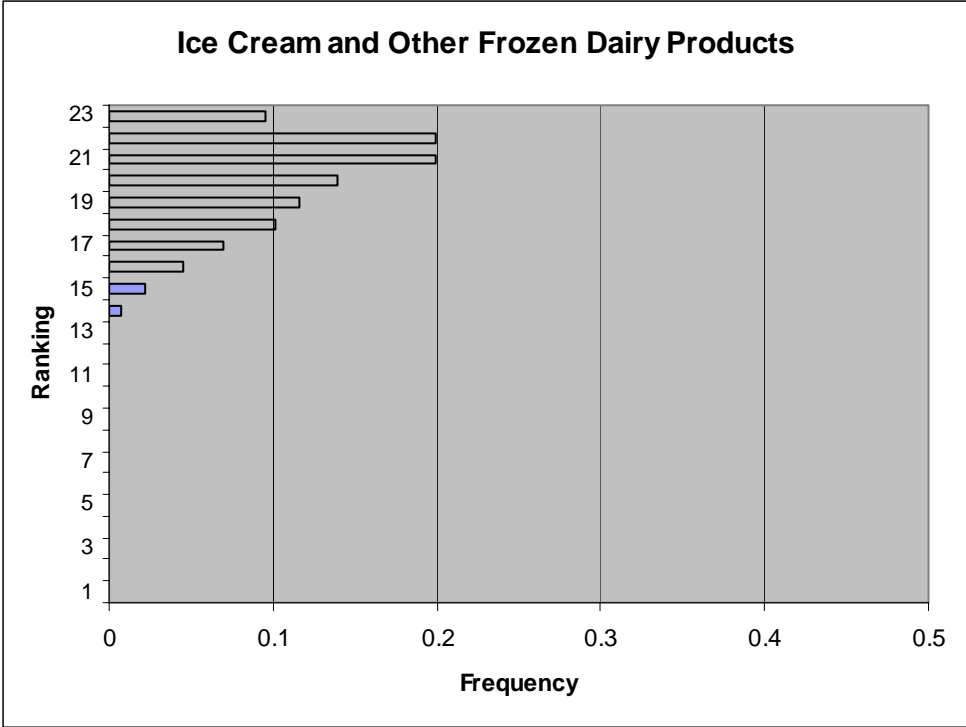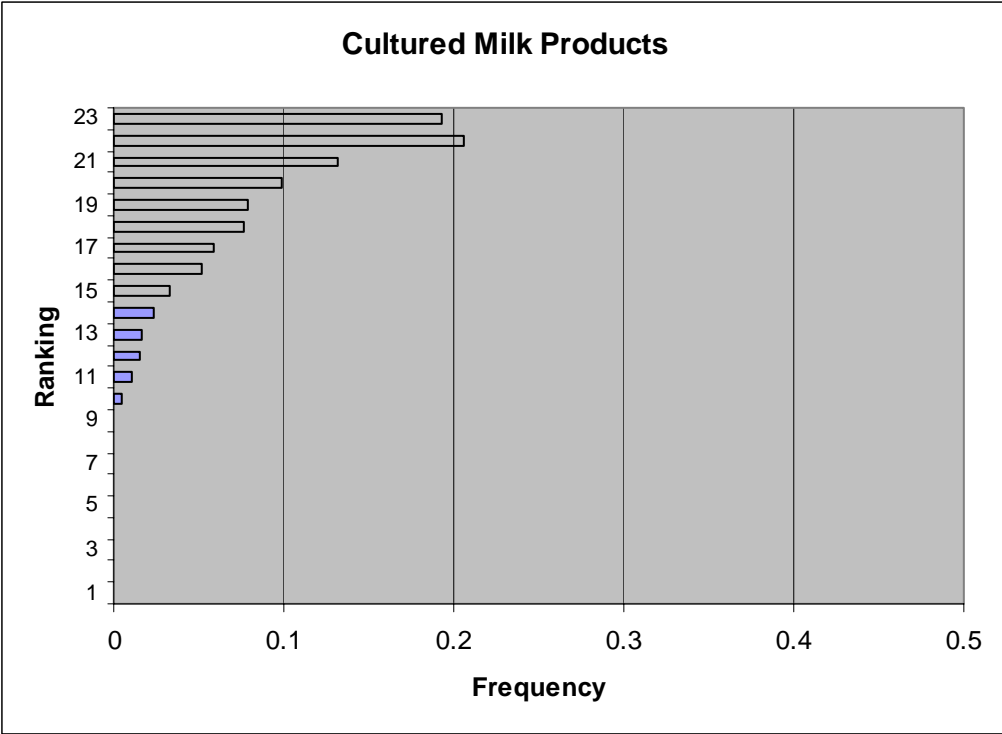
Figure V-19a. Rankings of Total Predicted Listeriosis Cases per Serving for Cultured Milk Products
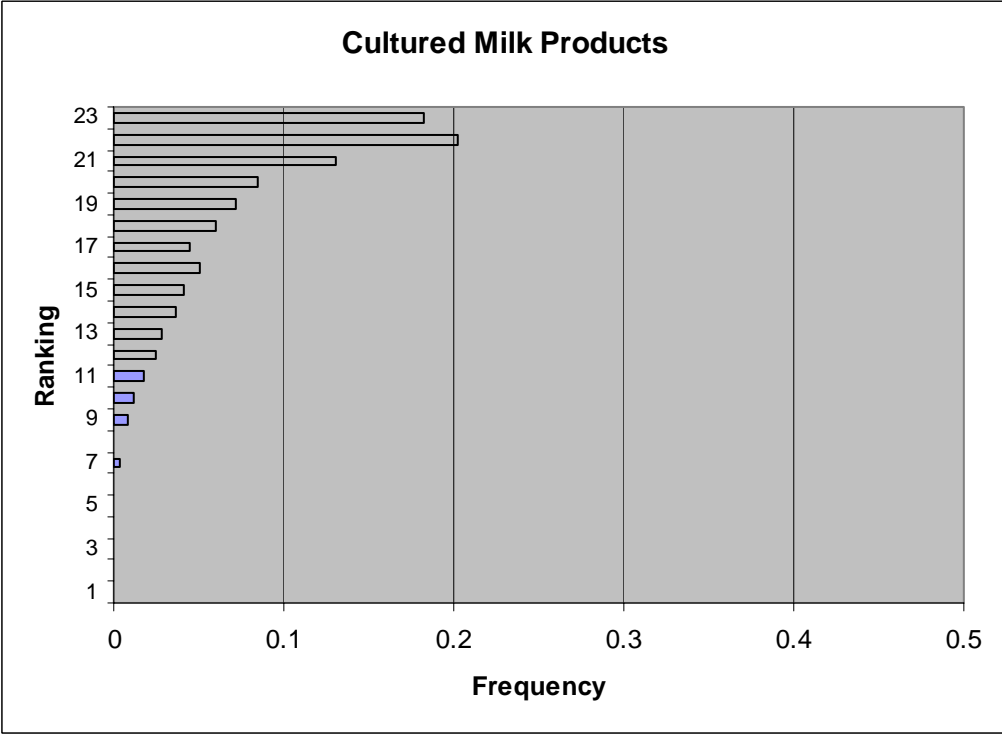
Figure V-19b. Rankings of Total Predicted Listeriosis Cases per Annum for Cultured Milk Products

Food Category: High Fat and Other Dairy Products

The High Fat and Other Dairy Products food category had a moderate predicted relative risk of listeriosis on a per serving basis. Although the High Fat and Other Dairy Products category had a relatively low contamination frequency and levels of contamination, *Listeria* can grow in these products and storage times are typically long. Because of the breadth of the category, there were a high number of servings annually, and the proportion of the population eating products from this category was high. This is offset to a degree by the low amount consumed per serving. These factors resulted in a high predicted relative risk on a per annum basis. Two products, pasteurized cream (in the U.K.) and butter (in the United States and Finland), have been linked to outbreaks of listeriosis (Ryser, 1999a; Lyytikäinen *et al.*, 2000).

The High Fat and Other Dairy Products category consists of high fat dairy products such as butter, cream, half and half, and other dairy products including shakes. These products had previously been grouped with Cultured Milk Products (referred to as the Miscellaneous Dairy Products) in the 2001 draft risk assessment. In this revised risk assessment, the cultured milk products and high fat milk products have been separated into two food categories based on product characteristics. Even with the removal of cultured dairy products from this group, the High Fat and Other Dairy Products category remains a relatively diverse group. Acquisition of additional data to address product-specific questions and subdivision of this category into smaller product groupings may be warranted in the future.

Consumption data for the High Fat and Other Dairy Products include many types of dairy products (milk shakes, cream, and butter). The median amount consumed per serving for this category is 13 g (a little less than 0.5 ounce), and the annual number of eating occasions is 2.1×10^{10}.

Twelve contamination data sets were available for this category, including four from the United States. Two studies (not from the United States) provided quantitative data. The studies comprised 18,169 samples of all types of dairy products, including some unspecified products. The specified products were butter and cream, primarily. It was not typically indicated whether these products, which generally have high water activity, were made from pasteurized or

unpasteurized milk. One set of cream samples was reported as being unpasteurized. Microbial analysis of dry milk products, casein, non-fat dried milk, and dry infant formula in their dry state were excluded. Over 40% of the samples were analyzed quantitatively. The percentage of samples with detectable contamination was about 1.3%, a low contamination rate, but the contamination levels were moderate.

Most of the foods in the High Fat and Other Dairy Products category support growth of *Listeria monocytogenes*. The six data sets ranged from –0.02 to 0.26 logs/day with an average of 0.11 logs/day, a moderate growth rate. The storage times for this category were long. The assumed distribution had a most likely time of 6 to 10 days and a maximum time of 15 to 45 days. The risk assessment did not attempt to estimate the fraction of butter servings left at room temperature; this practice could increase the predicted risks for this food category.

The median risk per serving for the High Fat and Other Dairy Product category of 2.7×10^{-9} corresponds to a predicted relative risk ranking of seventh for the total United States population. The per serving rankings for High Fat and Other Dairy Products was normally distributed and the range of the distribution was relatively narrow (Figure V-20a), indicating that there is a reasonable degree of certainty associated with the per serving ranking despite the broad range of foods in the category. The predicted median per annum relative risk ranking for the High Fat and Other Dairy Products category was third for the total United States population, representing a median of approximately 56 predicted cases of listeriosis for the total United States population. The range for the per annum ranking distribution is somewhat narrower (Figure V-20b) and shifted to the lower ranks (i.e., higher risk levels). This indicates that there was also a fair amount of certainty associated with the per annum predicted relative risk ranking for the High Fat and Other Dairy Products category. However, the degree of uncertainty associated with this food category must be considered in light of the category's diversity. If additional data became available, uncertainty would likely be reduced further if this food category was subdivided.

High Fat and Other Dairy Products
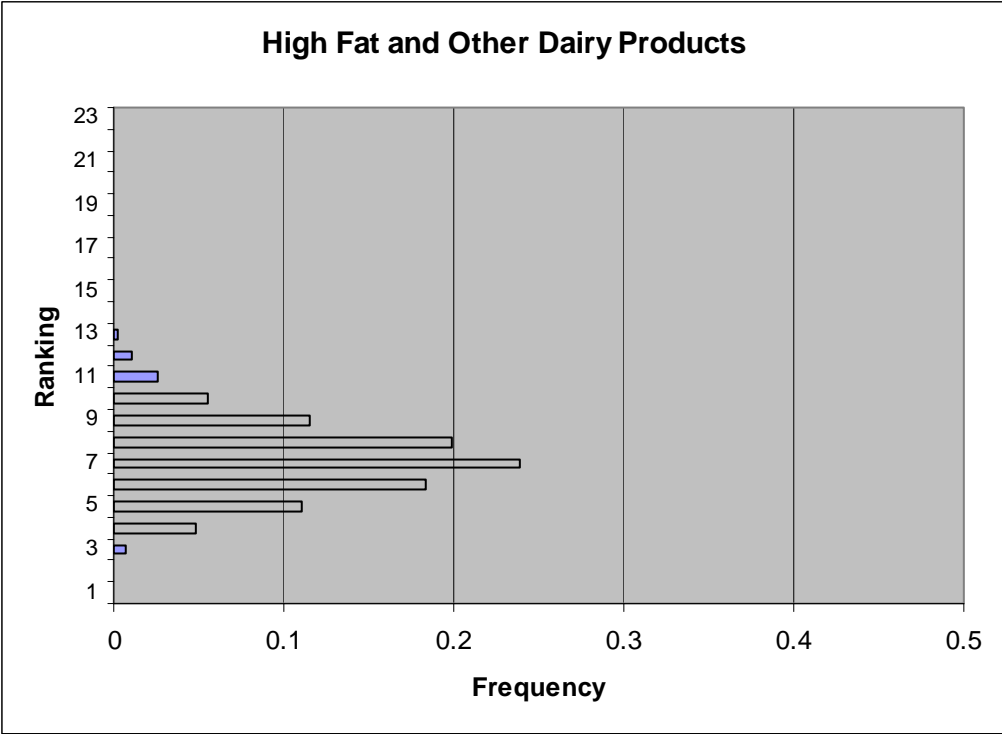
Figure V-20a. Rankings of Total Predicted Listeriosis Cases per Serving for High Fat and Other Dairy Products

High Fat and Other Dairy Products
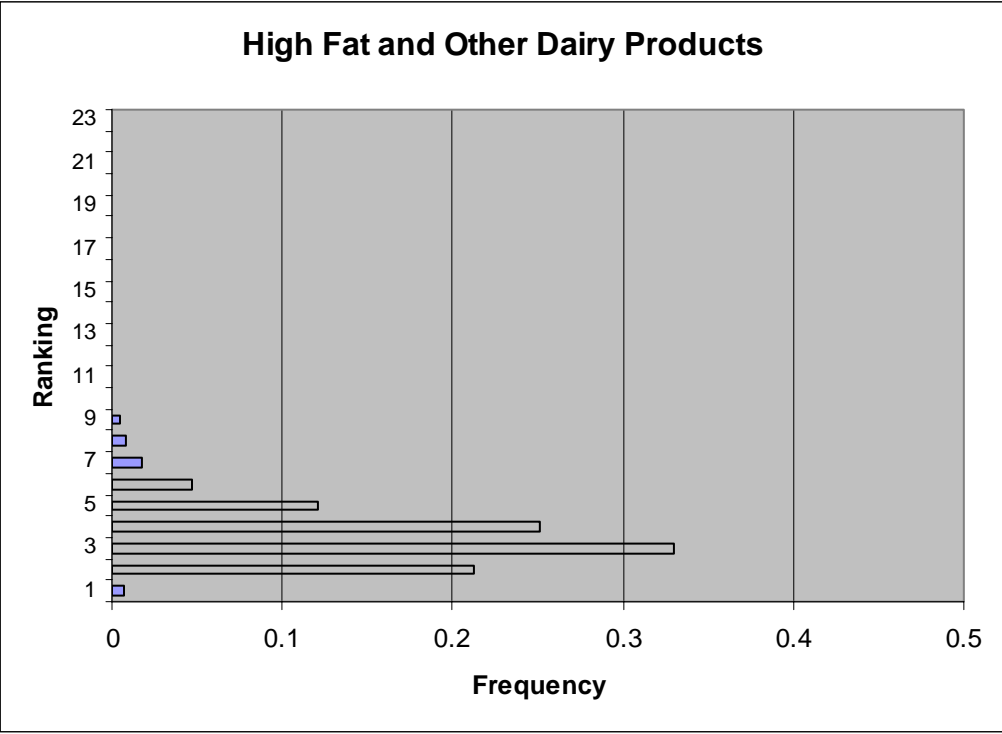
Figure V-20b. Rankings of Total Predicted Listeriosis Cases per Annum for High Fat and Other Dairy Products

Food Category: Frankfurters (Reheated)

Reheated Frankfurters had a low predicted relative risk of causing listeriosis in the United States on a per serving basis and per annum basis. The initial cooking of frankfurters during manufacture is sufficient to eliminate *Listeria monocytogenes* so contamination is associated with the post-processing recontamination of the product. Reheating the product just prior to consumption would be expected to produce a significant reduction in contamination. There is potential for frankfurters to serve as a source for cross contamination prior to reheating, however, there were no data available upon which this could be modeled in the current risk assessment. The number of annual servings, median amount consumed per serving, contamination frequency, and growth rate were moderate for reheated frankfurters.

In this risk assessment, the risk for frankfurters reheated before consumption was calculated separately from those eaten without reheating. Up to 7% of frankfurters are frozen by the consumer before reheating (AMI, 2001). The model assumed that growth would not occur in these frozen frankfurters. It was also assumed (based on survey data) that between 1 and 10% of the frankfurters are consumed without reheating (i.e., 90 to 99% are reheated). To account for the reduction of levels of *Listeria monocytogenes* in adequately reheated frankfurters, a thermal inactivation step was included in the risk assessment model. There have been two outbreaks in the United States of listeriosis linked to consumption of frankfurters or microwaved turkey franks (Ryser, 1999a; CDC, 1998a, 1999b; Farber and Peterkin, 1991). These were likely the results of breakdowns in food safety controls within the processing plant. The factor that has the greatest effect on the predicted health impact of frankfurters is the extent of post-retail reheating by the consumer.

Consumption data for the frankfurter category include the meat portion of various types of frankfurters. This excludes the bun, relish, and other condiments. Frankfurters made with chicken, turkey, all beef, and beef-pork products are included. Bologna, which is processed similarly to frankfurters, but has different retail and home handling practices, is included in the Deli Meat food category. The median amount consumed per serving for this category is 57 g

(approximately 2 ounces, the typical weight a single frankfurter), and the annual total number of servings is 6.1×10^9.

There were nine contamination studies with a total of about 3,763 samples for this food category. Six of the studies were conducted in the United States. One of the largest data sets used to develop the exposure rates for this food category was the result of the recent FSIS analyses of product taken soon after manufacture. These results were modified to take into account the likely increase in *Listeria monocytogenes* levels that would have resulted from storage conditions and times that would have been likely to have occurred between manufacture and purchase. The large size of this data set had a substantial influence on the overall calculated relative risk.

As introduced above, two underlying assumptions used in estimating the relative risk associated with this product are that *Listeria monocytogenes* was transmitted via the direct consumption of frankfurters, and that reheating of the product just prior to consumption is a generally effective means of eliminating the microorganism. Thus, to a large extent the primary factor controlling the risk is the percentage of individuals that do not adequately reheat the product. Nevertheless, if a substantial portion of frankfurter-associated listeriosis cases were the result of the product cross-contaminating other foods prior to reheating or if certain types of reheating were not fully effective in eliminating the pathogen, this would significantly alter the relative risk associated with the product. In such a case, the relative risk would be more accurately estimated by increasing the percentage of frankfurters consumed without adequate reheating. These possibilities are supported by the results of outbreak investigations where the victims reported reheating the product prior to consumption.

In general, the literature references did not indicate whether the frankfurters were made from beef or poultry meats. The percentage of samples with detectable contamination was a moderate 4.8%. The highest levels of *Listeria monocytogenes* were less than 100 cfu/g.

Five studies reported growth rates for *Listeria monocytogenes* in frankfurters, including beef/pork, turkey, and chicken frankfurters. The average growth rate at 5°C was 0.13 logs/day. As with most foods, the maximum growth was related to storage temperature. Based, in part, on

a survey sponsored by the American Meat Institute (AMI, 2001), the distribution of home storage times assumed that 91% of the frankfurters were consumed within 9 days and 99% were consumed within 26 days.

The predicted median per serving and per annum relative risk rankings for the Frankfurters (reheated) category were both eleventh for the total United States population. The range for the per serving ranking distribution for Frankfurters is moderately narrow (Figure V-21a) and the range for the per annum is similar and normally distributed (Figure V-21b). This indicates that there was a relatively low degree of uncertainty associated with the predicted relative risk ranking for the Frankfurters (reheated) category.

Figure V-21a. Rankings of Total Predicted Listeriosis Cases per Serving for Frankfurters (Reheated)
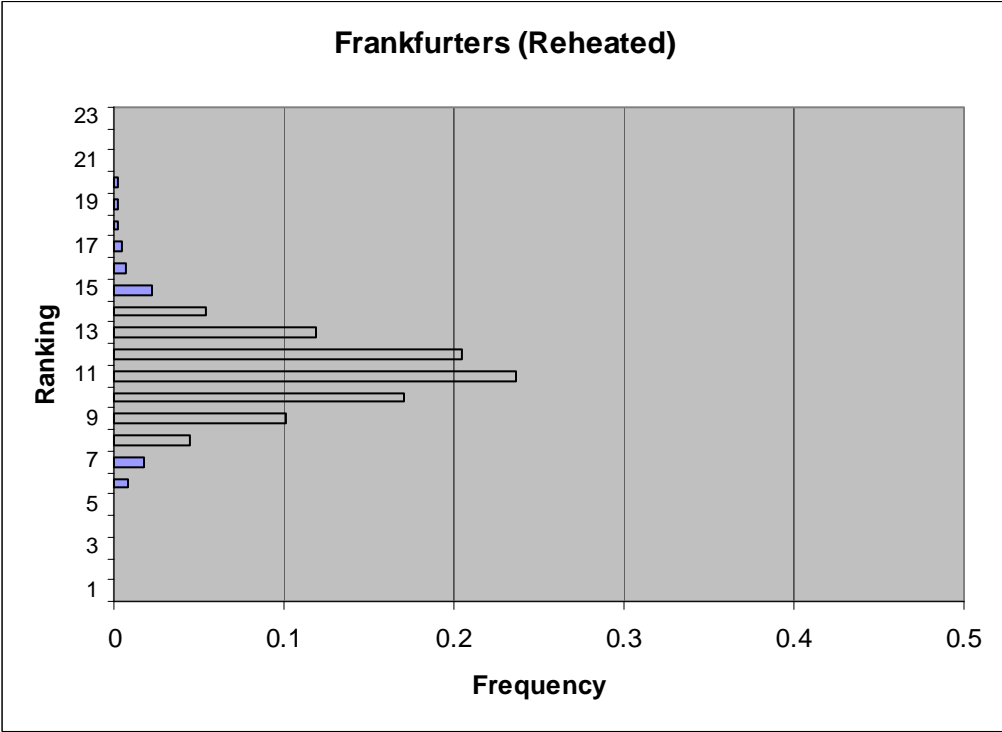
Figure V-21b. Rankings of Total Predicted Listeriosis Cases per Annum for Frankfurters (Reheated)

Food Category: Frankfurters (Not Reheated)

Frankfurters (not reheated) had a high predicted relative risk of causing listeriosis in the United States on a per serving basis. Comparison of the Frankfurter (reheated) and Frankfurter (not reheated) food categories indicates that post-retail reheating by the consumer significantly reduces contamination levels and the resulting predicted risk to public health.

In this risk assessment the risk for frankfurters reheated before consumption was calculated separately from frankfurters eaten without reheating. In the 2001 draft risk assessment the risk of frankfurters eaten without reheating was determined only on a per serving basis. This category includes the 1 to 10% of frankfurters that are stored in the refrigerator (not frozen) and consumed without reheating (i.e., no thermal inactivation step).

The Frankfurters (not reheated) and the Frankfurter (reheated) categories share the same contamination frequency, contamination levels, growth rates and storage times. See the section above for Frankfurters (reheated) for a discussion of these data. Consumption for the Frankfurter (not reheated) category is a proportion of the total frankfurters. The mean number of annual servings of not reheated frankfurters was 4.7×10^8 for the total United States population. Without the decrease in *Listeria monocytogenes* levels from heating, the frequency of contamination at consumption was high with 1.0% of the servings containing 10^3 to 10^6 cfu. This is in contrast to the reheated frankfurters, which had a comparative frequency of only 0.5%.

The predicted median per serving risk for Frankfurters (not reheated) category was 6.5×10^{-8} which corresponds to a relative risk ranking of second for the total United States population. This ranking is based on the assumption that 1% to 10% of frankfurters are consumed without reheating. The predicted median per annum relative risk ranking was fourth, representing a median prediction of 31 cases of listeriosis per year for the total United States population. The range for the per serving and per annum ranking distributions for Frankfurters are narrow (Figures V-22a and V-22b) and concentrated toward the lower ranks (higher risk). This indicates that there was a relatively low degree of uncertainty associated with the predicted relative risk ranking for the Frankfurters (not reheated) category.

Scenario testing: Reduction of the Estimated Consumption of Unreheated Frankfurters

Cooking is a post-retail intervention. Because cooking is an effective method of killing *Listeria monocytogenes*, the risk from unreheated frankfurters is much greater than from adequately reheated frankfurters. A simulation was run in order to simulate the consequence of an intervention that reduces the number of frankfurters consumed without adequate reheating. Reducing the number of frankfurters consumed without adequate reheating reduced the predicted median number of cases of listeriosis. (For additional details, see Chapter VI 'What-If' Scenarios.)
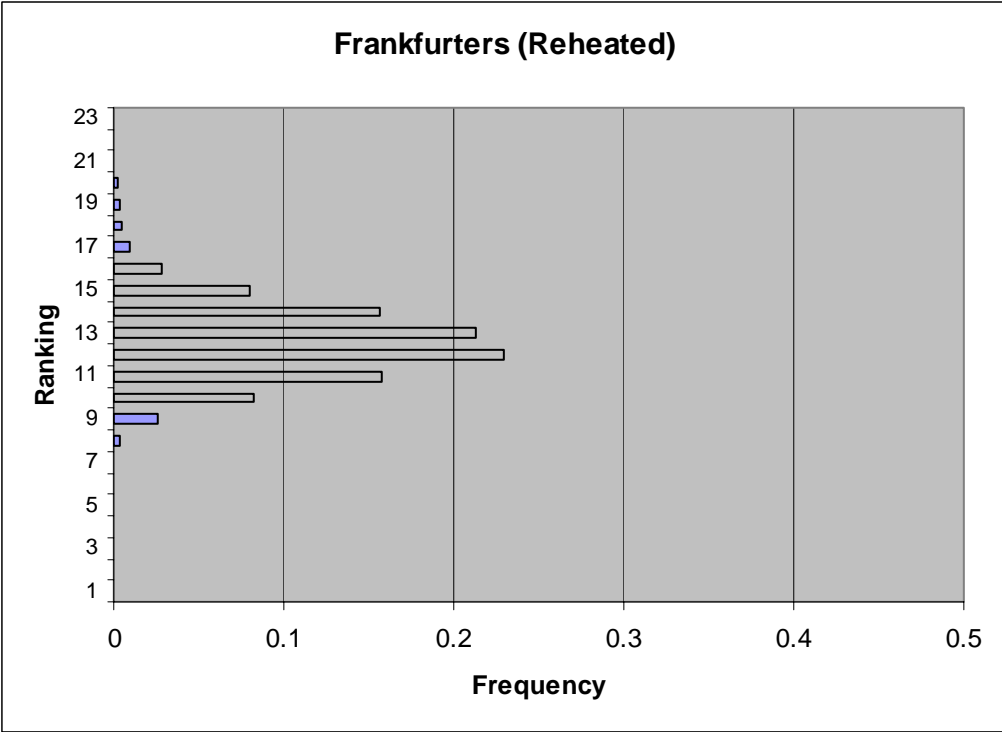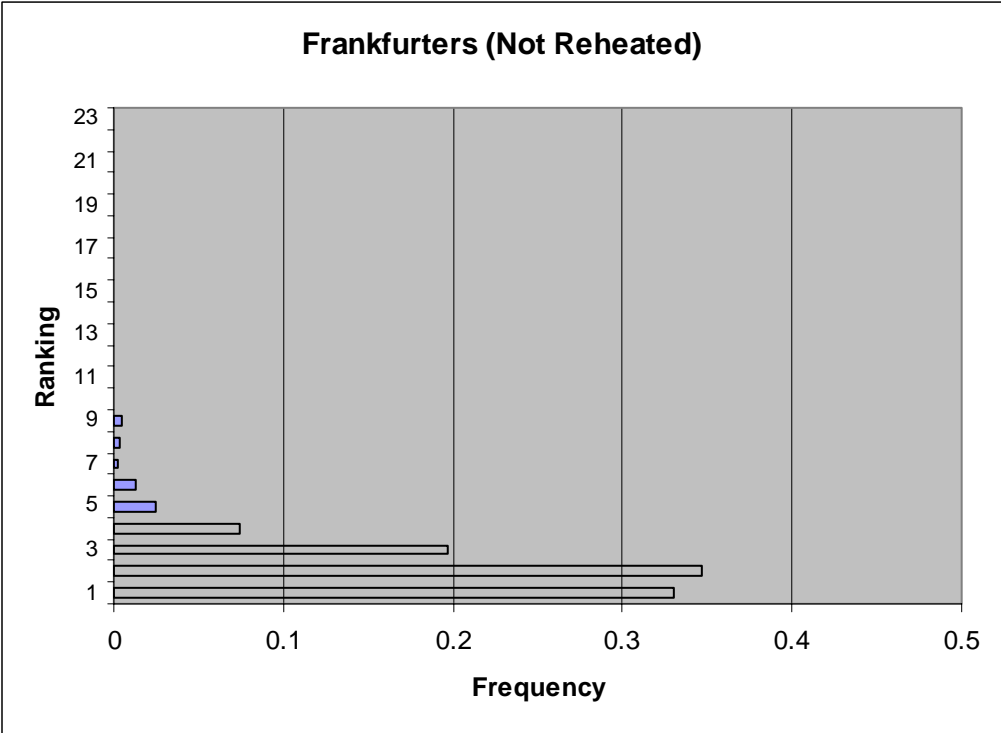
Figure V-22a. Rankings of Total Predicted Listeriosis Cases per Serving for Frankfurters (Not Reheated)
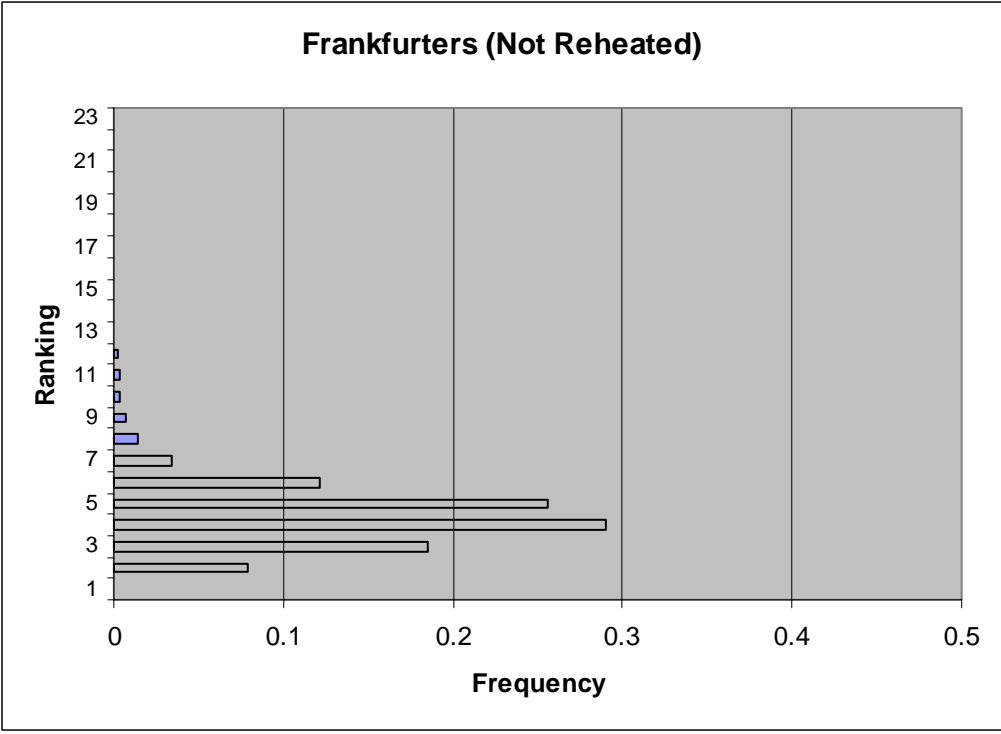
Figure V-22b. Rankings of Total Predicted Listeriosis Cases per Annum for Frankfurters (Not Reheated)

Food Category: Dry/Semi-Dry Fermented Sausages

The Dry/Semi-dry Fermented Sausages food category had a low predicted relative risk of causing listeriosis in the United States on a per serving basis. This reflects the fact that this is a food category that does not support growth, despite all other factors except contamination frequency storage time are at a moderate level. This food category included foods such as Lebanon bologna, mortadella, pepperoni, and salami. One outbreak and one sporadic case of listeriosis in the United States have been linked to the consumption of salami (Ryser, 1999a; Farber and Peterkin, 1991).

Consumption data for this category included samples of smoked beef sausage, Lebanon bologna, pepperoni, salami, and Thuringer sausage. The median amount consumed per serving for this category is 46 g (i.e., just over 1.5 ounces), and the total annual number of servings is 1.8×10^9. Both of these values are considered moderate.

There were 14 contamination studies, including 3 studies from the United States. Three studies provided quantitative data. Products tested included salami, cured chorizo, pepperoni, beef stick, and unspecified fermented, dry and other sausages. Two of the United States studies were from FSIS and included 1208 samples with 32 positives (2.6%). This contamination frequency is lower then the overall frequency for this food category (6.4%) and is a source of uncertainty. The quantitative data are from Europe, with 3 of the 41 positive samples containing 10^2 to 10^4 cfu/g.

Inoculated pack studies show *Listeria monocytogenes* decreases several logs during the manufacture of these meat products and then slowly declines with additional storage. The organism can grow during the early phase of the fermentation or if there has been a fermentation failure. Fermentation failures also have been linked to outbreaks caused by *Staphylococcus aureus* and *Salmonella* in products associated with this food category. Four data sets were used to model the rate of decline of *Listeria monocytogenes* in these foods during storage; the range was 0.0 to -0.036 logs decline/day. The length of storage is long, ranging from 0.5 to 90 days with the most likely 6 to 10 days.

The predicted median per serving relative risk rankings for the Dry/Semi-Dry Fermented Sausages category was fifteenth for the total United States population. The range for the per serving ranking distribution for Dry/Semi-Dry Fermented Sausages is broad (Figure V-23a) and concentrated in the middle ranks (moderate risk). This indicates that there was a high degree of uncertainty associated with the per serving predicted relative risk ranking for Dry/Semi-Dry Fermented Sausages category. The predicted median per annum relative risk ranking was thirteenth for the total United States population. The range of the per annum ranking distribution was broad (FigureV-23b), indicating substantial uncertainty associated with the per annum predicted relative risk ranking. The uncertainty may reflect the variability in the consumption patterns for this food category.

Figure V-23a. Rankings of Total Predicted Listeriosis Cases per Serving for Dry/Semi-Dry Fermented Sausages
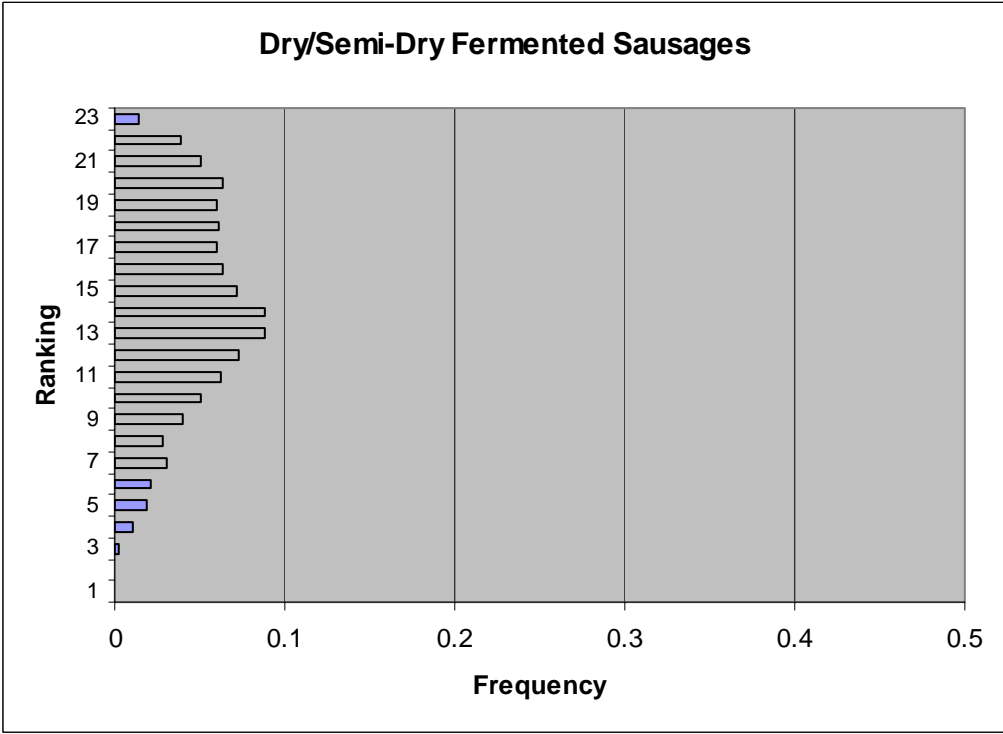
Figure V-23b. Rankings of Total Predicted Listeriosis Cases per Annum for Dry/Semi-Dry Fermented Sausages

Food Category: Deli Meats

Deli Meats had the highest predicted relative risk of causing listeriosis in the United States on both per serving basis and per annum basis. Though this category had a moderate contamination frequency with high contamination levels, there were a high number of servings consumed and a high growth rate, two of the primary factors that drive listeriosis risk in foods. Deli meats have been implicated in two United States outbreaks: a 1998-99 outbreak that was primarily linked to frankfurters but contaminated luncheon meats were also found, and a 2002 outbreak in the Northeastern United States which was linked to poultry products. There have been two outbreaks of listeriosis in France linked to pork tongue in jelly, and an outbreak in Western Australia linked to processed meats (Ryser, 1999a; CDC, 1998a, 1999b).

Consumption data were available for a number of deli meats, such as bologna, ham, turkey, roast beef, chicken, and the meat portion of sandwiches. Consumption databases (and most contamination studies) did not distinguish between pre-packaged and sliced deli products. The median amount consumed per serving for this category is 56 g (i.e., ~ 2 ounces), and the total annual number of servings is estimated to be 2.1×10^{10}.

This category of products encompasses a variety of processes and formulations that can affect contamination and growth. There were 19 contamination studies, including four from the United States. Three studies provided enumeration data. The overall percentage of positive samples from these 19 studies was 1.9%. The 2000 and 2001 surveys conducted by USDA/FSIS observed 2.1% positive samples (395 of 18,506) from collection at manufacturing, however, the 2002 NFPA survey observed a lower frequency of positive samples of only 0.8% from collection of 9,199 samples at the retail level.

The cooking steps that are used to produce Deli Meats are assumed to kill any *Listeria monocytogenes* present. It is generally assumed that *Listeria monocytogenes* present in the finished product is the result of recontamination. This is often associated with specific processing steps, such as slicing. Sliced Deli Meats are available in two forms: those that are sliced and then packaged for consumer purchase, and those that are produced in bulk and then sliced in retail stores. It is generally assumed that the latter group of products is more likely to

be recontaminated, but would also have a shorter storage time. The NPFA survey showed a prevalence rate of 1.2% for in-store packaged but only 0.4% for manufacturer packaged deli meats (Gombas *et al.*, 2003). Nevertheless, insufficient data were available to allow these two approaches to the marketing of Deli Meats to be distinguished in the risk assessment.

The Deli Meats were differentiated from the Dry/ Semi-dry Fermented Sausages category by higher pH values and water activities that allowed growth. There were nine growth studies conducted on a variety of deli meats including bologna, corned beef, ham, roast beef, poultry loaf, and breaded chicken fillets. Growth rates varied with product composition (e.g., salt, pH) and packaging (e.g., aerobic, vacuum). The average growth rate was 0.28 logs/day at 5°C, a rapid rate of growth. Storage times were relatively long compared with the other food categories. Storage times were based on the survey sponsored by the American Meat Institute (AMI, 2001).

The predicted median cases per serving (77×10^{-9}) and per annum (1,599) risks both correspond to relative risk rankings for the Deli Meats category of first (highest risk) for the total United States population. The ranges for the per serving and per annum ranking distributions for Deli Meats are narrow (Figures V-24a and V-24b). This suggests a low degree of uncertainty associated with the predicted relative risk rankings for this food category. Deli Meats are clearly the highest risk food category of those considered in this risk assessment.
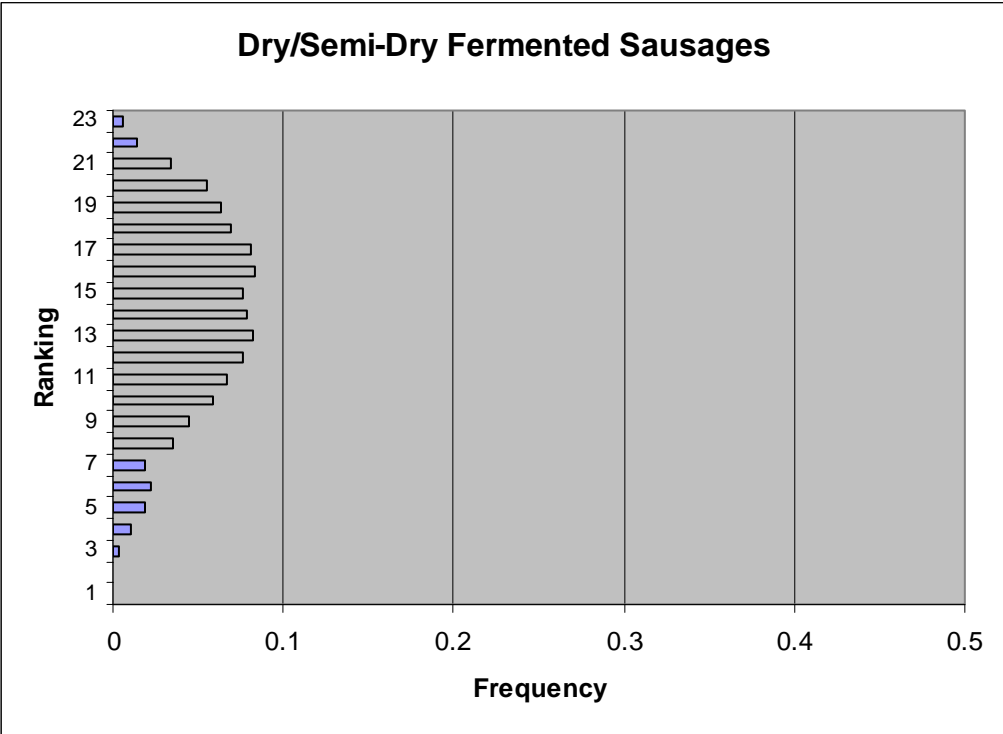
Figure V-24a. Rankings of Total Predicted Listeriosis Cases per Serving for Deli Meats
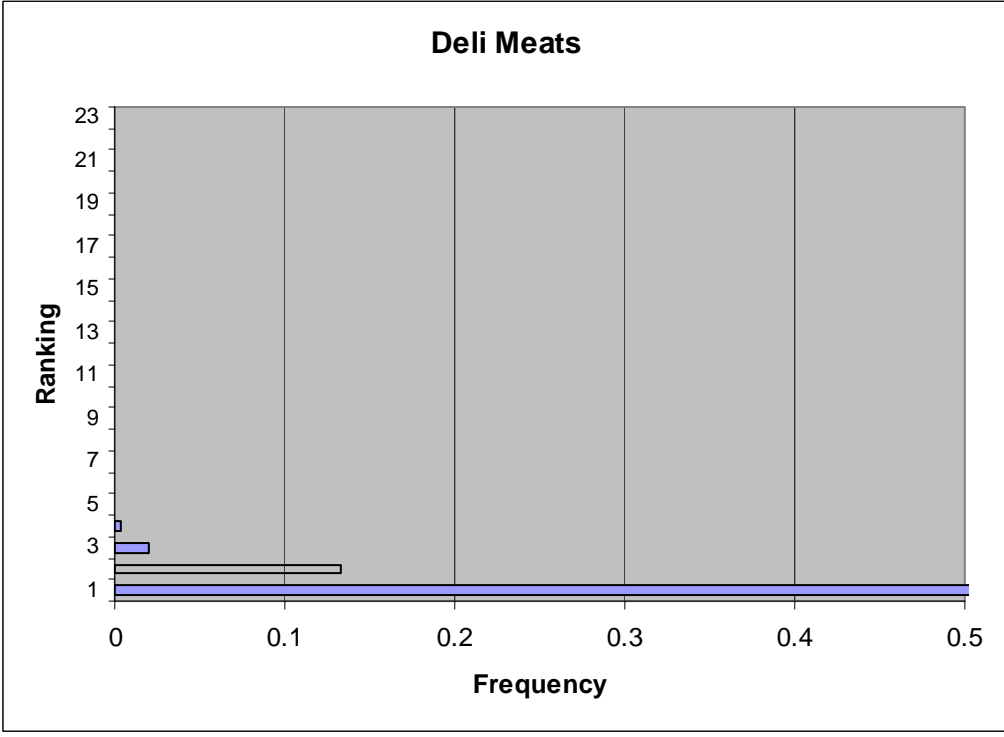
Figure V-24b. Rankings of Total Predicted Listeriosis Cases per Annum for Deli Meats

Food Category: Pâté and Meat Spreads

Foods in the Pâté and Meat Spreads category had a high predicted relative risk of causing listeriosis in the United States on a per serving basis. Foods in this category generally were consumed on an infrequent basis, with moderate serving sizes. The contamination frequency and the growth rates are high and the storage times long. Outbreaks in the U. K., France, and Western Australia have been linked to consumption of pâté (Ryser, 1999a; Goulet *et al.*, 1998).

Contamination data for this category included pâté (e. g., liver pâté) and meat spreads**.** The percentage of samples with detectable contamination was about 6.5%, which is in the moderate contamination range. Three of the twelve contamination studies were conducted in the United States, including the USDA/FSIS surveys conducted in 2001 and 2002 where 17 of the 721 samples were positive. In total, there were 208 positive enumerated samples (most from the U.K.) with high contamination levels including 3 samples greater than 10^5 cfu/g and 3 samples greater than 10^6 cfu/g. The modeled median amount consumed per serving for this category is 57 g (approximately 2 ounces) and the total annual number of servings is 1.2×10^8.

Pâté and Meat Spreads are known to support growth of *Listeria monocytogenes* and the two available studies reported high rates of growth (0.14 and 0.36 logs/day). Storage times were long, ranging from 0.5 to 45 days, with the most likely 6 to 10 days. The predicted percentage of servings with 10^3 to 10^6 cfu at retail was moderate. Post-retail levels are likely to increase prior to consumption due to a significant predicted post-retail growth.

The predicted median risk per serving for the Pâté and Meat Spreads category was 3.2×10^{-8} cases of listeriosis per serving which corresponds to a relative risk ranking of third for the total United States population. The range for the per serving ranking distribution for Pâté and Meat Spreads is relatively narrow (Figure V-25a) and concentrated in the lower ranks (higher risk). This indicates that the extent of variability and uncertainty affecting the predicted relative risk ranking for the Pâté and Meat Spreads category is minimal. The predicted median per annum relative risk rankings was sixth (approximately 4 cases of listeriosis per year) for the total United States population. The range of the per annum ranking distribution was normally distributed but

slightly broader than for the per serving distribution, indicating increased uncertainty associated with the predicted per annum ranking (Figure V-25b). The broadening of the distribution of the per annum rankings reflects the variability and uncertainty associated with the annual consumption of this food category.

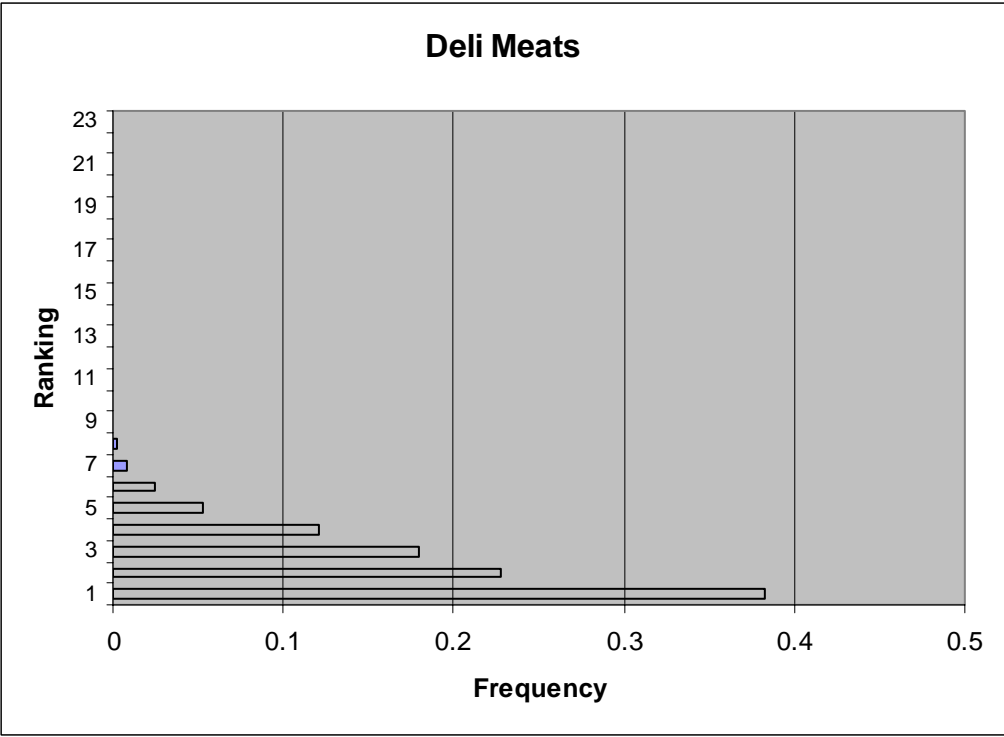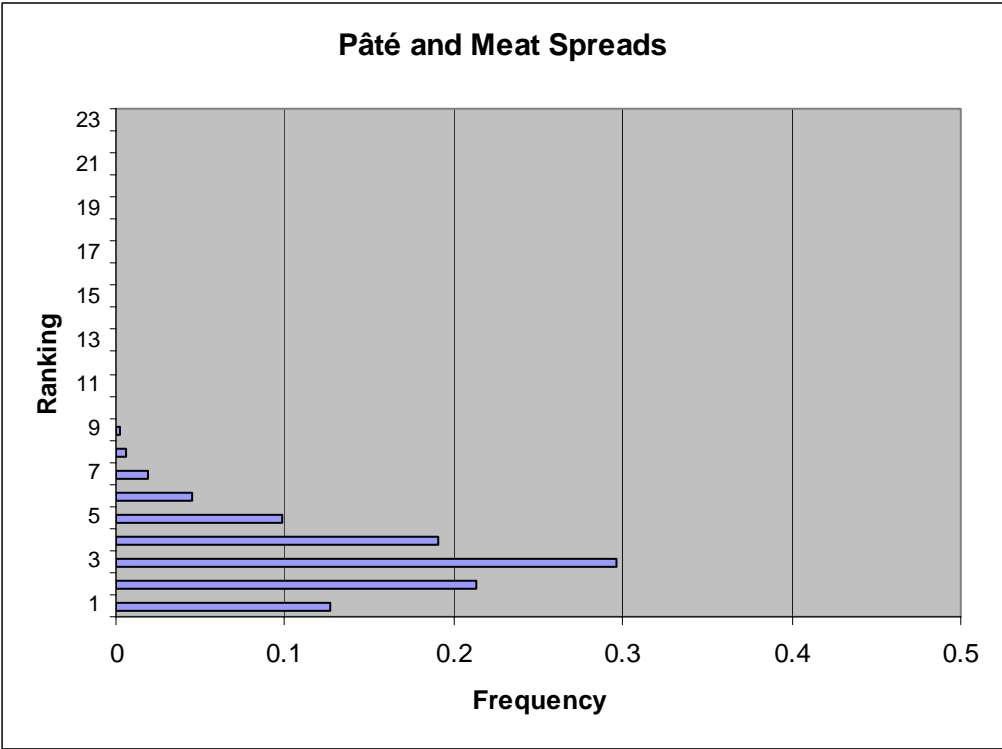**Figure V-25a. Rankings of Total Predicted Listeriosis Cases per Serving for Pâté and Meat Spreads**
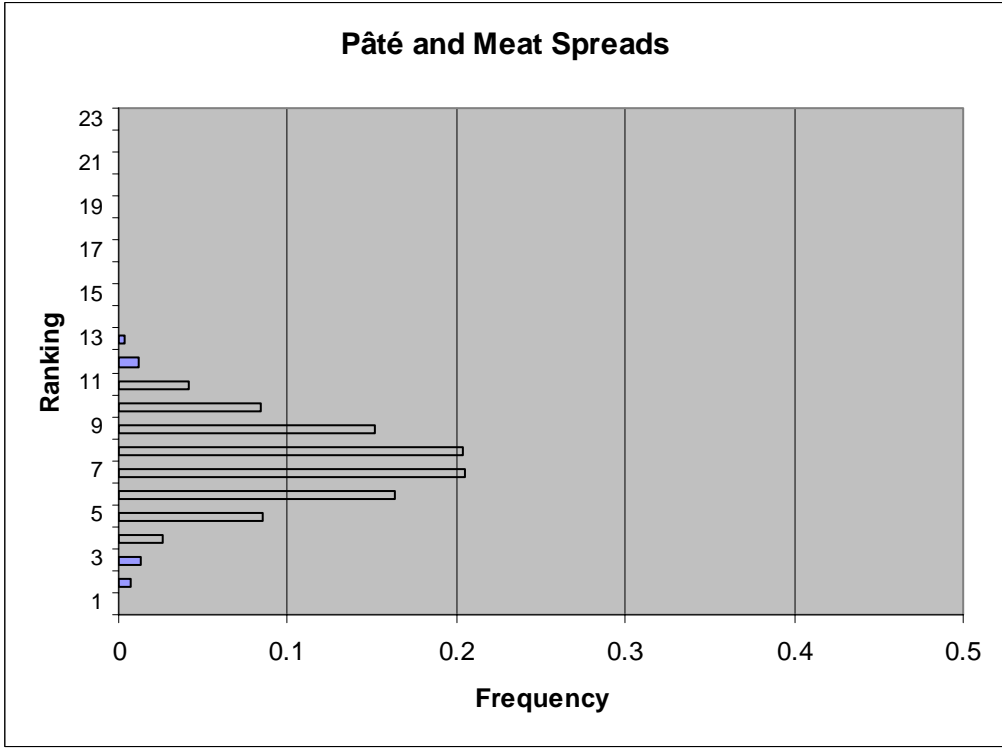
Figure V-25b. Rankings of Total Predicted Listeriosis Cases per Annum for Pâté and Meat Spreads

Food Category: Deli-type Salads

Foods in the Deli-type Salads category has a low predicted relative risk of causing listeriosis in the United States on a per serving basis. The predicted risk for this food category is much lower than the predication of risk in the 2001 draft risk assessment. Based on public comments and newly available data, there were two major changes to the Deli-type Salad food category for this revised risk assessment. First, the vegetable and fruit salads made with dressing were moved from the Vegetables and Fruits categories to the Deli-type Salads category. Secondly, new growth rate information (Johnson *et al.*, 1993; Eblen, 2002a) indicated that levels of *Listeria monocytogenes* actually decrease in most types of Deli Salads during storage instead of growing (as assumed in the 2001 risk assessment). The meat, seafood, eggs, and pasta salads from this category have not been linked to outbreaks or sporadic cases of listeriosis, but FDA has monitored recalls of seafood and egg salads because of the presence of *Listeria monocytogenes*. On the other hand, deli-type salads that are predominately composed of vegetables, have been linked to outbreaks. For example, coleslaw has been linked to an outbreak of listeriosis in Canada, potato salad in the United States and Australia, and sweet corn and rice salad in Italy (Ryser, 1999a).

Although the annual number of servings and median amount consumed are high, the levels of *Listeria monocytogenes* at retail are low and the storage times are short. Of most importance is that the *Listeria monocytogenes* has a low growth rate or declines in most of the foods in this category.

This category includes consumption data for a wide variety of meat, seafood, egg, and pasta salads, vegetable and fruit salads with salad dressing, as well as the salad portion of sandwiches. The median amount consumed per serving is 97 g (i.e., about 3.5 ounces, which is considered a high amount) and the total annual number of servings is 1.3×10^{10}.

Changes in *Listeria monocytogenes* populations were modeled using the newly available data (Johnson *et al.*, 1993; Eblen, 2002a). Decreases in *Listeria monocytogenes* populations are particularly evident in deli salads made by food processors where sufficient acidity and the

addition of preservatives (e.g., sorbate, benzoates) create an inhospitable environment for *Listeria monocytogenes*. In contrast, Deli-type Salads made fresh in the retail establishment typically were not made with preservatives, and could support growth. FDA research (Eblen, 2002a) showed that retail-made seafood-containing salads permitted growth. It is estimated that 85% of the deli-type salads are manufactured by food processors and do not support growth, and that shrimp and crab salads represent less than 10% of the total deli salad sales (Mitchell, 2001). Storage times were relatively moderate and ranged from 0.5 to 12 days with a most likely range of 3 to 4 days.

Sixteen studies, including six conducted in the United States provided contamination data for this food category. The NFPA (2002) survey, which analyzed 11,236 samples, observed that 3.9% were positive (443 positive samples). The contamination frequency was higher for seafood salads (4.5%) vs. non-seafood deli-type salads (2.4%). Of the positive samples, two contained between 100 and 1000 cfu/g and one contained between 10^3 and 10^4 cfu/g. The overall contamination rate for 16 studies in this food category was moderate at 3.8%.

The predicted median per serving relative risk ranking for the Deli-type Salads category was nineteenth for the total United States population. The range for the per serving ranking distribution for Deli Salads was broad (Figure V-26a) but clustered in the higher rankings (i.e., lower risk). The predicted median per annum relative risk rankings was seventeenth for the total United States population. The range for the per annum ranking distribution for Deli-type Salads was slightly wider (Figure V-26b) compared with the per serving ranking distribution. Overall, there was a relatively high degree of uncertainty associated with both the predicted per serving and per annum rankings. This likely reflects that fact that this category includes deli-type salads that do and do not support the growth of *Listeria monocytogenes*. If additional data become available, uncertainty would likely be reduced if this food category was subdivided.
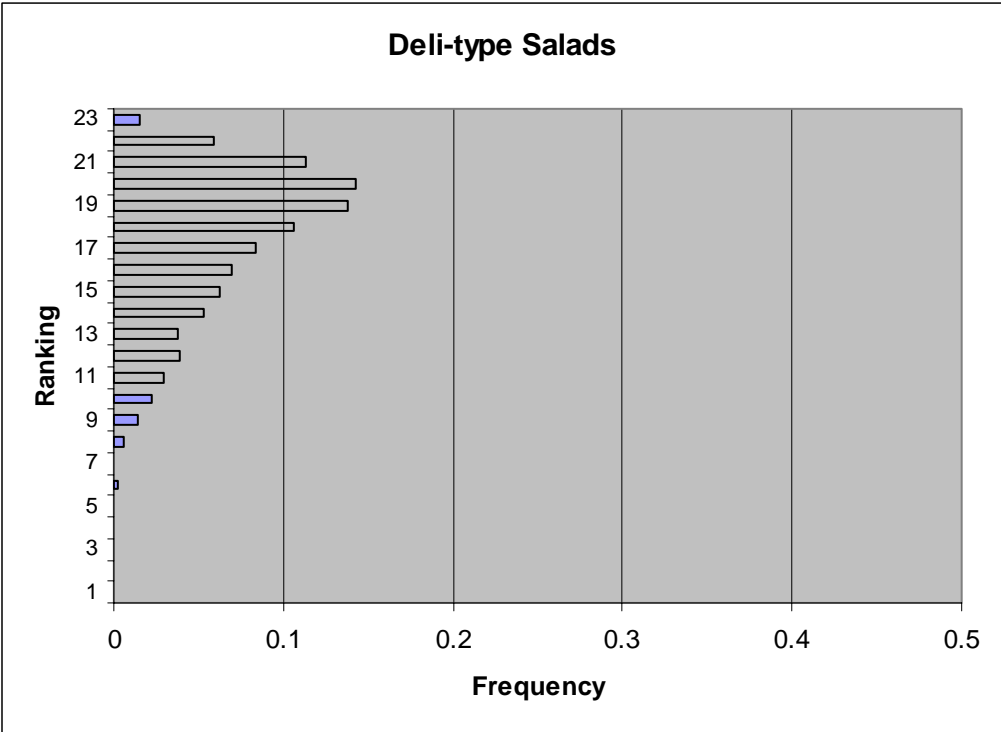
Deli-type Salads

Figure V-26a. Rankings of Total Predicted Listeriosis Cases per Serving for Deli-type Salads
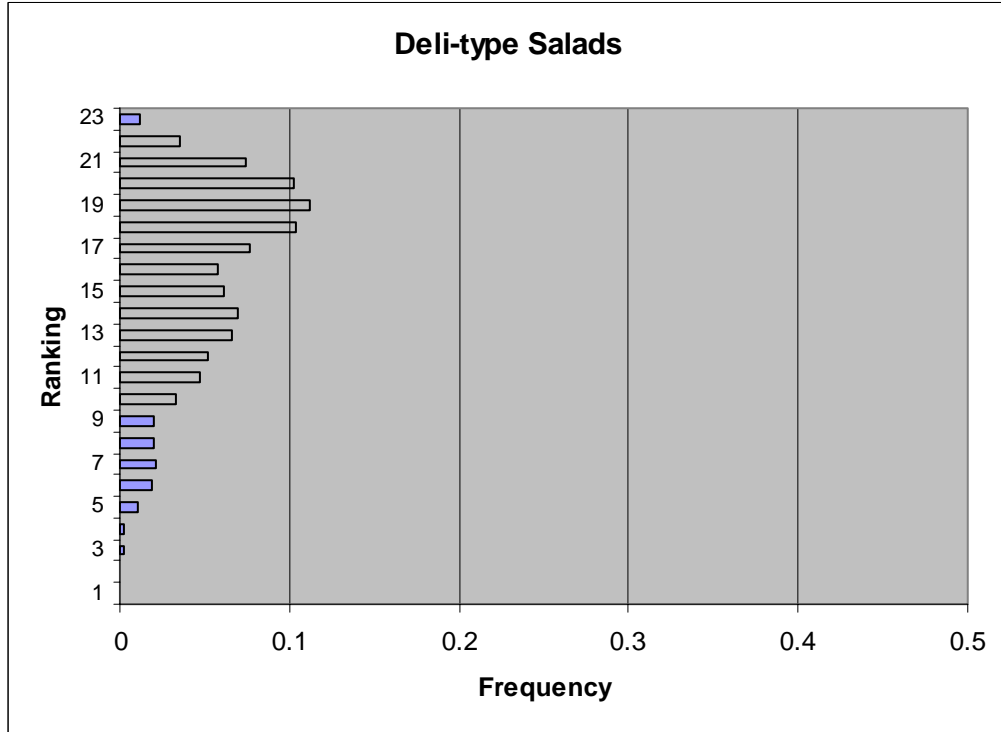
Deli-type Salads

Figure V-26b. Rankings of Total Predicted Listeriosis Cases per Annum for Deli-type Salads

VI. 'WHAT IF' SCENARIOS

The revised FDA/FSIS *Listeria monocytogenes* risk assessment model, taken in its entirety, describes the current status of knowledge about listeriosis and provides predications of disease incidence based on *Listeria monocytogenes* concentration in foods at retail, frequency of consumption, serving size, the microorganism's growth/survival characteristics, and storage conditions. This risk assessment model can be used to estimate the likely impact of intervention strategies by changing one or more input parameters and measuring the change in the model outputs. These changes to the model, which are commonly referred to as 'what if' scenarios, can be used to test the likely impact of new or different processing parameters or regulatory actions. These 'what if' scenarios can also be hypothetical, not necessarily reflecting achievable changes but designed instead to show how different components of the complex model interact. Modeling specific scenarios can assist in the interpretation of a complex risk assessment model by allowing a comparison of baseline calculations to new situations. The following scenarios are intended to simulate the consequence of a putative regulatory policy (i.e., a possible intervention strategy) that alter one or more of the input distributions. Post-retail, at retail and pre-retail interventions were evaluated.

Several simulations were constructed to illustrate the relationship between concentration at consumption or at retail and predicted disease rate. These simulations used exposure models with a range of fixed concentrations. Because a separate simulation was required for each concentration point at the range, a few selected food category/ subpopulation pairs were selected to serve as examples.

Post-Retail Interventions

This risk assessment indicates that most cases of listeriosis result from consuming high levels of *Listeria monocytogenes* from foods that permit growth. For a specific food, the growth is dependent on the characteristics of the food matrix and on the temperature and time allowed for growth. Microbial growth is exponential with time (e.g., linear when plotted on a logarithmic scale) until the stationary phase is approached. The levels of a microorganism after a period of growth also depend upon the initial levels. The following scenarios show how refrigeration temperature and storage time are interrelated using selected food categories and subpopulations.

The relationships demonstrated with these examples would generally apply to other foods and to the other subpopulations. Cooking is another post-retail intervention. The impact of consumers adequately cooking foods was evaluated to measure the impact of reducing the number of frankfurters consumed without adequate reheating on the predicted number of illnesses. The rate of illness as a function of the concentration levels of *Listeria monocytogenes* in food at the time of consumption was also examined.

Refrigerator Temperature Scenarios

These scenarios evaluate the impact of controlling refrigerators to eliminate temperature above various limits. The baseline model used the full empirical distribution of refrigerator temperatures reported by Audits International (1999). Two types of scenarios were run:

- Limit range of refrigeration temperatures for two food categories. The baseline model for Deli Meats and Pasteurized Fluid Milk were modified by limiting the range of refrigeration temperature values to a maximum of 4 to 16 ℃ (39 to 53 ℉) and calculating the resulting annual mortality.

- Truncate refrigeration temperatures for all food categories. The baseline model for all 23 food categories was modified by truncating the refrigeration temperature at 5 ℃ and 7 ℃ (41 and 45 ℉). This scenario allows a comparison of the impact of total cases of listeriosis if the maximum refrigerator temperatures could be regulated at these two specific temperatures.

Figures VI-1 and VI-2 show the estimated annual predicted mortality rate in the elderly subpopulation as a function of maximum temperature for Deli Meats and Pasteurized Fluid Milk, respectively. The median number of annual cases of listeriosis predicated by the baseline assumption (includes all refrigeration temperatures up to a maximum of 16 °C) is 228 for Deli Meats and 13 for Pasteurized Fluid Milk. As the refrigerators that have higher temperatures are removed from the distribution (i.e., moving from the right to the left on the curve) the number of predicted cases declines. This is a consequence of removing the higher temperature refrigerators where the fastest growth of *Listeria monocytogenes* would occur. The number of refrigerators with temperatures between 12 and 16 °C represent about 1% of the refrigerators from the Audits International survey, however, these refrigerators account for approximately 10% of the deaths

from consumption of deli meats. At 7 °C, the removal of 12.2% of the refrigerators reduces the median mortality from deli meat consumption to 71 cases (68.9% reduction). For milk, the decrease in mortality is more linear than for deli meats and occurs at higher limits than for deli meats. Removal of refrigerators above 7 °C reduces the predicted median number of cases from milk consumption from 13 to only 2 cases (84.6% reduction). It should be noted that the relationship between maximum temperature and case rate varies among food categories. However, both examples indicate that eliminating the minority of refrigerators operating above 7 °C would greatly reduce the incidences of listeriosis. The impact on the predicated total number of cases of listeriosis from all 23 food categories and total United States population by eliminating the refrigerators operating above 5 and 7 °C is shown in Table VI-1. By limiting the refrigerator temperature at 7 °C, the number of cases of listeriosis is reduced 69% from 2105 to 656 and limiting the refrigerator temperature at 5 °C further reduces the number of cases to 28 per year (>98% reduction). These scenarios indicate that controlling refrigerator temperature is a potentially effective means to reduce listeriosis.
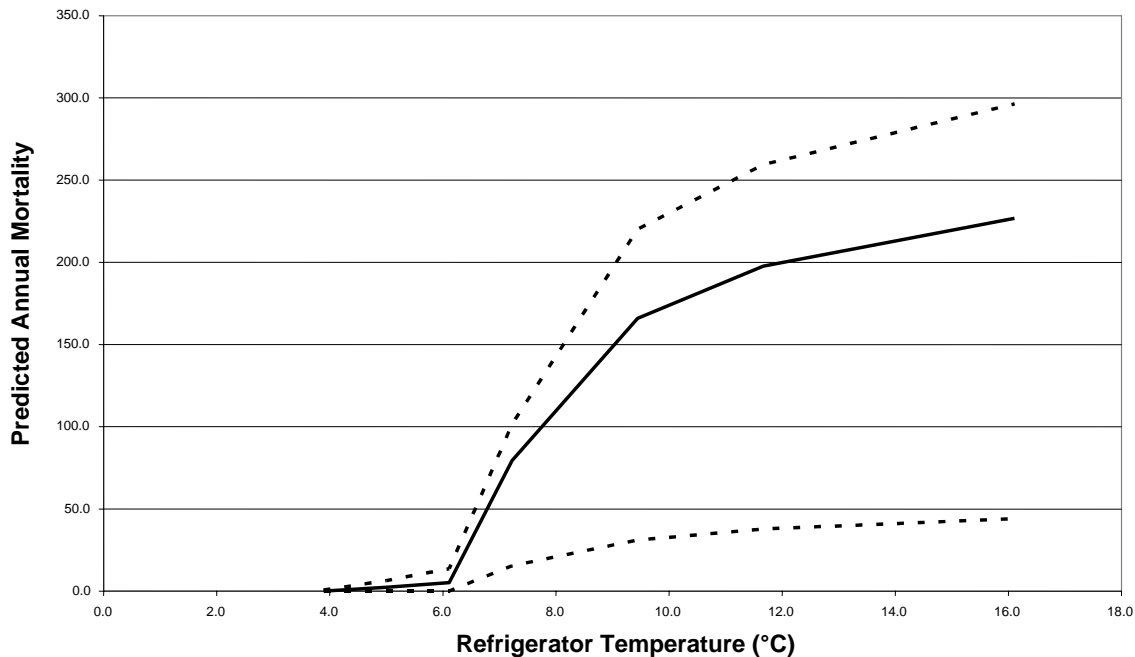
Figure VI-1. Predicted Annual Mortality in the Elderly Population Attributable to Deli Meat as a Function of Maximum Storage Temperaure
[The solid line represents the median estimate. The dotted lines represent the 5th and 95th percentiles of the uncertainty distribution.]
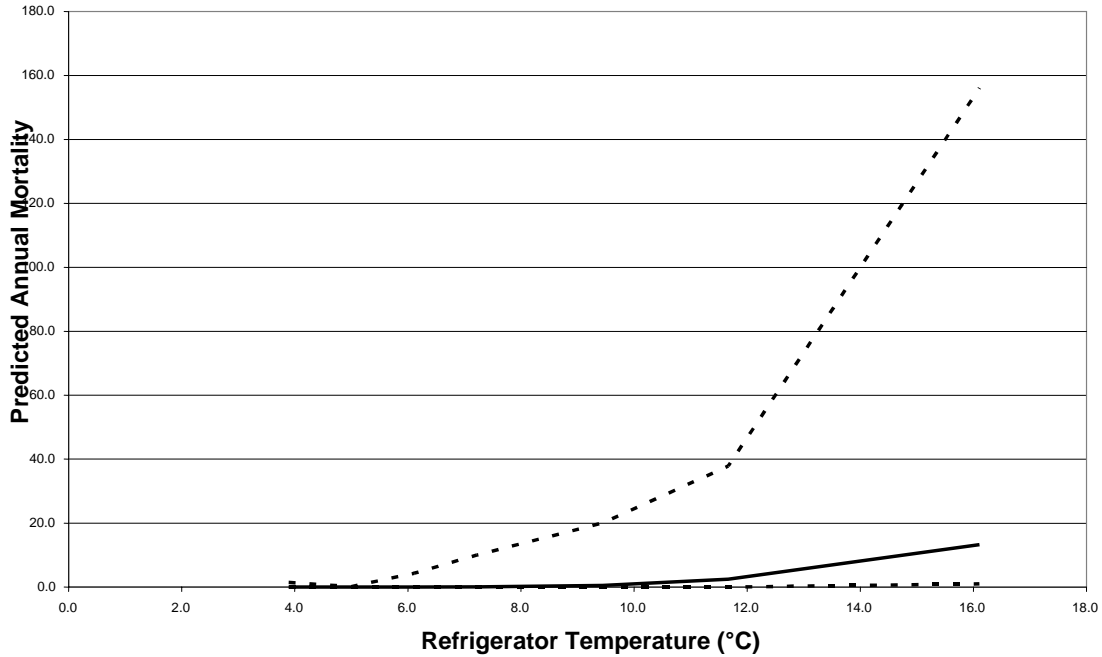
Figure VI-2. Predicted Annual Mortality in the Elderly Population Attributable to Pasteurized Milk as a Function of Maximum Storage Temperaure

[The solid line represents the median estimate. The dotted lines represent the 5th and 95th percentiles of the uncertainty distribution.]

Table VI-1. Estimated Reduction of Cases of Listeriosis from Limits on Refrigeration Temperatures

Maximum Refrigerator Temperature	Cases of Listeriosis[a]		
	Median	5th Percentile	95th Percentile
Baseline[b]	2105	—[c]	—[c]
7 ˚C (45 ˚F) maximum	656	331	761
5 ˚C (41 ˚F) maximum	28	1	126

[a]Values for the median, upper and lower uncertainty levels.
[b]The baseline uses the full empirical distribution of refrigerator temperatures from the Audits International (1999) survey.
[c]The baseline number of cases of listeriosis is fixed based on CDC surveillance data.

Storage Time Scenarios

These scenarios evaluate the impact of changing the maximum storage time (e.g., by labeling food with "consume-by" dates). In two scenarios using Deli Meats and Pasteurized Fluid Milk, the baseline model was modified by truncating the storage time at various maximum limits. In another scenario using Smoked Seafood, the impact of extending shelf life on the predicted risks was explored. The baseline distributions were modified BetaPert distributions defined by minimum, most likely and maximum times.

Limited Storage Times. In these scenarios, when a simulation chose a storage time longer than desired, that simulation was assigned the maximum storage time for that scenario. These simulations assume that the food is consumed during storage up to the maximum scenario storage time and the food is not discarded. Simulations were run for Deli Meats and Pasteurized Fluid Milk and the predicted annual mortality rate attributable to the group was calculated for the elderly subpopulation. The scenarios tested included seven maximum storage times for deli meats of 4, 7, 10, 14, 17, 21, and 28 days and four maximum storage times for milk of 4, 7, 10, and 14 days. The baseline maximum storage time is 28 days for deli meats and 14 days for milk.

Results from the simulations are presented in Figure VI-3 and Figure VI -4. The baseline risk assessment is shown on the right of the curve (28 days for deli meats and 14 days for milk). Limiting the storage time for deli meat from the 28 day baseline to 14 days reduces the median number of cases of listeriosis in the elderly population from 228 to 197 (13.6%) and shortening storage time to 10 days further reduces the cases to 154 (32.5%). For milk, reducing the maximum storage time from the 14 day baseline to 4 days reduced the annual number of listeriosis cases from 13.3 to 7.5 (43.6%). The dependence of predicted risk on storage time varies across food categories. Reducing maximum storage time appears to be less effective at reducing risk than reducing the refrigerator temperature for the deli meat and milk examples. Other storage time scenarios with other food categories would produce different results, for example, the reduction in cases of listeriosis might be greater if foods stored beyond the maximum scenario storage time are discarded instead of consumed on the last day.
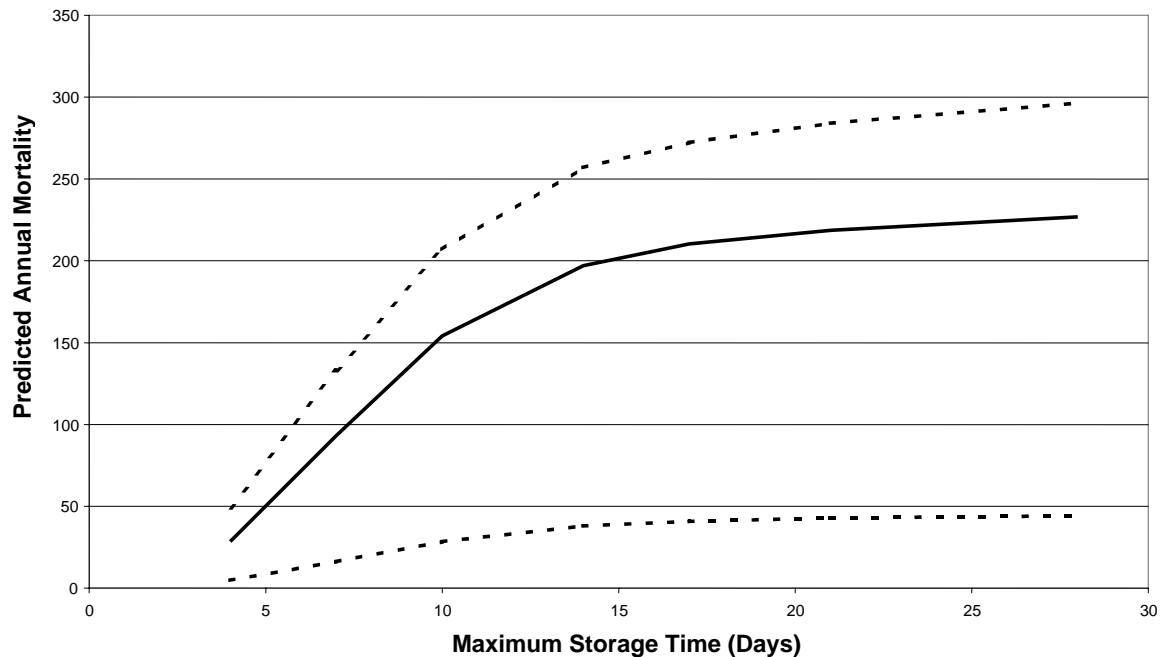
Figure VI-3. Predicted Annual Mortality in the Elderly Subpopulation Attributible to Deli Meats as a Function of Maximum Storage Time

[The solid line represents the median estimate. The dotted lines represent the 5th and 95th percentiles of the uncertainty distribution.]

Figure VI-4. Predicted Annual Mortality in the Elderly Subpopulation Attributible to Pasteurized Milk as a Function of Maximum Storage Time

[The solid line represents the median estimate. The dotted lines represent the 5th and 95th percentiles of the uncertainty distribution.]

Extended Storage Times. A storage scenario was conducted using Smoked Seafood to estimate the impact of a lengthened storage time on the predicted risks per serving and cases per annum for the elderly subpopulation. The estimates from the current 2003 risk assessment used the best estimates of the expert panel for the variation and uncertainty in the home storage times. A modified BetaPert distribution for the 2003 risk assessment had minimum, most likely and maximum values, with uniform uncertainty ranges, of 0.5 days, 3 to 5 days, and 15 to 30 days, respectively. For the extended storage time scenario, the modified BetaPert distribution was defined as 0.5 days (minimum), 6 to 10 days (most likely), and 15 to 45 days (maximum).

The distribution for the extended storage scenario is the same one used in the 2001 draft risk assessment for Smoked Seafoods. However, the calculated values are not the same as in the draft risk assessment because other data sets that are part of the calculation (such as contamination and growth data) have been revised and updated for the 2003 risk assessment.

The median and mean risks per serving and cases per annum are given on Table VI-2 with 5th and 95th values indicating the uncertainty distributions for the calculated risks. The median risk per serving for the elderly subpopulation increased from the baseline value of 1.9×10^{-8} to the extended storage time value 5.0×10^{-8} cases per serving, an increase of about 2.5 times. The median storage time increased from 5.3 to 9.3 days and the percentage of servings that exceeded 10 days of storage increased from 9 to 43%. The uncertainty range for the baseline scenario from the 5th to 95th percentile was approximately three logarithms. The mean risk per serving increased about 58% with the longer storage times. The estimates of the cases per annum follow the changes in risks per serving because the same dose-response relationship and number of servings are used in each scenario. The median number of cases per annum increases from 0.8 with the baseline scenario to 2.1 with the extended storage time scenario and the mean number of cases per annum increased from 10.6 to 17.

The difference between the median and mean reflect the skewed shape of the uncertainty distributions. The median indicates where the center of the distribution is and where the values tend to congregate. The mean will be larger because it is more affected by the few high values than the median, however, it does indicate the central tendency of repeated samplings of the distribution and can be viewed as the "average" value if the cases per annum were tracked over a number of years. The mean risk per serving and risk per annum for each food category is provided in Appendix 10.

The comparison for Smoked Seafood agrees with the truncated storage time scenarios used in the Deli Meats and Pasteurized Fluid Milk examples. Extending the storage times of a food that supports growth increase the probability that listeriosis will occur.

Table VI-2. Impact of Home Refrigerator Storage Times on the Number of Predicted Cases of Listeriosis Attributed to Smoked Seafood for the Elderly Subpopulation

Parameter	Number of Predicted Cases of Listeriosis	
	Current 2003[a]	'What if' Scenario[b]
Per Serving Basis		
Median	1.9×10^{-8}	5.0×10^{-8}
Lower bound (5th percentile)	9.7×10^{-10}	2.7×10^{-9}
Upper bound (95th percentile)	1.0×10^{-6}	1.8×10^{-6}
Mean	2.6×10^{-7}	4.1×10^{-7}
Per Annum Basis		
Median	0.8	2.1
Lower bound (5th percentile)	<0.1	0.1
Upper bound (95th percentile)	43	74
Mean	10.6	17

[a]For the current 2003 risk assessment, the assumed storage time was a distribution with minimum of 0.5 days, most likely of 3 to 5 days, and maximum of 15 to 30 days.
[b]For the 'What if' Scenario, the assumed storage time was a distribution with minimum of 0.5 days, most likely of 6 to 10 days, and maximum of 15 to 45 days. [Note this was the storage time used for the draft 2001 risk assessment.]

Storage Time and Temperature Interaction Scenario

As an example of the potential impact of dual interventions, the interaction modifying both storage time and temperature on the predicted annual mortality rate in the elderly subpopulation attributed to Deli Meats was simulated. The baseline models were adjusted in the same manner as the individual interventions. Results for the temperature and time interaction are shown in Figure VI-5.

The median estimates from the uncertainty distribution are plotted for each storage duration series. The baseline model estimated the upper right value, 228 cases as shown in Figure VI-5. Each line represents a range of maximum storage times at maximum refrigerator temperatures. Achieving a 50% reduction in cases of listeriosis from consumption of deli meats would require eliminating storage above approximately 8 °C or all storage times longer than 8 days. An example of a combination that would reduce cases of listeriosis by 50% is 10 °C and 11 days.
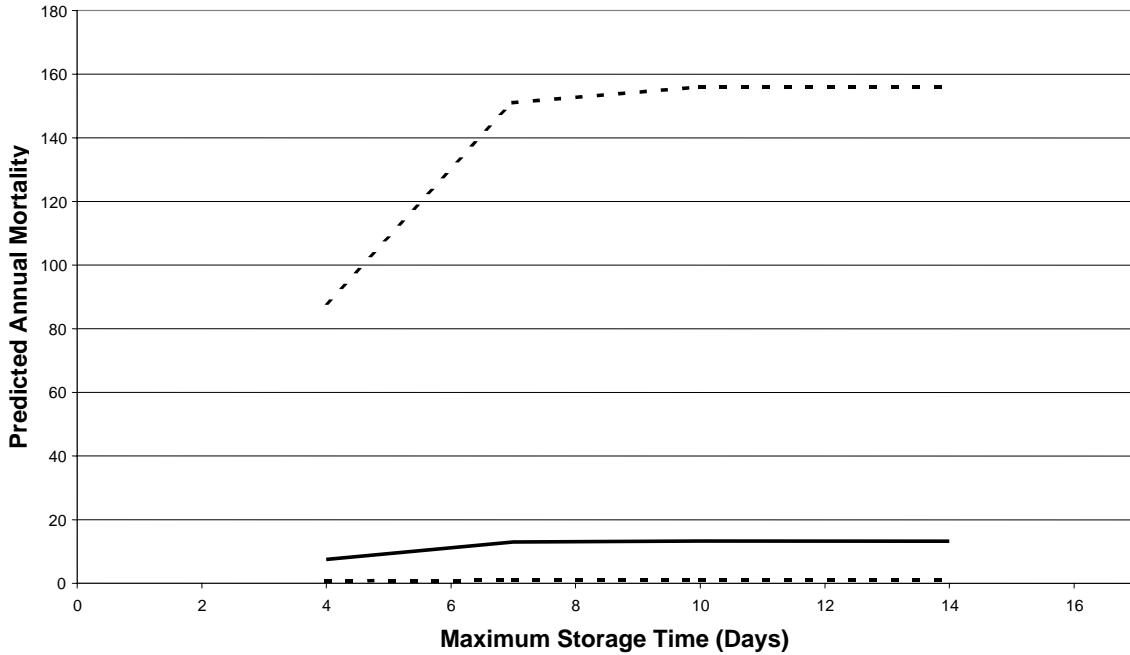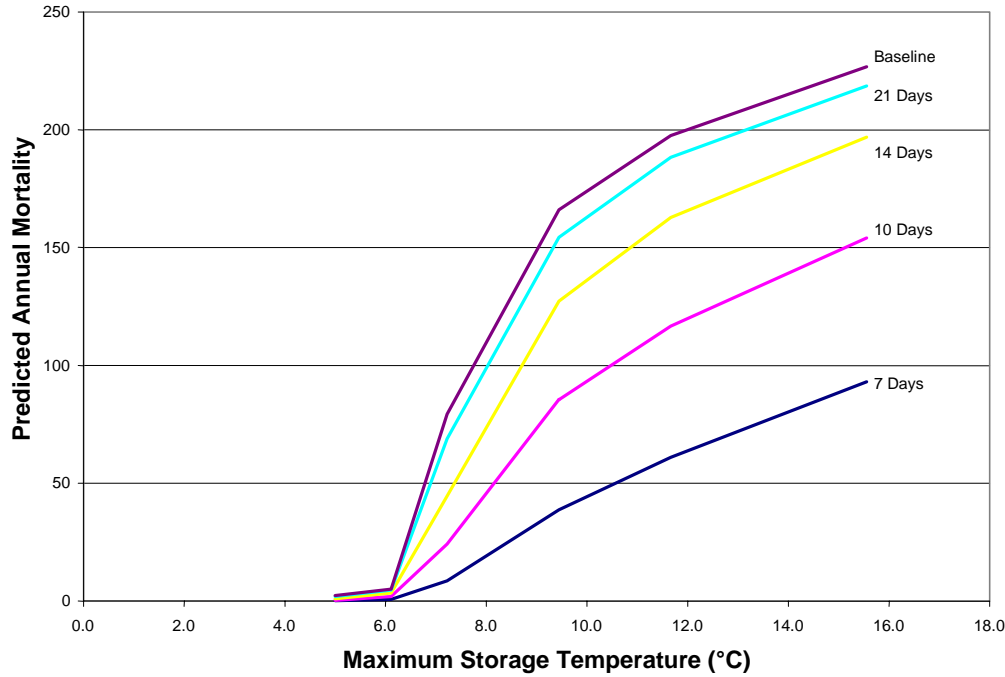
Figure VI-5. Predicted Annual Mortality in the Elderly Subpopulation Attributible to Deli Meats as a Function of Maximum Storage Time and Maximum Storage Temperature

Cooking Scenario

Cooking is a post-retail intervention. Because cooking is an effective method of killing *Listeria monocytogenes*, the risk from unreheated frankfurters is much greater than from adequately reheated frankfurters. A simulation was run in order to simulate the consequence of an intervention that reduces the number of frankfurters consumed without adequate reheating. The baseline assumption, a triangle distribution with an uncertainty range (minimum 4, most likely 7, and maximum 10), was replaced with values of 2, 4, and 6 (minimum, most likely, maximum, respectively). The impact of this change was to reduce the predicted median number of cases of listeriosis by approximately 58% (Table VI-3).

Table VI-3. Scenario testing: Reducing the Estimated Consumption of Unreheated Frankfurters

Scenario	Predicted Number of Cases of Listeriosis		
	Median	**5th Percentile**	**95th Percentile**
Baseline[a]	31	3.3	250
Reduced Consumption[b]	18	2.2	133

[a]Baseline model uses triangular distribution with minimum of 4%, most likely of 7%, and maximum of 10% frankfurters are consumed without reheating.
[b]Reduced consumption scenario assumes a triangular distribution of minimum of 2%, most likely of 4%, and maximum of 6% frankfurters are consumed without reheating.

<u>Disease Rate as a Function of Concentration Levels at the Time of Consumption</u>
This simulation utilizes the main elements of the dose-response simulation and the serving size component from the exposure simulation. Figure VI-6 illustrates the relationship between *Listeria monocytogenes* concentration at the time of consumption and mortality for Deli Meats, it is derived from Figure IV-8 and the serving size distribution for deli meats. Since the only food category specific component is serving size, a similar relationship would be expected for other food categories.
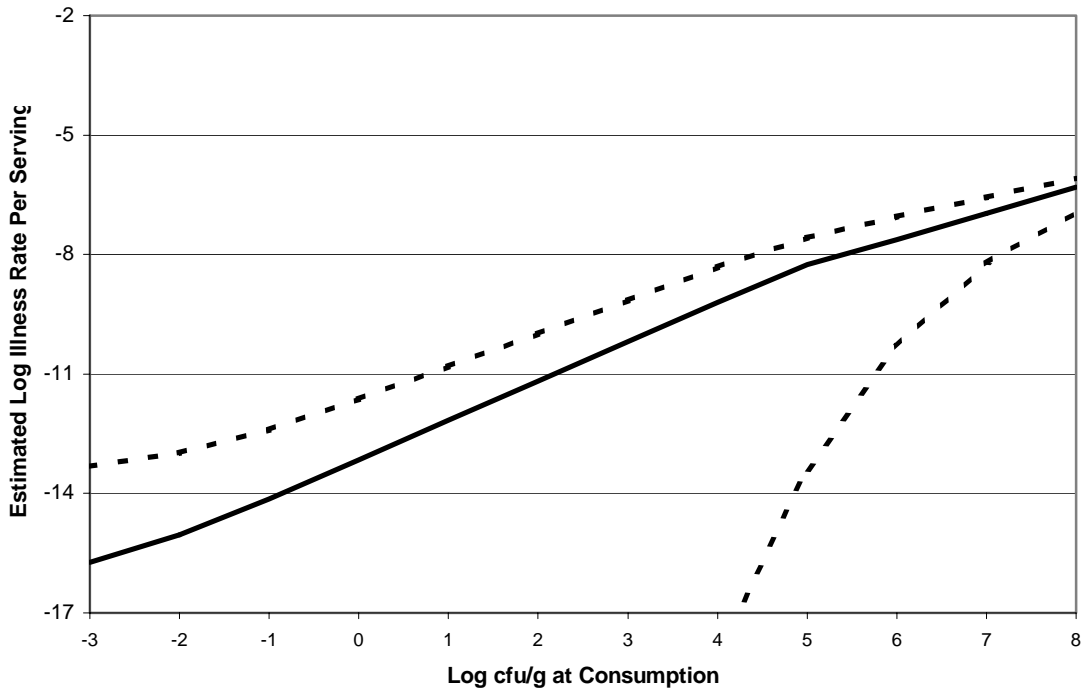
Figure VI-6. Cases of Listeriosis (per serving basis) for the Elderly Population as a Function of *Listeria monocytogenes* **Concentration at Consumption in Deli Meats**

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Pre-Retail and At Retail Interventions

Reduction of the Number of Organisms Scenarios

Interventions might also be designed to reduce the number of *Listeria* in food before it is sold. There are a variety of ways in which this might be done. Effectively modeling a pre-retail intervention may require expanding the modeling effort to include the step at which the intervention takes place. However, a common method of representing or measuring an intervention that kills bacteria (e.g. pasteurization, cooking) is to calculate the number of surviving bacteria as a fraction of the initial number. Since the surviving fraction may be very small, the effectiveness of a kill step may be represented as a log reduction of cfu, where 10% survival represents a 1 log reduction, 1% survival a 2 log reduction, etc. To model an intervention that is measured this way, scenarios were run to calculate the predicted reduction in the number of cases in the elderly population attributable to deli meats as a function of the reduction in cfu prior to retail. This means that for a one log reduction, the distribution of servings containing a given number of *Listeria monocytogenes* at retail was shifted to values one log lower. For example, the 10^3 cfu/g level, which represented 0.5% of the servings, was shifted to 10^2 cfu/g. The contamination was not truncated at any specific cfu/g level, so high contamination levels could still occur but they would be observed less frequently compared to the baseline simulations. The growth after retail was modeled in the same manner as in the baseline model. The results are displayed in Figure VI-7.
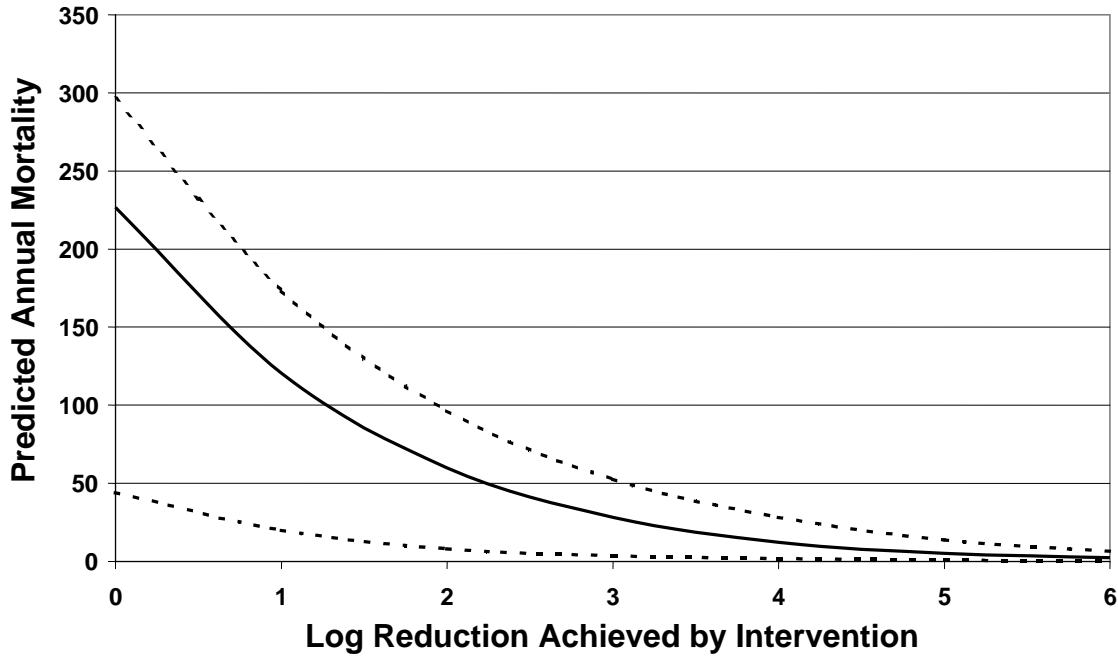
Figure VI-7. Reduction of Predicted Annual Mortality in the Elderly Subpopulation Attributible to Deli Meats as a Function of Log Kill Achieved by the Inclusion of a Lethal Intervention Prior to Retail
[The solid line represents the median estimate. The dotted lines represent the 5th and 95th percentiles of the uncertainty distribution.]

The scenarios shown in Figure VI-7 indicate that inclusion of treatment that produced a one log reduction in contamination at retail would reduce the number of predicted deaths in the elderly population attributed to Deli Meats nearly 50%, from 227 to 120. Reducing contamination two logs would result in a 74% reduction. This reduction could be achieved by a number of different means such as reduced contamination of raw materials, more effective sanitation, or a process step that results in some lethality.

Estimations of risk per serving from specific cfu/g at retail scenarios
The ability of *Listeria monocytogenes* to grow in a food is associated with the likelihood of that food causing illness. The following scenario provides insight on how the contamination level at retail in a food that supports growth affects the risk of listeriosis per serving. This example is based on Deli Meat and the elderly population where the contamination level is a single value, not a distribution with variation and uncertainty as in the other examples (Figure VI-8). Since

the actual number of cases depends on the number of servings, only the case rate per serving is used as the endpoint.

There is a wide variation in growth resulting from the combination of exponential growth rate, temperature, time and maximum levels but some servings will grow to populations having high likelihood of causing illness. The level of *Listeria monocytogenes* is the determining factor in the resulting risk per serving. For example, if a 56-g serving that has one *Listeria monocytogenes* per gram at retail (i.e., 0 $\log_{10}$ cfu/g or approximately 56 total *Listeria monocytogenes* per serving) grows as described by the baseline model, it will result in a risk per serving of 1.1 x 10^{-6} (-5.96 $\log_{10}$ or approximately 1 death per million servings). For a 56-g serving with 100 cfu/g at retail, the model predicts a modest increase in the likelihood of death (1.3 x 10^{-6} deaths per serving). Conversely, if a 10-g serving has one *Listeria monocytogenes* per g, the model predicts a risk of 1.0 x 10^{-6} (-6.0 $\log_{10}$) and for a 100-g serving, the model predicts a reduction of the risk to 0.71 x 10^{-6} (-6.15 $\log_{10}$). These relatively small changes in risk despite a ten-fold change in contamination level are a consequence of the expected post-retail growth of *Listeria monocytogenes* in food before consumption.

Given the refrigerator temperature and storage time distributions, the relatively low numbers at retail have the potential to grow to levels at the time of consumption in a sufficient fraction of servings that the overall risk is in the range of 10^{-6} per serving. Reducing the levels from 10^{3} to 10^{2} and to even 10^{0} cfu/g reduces the risk, but not very much. Only when the contamination level decreases to less than one *Listeria monocytogenes* per package does the risk fall in proportion to the frequency of contamination (10^{-3} cfu/g decreases the risk to 0.02 x 10^{-6} per serving). What this implies is that in foods that support growth, reducing contamination to some specified level (but not zero) is not adequate by itself in controlling the risk of listeriosis.
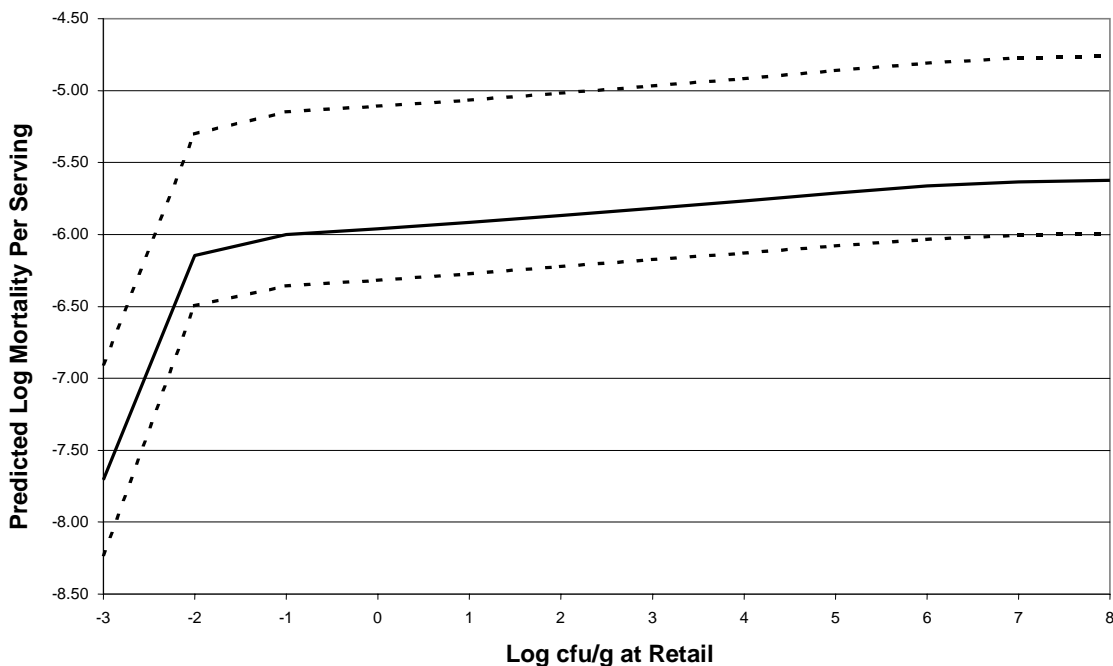
Figure VI-8. Predicted Mortality per Serving for the Elderly Subpopulation When Specific Concentrations of *Listeria monocytogenes* in Deli Meats at Retail are Allowed to Grow Before Consumption

Fresh Soft Cheese Made from Unpasteurized Milk Scenario

Unlike the 2001 draft risk assessment, the revised risk assessment indicates that the risk from Fresh Soft Cheese is low. This change is largely attributable to the inclusion of additional new data indicating a very low prevalence rate in this food category. However, there is a strong epidemiological correlation between Hispanic-style fresh soft cheese (Queso Fresco) and listeriosis. A likely explanation for this discrepancy is that the data collected for this category is not representative of the cheese linked to the disease (i.e., fresh soft cheese made from raw, unpasteurized milk). In particular, although most commercial sources of fresh soft cheese are manufactured from pasteurized milk, some sources of queso fresco are made from raw milk.

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To characterize the risk from queso fresco made from raw milk, the exposure model was constructed using the same analog as in the 2001 draft risk assessment – soft unripened cheese made from raw milk (Loncarevik, *et al*., 1995), where 50% of the samples tested were positive. A data set for the contamination distribution was developed using the methodology described in the Exposure Assessment chapter using the default range of 2 to 5 geometric standard deviations and applying a correction factor for overestimation from older data. The same growth and storage parameters were used as in the baseline estimation.

The estimated risk per serving for two sensitive populations is presented in Table VI-4. The risk per serving was 43 times greater for the perinatal population and 36 times greater for the elderly population when cheeses were assumed to be made from unpasteurized milk compared to manufacture with pasteurized milk. The tested 'high prevalence' scenario increased the predicted risk on a per serving basis from low to a high risk.

Table VI-4. Comparison of Baseline and a High Prevalence Scenerio Risk per Serving for Fresh Soft Cheese for Two Subpopulations

| Population | Median Predicted Risk per Serving (5th and 95th percentiles) | |
	Baseline[a]	High Prevalence[b]
Perinatal	4.7×10^{-9} (3.0×10^{-11}, 9.8×10^{-8})	2.0×10^{-7} (5.1×10^{-9}, 5.3×10^{-6})
Elderly	2.8×10^{-10} (1.3×10^{-12}, 4.5×10^{-9})	1.0×10^{-8} (3.2×10^{-10}, 2.3×10^{-7})

[a]Baseline uses a prevalence distribution based on available survey data.
[b]High Prevalence scenarios assumes that 50% of the samples tested are positive.

Disease Rate as Function of Concentration Levels Measured at Retail

To simulate the relationship between *Listeria monocytogenes* concentration at retail and public health, the growth component of the exposure assessment is also included. Since the growth model differs significantly across food categories, examples for both high (Deli Meats) and low (Hard Cheese) growth are shown in Figures VI-9, VI-10, and VI-11. Comparison of Figures VI-9 (elderly) and VI-10 (neonatal) suggests that similar dose-response relationships may be expected for different subpopulations. However, the comparison of Figure VI-9 (Deli Meat) and VI-11 (Hard Cheese) indicates that the growth component of the model for a particular food category can have a large influence on the relationship between concentration at retail and the rate of listeriosis. Foods with high growth rates (such as Deli Meats) exhibit a relatively flat

curve that suggests that the number of cases is only slightly dependent on initial concentration. On the other hand, low growth foods (such as Hard Cheese) indicate a substantial increase in the disease rate as the concentration increases. This suggests that for foods that support growth, above some minimum concentration the risk is largely determined by the growth that occurs subsequent to purchase.
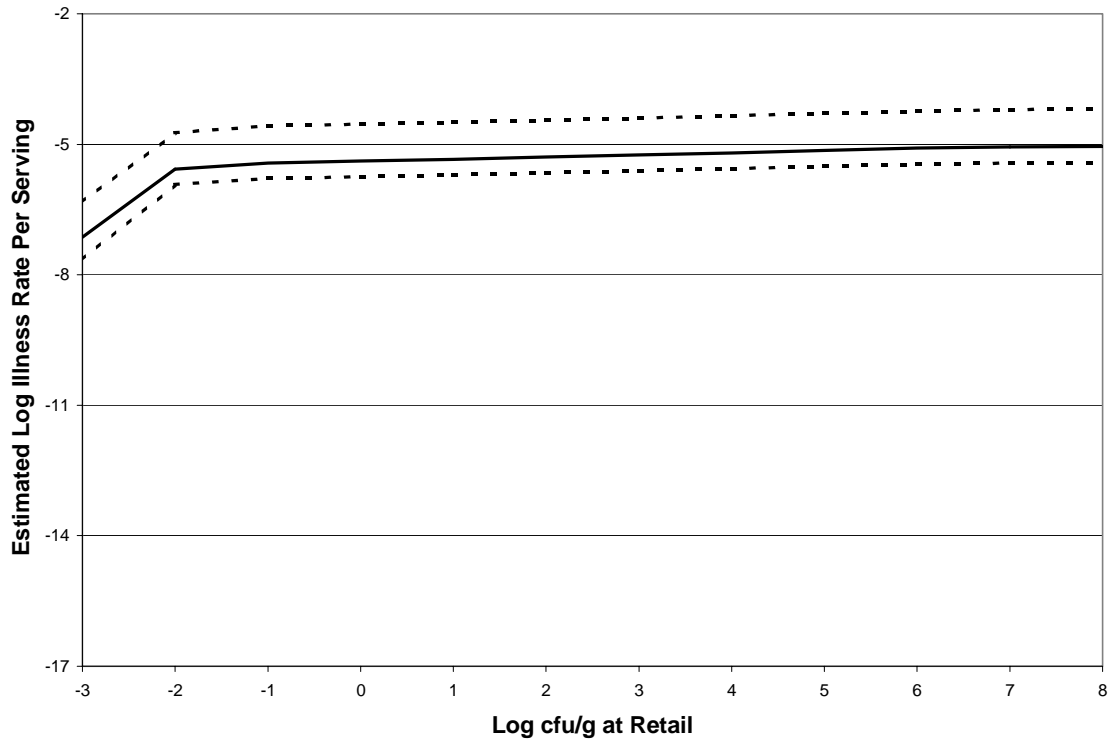
Figure VI-9. Cases of Listeriosis (per serving basis) for the Elderly Subpopulation as a Function of *Listeria monocytogenes* Concentration at Consumption for Deli Meat
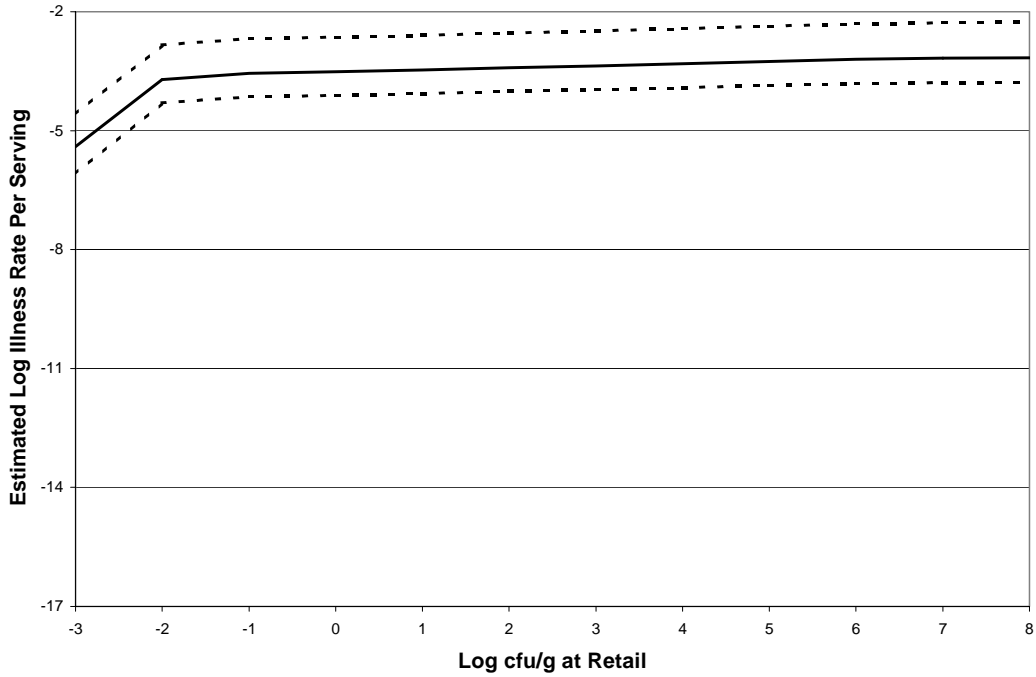
Figure VI-10. Cases of Listeriosis (per serving basis) for the Neonatal Subpopulation as a Function of *Listeria monocytogenes* **Concentration at Retail for Deli Meat.**
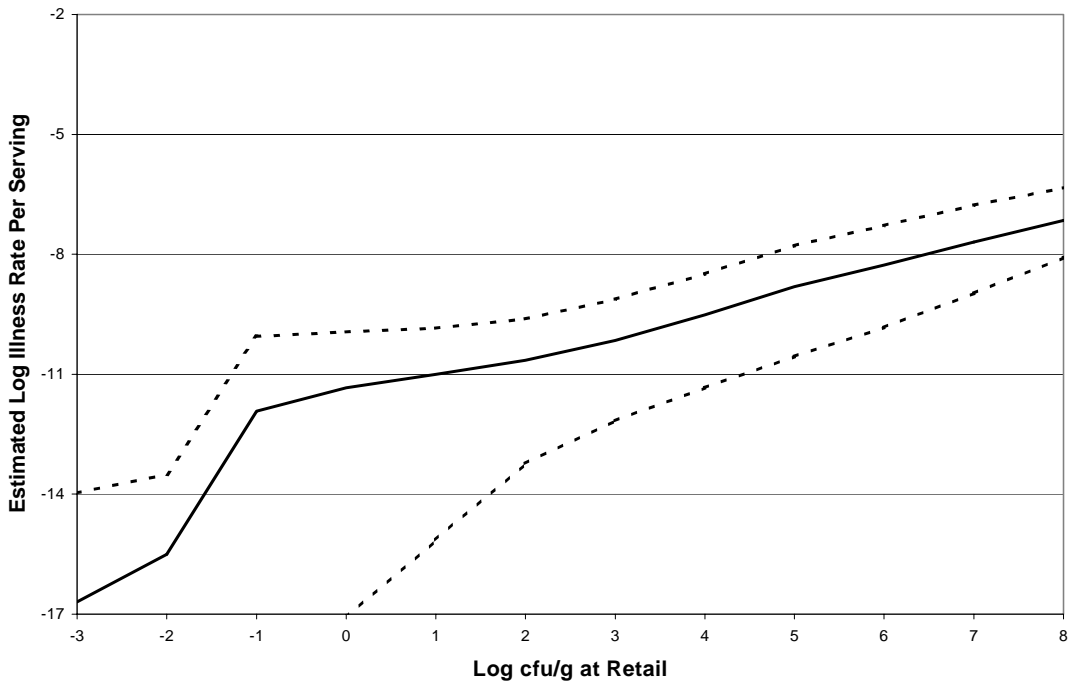
Figure VI-11. Cases of Listeriosis (per serving basis) for Elderly Subpopulation as a Function of *Listeria monocytogenes* **Concentration at Retail for Hard Cheese**

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Pasteurized Fluid Milk Scenarios

The primary intervention for milk is pasteurization. Differences in pasteurization requirements and handling practices among different countries could result in different levels of frequency and amounts of *Listeria monocytogenes* in milk at consumption. The Pasteurized Fluid Milk food category contains 30 studies including 3 studies conducted in the United States. There are a total of 12,407 fluid milk samples including whole milk, low fat, skim milk, and chocolate milk. All of the milk samples are from cows, except for a single sample of goat milk. The average percent of positive samples across the 30 studies is 0.4%. As with all of the food categories, the data were weighted for location, study age, and study size.

A "what-if" analysis was conducted to evaluate the impact of including non-U.S. studies and chocolate milk in this food category. The results for the three subpopulations and the total U.S. population are presented below in Tables VI-5 And VI-6. Excluding non-U.S. milk and chocolate milk has little impact on the predicted number of cases of listeriosis attributed to Pasteurized Fluid Milk on both per serving and per annum basis.

Table VI-5. Impact of Excluding Non-U.S. and Chocolate Milk from the Pasteurized Fluid Milk Food Category on the Number of Cases of Listeriosis per Serving Basis

Scenario	Median Cases of Listeriosis per Serving (5th, 95th percentile)			
	Intermediate-Age	Perinatal	Elderly	Total
Baseline	4.4×10^{-10} $(2.8 \times 10^{-11}, 5.7 \times 10^{-9})$	1.6×10^{-8} $(1.3 \times 10^{-9}, 1.9 \times 10^{-7})$	3.4×10^{-9} $(2.5 \times 10^{-10}, 3.9 \times 10^{-8})$	1.0×10^{-9} $(7.5 \times 10^{-11}, 1.3 \times 10^{-8})$
Domestic Milk Only	3.7×10^{-10} $(2.8 \times 10^{-11}, 3.5 \times 10^{-9})$	8.0×10^{-7} $(7.1 \times 10^{-8}, 6.5 \times 10^{-6})$	2.9×10^{-9} $(2.6 \times 10^{-10}, 2.5 \times 10^{-8})$	8.8×10^{-10} $(7.5 \times 10^{-11}, 7.7 \times 10^{-9})$
Domestic Milk (excluding chocolate milk)	3.8×10^{-10} $(3.0 \times 10^{-11}, 3.4 \times 10^{-9})$	8.4×10^{-7} $(7.6 \times 10^{-8}, 6.0 \times 10^{-6})$	3.0×10^{-9} $(2.7 \times 10^{-10}, 2.3 \times 10^{-8})$	9.3×10^{-10} $(8.2 \times 10^{-11}. 7.5 \times 10^{-9})$
Domestic Chocolate Milk Only	4.2×10^{-10} $(2.9 \times 10^{-11}, 7.9 \times 10^{-9})$	9.4×10^{-7} $(7.6 \times 10^{-8}, 1.5 \times 10^{-5})$	3.4×10^{-9} $(2.6 \times 10^{-10}, 6.1 \times 10^{-8})$	5.8×10^{-10} $(4.3 \times 10^{-11}, 1.1 \times 10^{-8})$

Table VI-6. Impact of Excluding Non-U.S. and Chocolate Milk from the Pasteurized Fluid Milk Food Category on the Number of Cases of Listeriosis per Annum Basis

Scenario	Median Cases of Listeriosis per Annum (5th, 95th percentile)			
	Intermediate-Age	Perinatal	Elderly	Total
Baseline	31.4 (2.0, 410.1)	8.0 (0.7, 95.8)	49.8 (3.7, 584.4)	90.8 (6.5, 1084.6)
Domestic Milk Only	27 (2.0, 250)	6.7 (0.65, 55)	43 (3.8, 360)	77 (6.5, 670)
Domestic Milk (excluding chocolate milk)	26 (2.1, 240)	6.9 (0.7, 52)	45 (4.0, 340)	78 (6.9, 630)
Domestic Chocolate Milk Only	1.2 (0.8, 23)	0.3 (0.2, 4.7)	0.2 (0.2, 4.1)	1.7 (0.1, 32)

Summary

In these scenarios, selected food categories (Deli Meats, Frankfurters, Fresh Soft Cheese, Pasteurized Fluid Milk, Smoked Seafood, and Hard Cheese) were used as examples. Other foods which permit different rates of growth and are stored for different lengths of time may have different results, but the general interrelationships are representative of other food categories. These scenarios compared with the baseline estimations of risk illustrate the impact of storage time, storage temperature, and contamination level on the risks per serving.

- Reducing the ranges of refrigerator temperatures by eliminating storage at the high temperatures reduced the predicted cases of listeriosis by reducing growth of *Listeria monocytogenes* in the foods that permit growth.

- Eliminating the longest storage times reduced the number of cases of listeriosis, even with the full range of storage temperatures and contamination levels. However, reducing a percentage of the longest storage times appeared to be less effective than reducing the corresponding percentage of highest storage temperatures, unless the storage time is reduced to very short duration between retail and consumption.

- Reducing the overall frequency of high levels of contamination will reduce the number of cases, particularly when frequencies of the highest contamination levels are reduced. However, growth can occur from relatively low contamination levels at retail to levels at consumption that are likely to cause illness. Thus, in foods that permit growth, reducing the *Listeria monocytogenes* at or before retail to less than some specified level other than zero will not result in the elimination of the risk.

VII. INTERPRETATION AND CONCLUSIONS

This risk assessment included analysis of the available scientific information and data in the development of exposure assessment and dose-response models to predict the relative public health impact of foodborne *Listeria monocytogenes* from 23 food categories. The assessment focuses on predicting the comparative risk among ready-to-eat foods that have a history of either *Listeria monocytogenes* contamination or were implicated epidemiologically. The risk assessment demonstrates the predicted relative risk associated with these foods in relation to the overall incidence of listeriosis including both apparently sporadic illnesses and illnesses associated with outbreaks. Illnesses attributed to documented outbreaks are a small proportion of the total estimated annual cases of listeriosis. Outbreaks frequently represent a breakdown in the food safety controls that have been established to prevent such occurrences. For example, outbreaks of listeriosis have been linked to failure to protect a frankfurter processing line from environmental contamination caused by plant renovations (1998-99), use of defective processing equipment in the production of chocolate milk (1994), and inadequate pasteurization of milk used to make fresh soft Mexican-style cheese (1987). Thus, continued vigilance of current food safety control systems and the targeted initiation of new controls will likely be needed to achieve further reductions of the incidence of listeriosis.

The scientific evaluations and the mathematical models developed during the risk assessment, provide a systematic assessment of the scientific knowledge needed to assist both in reviewing the effectiveness of current policies, programs, and practices, and identifying new strategies to minimize the public health impact of foodborne *Listeria monocytogenes*. This systematic assessment provides a foundation to assist future evaluations of the potential effectiveness of new strategies for controlling foodborne listeriosis. The risk assessment provides a means of comparing the relative risks associated with these foods on a per serving and a per annum basis. However, overall interpretation of the risk assessment requires more than just a simple consideration of only the relative risk rankings associated with the various food categories. As discussed above, the results must also be evaluated in relation to the degree of variability and uncertainty inherent in the predicted relative risk, and interpreted in relation to available

scientific knowledge of the production, marketing, and consumption of the various food
categories. Likewise, the results must be evaluated in relation to the available epidemiological
record. A detailed consideration of the quantitative and qualitative findings for each food
category is provided in the risk assessment and its appendices.

As part of the evaluation and interpretation of the predicted risk estimates and the accompanying
relative risk rankings, the risk assessment considered various qualitative and quantitative
methods of grouping the results that may be useful for risk management or risk communication
purposes. For example, Table V-6 includes an arbitrary grouping of the per serving and per
annum results into very high, high, medium, and low risk categories based on the criteria
provided in the table's footnotes. In this instance, six food categories were considered to be high
risk on a per serving basis: Deli Meats, Frankfurters (not reheated), Pâté and Meat Spreads,
Unpasteurized Fluid Milk, Smoked Seafood, and Cooked Ready-to-Eat Crustaceans. Three food
categories are considered to be moderate risk and the remaining 14 food categories are
considered to be low risk on a per serving basis. On a per annum basis, the majority of the cases
are predicted to be attributable to Deli Meats. The high-risk food categories included
Pasteurized Fluid Milk, High Fat and Other Dairy Products, and Frankfurters (not reheated).
Five food categories are considered to be moderate risks and the remaining 14 food categories
are considered to be low risk on a per annum basis.

A number of methods for objectively grouping the results were evaluated, and are discussed in
detail within the risk assessment. One approach that appears to be very useful for risk
management/communication purposes is the evaluation of the relative risk ranking results using
cluster analysis (see Appendix 12). When performed at the 90% confidence level, this analysis
groups the per serving rankings into four clusters and the per annum rankings into five clusters
(Table VII-1). These clusters are used, in turn, to develop a two-dimensional matrix of per
serving vs. per annum rankings (see Figure VII-1) of the food categories. In this approach, the
four per serving clusters were arrayed against the per annum clusters (A and B, C and D, and E).
The matrix was then used to depict five overall risk designations: Very High, High, Moderate,
Low, and Very Low. For example, as shown in Table VII-1, Deli Meats is included in the 'per

serving' Cluster 1 and in the 'per annum' Cluster A, so it is placed in the two-dimensional matrix cell, Very High Risk, Cluster 1-A (See Summary Figure VII-1). Frankfurters (not reheated) is in the 'per serving' Cluster 1 and in the 'per annum' Cluster B, so it is also placed in the Very High Risk cell, representing Cluster 1-B. No food categories are in the Moderate Risk cell for Clusters 3-A and 3-B because there are no foods in the 'per serving' Cluster 3 that match with the 'per annum' Cluster A or Cluster B.

Table VII-1. Results of Cluster Analysis at the 0.1 Level

Risk per Serving	Risk per Annum
CLUSTER 1 Deli Meats Frankfurters, not reheated Pâté and Meat Spreads Unpasteurized Fluid Milk Smoked Seafood	**CLUSTER A** Deli Meats
CLUSTER 2 Cooked RTE Crustaceans High Fat and Other Dairy Products Pasteurized Fluid Milk Soft Unripened Cheese	**CLUSTER B** High Fat and Other Dairy Products Frankfurters, not reheated Pasteurized Fluid Milk Soft Unripened Cheese
CLUSTER 3 Deli-type Salads Dry/Semi-dry Fermented Sausages Fresh Soft Cheese Frankfurters, reheated Fruits Preserved Fish Raw Seafood Semi-soft Cheese Soft Ripened Cheese Vegetables	**CLUSTER C** Cooked RTE Crustaceans Fruits Pâté and Meat Spreads Unpasteurized Fluid Milk Smoked Seafood
CLUSTER 4 Cultured Milk Products Ice Cream and Frozen Dairy Products Processed Cheese Hard Cheese	**CLUSTER D** Deli-type Salads Dry/Semi-dry Fermented Sausages Frankfurters, reheated Fresh Soft Cheese Soft Ripened Cheese Semi-Soft Cheese Vegetables
	CLUSTER E Cultured Milk Products Hard Cheese Ice Cream and Frozen Dairy Products Preserved Fish Processed Cheese Raw Seafood

Decreased Risk per Annum

→

Clusters A and B	Clusters C and D	Cluster E	
Very High Risk (Clusters 1-A, 1-B) Deli Meats Frankfurters (not reheated)	**High Risk (Clusters 1-C, 1-D)** Pâté and Meat Spreads Unpasteurized Fluid Milk Smoked Seafood	**Moderate Risk (Cluster 1-E)** No food categories	**Cluster 1**
High Risk (Clusters 2-A, 2-B) High Fat and Other Dairy Products Pasteurized Fluid Milk Soft Unripened Cheese	**Moderate Risk (Clusters 2-C, 2-D)** Cooked RTE Crustaceans	**Moderate Risk (Cluster 2-E)** No food categories	**Cluster 2**
Moderate Risk (Clusters 3-A, 3-B) No food categories	**Moderate Risk (Clusters 3-C, 3-D)** Deli-type Salads Dry/Semi-dry Fermented Sausages Frankfurters (reheated) Fresh Soft Cheese Fruits Semi-soft Cheese Soft Ripened Cheese Vegetables	**Low Risk (Cluster 3-E)** Preserved Fish Raw Seafood	**Cluster 3**
Moderate Risk (Clusters 4-A, 4-B) No food categories	**Low Risk (Clusters 4-C, 4-D)** No food categories	**Very Low Risk (Cluster 4-E)** Cultured Milk Products Hard Cheese Ice Cream and Other Frozen Dairy Products Processed Cheese	**Cluster 4**

*(Left vertical axis label: **Decreased Risk per Serving** ↓)*

Figure VII-1. Two-Dimensional Matrix of Food Categories Based on Cluster Analysis of Predicted per Serving and per Annum Relative Rankings
[The matrix was formed by the interception of the four per serving clusters vs. the per annum clusters A and B, C and D, and E. For example, Cluster 3-E (Low Risk) refers to the food categories that are in both Cluster level 3 for the risk per serving and Cluster level E for the risk per annum. See Table VII-1.]

The risk characterization combines the exposure and dose-response models to predict the relative risk of illness attributable to each food category. While the risk characterization must be interpreted in light of both the inherent variability and uncertainty associated with the extent of contamination of ready-to-eat foods with *Listeria monocytogenes* and the ability of the microorganism to cause disease, the results provide a means of comparing the relative risks among the different food categories and population groups considered in the assessment and should prove to be a useful tool in focusing control strategies and ultimately improving public health through effective risk management. As described above, cluster analysis techniques are employed as a means of discussing the food categories within a risk analysis framework. The food categories are divided into five overall risk designations (see Figure VII-1), which are likely to require different approaches to controlling foodborne listeriosis.

Risk Designation Very High. This designation includes two food categories, Deli Meats and Frankfurters, Not Reheated. These are food categories that have high predicted relative risk rankings on both a per serving and per annum basis, reflecting the fact that they have relatively high rates of contamination, support the relative rapid growth of *Listeria monocytogenes* under refrigerated storage, are stored for extended periods, and are consumed extensively. These products have also been directly linked to outbreaks of listeriosis. This risk designation is one that is consistent with the need for immediate attention in relation to the national goal for reducing the incidence of foodborne listeriosis. Likely activities include the development of new control strategies and/or consumer education programs suitable for these products.

Risk Designation High. This designation includes six food categories: High Fat and Other Dairy Products, Pasteurized Fluid Milk, Pâté and Meat Spreads, Soft Unripened Cheeses, Smoked Seafood, and Unpasteurized Fluid Milk. These food categories all have in common the ability to support the growth of *Listeria monocytogenes* during extended refrigerated storage. However, the foods within this risk designation appear to fall into two distinct groups based on their rates of contamination and frequencies of consumption.

- Pâté and Meat Spreads, Smoked Seafood, and Unpasteurized Fluid Milk have relatively high rates of contamination and thus high predicted per serving relative risks. However,

these products are generally consumed only occasionally in small quantities and/or are eaten by a relatively small portion of the population, which lowers the per annum risk. All three products have been associated with outbreaks or sporadic cases, at least internationally.

These foods appear to be priority candidates for new control measures (i.e., Smoked Seafood, Pâté and Meat Spreads) or continued avoidance (i.e., Unpasteurized Fluid Milk).

- High Fat and Other Dairy Products, Pasteurized Fluid Milk, and Soft Unripened Cheeses have low rates of contamination and corresponding relatively low predicted per serving relative risks. However, these products are consumed often by a large percentage of the population, resulting in elevated predicted per annum relative risks. In general, the predicted per annum risk is not matched with an equivalent United States epidemiologic record. However, the low frequency of recontamination of individual servings of these products in combination with their broad consumption makes it likely that these products are primarily associated with sporadic cases and normal case control studies would be unlikely to lead to the identification of an association between these products and cases of listeriosis.

  These products (High Fat and Other Dairy Products, Pasteurized Fluid Milk, and Soft Unripened Cheeses) appear to be priority candidates for advanced epidemiologic and scientific investigations to either confirm the predictions of the risk assessment or identify the factors not captured by the current models that would reduce the predicted relative risk.

Risk Designation Moderate. This risk designation includes nine food categories (Cooked Ready-to-Eat Crustaceans, Deli Salads, Dry/Semi-Dry Fermented Sausages, Frankfurters-Reheated, Fresh Soft Cheese, Fruits, Semi-soft Cheese, Soft Ripened Cheese, and Vegetables) that encompass a range of contamination rates and consumption profiles. A number of these foods include effective bactericidal treatments in their manufacture or preparation (e.g., Cooked Ready-to-Eat Crustaceans, Frankfurters-Reheated, Semi-soft Cheese) or commonly employ

conditions or compounds that inhibit the growth of *Listeria monocytogenes* (e.g., Deli Salads, Dry/Semi-dry Fermented Sausages). The risks associated with these products appear to be primarily associated with product recontamination, which in turn, is dependent on continued, vigilant application of proven control measures.

It is worth noting that two food categories, Fresh Soft Cheese and Soft Ripened Cheese, were previously classified as higher risk products in the draft 2001 version of the risk assessment. This change reflects the acquisition of extensive new exposure data that indicate a significant reduction in contamination rates. The changes in contamination rates, in turn, appear to be the result of increased use of pasteurized or otherwise heat-treated milk, and reflect how relative risk can change as a result of effective food safety control programs.

Risk Designation Low. This risk designation includes two food categories, Preserved Fish and Raw Seafood. Both products have moderate contamination rates but include conditions (e.g., acidification) or consumption characteristics (e.g., short shelf-life) that limit *Listeria monocytogenes* growth and thus limit predicted per serving risks. The products are generally consumed in small quantities by a small portion of the population on an infrequent basis, which results in low predicted per annum relative risks. Exposure data for these products are limited so there is substantial uncertainty in the findings. However, the current results predict that these products, when manufactured consistent with current good manufacturing practices, are not likely to be a major source of foodborne listeriosis.

Risk Designation Very Low. This risk designation includes four food categories: Cultured Milk Products, Hard Cheese, Ice Cream and Other Frozen Dairy Products, and Processed Cheese. These products all have in common the characteristics of being subjected to a bactericidal treatment, having very low contamination rates, and possessing an inherent characteristic that either inactivates *Listeria monocytogenes* (e.g., Cultured Milk Products, Hard Cheese) or prevents its growth (e.g., Ice Cream and Other Frozen Dairy Products, Processed Cheese). This results in a very low predicted per serving relative risks. The predicted per annum relative risks are also low despite the fact that these products are among the more commonly consumed ready-

to-eat products considered by the risk assessment. The results of the risk assessment predict that unless there was a gross error in their manufacture, these products are highly unlikely to be a significant source of foodborne listeriosis.

The following conclusions are provided as an integration of the results derived from the models, the evaluation of the variability and uncertainty underlying the results, and the impact that the various qualitative factors identified in the hazard identification, exposure assessment, and hazard characterization have on the interpretation of the risk assessment.

- The risk assessment reinforces past epidemiological conclusions that foodborne listeriosis is a moderately rare although severe disease. United States consumers are exposed to low to moderate levels of *Listeria monocytogenes* on a regular basis.

- The risk assessment supports the findings of epidemiological investigations of both sporadic illness and outbreaks of listeriosis that certain foods are more likely to be vehicles for *Listeria monocytogenes*.

- Three dose-response models were developed that relate the exposure to different levels of *Listeria monocytogenes* in three age-based subpopulations [i.e., perinatal (fetuses and newborns), elderly, and intermediate-age] with the predicted number of fatalities. These models were used to describe the relationship between levels of *Listeria monocytogenes* ingested and the incidence of listeriosis. The dose of *Listeria monocytogenes* necessary to cause listeriosis depends greatly upon the immune status of the individual.

  1. Susceptible subpopulations (such as the elderly and perinatal) are more likely to contract listeriosis than the general population.

  2. Within the intermediate-age subpopulation group, almost all cases of listeriosis are associated with specific subpopulation groups with increased susceptibility (e.g., individuals with chronic illnesses, individuals taking immunosuppressive medication).

3. The strong association of foodborne listeriosis with specific population groups suggests that strategies targeted to these susceptible population groups, i.e., perinatal (pregnant women), elderly, and susceptible individuals within the intermediate-age group, would result in the greatest reduction in the public health impact of this pathogen.

- The dose-response models developed for this risk assessment considered, for the first time, the range of virulence observed among different isolates of *Listeria monocytogenes*. The dose-response curves suggest that the relative risk of contracting listeriosis from low dose exposures could be less than previously estimated.

- The exposure models and the accompanying 'what-if' scenarios identify five broad factors that affect consumer exposure to *Listeria monocytogenes* at the time of food consumption.

  1. Amounts and frequency of consumption of a ready-to-eat food

  2. Frequency and levels of *Listeria monocytogenes* in a ready-to-eat food

  3. Potential of the food to support growth of *Listeria monocytogenes* during refrigerated storage

  4. Refrigerated storage temperature

  5. Duration of refrigerated storage before consumption

Any of these factors can affect potential exposure to *Listeria monocytogenes* from a food category. These factors are 'additive' in the sense that factors where multiple factors favor high levels of *Listeria monocytogenes* at the time of consumption are typically more likely to be riskier than foods where a single factor is high. These factors also suggest several broad control strategies that could reduce the risk of foodborne listeriosis such as reformulation of products to reduce their ability to support the growth of *Listeria monocytogenes* or encouraging consumers to keep refrigerator temperatures at or below 40 ºF and reduce refrigerated storage times. For example, the 'what-if' scenarios using Deli Meats predicts that consumer education and other

strategies aimed at maintaining home refrigerator temperatures at 40 ºF could substantially reduce the risks associated with this food category. Combining this with pre-retail treatments that decrease the contamination levels in Deli Meats would be expected to reduce the risk even further.

The models generated as the basis for this risk assessment can be used to further evaluate the impact of listeriosis on the public health. For example, the FAO/WHO risk assessment on *Listeria monocytogenes,* which is largely based on the approaches used in the current risk assessment, is being developed to consider several risk management questions posed by Codex Alimentarius. It is anticipated that additional risk assessments on individual foods within specific food categories will be conducted to help answer specific questions about how individual steps in their production and processing impact public health, including the likely effectiveness of different preventive strategies. The models may also be used to evaluate the expected public health impact of preventative controls such as storage limits, sanitation improvements, or new processing technologies. Sources of contamination during food production and retail conditions can also be added to the model to provide more detailed examination of factors contributing to the risk of listeriosis from the final product. For example, the FSIS *Listeria* Risk Assessment in Deli Meats, used portions of the exposure and dose-response models from the current risk assessment to develop information about the effects of combining testing, sanitation, and post-lethality processing interventions to reduce cases of listeriosis.

The models may also be used to evaluate the impact of hypothetical changes in a process such as limits on storage time or temperature to provide insight in how the different components of the model interact. The 'what if' scenarios modeled in this risk assessment provide insight to the impact on public health of limiting storage times, avoiding high temperature refrigeration storage, and reducing contamination levels. Scenario testing emphasizes that the results of any risk assessment are influenced by the assumptions and data sets that were used to develop the exposure assessment and hazard characterization. The results of this revised *Listeria monocytogenes* risk assessment, particularly the predicted relative risk ranking values, could

change as a result of the availability of new information, changes in scientific approaches, or data.

This risk assessment significantly advances our ability to describe our current state of knowledge about this important foodborne pathogen, while simultaneously providing a framework for integrating and evaluating the impact of new scientific knowledge on public health enhancement.

NEW REFERENCES

[The references below are additional, new references that were not cited in the 2001 Draft *Listeria monocytogenes* Risk Assessment. Copies of these references are available in the public docket. FDA Docket No 99N-1168: Food and Drug Administration, Dockets Management Branch (HFA-305), 5630 Fishers Lane, Room 1061, Rockville, MD 20852 and in the FSIS Docket No 00-048N: FSIS Docket Clerk, U.S. Department of Agriculture, Food Safety and Inspection Service, Room 102, Cotton Annex, 300 12th Street, SW., Washington, DC 20250-3700.]

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