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 *L. monocytogenes*, the overall incidence of illness, its severity, and the differential risk to immunocompromised subpopulations are well characterized. In contrast, the relationship between the amount of *L. 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 *L. 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 are available from humans (outbreaks, case reports, case-controlled studies), animals (mice, rats, primates, and other species), and *in vitro* (e.g., tissue culture) studies. Surveillance data were used to describe the magnitude and the incidence of severe disease. Human data from surveillance studies and data from surrogate studies using animals were used to establish the dose-response relationship for the intermediate-age subpopulation.

An adjustment factor was applied to the elderly and perinatal subpopulations to account for increased host susceptibility. This adjustment factor used animal data to establish a susceptibility range, and human epidemiological surveillance data to adjust for increased susceptibility of these subpopulations.

Neonatal deaths result from foodborne infection of a pregnant woman that is then transmitted to the fetus before or during birth. Neonatal deathrates 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 available and had to be estimated based on the reporting of listeriosis infections for the mother. An adjustment is made in the risk characterization section to include all perinatal deaths (both prenatal and neonatal deaths).

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.

Dose-Response Modeling

The primary variables involved in constructing dose-response models for *L. 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 *L. 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 *L. 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 enhance interaction with intestinal mucosa, increasing the likelihood of infection. While *L. 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 *L. 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 *L. 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 *L. 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 *L. 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 *L. 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 (described later in this section) that provided quantitative data.

Comparison to Other Dose-Response Models for *Listeria monocytogenes*

Previously published risk assessments for *L. monocytogenes* included dose-response models (Farber *et al.*, 1996; Buchanan *et al.*, 1997; Haas *et al.*, 1999; Lindquist and Westoo, 2000). All of these efforts share some similarities with the dose-response evaluation used in this 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 present exercise 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 for the current risk assessment modeled mortality as an outcome. The total number of listeriosis cases is estimated from mortality data and was not predicted directly from the dose-response model.

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 *L. monocytogenes*. The dose-response model in this risk assessment used 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 U. S.

Both Farber *et al.* (1996) and this risk assessment generate separate equations for susceptible and non-susceptible groups. Farber *et al.* (1996) employed a Weibull-Gamma model with a different set of parameters, while this risk assessment included a dose adjustment that modified the effective dose used with the primary dose-response model. 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.

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 Risk Assessment (Current)	Mouse, Epidemiology	Lethality and Infection	Multiple	Beta-Poisson Gompertz-Log Gompertz-Power Weibull-Gamma	Explicit, Epidemiology	Explicit, Strain virulence or potency equivalent for mice and humans

Dose-Response in Animal Surrogates

The virulence factors of *L. 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 *L. monocytogenes*. In humans, both food contamination data and fecal carriage studies suggest that exposure to *L. 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 proportion of positive isolates of *L. 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 *L. 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 *L. 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, due to the fact that the surrogates (mice) are uniformly immunologically naïve while the normal human population probably encompasses various degrees of immune sensitization.

In laboratory dose-response studies with mice, two methods of administering *L. 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 *L. 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 *L. 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 perinatal 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 additional surrogates. 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.

Modeling: Dose-Response in Surrogates

The relationship between the number of *L. monocytogenes* consumed and the occurrence of death (mortality) was modeled by using data obtained from mice with a single strain of *L. 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-1), 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 (Appendix 6). The best four models (Beta-Poisson, Exponential, Logistic, and Gompertz-Log) were used to characterize the uncertainty in the shape of the dose-response curve. The exponential model provided the best fit and received the most weight (Figure IV-1).

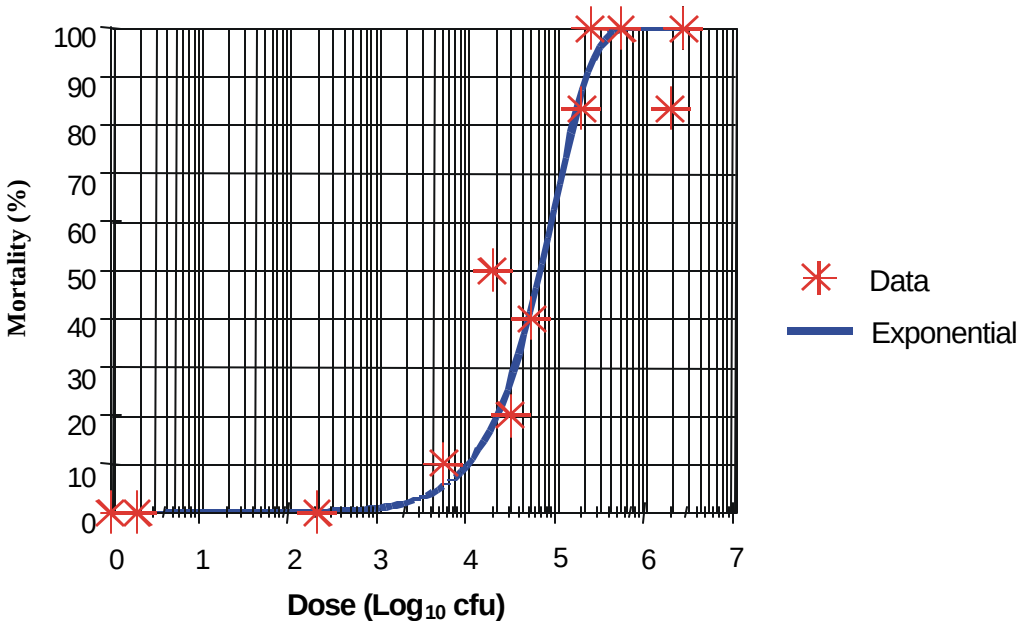

Figure IV-1. *Listeria monocytogenes* Dose vs. Mortality in Mice

Variability in Virulence

Variation in virulence is demonstrable among *L. 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 well investigated, and a large number of virulence components of *L. monocytogenes* have been discovered. Studies on *L. monocytogenes* virulence have, of necessity, been conducted using well-characterized strains of *L. 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 *L. monocytogenes*.

There is also epidemiological evidence for variability in virulence among foodborne isolates of *L. monocytogenes.* Most outbreaks are associated with a restricted number of serotypes such as 1/2a, 1/2b, and 4b. Serotype 4b occurs most frequently (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). The frequency

with which these serotypes are isolated from foods does not parallel the disease distribution. For example, serotype 4b strains are the primary illness producing serotype, but are not the strains most frequently found in foods (Pinner *et al.*, 1992). Contamination of frankfurters by two serotypes of *L. monocytogenes* (1/2a and 4b) resulted in disease associated only with the 4b strain, which was present at apparently much lower concentrations (Mead, 1999). Ribotyping has also been used to identify subtypes of *L. monocytogenes* primarily associated with large outbreaks, sporadic cases, or animal disease (Wiedman *et al.*, 1997).

Animal surrogate studies also show a range of virulence among food isolates of *L. 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, 1991).

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 *L. 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 *L. 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-2 describes the LD_{50} values from three studies in which *L. 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-2. 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-3 presents the results of a study by Pine *et al.* (1990) in which *L. 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-3. 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-2 were modeled by fitting nine distributions with ParamFit (see Appendix 6). Figure IV-2 displays all nine distributions. The best five models were used to characterize the dose-response model uncertainty associated with the distribution. Output from the resulting function is given in Table IV-4. This output was used to describe 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}.

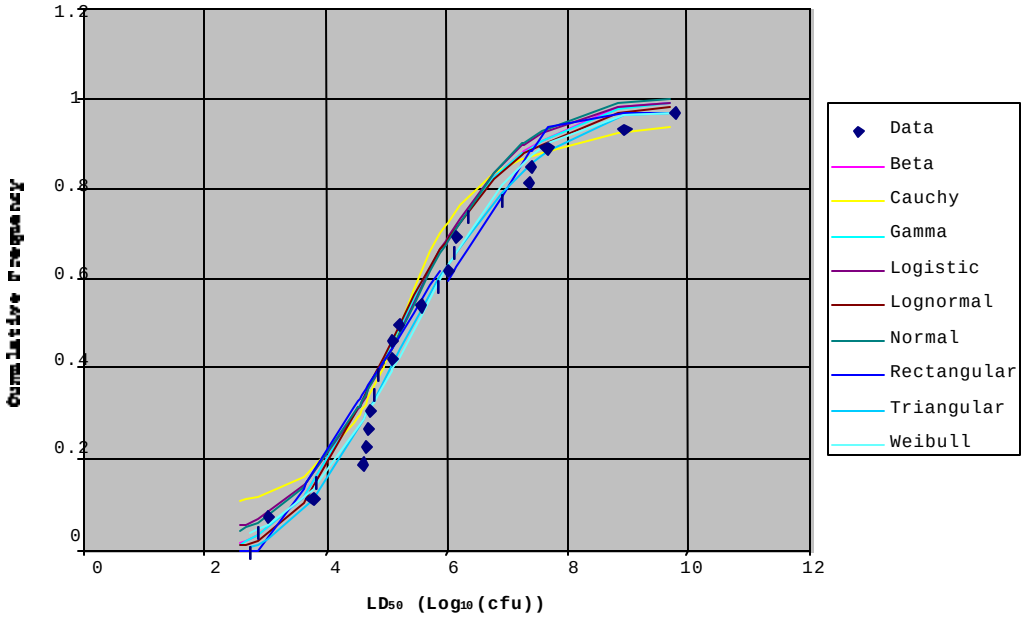

Figure IV-2. Variation in *Listeria monocytogenes* Strain Virulence: Nine Distributions

Table IV-4. Model Output for *Listeria monocytogenes* Strain Virulence

Percentile	Median LD_{50} Log_{10}(cfu)[a]
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] The confidence intervals for the 5th and the 95th percentiles of the uncertainty distribution are given in parentheses. LD_{50} (50% of the lethal dose)

Dose-Response Adjustment Factor

Dose-response modeling is a complex process and involves numerous parameters. Some parameters, like 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 *L. 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 adjustment factor, was used to account for these factors, 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 *L. 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 exposure data indicate that exposure to this number of *L. 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 *L. 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 *L. monocytogenes* that may serve to develop immunity to challenges with larger numbers.
- Route of exposure: The *L. monocytogenes* dosing in the animal studies was not introduced by the dietary consumption route. The consumption of *L. 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 *L. monocytogenes*-contaminated food may vary depending on fat content or other factors.
- Exposure: A gross overestimate of the occurrence and growth from the exposure calculations would overestimate the risk.

Since there are currently no available quantitative data related to *L. monocytogenes* for the factors listed above, a dose-response adjustment 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 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. Table IV-5a shows that, because of differences in the behavior of the dose-response model distributions at low doses for the intermediate-age subpopulation, the magnitude of the adjustment factor was model-dependent. Table IV-5b provides the range of dose-response adjustment factors for each of the three subpopulations.

Table IV-5a. Model-Dependence of the *Listeria monocytogenes* Dose-Response Adjustment Factor for the Intermediate-Age Subpopulation

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

Table IV-5b. Model-Dependence of the *Listeria monocytogenes* Dose-Response Adjustment Factor Ranges for the Three Subpopulations

Subpopulation	Dose-Response Adjustment Factor Range (Log_{10} cfu)	
	Minimum	Maximum
Intermediate-Age	11.85	12.45
Neonatal[a]	7.8	8.4
Elderly	1.85	11.45

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

This single dose-response adjustment 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 adjustment 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 *L. monocytogenes* contamination levels, growth rates, strain virulence, host susceptibility, or the annual number of reported cases would affect the magnitude of the adjustment factor. A demonstration of this effect can be found in the hazard characterization section entitled Modeling: Outbreak Data.

Results: Intermediate-Age Dose-Response Curve

After applying the virulence distribution (Table IV-2) to the normal mouse dose-response mortality curve (Figure IV-1), the dose-response adjustment factor is used to shift the curve towards higher doses necessary for lethality estimates similar to surveillance data. Figure IV-3 depicts the results of applying this factor to the intermediate-age subpopulation. It describes the dose (in colony forming units) required to produce death from a series of servings contaminated with different (or variable) *L. 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 and uncertainty in the animal data used to characterize those strains; 2) uncertainty in the primary mouse model curve; and 3) uncertainty in the dose-response adjustment factor.

An example of how the dose-response curve relates exposure to public health impact can be examining an example using Figure IV-3. 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 x 10^{10} cfu/serving, the dose-response model predicts a median death rate of 1 in 100,000 servings. The uncertainty results in a lower bound prediction of 1 death in 260 billion servings and an upper bound prediction of 1 in approximately 11,000 servings. Similar predictions can be made for any dose.

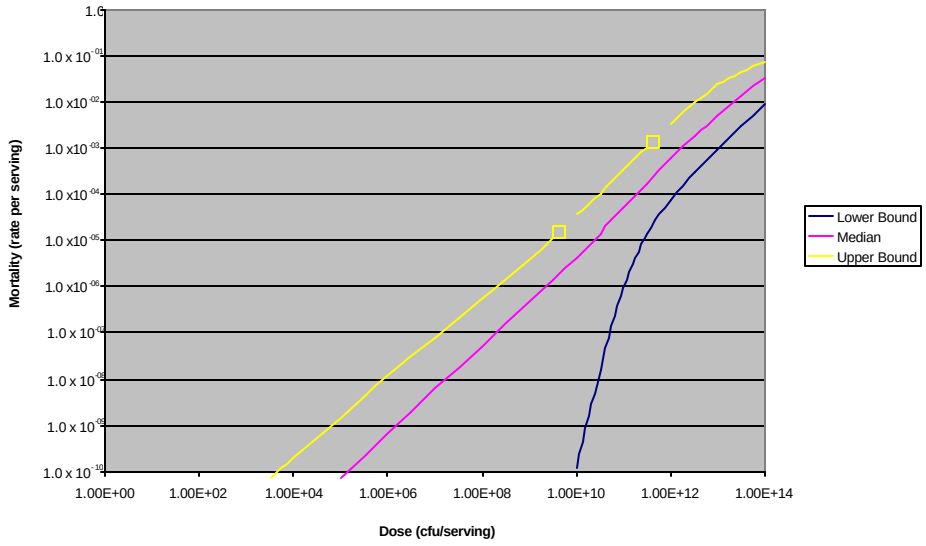

Figure IV-3. *Listeria monocytogenes* Dose-Response with Variable Strain Virulence for the Intermediate-Age Subpopulation

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 *L. 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 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 *L. monocytogenes* animal model was characterized with respect to the role of many specific immune defects. Host resistance mechanisms to *L. 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 *L. monocytogenes* (Czuprynski and Brown, 1986; Cheers and McKenzie, 1978, Unanue, 1997a*)*.

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 *L. 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 *L. 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 *L. monocytogenes*. The mouse study showed no significant effect with drug treatment (Golnazarian *et al.*, 1989), while the rat study showed increased infectivity of *L. 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.

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-4 shows listeriosis incidence by age using 1997 FoodNet data (CDC, 1998a). 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 U. S. 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 *L.*

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 3.

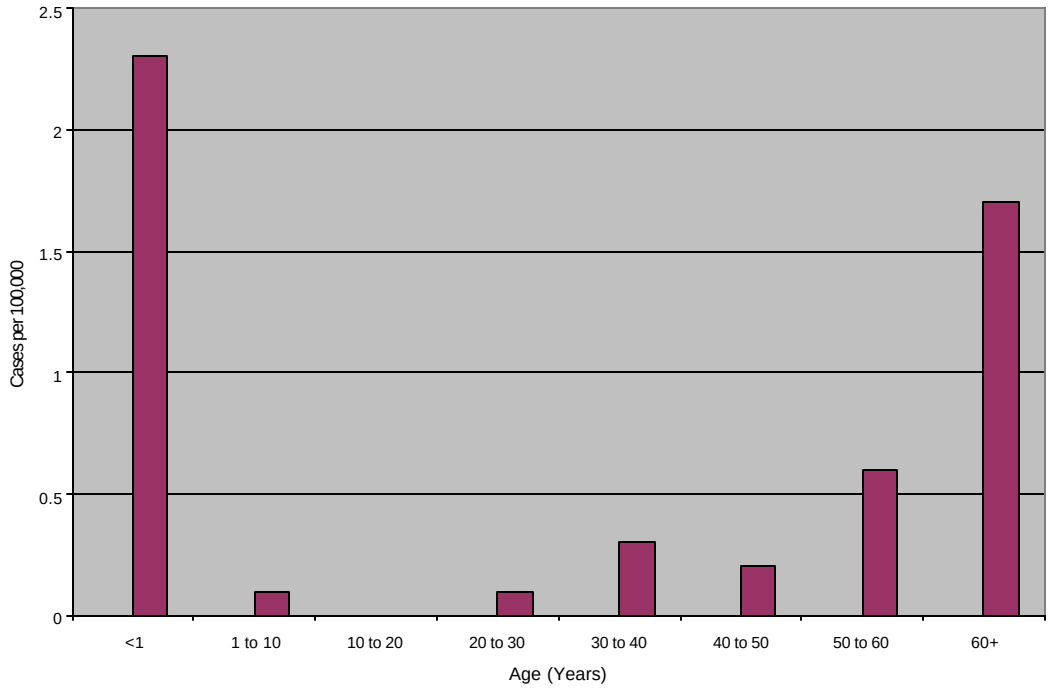

Figure IV-4. 1997 FoodNet Estimates of Listeriosis Incidence, by Age

Table IV-6 shows the number of listeriosis isolates by age and the total number of *L. monocytogenes* isolates per year from FoodNet, 1997-1999 (CDC, 1998a, 1999a, 2000; Wong, 2000). In 1997, FoodNet surveillance identified 77 listeriosis cases from five states. Of these cases, five (6%) were considered perinatal cases (aged 30 days or less) and 42 (55%) were 60 years of age or older. Hospitalization occurred in 68 (88%) of cases. Of the 75 cases with known outcome, 15 (20%) died. In 1998, FoodNet surveillance was expanded to eight states, in which 120 *L. monocytogenes* cases were reported. Ten cases (8%) were perinatal cases (aged 30 days of less) and 64 (53%) were patients aged 60 years or older. Hospitalization occurred in 91(76%) of cases and 14 (12%) died. Summarization of the number of perinatal isolates, as well as resultant hospitalizations and deaths, has not been completed for the 1999 data.

Table IV-6. 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]
<30 days old	5	10	12[d]
1 to 9 years old	2	1	3
10 to 19 years old	1	2	1
20 to 29 years old	3	6	5
30 to 39 years old	9	13	7
40 to 49 years old	6	6	8
50 to 59 years old	9	13	16
≥60 years old	42	61	48
Unknown age	0	0	14
Total	77	112	114

[a] CDC, 1998a (from five states).
[b] CDC, 1999a (from seven states).
[c] CDC, 2000 (from seven states) and Wong, 2000 (Unpublished data).
[d] For patient age less than 1 year.

Modeling: Dose-Response in Susceptible Subpopulations

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 for the three distributions were 1, 2.5, and 3.5 $\log_{10}$ cfu for the Low, Medium, and High Variability populations, 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 on Modeling: Dose-Response in Surrogates). In conjunction with a population-specific dose-response adjustment 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 highly immune individuals, 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 adjustment 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 (Log_{10}(cfu))	Medium Variability Adjustment (Log_{10}(cfu))	High Variability Adjustment (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 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. Since the susceptibility of the elderly or immunocompromised individual may be a matter of degree, wider ranges are assigned to these groups. The perinatal 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.

Results: Perinatal Dose-Response Curve

Figure IV-5 depicts the neonatal subpopulation dose-response curve. It describes the dose (in colony forming units) required to produce death from a series of servings, consumed maternally, that are contaminated with different (or variable) *L. monocytogenes* strains. The distribution (indicated by the lower and upper bound lines) accounts for the uncertainty from four primary sources: 1) the variability in the virulence of different strains and the uncertainty in the animal data used to characterize those strains; 2) the variability in animal susceptibility and the uncertainty in the animal data; 3) the variability and uncertainty in the primary mouse model curve; and 4) the uncertainty in the dose-response adjustment factor.

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 77 servings. However, the uncertainty introduced by the variability in virulence and in host susceptibility provides a lower bound prediction of 1 death in 370 servings and an upper bound prediction of 1 death in approximately 23 servings. Similar predictions can be made for any dose.

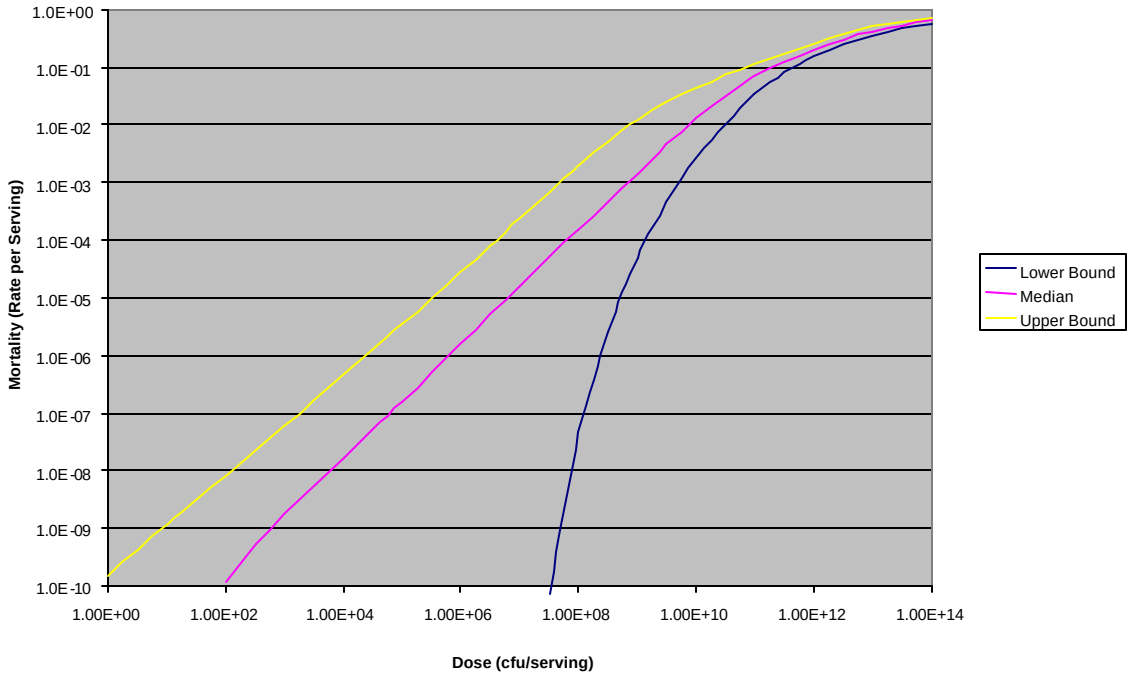

Figure IV-5. *Listeria monocytogenes* Dose-Response with Variable Strain Virulence for the Neonatal Subpopulation

Data reported to FoodNet are the only national data available for estimating cases of perinatal infection and death. However, FoodNet surveillance data are affected by ambiguities in the methods used to characterize prenatal infection resulting in premature termination of pregnancy. Reporting procedures may have resulted in positive cultures from prenatal infection being assigned to groups other than the perinatal group (*e. g*., the age group of the mother, or the unknown age group). This would result in an underestimate of the number of deaths associated with prenatal infection, because only those infections resulting in a live birth would be reported. To compensate for this potential underreporting, data from the

California State 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 *L. monocytogenes* isolates from clinical laboratories indicating that the combined prenatal and neonatal deaths were 2.5 times the neonatal deaths (Buchholz, 2000). The final risk characterization describes the perinatal deaths as both prenatal and neonatal.

Results: Elderly Dose-Response Curve

Figure IV-6 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) *L. monocytogenes* strains. The lower and upper lines delineate the uncertainty bounds from four primary sources: the variation in the virulence of different strains and the uncertainty in the animal data used to characterize those strains; the variability in animal susceptibility and the uncertainty in the animal data; the variability and uncertainty in the primary mouse model curve; and the uncertainty in the dose-response adjustment factor.

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 27,000 servings. However, the uncertainty introduced by the variability in virulence and in host susceptibility provides a lower bound prediction of 1 death in 2 million servings and an upper bound prediction of 1 death in approximately 4,300 servings.

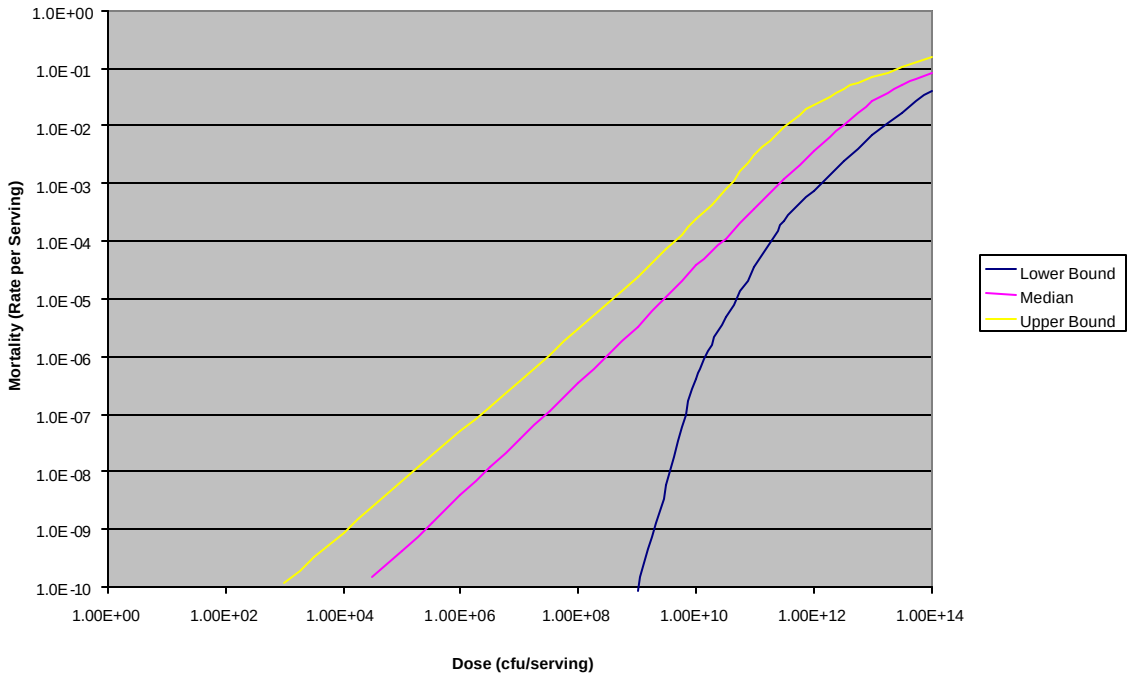

Figure IV-6. *Listeria monocytogenes* Dose-Response with Variable Strain Virulence for the Elderly

Table IV-9 provides a summary of the data presented in the preceding figures for the general, perinatal, and elderly subpopulations. Dose is described in colony forming units per serving. The death rate per serving is presented as the median and the upper and lower boundaries of the uncertainty. The data in Table IV-9 show a general decrease in the dose necessary to cause listeriosis for the perinatal and elderly subpopulations.

Table IV-9. Dose-Response with Variable *Listeria monocytogenes* Strain Virulence for Three Age-Based Subpopulations

Median Mortality Rate per Serving[a]			
Dose (cfu/serving)	**Intermediate-Age**	**Neonatal[b]**	**Elderly**
1	$1.6x10^{-15}$ ($1.9x10^{-134}$, $2.7x10^{-13}$)	$1.7x10^{-15}$ ($7.5x10^{-84}$, $1.5x10^{-10}$)	$6.2x10^{-15}$ ($4.3x10^{-112}$, $3.9x10^{-13}$)
10^3	$1.6x10^{-12}$ ($3.9x10^{-83}$, $7.9x10^{-11}$)	$1.7x10^{-9}$ ($1.3x10^{-44}$, $6.2x10^{-8}$)	$5.2x10^{-12}$ ($2.2x10^{-65}$, $1.2x10^{-10}$)
10^6	$1.3x10^{-9}$ ($1.2x10^{-43}$, $2.9x10^{-8}$)	$1.6x10^{-6}$ ($2.1x10^{-18}$, $2.8x10^{-5}$)	$4.0x10^{-9}$ ($8.6x10^{-32}$, $5.0x10^{-8}$)
10^9	$1.0x10^{-6}$ ($7.1x10^{-18}$, $1.2x10^{-5}$)	$1.4x10^{-3}$ ($5.0x10^{-5}$, $1.3x10^{-2}$)	$3.3x10^{-6}$ ($3.6x10^{-11}$, $2.3x10^{-5}$)
10^{12}	$1.1x10^{-3}$ ($3.5x10^{-5}$, $5.7x10^{-3}$)	$2.0x10^{-1}$ ($1.5x10^{-1}$, $2.6x10^{-1}$)	$3.6x10^{-3}$ ($7.8x10^{-4}$, $2.3x10^{-2}$)

[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.

Dose-Response Curves for Infection and Serious Illness

Dose-response curves for infection and serious illness in humans were not included in the Hazard Characterization section of the risk assessment. However, mouse data were used to develop a dose-response curve for infection (Figure IV-7).

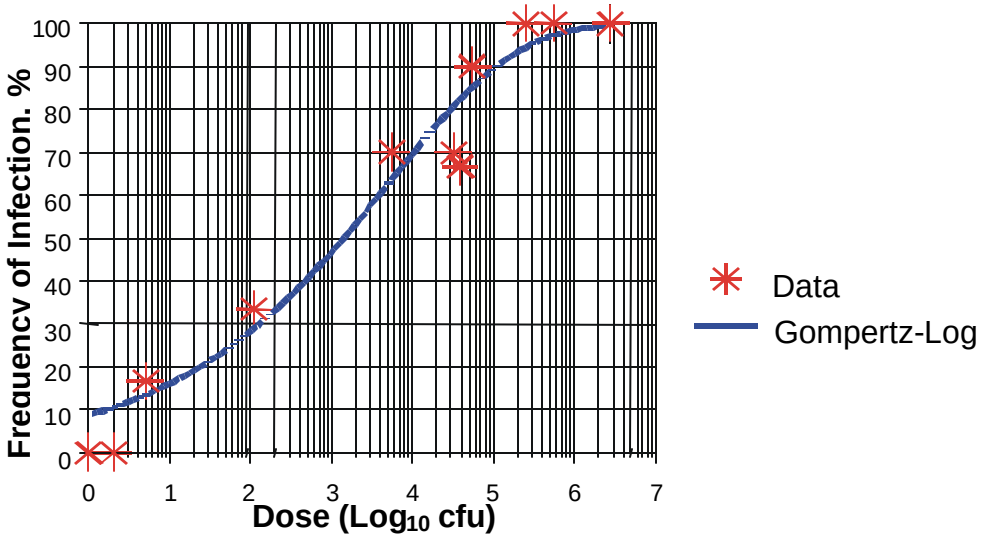

Figure IV-7. Dose vs. Frequency of Infection in Mice

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, which came from two different experiments, were fit with six different models using the Dose Frequency curve-fitting procedure. (See Appendix 6 for information about this procedure and more details about

modeling and software.) The best five models (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-7). The shape of the curve for infection is very shallow and rises gradually, whereas the curve for lethality (Figure IV-1) 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 *L. 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 *L. 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.

The FoodNet data estimated the numbers of serious illness to be five times the number of deaths. This factor is used to estimate the number of serious illnesses (including deaths) in the Risk Characterization section, because it more accurately reflects the total number of food borne listeriosis cases. 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 group, but not the relative risk ranking of the food groups. 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 infection dose response curve in mice is much less than that for mortality so that the factor 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 mortality and serious illness across the entire dose range.

The Dose-Response Model as a Predictive Tool

This model can be used to make predictions about the public health impact of *L. monocytogenes* isolated from a food source. For example, in the best case, a clinical isolate may be traced to the source food. If the source of the food is known or suspected, the number of servings of the source food that are likely to be consumed can be estimated. If the isolate strain can or has been tested in a manner consistent with the LD_{50} mouse experiment, the virulence potential can be established. With these inputs, the dose-response model can predict, with minimal uncertainty, the median number of deaths for different populations. If not all of this information is available, the dose-response model still can predict the probability of death for different populations and different consumption scenarios, and provides an estimate of the uncertainty for that prediction.

Outbreak Investigations in Quantitative Risk Assessment

There have been many foodborne listeriosis outbreaks and reports of sporadic individual cases in the past 15 years, with a variety of foods implicated as vehicles of infection. Those outbreak and individual case reports that identify the food vehicle for infection have been very helpful to the hazard identification phase of the *L. monocytogenes* risk assessment. However, identification of the food source of *L. monocytogenes* contamination is infrequent.

To be informative for quantitative risk assessment, outbreak reports must include quantitative exposure data linked to individuals. Unfortunately, outbreak reports with information about the dose and attack rate are rare. Many reports have provided information about the food source, serotype, and contamination in food, but the amount of food consumed by each person and the number of people exposed is usually not reported. For example, an outbreak of listeriosis occurred in Massachusetts in 1983 involving 49 cases (Fleming *et al.*, 1985). Illness was strongly associated with the consumption of pasteurized milk. The investigation revealed attack rate, food source, and serotype data, but information was not provided about the level of contamination and amount of milk consumed.

Tables IV-10 and IV-11 list examples of outbreak and sporadic case reports where data on contamination level in the implicated food were reported. These tables also provide the

location and year of occurrence, the implicated food source(s), *L. monocytogenes* serotype, level of contamination of implicated food(s), amount of food consumed, number of persons affected, and the attack rate. Of the outbreaks listed in Table IV-10, only the report of the outbreak in 1994 associated with chocolate milk contained the critical details necessary to estimate the dose-response relationship. However, the primary endpoint of this outbreak (gastrointestinal illness) makes it of minimal usefulness in characterizing outbreaks of severe listeriosis. None of the other reports contained information on the amount of either the food or *L. monocytogenes* consumed per serving by individuals or the attack rate. However in two cases, the Mexican-style soft cheese and the Finnish butter outbreaks, an attack rate and dose range could be estimated. The Mexican-style soft cheese would be similar to the Fresh Soft Cheese food category used in this risk assessment. Butter is one of the foods included in the Miscellaneous Dairy Products category.

Table IV-10. Location, Year of Occurrence, and Epidemiologic Data for Outbreaks of Listeriosis

Location, Year (Reference)	Food Source	Serotype	Contamination Level (cfu/g)	Amount Consumed	No. Ill	Attack Rate
LA County, 1985 (Linnan *et al.*, 1988)	Mexican-style soft cheese	82% 4b	$1.4x10^4$ to $5x10^4$	NA [a]	142	NA
Switzerland, 1983-87 (Bula *et al.*, 1995)	Soft smear-ripened cheese	75% 4b	$1x10^4$ to $1x10^6$	NA	122	NA
IL, MO, WI, 1994 (Dalton *et al.*, 1997)	Chocolate milk	1/2b	$1x10^9$ (cfu/mL)	240 mL	45	45/60 (median)
Italy, 1993 (Salamina *et al.*, 1996)	Cream cheese fruit tart Rice Salad [b]	1/2b 1/2b NA	460 0.93 NA	NA NA NA	18	18/39
Finland, 1998-99 (Lyytikäinen *et al.*, 2000)	Butter	3a	<100 [c]	NA	25	NA
Multistate, 1998-99 (CDC, 1998b)	Frankfurters	4b	<0.3	NA	101	NA

[a] NA = Not available
[b] Rice salad implicated by epidemiology; $p<0.001$
[c] One sample contained 11,400 cfu/g

Table IV-11. Location, Year of Occurrence, and Epidemiologic Data for Sporadic Cases of Listeriosis not Reported in Outbreaks

Location (Reference)	Year	Confirmed Food Source	Serotype	Contamination Level (cfu/g)	Amount Consumed	Number Ill	Attack Rate
England (Azadian *et al.*, 1989)	1988	Goat Cheese	4b	4×10^7	85 g	1	NA [a]
Oklahoma (Barnes *et al.*, 1989)	1988	Frankfurter Sausage	NA	>1100[b]	1 per day	1	NA
Italy (Cantoni, 1989)	1989	Homemade Sausage	NA	2.7×10^6	NA	1	NA
New Jersey (Ryser, 1999b)	NA	Ricotta Cheese	NA	100 to 10^6	NA	1	NA
Finland (Juntilla and Brander, 1989)	1989	Salted Mushrooms	4b	1×10^6	NA	1	NA
Belgium (Andre *et al.*, 1990)	1989-90	Commercial Ice Cream	4b	1×10^4	NA	1	NA
Tasmania (Brett *et al.*, 1998)	1991	Smoked Mussels	NA	1.6×10^7	90 g	2	NA
Canada (Farber, 1997)	1997	Imitation Crabmeat	NA	2.1×10^9	NA	2	NA

[a] NA= Not Available
[b] >1100 cfu/g, in opened package, patient refrigerators; <0.3 cfu/g in closed package, retail.

Estimating Dose-Response for Pregnant Females: 1985 Los Angeles Mexican-Style Soft Cheese Outbreak

Between January 1 and August 15, 1985, 142 cases of listeriosis were reported in Los Angeles County. There were 48 deaths (including 19 fetal and 10 neonatal). The overall case fatality rate was 33%. Of the 142 cases, 93 (65%) were perinatal. The mean age of mothers was 26 years and the mean gestational age of fetus or neonate was 32 weeks. Eighty-six percent of the cases were Hispanic individuals. Of the remaining non-perinatal cases, the mean age was 58 years and only 29% were Hispanic individuals. Mexican-style soft cheese was epidemiologically and bacteriologically associated with the occurrence of disease.

At the time the Los Angeles County Department of Public Health reported on the outbreak, their report did not contain information on either the amount of cheese consumed per serving by individuals or the attack rate. Fortunately, consumption data by individuals were collected and records from the outbreak were saved. Therefore, in 1998, an attack rate was estimated (Buchholz, 2000) based upon information in the outbreak records and Los Angeles County demographics. Table IV-12 shows the calculation of the attack rate for the listeriosis outbreak among pregnant Hispanic females in Los Angeles County in 1985 associated with consumption of Mexican-style soft cheese.

There were a total of 81 listeriosis cases among pregnant Hispanic females. Only cases infected with the outbreak phage type were used in the analysis (63 of 142). Two matched case-control studies (n=39) were conducted during the outbreak (Linnan *et al.*, 1988). The total number of controls (31) and the number of controls that were exposed to the implicated food (11) were determined by re-construction of the odds ratio table. This allowed for an estimate of the proportion of the population that consumed the implicated food (11/31=35%).

To estimate the dose-response for pregnant females, it was assumed that the same percentage of pregnant Hispanic females as in the case-control studies ate the implicated cheese. The total number of pregnant Hispanic females within the marketing area during the time interval of interest (33,642) was multiplied by the calculated proportion of the population that consumed the implicated food (35%), providing an estimate of the number of pregnant Hispanic women who ate the implicated food (11,775).

Two studies were used to determine the Mexican-style soft cheese contamination rate. Laboratory data from one study were examined to determine the total number of food samples qualitatively tested (85) and the number of samples that were positive (22) outbreak (Linnan *et al.*, 1988). The number of positive tests divided by the number of foods sampled yielded an estimate of the proportion of food contaminated (22/85 = 26%). That proportion multiplied by the estimate of the number of pregnant women who ate the implicated food (11,775), provides one estimate of the total number of pregnant women who were exposed to *L. monocytogenes* (3,061).

A second estimate of the proportion of food contaminated was determined based on the second contamination study (Ryser, 1999a). Samples (665) were tested, with 56 positive results, to give an estimated contamination frequency of 8.4%. It should be noted that these outbreak related cheese samples were recovered from a landfill after disposal, which had an unknown impact on the results. The low estimate of pregnant Hispanic women who ate contaminated cheese (989) was derived by multiplying this contamination rate (8.4%) by the total number of Hispanic females who ate the implicated cheese (11,775).

For the high estimate, the estimated attack rate (2.1%) is equal to the number of cases that developed listeriosis from the outbreak phage type (63) divided by the high estimate of the total number of pregnant Hispanic females who were exposed (3,061). The proportion of actual cases that were identified during the outbreak was then estimated based on 100% of cases identified (best case scenario) and 75% of cases identified (based on estimates from health care workers in Los Angeles at the time of the outbreak).

For the low estimate, the estimated attack rate (6.4%) is equal to the number of cases that were infected with the outbreak phage type (63) divided by the low estimate of the total number of exposed persons in the population (989). The proportion of actual cases that were identified during the outbreak was again estimated based on 75% and 100% of cases identified.

Using this strategy, the estimated attack rate during the Mexican-style soft cheese outbreak ranges between a low of 2.1-2.7% and a high of 6.4 to 8.5%.

From the outbreak records, it was possible to estimate the one-day consumption of the implicated cheese from 39 of 63 pregnant Hispanic females infected. The consumption ranged from 0.5 ounces/day to 21 ounces/day (median about 5.5 ounces/day). In addition to reporting consumption for one day, about 38% of the females reported their usual consumption of cheese for more than one day. The effect of cumulative doses on the attack rate and pathogenesis is not well understood and was not estimated.

L. monocytogenes contamination levels in this outbreak were reported twice, at 1,000 to 10,000 cfu/g (NACMCF, 1991) and 14,000 to 50,000 cfu/g (Ryser, 1999a). The dose of *L.*

monocytogenes consumed in the contaminated cheese in one day was calculated to range between 15,000 cfu/day (0.5 oz/day x 30 g/oz x 1,000 cfu/g) and 31,500,000 cfu/day (21 oz/day x 30 g/oz x 50,000 cfu/g). It was therefore estimated that approximately 2.1%-8.5% of pregnant Hispanic females who consumed between 1.5×10^4 and 3.15×10^7 *L. monocytogenes* 4b organisms in a single day became ill.

Table IV-12. Calculation of Attack Rate for an Outbreak of *Listeria monocytogenes* 4b in Pregnant Hispanic Females Using Data from the 1985 Mexican-style Soft Cheese Outbreak

Hispanic births (January - June, 1985), LA County	33628
Hispanic fetal and neonatal deaths (January - June, 1985)	+ 350
Proportion of multigestational births (1%)	- 336
Population giving rise to cases (Total Hispanic pregnant females, January - June, 1985)	33642
Total Hispanic pregnant females who ate the implicated cheese (based on an estimate that 35% of controls ate implicated cheese)	11775
High estimate of Hispanic pregnant females who ate contaminated cheese (based on a estimate of 26% product contamination x 11775)	3061
Total Listeriosis cases among Hispanic pregnant females	81
Cases with outbreak phage type	63
Attack rate if all cases were identified (63 x 100 /3061)	2.1%
Attack rate if 75% of cases identified (63 x 100/3061)/0.75	2.7%
Low estimate of Hispanic pregnant females who ate contaminated cheese (based on an estimated rate of 8.4% product contamination x 11775)	989
Attack rate if all cases were identified (63 x 100 /989)	6.4%
Attack rate if 75% of cases identified (63 x 100/989)/0.75	8.5%

Modeling: 1985 Mexican-style Soft Cheese Outbreak

A dose-response model of this outbreak was developed using the same structure as the dose-response model for the national estimates (Figure IV-8). However, two of the components of the outbreak dose-response model were modified to reflect specific information from the outbreak.

First, a distribution ranging from 10^2 to 10^4 was used as an estimate of the *L. monocytogenes* concentration in the contaminated cheese. Because the cheese samples were obtained from consumer refrigerators, it is reasonable to assume that the measurements producing these estimates included growth during storage. Therefore, no additional growth was modeled.

Second, the strain virulence model was modified to include only mouse LD_{50} values for the single strain associated with the outbreak. Attribution of all the cases to a single strain implies that strain virulence is no longer a source of variability in the cause of illness. The frequency distribution in the national model is therefore replaced by a single uncertain value. Since mouse LD_{50} values are available, the uncertainty in the virulence is much less than it otherwise would be (a range of one log rather than eight). A normal distribution of the three LD_{50} values was used to represent the uncertainty; since the doses were measured in logs and the distribution is essentially Lognormal.

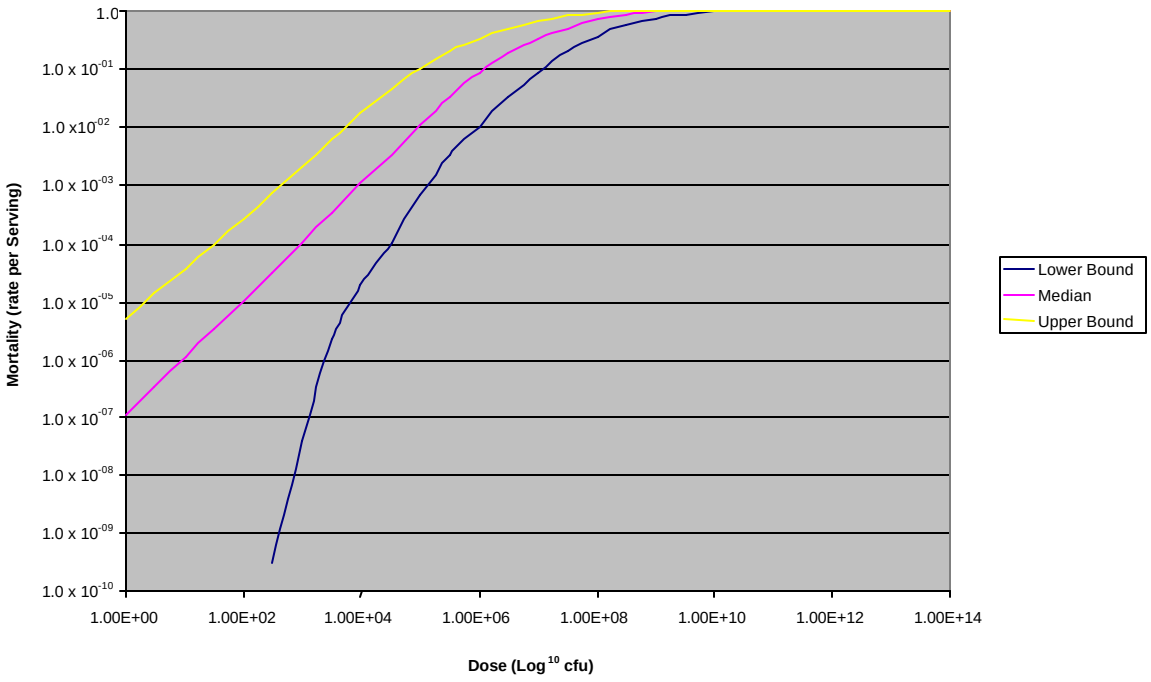

Figure IV-8. *Listeria monocytogenes* Dose-Response with Single Strain Virulence Derived from 1985 Los Angeles Mexican-style Soft Cheese Outbreak

Without a dose-response adjustment factor, the Los Angeles model also overestimated the number of cases expected in the epidemic. However, a much smaller adjustment factor (3.5

to 4.5 logs) was required to produce an estimate that roughly corresponded to the observed number of cases than was required for the national model for perinatal (10 to 12 logs). Since the main difference between the national and Los Angeles outbreak models are the estimates of *L. monocytogenes* concentrations and strain virulence, it appears that a major portion of the uncertainty described by the dose-response adjustment factor may be attributed to these two model components. More specifically, the national estimates for *L. monocytogenes* concentrations are too high or the Los Angeles estimates are low, and/or the number of low-virulence *L. monocytogenes* strains may be underrepresented in the frequency distributions for strain virulence. The Los Angeles model, based on our limited data, primarily provides assurance that the population-based model can be useful as a predictive tool that is reasonably accurate (within a several log range) when *L. monocytogenes* concentrations and strain virulence information are known. The dose response model using the 1985 Mexican-style soft cheese outbreak represents an application of the model to a specific set of circumstances. The data were not used in this *L. monocytogenes* risk assessment.

Estimating Dose-Response for the Immunocompromised: 1999 Finland Pasteurized Butter Outbreak

The Finland pasteurized butter outbreak attack rate and dose was calculated in a manner similar to the method used to calculate these parameters for the Mexican-style soft cheese outbreak. Between December 1998 and February 1999, an increase in cases of listeriosis due to *L. monocytogenes* serotype 3a in Finland was recognized (Lyytikäinen *et al.*, 2000). Review of national laboratory surveillance data from June 1, 1998 to March 31, 1999 identified 25 *L. monocytogenes* 3a cases, which included six deaths. Cultures of blood, cerebrospinal fluid, and samples from other sterile sites were used to identify cases of listeriosis. Most of the cases were hematological or organ transplant patients. The median age of cases was 53 years (range 12-85). Ten males and no pregnant females or newborns were identified as listeriosis cases. The average annual number of hematological and organ transplant patients admitted to the hospital is 410. Therefore, the number of persons at risk for the 9-month period of concern was approximately 308.

Butter was implicated as the vehicle of infection. Isolates of *L. monocytogenes* 3a from the butter and from the 25 cases were indistinguishable by PFGE. At the tertiary care hospital where most cases (15/25) occurred, only one brand of butter was consumed during the

outbreak period. The hospital is the only site in Finland for organ transplants and is where most bone marrow transplants are performed.

Thirteen butter samples were obtained from the hospital kitchen and 139 butter samples were obtained from the dairy and a wholesale store, and were then analyzed for the presence of *L. monocytogenes*. The outbreak strain was detected in all thirteen hospital-kitchen samples. The outbreak strain was also detected in several lots from the dairy and wholesale store. The level of *L. monocytogenes* contamination was <100 cfu/g (range 5 to 60 cfu/g) for all positive butter samples, except for one sample that contained 11,000 cfu/g. A complete description of the environmental investigation is described in Lyytikäinen *et al.* (2000).

It was possible to estimate butter consumption for five case patients based on interviews about dietary practices (number of times per week and per day). Researchers assumed that patients ate one package of butter per meal (7 g). The estimated consumption was divided by 31 days (median hospital stay) to estimate daily butter consumption. To determine the median dose range, the minimum butter consumption (1.1 g/day) was multiplied by the minimum contamination level for the hospital kitchen samples (5 cfu/g) and the maximum butter consumption (55 g/day) was multiplied by the maximum contamination level for the hospital samples (60 cfu/g). Using the hospital samples, the consumed dose would be 5.5 to 3,300 cfu/day. By using the maximum contamination level found in the wholesale samples (11,000 cfu/g), then the daily dose consumed would range between 1.21×10^{4} to 6.6×10^{5} cfu/day.

Table IV-13 shows the calculation for the attack rate of the 1999 Finland butter outbreak. Approximately 6.4% to 10.7% of the hematological/transplant patients at the hospital who consumed between 5.5 cfu/g and 6.6×10^{5} cfu/g developed listeriosis. We assumed that hospitalized patients ate the implicated butter on each of 31 days (median hospital stay) while hospitalized. The majority of the illnesses were associated with severe symptoms. The effect of cumulative doses on the attack rate and pathogenesis was not estimated.

Table IV-13. Calculation of Attack Rate for an Outbreak of *Listeria monocytogenes* Serotype 3a Infections from Butter in Finland for Hematological and Transplant Patients

Annual number of new diagnoses for acute leukemias/lymphomas plus annual number of kidney/liver transplants performed at the hospital.	410
Total persons at risk (time interval x annual new diagnoses, time interval: June 1998 to February 1999, 9/12 months)	308
Estimated number of hematological and transplant patients in the population that ate the butter (estimated proportion of controls that ate implicated butter, 76%)	234
Number of cases during the outbreak	25
Number of cases at tertiary care hospital	15
High estimate of product contamination (100%)	
Population at risk (100%)	234
Attack rate (15 x 100/234)[1]	6.4%
Mid-estimate of product contamination (80%)	
Population at risk 80%	187
Attack rate (15 x 100/187)	8.0%
Low estimate of product contamination (60%)	
Population at risk 60%	140
Attack rate (15 x 100/140)	10.7%

The Finland outbreak demonstrates the serious consequences of even low dose exposure when the severely immunocompromised are exposed. Because this outbreak currently lacks strain information and the relative susceptibility of transplant patients is unknown, it is not possible to calculate a new dose-response curve for this population. However, intensive outbreak investigations such as this one are applauded. They help to explain the sources of uncertainty in our dose-response model and they provide controlled environments in which accurate dose and attack rate data may be collected.

Buchanan and Lindqvist (2000) used the same two outbreak data sets in conjunction with the single parameter exponential model to develop dose-response models. With this simple model, the doses that would lead to half the two populations associated with the cheese and butter outbreaks acquiring severe listeriosis was estimated to be1.9×10^6 cfu and 6.8×10^4 cfu, respectively. However, confidence intervals around these estimates were not provided.