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.

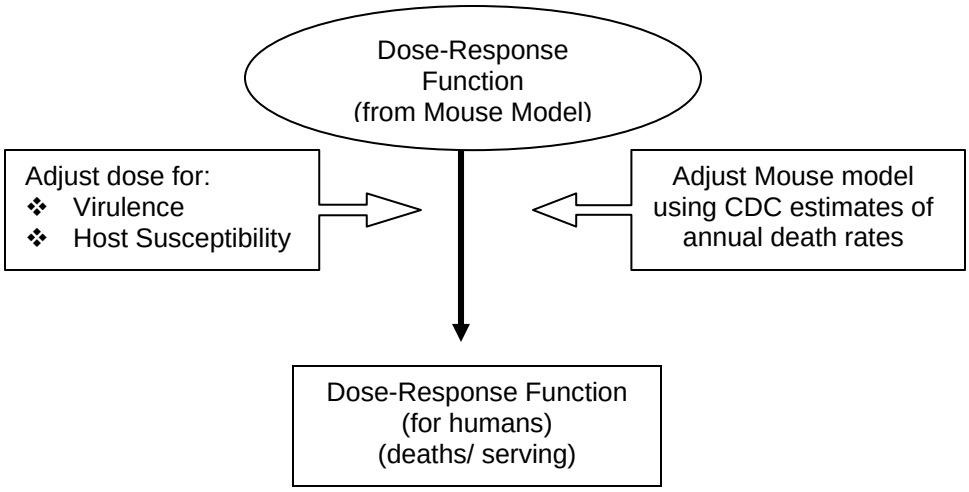

Figure IV-1. Components of the Dose-Response Model

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).

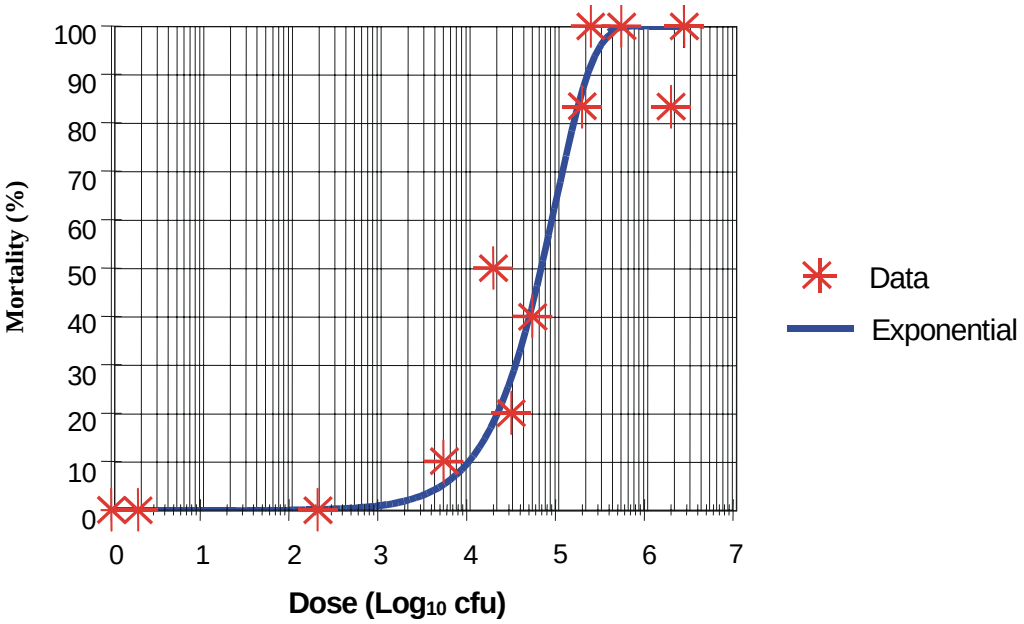

Figure IV-2. *Listeria monocytogenes* Dose vs. Mortality in Mice

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.

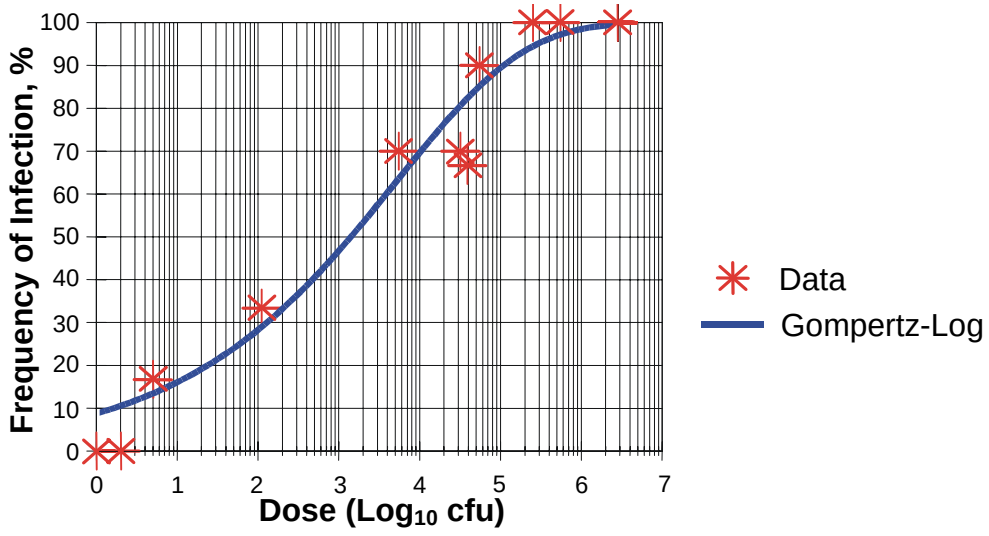

Figure IV-3. Dose vs. Frequency of Infection in Mice

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}.

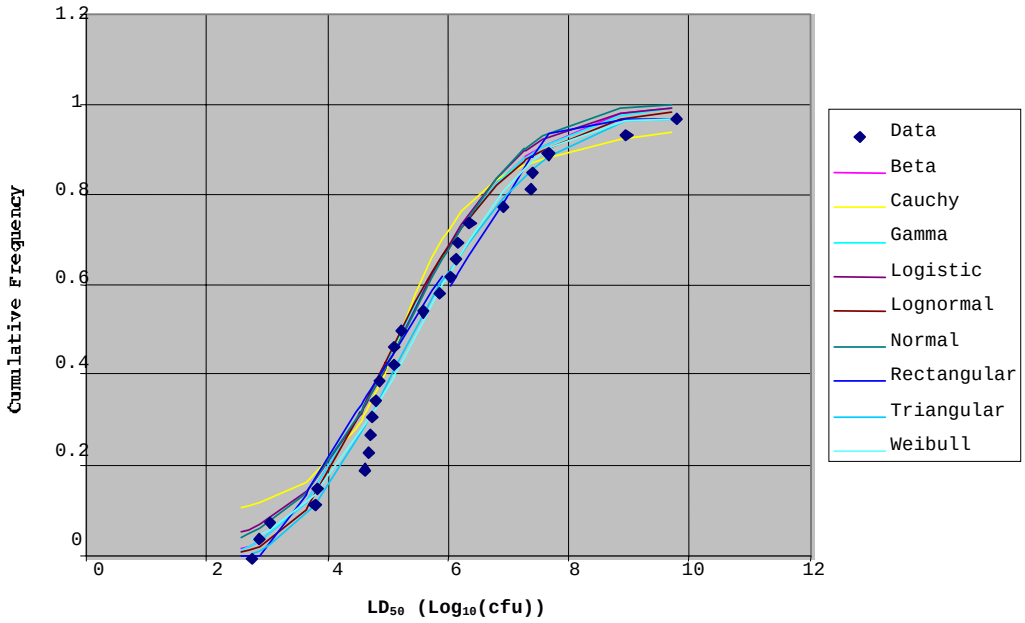

Figure IV-4. Variation (Cumulative Frequency) of *Listeria monocytogenes* Strain Virulences: Nine Distributions

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.

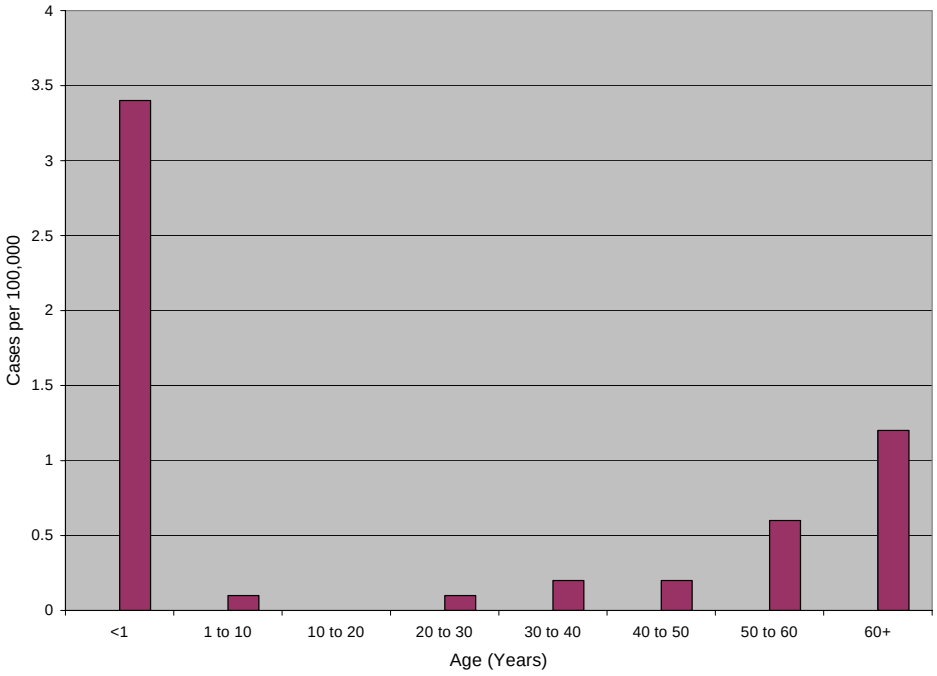

Figure IV-5. 1999 FoodNet Estimates of Listeriosis Incidence, by Age

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.

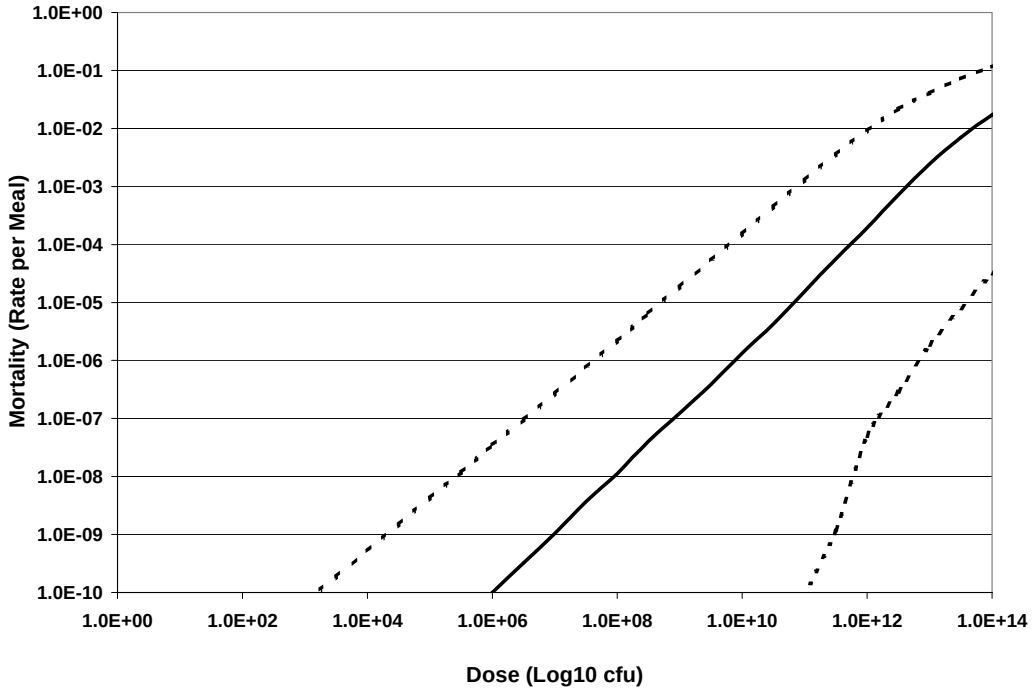

Figure IV-6. *Listeria monocytogenes* Dose-Response for Mortality with Variable Strain Virulence for the Intermediate-Age Subpopulation

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.

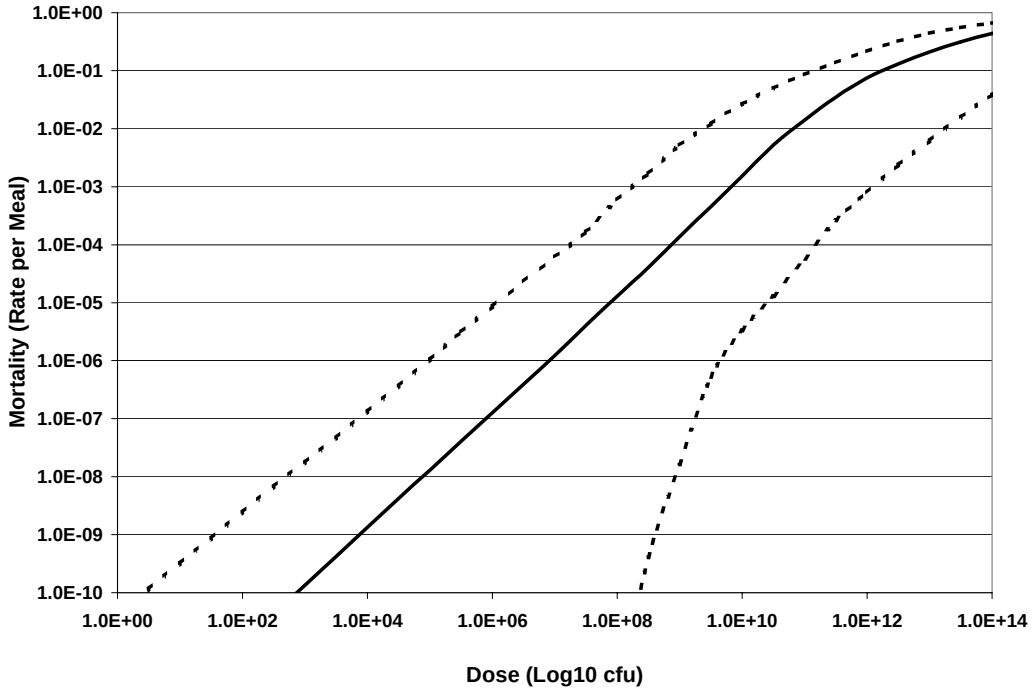

Figure IV-7. *Listeria monocytogenes* Dose-Response for Mortality with Variable Strain Virulence for the Neonatal Subpopulation

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 (95^{th}) and lower (5^{th}) 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.

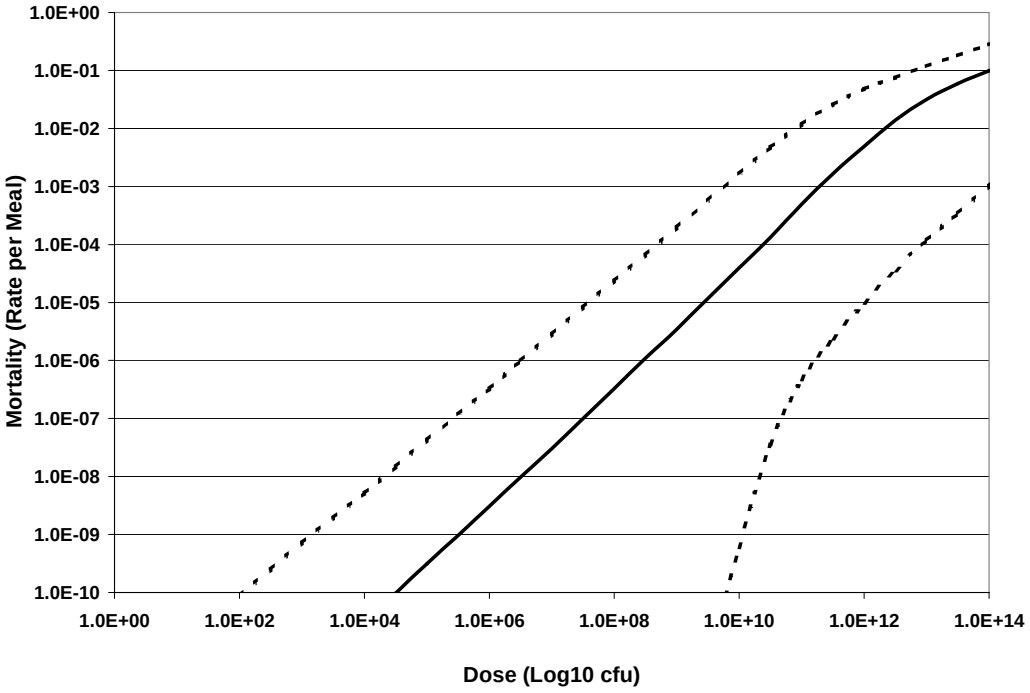

Figure IV-8. *Listeria monocytogenes* Dose-Response for Mortality with Variable Strain Virulence for the Elderly

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

	Median Mortality Rate per Serving[a]		
Dose (cfu/serving)	**Intermediate-Age**	**Neonatal[b]**	**Elderly**
1	$1.5x10^{-16}$ ($1.2x10^{-146}$, $1.9x10^{-13}$)	$1.6x10^{-13}$ ($1.2x10^{-99}$, $4.0x10^{-11}$)	$4.0x10^{-15}$ ($6.3x10^{-124}$, $1.8x10^{-12}$)
10^3	$1.2x10^{-13}$ ($5.4x10^{-92}$, $6.8x10^{-11}$)	$1.3x10^{-10}$ ($4.3x10^{-56}$, $1.7x10^{-8}$)	$3.6x10^{-12}$ ($2.2x10^{-74}$, $7.2x10^{-10}$)
10^6	$1.0x10^{-10}$ ($1.9x10^{-50}$, $3.5x10^{-8}$)	$1.3x10^{-7}$ ($1.2x10^{-25}$, $8.6x10^{-6}$)	$3.1x10^{-9}$ ($5.7x10^{-38}$, $3.3x10^{-7}$)
10^9	$1.2x10^{-7}$ ($6.0x10^{-22}$, $1.9x10^{-5}$)	$1.4x10^{-4}$ ($1.6x10^{-8}$, $5.1x10^{-3}$)	$3.4x10^{-6}$ ($1.3x10^{-14}$, $1.9x10^{-4}$)
10^{10}	$1.3x10^{-6}$ ($2.5x10^{-15}$, $1.5x10^{-4}$)[c]	$1.5x10^{-3}$ ($3.3x10^{-6}$, $2.7x10^{-2}$)	$3.9x10^{-5}$ ($6.0x10^{-10}$, $1.7x10^{-3}$)
10^{12}	$1.9x10^{-4}$ ($4.9x10^{-8}$, $9.2x10^{-3}$)	$7.4x10^{-2}$ ($7.8x10^{-4}$, $2.2x10^{-1}$)	$4.9x10^{-3}$ ($9.8x10^{-6}$, $4.8x10^{-2}$)

[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 $1.3x10^{-6}$ for the intermediate-age subpopulation at the 10^{10} cfu/serving dose level, corresponds to 1 death in approximately 769,231 servings ($1/1.3x10^{-6}$).

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).

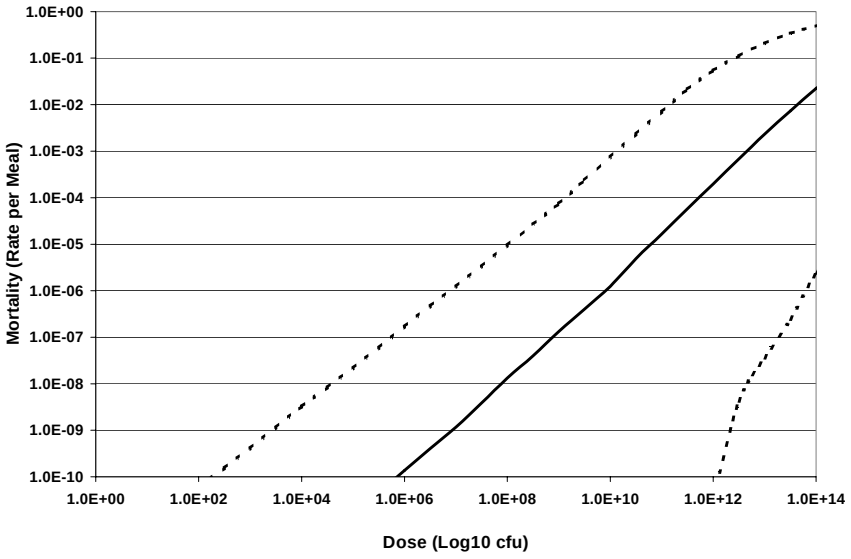

Figure IV-9. Dose Frequency Function for Elderly Population with a Single Strain of Unknown Virulence