

Use of Epidemiologic and Food Survey Data To Estimate a Purposefully Conservative Dose-Response Relationship for *Listeria monocytogenes* Levels and Incidence of Listeriosis†

ABSTRACT

The development of effective quantitative microbial risk-assessment models for foodborne pathogens depends on the availability of data on the consumers' exposure to a biological agent and the dose-response relationship that relates levels of the biological agent ingested with frequency of infection or disease. Information on the latter has historically been acquired from human volunteer feeding studies. However, such studies are not feasible for pathogens that either have a significant risk of being life threatening or for which morbidity is primarily associated with high-risk populations (i.e., immunocompromised persons). For these pathogens, it is proposed that purposefully conservative dose-response relationships can be estimated on the basis of combining available epidemiologic data with food-survey data for a ready-to-eat product. As an example, data on the incidence of listeriosis in Germany were combined with data on the levels of *Listeria monocytogenes* in smoked fish to generate a dose-response curve for this foodborne pathogen.

Key words: Smoked fish, immunocompromised, risk assessment

The development of effective quantitative risk-assessment techniques can greatly enhance the ability to make scientifically supportable decisions pertaining to the microbiological safety of foods, including enhancing the adoption of hazard analysis critical control point (HACCP) systems by the food industry worldwide (2, 7, 15, 16). However, development of quantitative microbial risk-assessment models is dependent on the availability of data for both the exposure of the population to the biological agent and the relationship between the levels of the biological agent ingested and the frequency of disease. In the latter case, information on dose-response relations has been historically acquired through feeding studies with human volunteers.

However, the number of these studies is severely limited, and they have inherent biases that have to be factored into their interpretation. Further, feeding studies are not feasible for a number of biological agents. For example, if the disease (e.g., the disease caused by *Escherichia coli* O157:H7) has a significant potential for being life threatening or having severe sequelae or if the disease (e.g., listeriosis, caused by *Listeria monocytogenes*) is restricted largely to specific high-risk populations, the use of volunteer studies is contraindicated.

The inability to conduct volunteer feeding studies requires that alternative approaches be used to estimate dose-response relations. Animal models are one potential approach. However, without some knowledge of human susceptibility, it is difficult to relate quantitatively the effects observed in animals with those in humans. Alternatively, data acquired from outbreak investigations could be used. However, epidemiological investigations are not conducted with the degree of clinical and food microbiological evaluations that would be needed to use the results of a single outbreak to calculate a dose-response relationship. Typically, there are insufficient data on the levels of the biological agent ingested and the numbers of individuals that consumed the contaminated food but remained disease-free. Further, for several foodborne biological agents, e.g., *Campylobacter* spp., sporadic cases may be more important than outbreaks.

Since use of these historical approaches is unlikely with these pathogens, alternative approaches are needed that can provide reasonable estimates of their dose-response relationships. The purpose of the present exercise was to determine if data on the annual incidence of a foodborne disease and the levels of the biological agent in a ready-to-eat food known to occasionally harbor relatively high levels of the microorganism at the time of consumption could be used to develop a purposefully conservative estimate of the dose-response relationship for a foodborne pathogen that is not amenable to human volunteer feeding studies. Listeriosis in immunologically high-risk populations resulting from the consumption of smoked fish was selected as an example.

MATERIALS AND METHODS

Data sources and assumptions

Smoked fish was selected because surveys have indicated that low levels of *L. monocytogenes* are common, and most smoked fish is a ready-to-eat product (i.e., does not receive a listericidal treatment before consumption). The specific quantitative data employed are those of Teufel and Bendzulla (13), who summarized the results of a nationwide survey that qualitatively and quantitatively examined a wide variety of foods (>14,000 samples) in Germany for *L. monocytogenes*. Their data for smoked fish were selected for the current calculations. The survey indicated that this food was a major source of *L. monocytogenes* in the German diet because a significant percentage of the product consumed by the public had occasional portions with elevated levels of the pathogen (12). Since this product is consumed without cooking, the levels of *L. monocytogenes* ingested by consumers would be expected to be as great or greater than those detected during the survey. These data were also selected because Germany is one of several countries that have actively sought to accurately estimate the nationwide extent of listeriosis. In Germany, there are an estimated 200 cases of listeriosis per year for a population of roughly 80 million (Teufel, personal communication). Although one-third of the cases are neonates (12), all cases were assumed to be food associated and were included as part of the total national case load. This estimated incidence of approximately 3 cases per 1,000,000 inhabitants is similar to the 2 to 3 and 4 to 5 cases per 1,000,000 reported for England and Wales (6) and the United States (11), respectively. It was assumed that listeriosis cases were restricted to individuals at increased risk resulting from impairments of the immune system. While cases in otherwise healthy individuals have been reported, it is generally accepted that foodborne listeriosis is primarily a disease associated with specific high-risk populations. Restricting consideration to this subpopulation also helps ensure a conservative dose-response estimate. It has been estimated that in the United States as much as 20% of the population may be at increased risk due to immune system impairment (3, 10). A similar value is assumed for Germany and was used for the current calculations. The effect of susceptible population size on the calculated dose-response relationship is discussed more fully later. The annual per capita consumption of smoked fish is approximately 1 kg (1). It was assumed that the average serving size for smoked fish is 50 g; thus, the average number of servings per year is 20. It is further assumed that individuals with impaired immune status consume smoked fish at the same rate as the general population.

The key assumption in the current exercise is that the dose-response relationship for foodborne human *L. monocytogenes* infections fits the exponential dose-response model. This model relates the number of cells of a biological agent consumed with the probability of an adverse effect in the consuming population,

$$P = 1 - e^{-RN}, \quad (1)$$

where P is the probability of an adverse effect, N is the number of biological agent consumed (CFU), and R is a constant specific to each pathogen that helps define the shape of dose-response curve. This model and the closely related beta-Poisson model have been effectively used to describe dose-response relations for a number of other foodborne and waterborne infectious agents (4, 5, 9). By selecting different biological end points (e.g., infection, morbidity, mortality) the model can be used to describe different aspects of dose-response relations and thus provide estimates of severity. In the current example, we will focus on morbidity, i.e., the incidence of symptomatic disease.

Concept of purposefully conservative dose-response relationship

If *L. monocytogenes* dose-response relations can be described by the exponential model, the key question is whether the R value for *L. monocytogenes* can be deduced. Mathematically, this is a simple transformation of equation 1:

$$R = -[\ln(1 - P)]/N. \quad (2)$$

Equation 2 implies that if sufficient data could be acquired on the levels of the organism consumed in a food and the percentage of individuals that subsequently developed listeriosis, then the R value could be estimated. Since R is a constant for the specific pathogen of concern, once established it could be used to estimate the dose-response relationship at other values of N .

Establishing the exact value for R is beyond the scope of the data available currently. However, it is proposed that current data are sufficient to calculate an initial conservative estimate of R based on the assumption that all cases of listeriosis are attributable to a single ready-to-eat food for which there are quantitative data relating to the frequency and extent of *L. monocytogenes* contamination. Such an estimate should be inherently conservative; however, it helps define a probable upper limit of risk, i.e., if this purposefully conservative estimate of the probability of acquiring listeriosis is low, then the actual risk is even lower.

In the present example, it is assumed that the all human listeriosis resulted from the consumption of ready-to-eat smoked fish. Two approaches were used to estimate the R value for *L. monocytogenes*. In the first, a single dose was used to estimate R , while the second employed a multiple-dose calculation.

RESULTS

Estimate based on a single dose

Since we have assumed that listeriosis is restricted to high-risk individuals, then the population of concern is that portion of the total population that is immunocompromised (IC). If we employ the upper value (i.e., 20%) for the proportion of the population that is immunocompromised, then the high-risk population in Germany is $\text{Pop}_{\text{IC}} = 0.2 \times 80 \times 10^6 = 16 \times 10^6$ persons, and the case rate for listeriosis in Germany is $200/(16 \times 10^6) = 12.5 \times 10^{-5}$ cases per person per year, or 12.5 cases per 1×10^6 immunocompromised inhabitants.

The population at increased risk was assumed to consume smoked fish at the same rate as the general population, so each susceptible individual consumes an

TABLE 1. The levels of *Listeria monocytogenes* observed in samples of smoked fish

Levels of <i>Listeria monocytogenes</i> observed ^a (CFU/g)	No. of samples at this level	Total samples (%)
<1/25	353	92.89
1/25-1	5	1.32
>1-100	14	3.68
>100-10,000	4	1.05
>10,000	4	1.05

^a Source: reference (13).

average of 20 50-g portions per year. Thus, the total number of portions consumed by the subpopulation of concern is $\text{Pop}_{\text{IC}} \times 20$ servings per person = $16 \times 10^6 \times 20 = 320 \times 10^6$ servings.

With the single-dose approach, it was assumed that all cases of listeriosis were restricted to that proportion of smoked fish that had the highest level of *L. monocytogenes* contamination ($\geq 10 \times 10^3$ CFU/g). This was 1.05% (4 of 380) of the smoked fish samples. The level of *L. monocytogenes* in these products was assumed to be 10×10^3 CFU/g, further ensuring a conservative *R* value. The rationale underlying the use of a single dose is that because of the large differences in the *L. monocytogenes* levels in the survey's contamination categories, the exponential model predicts that the highest concentration would account for essentially all of the effect observed in a population. The total number of portions consumed by the high-risk subpopulation with this high level of *L. monocytogenes* would be 320×10^6 servings $\times 105 \times 10^{-4} = 336 \times 10^4$ servings. Thus, the probability of acquiring a listeriosis infection from consuming one of these portions would be $P = 200$ cases per 336×10^4 servings = 595×10^{-7} cases per serving.

The level of *L. monocytogenes* ingested when these products are consumed is calculated by multiplying the pathogen content of the smoked fish times the serving size:

$N = 10 \times 10^3$ CFU/g $\times 50$ g = 500×10^3 CFU. Once values for *P* and *N* have been estimated, the *R* value can be calculated by substituting into equation 2: $R = -[\ln(1 - P)]/N = -[\ln(1 - 595 \times 10^{-7})]/500 \times 10^3 = 1.190 \times 10^{-10}$. Conceptually, the *R* value can be viewed as the probability that ingesting a single *L. monocytogenes* cell would produce an active case of listeriosis. Once derived, the *R* value can then be used to estimate the relationship between the levels of *L. monocytogenes* ingested and the incidence of listeriosis (Figure 1). Plotting the results as *P* versus $\log(N)$ (Fig. 1A) produces a sigmoidal curve that historically has been interpreted as being indicative of the presence of a threshold level below which there is no response. However, plotting $\log(P)$ versus $\log(N)$ (Fig. 1B) demonstrates that the exponential model does not assume a threshold. It is also worth noting that over much of the dose range, *P* approaches a linear relation to *N* (5).

ESTIMATE BASED ON MULTIPLE DOSES

The *R* value for symptomatic listeriosis was recalculated employing a technique that took advantage of all of the *L. monocytogenes* data. The general approach was to calculate the number of cases of listeriosis that would be expected for the different levels of *L. monocytogenes* enumerated in the smoked fish survey, given a specific *R* value. The number of expected cases for the different levels were then summed to obtain the total number of expected listeriosis cases. Different *R* values were then substituted iteratively until an *R* value was obtained that yielded the reported number of listeriosis cases (i.e., 200 per year). These iterations were performed readily by setting up the equations on a spreadsheet (Table 2).

In setting up the spreadsheet, the lowest value of *L. monocytogenes* for each of the prevalence ranges (0, 0.04, 1, 100, and 10,000 CFU/g) from the survey data was again used to provide a conservative estimate. The calculations used to determine the number of cases associated with each of the *L. monocytogenes* levels in smoked fish were conceptually similar to those used for the single-dose estimate. Within any prevalence category, the number of cases of listeriosis is the product of the number of servings consumed times the probability of listeriosis from one such serving.

$$L_{ci} = S_{ci} \times P_{ci}, \quad (3)$$

where L_{ci} = number of cases of listeriosis associated with smoked fish having prevalence category of *L. monocytogenes*; S_{ci} = number of servings consumed within category *i*; and P_{ci} = Probability that one would acquire listeriosis from one such serving.

The number of servings belonging to any single category is a product of the total number of servings times the probability that a given serving falls within that category. The latter was estimated using the survey data. So, for example, the probability that a serving of smoked fish has $>10,000$ CFU/g is $P_{Sci} = 4/(353 + 5 + 14 + 4 + 4) = 4/380 = 0.0105$. Since the total number of servings in

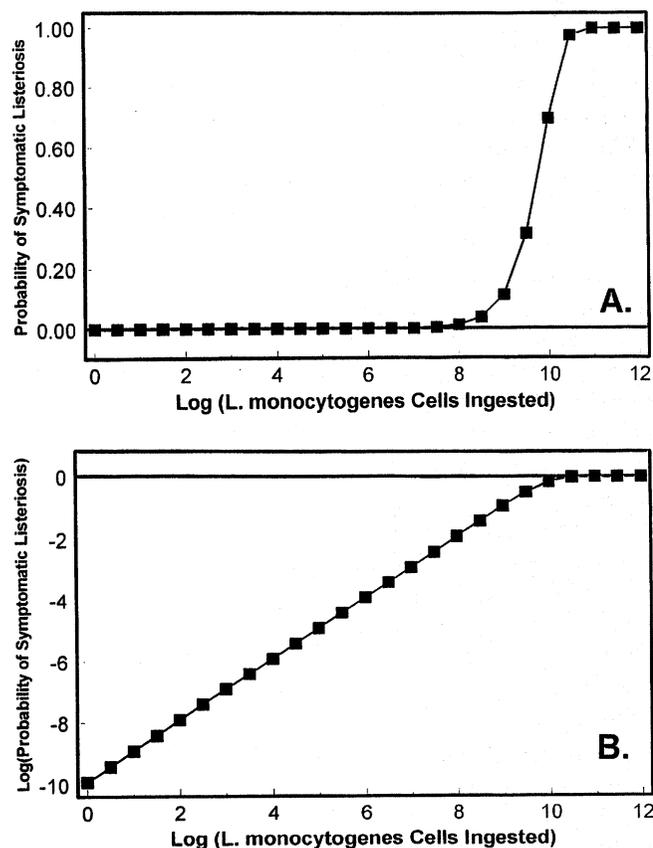


FIGURE 1. The dose-response curve predicted by the exponential model using an *R* value calculated using a single dose-derived estimate of *R* based on data for the *L. monocytogenes* levels in smoked fish and the annual incidence of listeriosis in Germany. The dose-response relationship is presented both as probability vs. \log (dose) (A) and as \log (probability) vs. \log (dose) (B).

TABLE 2. Spreadsheet used to calculate the *R* value for the dose-response relationship for symptomatic listeriosis based on the prevalence of *Listeria monocytogenes* in smoked fish

R value	<i>Listeria monocytogenes</i> (CFU/g)	Number of cells ingested (<i>N</i> , CFU)	Probability of listeriosis per serving	% smoked fish at this level of <i>Lm</i>	Number of servings consumed	Predicted number of listeriosis cases	Total probability of acquiring listeriosis
1.179×10^{-10}	0.00	0	0	92.89	3.0×10^8	0.000	0
	0.04	2	2.4×10^{-10}	1.32	4.2×10^6	0.001	3.1×10^{-12}
	1.00	50	5.9×10^{-9}	3.68	1.2×10^7	0.069	2.17×10^{-10}
	100.00	5000	5.9×10^{-7}	1.05	3.4×10^6	1.981	6.19×10^{-9}
	10000.00	500000	5.9×10^{-5}	1.05	3.4×10^6	198.066	6.19×10^{-7}
Totals:				99.99	3.2×10^8	200.117	6.25×10^{-7}

Germany is 80×10^6 individuals \times 20% immunocompromised \times 20 servings per year per individual, the number of servings in the highest contamination group is $S_{ci} = 0.0105 \times 80 \times 10^6 \times 0.2 \times 20 = 3.36 \times 10^6$.

The probability that one would acquire listeriosis from a single serving is calculated using the exponential dose-response model, where *N* is the level of *L. monocytogenes* for that category and the *R* value is assumed. For example, if $R = 1.2 \times 10^{-10}$, the single dose estimate, was assumed, then $P_{ci} = 1 - \exp[(-1.2 \times 10^{-10}) \times 5.0 \times 10^5] = 6 \times 10^{-5}$. When this value is multiplied by S_{ci} , the total number of cases of listeriosis anticipated for this category is $L_{ci} = S_{ci} \times P_{ci} = (6 \times 10^{-5}) \times (3.36 \times 10^6) = 202$ cases. Finally, the total number of cases is determined by summing the cases for each of the categories: $L_{Total} = L_{c1} + L_{c2} + L_{c3} + L_{c4} + L_{c5}$. The final estimate of *R* using this method was 1.179×10^{-10} (Table 2), a value that is almost identical with the estimate based on a single-dose calculation.

DISCUSSION

The current calculations indicate that given sufficient data on the incidence of listeriosis and the levels of *L. monocytogenes* in a ready-to-eat food that is likely to be a major source of the pathogen, it is possible to generate a conservative estimate of the relationship between exposure and morbidity. Presumably, similar calculations could be performed for other biological end points to determine, for example, the dose-response relationship between exposure estimates (i.e., levels in a food) and infection rates (i.e., colonization of the intestinal tract). Presumably this could be done if sufficient data were available on the percentage of symptomatic and asymptomatic individuals that are actively colonized by the microorganism.

The current estimate of the *R* value for symptomatic listeriosis had to be based on a number of assumptions pertaining to factors that could impact its magnitude. Thus, the dose-response estimate should be taken as an initial attempt to demonstrate the approach and not as a definitive value. For example, reducing the size of the high-risk subpopulation to a value less than 20% increases the *R* value and alters the derived dose-response relationship. However, the magnitude of this change does not appear great unless the subpopulation is reduced greatly. For example, even if the potential high-risk subpopulation was 0.5% instead of

20%, the *R* value (based on the single-dose model) would only increase from 1.19×10^{-10} to 4.77×10^{-9} . Other factors, such as the true incidence of listeriosis, the number of servings per year, the average serving size, or levels of *L. monocytogenes* in the product also affect the final estimate to varying degrees.

The accuracy of the estimated *R* value can be enhanced by the availability of additional epidemiologic and microbiological survey data. Any estimate of *R* is dependent on having a reasonable assessment of the true incidence of listeriosis. One of the reasons that *L. monocytogenes* was selected as an example was that there has been a concerted effort in the past several years to acquire realistic estimates of the incidence of this disease. Better estimates of the levels of *L. monocytogenes* in the food being evaluated would also enhance the estimation of *R*. For example, quantitative data on the actual levels of *L. monocytogenes* detected in the food product (as opposed to the use of categories such as $>10,000$ CFU/g) would permit frequency distributions to be used in assessing pathogen levels. Similar profiles could also be generated for factors such as serving sizes and consumption patterns. Such frequency distributions would allow the use of advanced modeling techniques such as Monte Carlo simulations to better assess the risk associated with consumption. Initial trials (not shown) indicated that given sufficient data, such modeling techniques can be readily applied to the steps in the above calculations where averages were employed.

It should be emphasized that *R* values calculated in the manner above are inherently conservative. The procedure overestimates the risk of listeriosis by assuming that all listeriosis is the result of consuming a single ready-to-eat food. Further, all calculations and assumptions were purposefully conservative. However, even with this conservative estimate for *R*, it is apparent that the probability that a high-risk individual will acquire symptomatic listeriosis is extremely low unless high levels of the pathogen are consumed (Fig. 1). The ability to generate a quantitative relationship between exposure and response dramatically supports the conclusion that the initial focus for risk-management decisions should be the prevention of the growth of this pathogen in food to high levels (8). This would have the greatest public-health impact on a cost-benefit basis. It would appear that if tolerable levels of risk can be agreed upon, then estimates similar to those presented

above may be a key to establishing truly risk-based microbiological criteria for foods (14). For example, calculations using the estimated *R* value for *L. monocytogenes* predict that the risk associated with a microbiological criterion for smoked fish of ≤ 1 CFU/g at the current frequency of contamination would result in only one case of listeriosis in Germany per decade.

In summary, the use of epidemiologic and microbiologic food survey data in conjunction with the exponential model appears to be an alternative means of estimating dose-response relationships for foodborne pathogenic bacteria that are not amenable to human volunteer feeding studies. In fact, estimates based on this alternative approach may have an advantage over those derived from feeding trials because the entire population can be considered, not just a small group of individuals who are likely to represent the most resistant segment of the population. A more detailed examination of this approach will be conducted as soon as additional epidemiologic and food-survey databases can be acquired.

REFERENCES

1. Anonymous. 1996. Geschäftsbericht des Bundesverbandes der deutsche Fischindustrie und des Fischgrosshandels e.V. Hamburg, Germany.
2. Buchanan, R. L. 1995. The role of microbiological criteria and risk assessment in HACCP. *Food Microbiol.* 12:421-424.
3. CAST. 1994. Foodborne pathogens: risks and consequences, p. 25-26. Task force report #122. Council for Agricultural Science and Technology, Ames, IA.
4. Crockett, C. S., C. N. Haas, A. Fazil, J. B. Rose, and C. P. Gerba. 1996. Prevalence of shigellosis in the U.S.: consistency with dose-response information. *Int. J. Food Microbiol.* 30:87-99.
5. Haas, C. N. 1983. Estimation of risk due to low doses of microorganisms: a comparison of alternative methodologies. *Am. J. Epidemiol.* 118:573-582. J. 1996.
6. McLauchlin, J. The relationship between *Listeria* and listeriosis. *Food Contr.*, 7:187-193.
7. Notermans, S., G. Gallhoff, M. H. Zwietering, and G. C. Mead. 1995. The HACCP concept: specification of criteria using quantitative risk assessment. *Food Microbiol.* 12:81-90.
8. Pinner, R. W., A. Schuchat, B. Swaminathan, P. S. Hayes, K. A. Deaver, R. E. Weaver, B. D. Plikaytis, M. Reeves, C. V. Broome, J. D. Wenger, and the Listeria Study Group. Roles of foods in sporadic listeriosis. II. Microbiologic and epidemiologic investigation. *JAMA* 267:2046-2050.
9. Rose, J. B., and C. P. Gerba. 1991. Use of risk assessment for development of microbial standards. *Water Sci. Technol.* 24(2): 29-34.
10. Smith, J. L. Long term consequences of foodborne toxoplasmosis: effects on the unborn, the immunocompromised, the elderly and the immunocompetent. *J. Food Prot.*, in press.
11. Tappero, J. W., A. Schuchat, K. A. Deaver, L. Mascola, and J. D. Wenger. 1995. Reduction in the incidence of human listeriosis in the United States. *JAMA* 273:1118-1122.
12. Teufel, P. 1994. European perspectives on *Listeria monocytogenes*. *Dairy Food Environ. Sanit.* 14(4):212-214.
13. Teufel, P., and C. Bendzulla. 1993. Bundesweite Erhebung zum Vorkommen von *L. monocytogenes* in Lebensmitteln. Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, Berlin.
14. van Schothorst, M. 1996. Setting of criteria for *Listeria monocytogenes* based on risk assessment, p. 157-168. In A. Amgar (ed.), *Food safety '96*. ASEPT, Laval, France.
15. Whiting, R. C., and R. L. Buchanan. 1997. Development of a quantitative model for *Salmonella enteritidis* in pasteurized liquid egg. *Int. J. Food Microbiol.*, in press.
16. WHO/FAO. 1995. Application of risk analysis to food standards issues: report of the joint FAO/WHO expert consultation. World Health Organization, Geneva, Switzerland.