NFPA
The Food Safety People

NATIONAL FOOD PROCESSORS ASSOCIATION

April 25, 2003

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Jenny Scott

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Docket No 03-005N
Room 102, Cotton Annex
300 12th Street, SW
Washington, DC 20250-3700

[Docket No. 03-005N] *Listeria* Risk Assessmei t Technical Meeting; 68 *Federal Register* 6109; Febru ıry 6, 2003

Dear Ms. Riley:

The National Food Processors Association (NFPA) is th : voice of the $500 billion food processing industry on scientific and public policy ssues involving food safety, food security, nutrition, technical and regulatory matters and consumer affairs. NFPA's three scientific centers, its scientists and profes: ional staff represent food industry interests on government and regulatory affairs ɛ nd provide research, technical services, education, communications and crisis management support for the association's U.S. and international members. NFPA m :mbers produce processed and packaged fruit, vegetable, and grain products, meat, poultry, and seafood products, snacks, drinks and juices, or provide supplies ; nd services to food manufacturers.

On February 24, 2003 we submitted galley proofs of tw‹ ı publications to this docket. Attached you will find reprints of the final publications:

Gombas, D. E., Y. Chen, R.S. Clavero and V.N. Scott. 2003. Survey of *Listeria monocytogenes* in ready-to-eat foods. J. Food Protectio ı 66: 559-569.

Chen, Y., W.H. Ross, V.N. Scott and D.E. Gombas. 20(3. *Listeria monocytogenes*: Low levels equal low risk. J. Food Protection 66: 570-577.

The survey data have also been made available on the Iı ternet through the JIFSAN Risk Analysis Clearinghouse.

Respectfully submitted,

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Survey of *Listeria monocytogenes* in Ready-to-Eat Foods

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ABSTRACT

The purpose of this study was to develop data on the risk of listeriosis to support a science-based strategy for addressing *Listeria monocytogenes* in foods in the United States. Eight categories of ready-to-eat foods were collected over 14 to 23 months from retail markets at Maryland and northern California FoodNet sites. The product categories included luncheon meats, deli salads, fresh soft "Hispanic-style" cheeses, bagged salads, blue-veined and soft mold-ripened cheeses, smoked seafood, and seafood salads. The presence and levels of *L. monocytogenes* in the samples were determined by rapid DNA-based assays in combination with culture methods. Of 31,705 samples tested, 577 were positive. The overall prevalence was 1.82%, with prevalences ranging from 0.17 to 4.7% among the product categories. *L. monocytogenes* levels in the positive samples varied from <0.3 MPN (most probable number) per g to 1.5 × 10^5 CFU/g, with 402 samples having levels of <0.3 MPN/g, 21 samples having levels of >10^2 CFU/g, and the rest of the samples having intermediate levels. No obvious trends with respect to seasonality were observed. Significant differences ($P < 0.05$) between the sampling sites were found, with higher prevalences for threes categories in northern California and for two categories in Maryland. Significantly ($P < 0.001$) higher prevalences were found for in-store-packaged samples than for manufacturer-packaged samples of luncheon meats, deli salads, and seafood salads, while 16 of the 21 samples with higher counts were manufacturer packaged. The data collected in this study help to fill gaps in the knowledge about the occurrence of *L. monocytogenes* in foods, and this new information should be useful in the assessment of the risk posed by *L. monocytogenes* to consumers.

Listeria monocytogenes has been recognized as a human pathogen for >70 years. Only within the past 2 decades, however, has *L. monocytogenes* been associated with food and classified as a foodborne pathogen. The Centers for Disease Control and Prevention (CDC) have estimated that up to 2,500 cases of listeriosis, resulting in 500 deaths *(17)*, occur each year in the United States. Several large outbreaks in the early 1980s prompted the U.S. Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) to establish a policy under which ready-to-eat foods contaminated with *L. monocytogenes* at a detectable level are deemed adulterated. This "zero tolerance" policy was established on the basis of very few data concerning the prevalence and control of the organism in food and represented a conservative approach based on the limited information available at the time. According to the policy, which assumes that all *L. monocytogenes* strains are pathogenic, the presence of the organism at a detectable level (e.g., 0.04 CFU/g) in a ready-to-eat (RTE) food renders that product adulterated *(23)*. Since the establishment of the policy, more information about the prevalence of the organism in food-manufacturing plants and in foods has become available. Also, there exists a better understanding about which types of foods are more likely to be involved in illnesses, which foods support the growth of the organism, and what segments of the population are likely to be adversely affected by the consumption of food containing *L. monocytogenes*.

Despite efforts to eradicate the organism from RTE foods *(25, 26)*, *L. monocytogenes* contamination continues to occur. Surveillance and monitoring activities of the FDA and the USDA have indicated that as much as 5% of some RTE foods, such as prepared deli-style salads and sliced luncheon meats, contain *L. monocytogenes (11, 15)*. Such a prevalence of the organism in these frequently consumed products implies that consumers are exposed to detectable levels of *L. monocytogenes* billions of times each year. This finding appears inconsistent with the relatively low level of listeriosis cases reported by the CDC. There are several possible explanations for the discrepancy: (i) only some of the population are sensitive to *L. monocytogenes*; (ii) only exposure to high levels of *L. monocytogenes* causes listeriosis, and/or (iii) only some subtypes of *L. monocytogenes* cause listeriosis. We know that the first hypothesis provides a partial explanation; listeriosis occurs most frequently in immunocompromised individuals, pregnant women, neonates, and elderly people. However, this factor does not account for all of the discrepancy. A risk assessment is needed to reveal and rank the factors contributing to listeriosis.

In a risk assessment, one typically characterizes risk by correlating an exposure assessment for the hazard with a dose-response model *(13, 18)*. A risk assessment for *L. monocytogenes* presents some difficulties. First, an accurate exposure assessment is unavailable. The FDA, the USDA,

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where n is the number of positive samples, P is the actual value of the proportion or percentage positive for the population, d is the desired upper bound on the absolute error (i.e., margin of error), and $z = 1.96$, corresponding to a 95% confidence level for the probability that the estimate is within $\pm d$ of the population value *(9)*. For a fixed value of d, the sample size is at its maximum when $P = 0.5$ (50%). To be conservative, and since we did not know the values of the percentages to be estimated, we assumed that $P = 50\%$. For this approach, n values of 125, 250, and 500 correspond to upper absolute error bounds of 8.8, 6.2, and 4.4%, respectively. Assuming that 5% of the total samples tested would be positive for *L. monocytogenes,* the corresponding total sample sizes required would be 2,500, 5,000, and 10,000, respectively. On the basis of this analysis, we decided to collect 2,500 samples of each of two types of products, luncheon meats and deli salads, at each of two FoodNet sites (see "Sampling Site Selection" section), for a total of 5,000 samples per food type, or 10,000 samples. In the course of the study, we found that prevalence levels for *L. monocytogenes* in luncheon meats and deli salads were considerably lower than had been expected, and thus we decided to double the total number of luncheon meat and deli salad samples collected. For the other product categories, we targeted 2,500 to 3,000 samples per food type.

For luncheon meats and deli salads, we weighted the number of samples by consumption of the product. For example, with luncheon meats, the proportions of ham, bologna, and chicken-turkey samples were based on the frequency of consumption of these meats in the geographical area involved (West for California and South for Maryland) according to the CSFII. Thus, for Maryland, 50% of the luncheon meat samples were ham, 30% were bologna, and 20% were turkey-chicken; for California, 43% of the samples were ham, 30% were bologna, and 27% were turkey-chicken. Owing to the diverse nature of the additional product categories, there was no weighting within those categories.

Sampling site selection. The CDC conduct active surveillance for listeriosis at nine FoodNet sites *(2)*, and this surveillance provides the most accurate estimate of listeriosis possible. In addition, while the present study was in progress, the CDC performed case-control studies for listeriosis at these sites, which provided a potential opportunity to obtain *L. monocytogenes* isolates from listeriosis patients and compare them with isolates from foods in the same geographical area. Thus, in order to relate exposure data and food isolates to illness, food samples were collected at the northern California and Maryland FoodNet sites. The rationale for selecting these sites was as follows. Although FoodNet data indicated that the incidence of listeriosis was similar for all sites (approximately 0.5 cases per 100,000 people), the 1997 FoodNet final report *(2)* indicated a slightly higher rate at the northern California site (0.7 cases per 100,000 people). The potential for a larger number of cases of listeriosis, and therefore more isolates for comparison with food isolates, at the northern California site was one factor in the selection of this site as one of our sampling sites. In addition, the limited geographical area (Alameda and San Francisco counties) simplified the sampling procedure (compared with a sampling area encompassing an entire state, such as Minnesota or Georgia). We selected Maryland because its FoodNet site was relatively small (comprising five counties plus Baltimore City) and because it was geographically far removed from the northern California site. However, in Maryland, listeriosis data for FoodNet are collected statewide, so we elected to sample all counties containing more than 2% of the population (10 counties) plus Baltimore City, covering 87.5% of the population. Counties in which products to be investigated (e.g., fresh soft Hispanic-style cheeses) were not available were omitted from the sampling list.

Selection of sampling locations within the FoodNet sites. Sampling within the sites was weighted by the populations in the counties involved (1 July 1998 estimate from www.census.gov). For example, since it was determined that approximately 65% of the population resided in Alameda County and 35% resided in San Francisco County, the study was designed so that 65% of the samples would be collected in Alameda County and 35% would be collected in San Francisco County. The sampling strategy for fresh soft Hispanic-style cheeses was based on the Hispanic population in the sampling area. In order to simplify sample collection and minimize travel costs, samples were generally collected in only one county on each sampling day. The order in which the counties were sampled within a site was determined with the use of a random number table *(12)*.

Selection of collection sites within counties. Given the ubiquitous nature of the organism, foods may be contaminated with *L. monocytogenes* at food service establishments or in the home, but we focused on evaluating levels of *L. monocytogenes* in foods purchased at retail stores. Purchasing products at retail stores allowed us to sample a variety of products (brand-name products and unbranded products) representative of what the consumer would purchase and consume in the areas of the study. Logistically, it was easier to sample at retail stores than at food service establishments or to obtain foods from consumers' homes, and the packages and containers for the samples obtained made these samples easier to ship to the laboratories than, for example, food service meals would have been. Furthermore, by collecting retail samples, we avoided the potential for cross-contamination via handling by consumers.

Lists of large and small retail markets were created with the use of current telephone directories accessed at the Library of Congress. For each county, the list of stores was divided into list A (major supermarkets) and list B (other grocers). It was assumed that list A stores would carry luncheon meats and deli salads. All list B stores were contacted by telephone to determine whether they carried the specific product to be sampled and to verify their addresses; stores were deleted from the list if they did not respond to three phone calls during business hours, if their phone number was incorrect, or if they did not carry the product to be sampled. For the additional product categories, list A stores were also contacted to verify product availability. The stores on the lists were numbered, and the random number table *(12)* was used to select stores for each collection week (5 major supermarkets and 10 other grocers). It was assumed (on the basis of our experiences and the experiences of others in the retail industry) that 75% of shopping is done at major supermarket chains and 25% is done at other grocers, and the number of samples from lists A and B were weighted accordingly. Supplementary lists of stores reported to have specific products (smoked seafood, seafood salads, soft cheeses, and bagged salads) were provided for use as needed to obtain the selected numbers of samples for these products.

Collection of samples. The NFPA RF contracted with an independent third party to collect samples of all products except fresh soft Hispanic-style cheese at retail markets; fresh soft Hispanic-style cheese samples were collected by a second independent third party.

Collection of samples: luncheon meats and deli salads. For luncheon meats and deli salads, 120 samples were collected in northern California and Maryland for approximately 90 weeks (each week, in alternating weeks) over 23 months. The NFPA RF

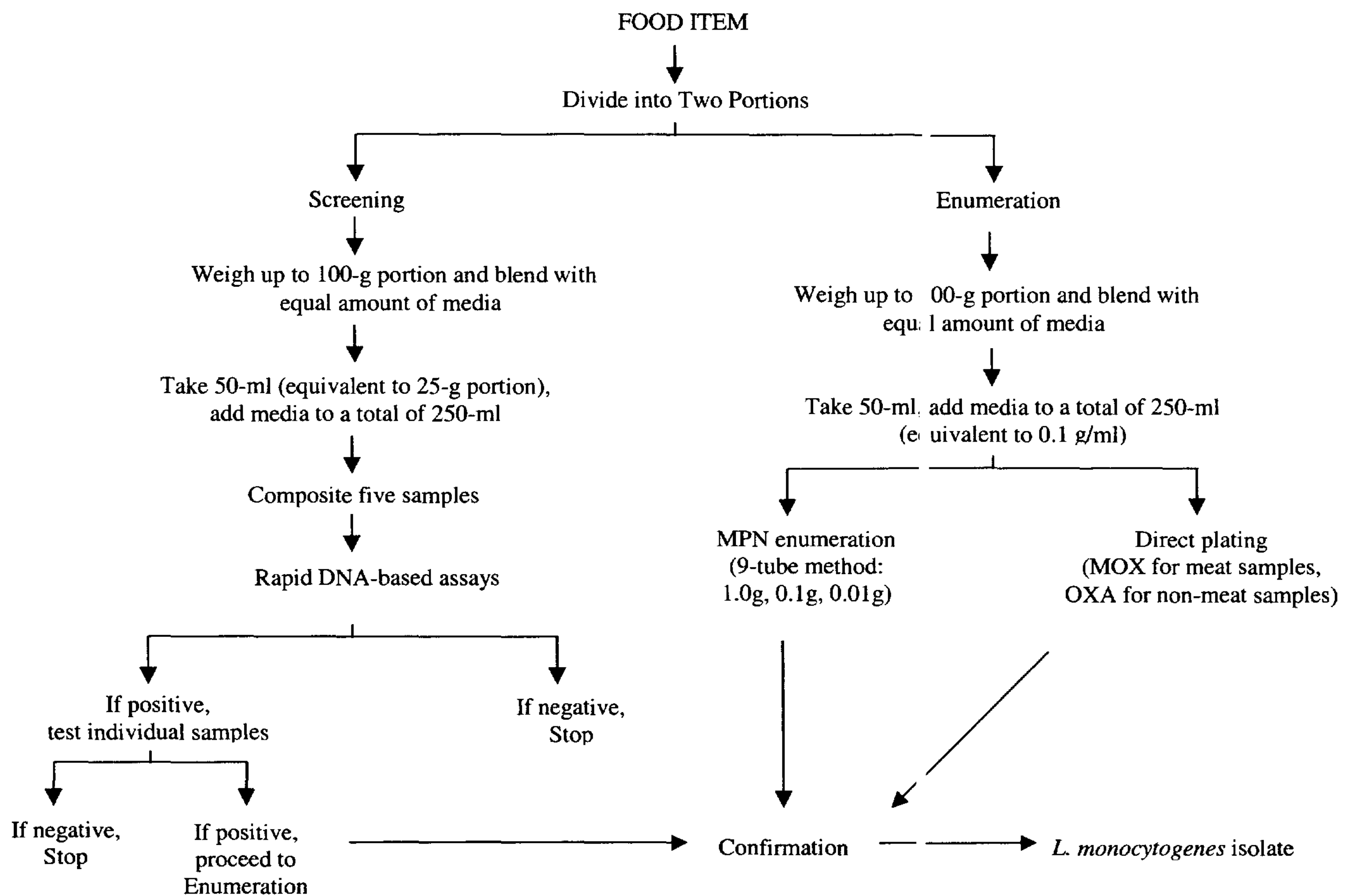

FIGURE 1. *Detection and enumeration of* L. monocytogenes *in RTE food samples.*

meats and deli salads, 100 samples were selected for testing. For the other product categories, 50 samples were selected for the first several weeks of sampling, and 25 samples were selected each week thereafter for testing. In a week when <100 or <25 samples were purchased (owing to product shortages in the stores), all acceptable samples were tested.

Samples were assigned codes, and the following product information was recorded for the luncheon meats and deli salad samples: sampling location (northern California or Maryland), date of receipt at the laboratory, and whether the sample appeared to be packaged in-store or in the original manufacturer's packaging. Additional product information was recorded for the other product categories; depending on the product, information included type of ingredient, whether pasteurized milk was listed as an ingredient, whether the product was domestic or imported, whether or not the product was vacuum packaged, and the use-by or sell-by date code, if present.

The laboratories were instructed to transfer samples aseptically into individual sterile plastic bags and discard the original retail packages. For Maryland samples, the selection of the 100 or 25 samples to be tested occurred after all samples had been transferred into plastic bags. For northern California samples, the selection occurred prior to the transfer of the samples. The selected samples were stored at 2 ± 2°C until they were used. Sample testing was initiated within 24 h of the receipt of samples.

Testing procedures. The general scheme for sample testing is shown in Figure 1. Four combinations of testing procedures were used to screen the samples for *L. monocytogenes.* The choice of screening procedure was based on product type and which laboratory performed the testing (see below). Samples were screened by recognized methods typically used by the laboratory for the detection and enumeration of *L. monocytogenes.* The Gene-Trak assay (Neogen, Lansing, Mich.) and the BAX assay (DuPont Qualicon, Wilmington, Del.) were used to screen samples collected in Maryland and northern California, respectively. In a prestudy evaluation of the laboratories, both DNA-based assays generated comparable results for the detection of *L. monocytogenes* in samples provided by the NFPA (data not shown). USDA or FDA testing procedures for *L. monocytogenes* were modified for use in enumeration and isolation. Methods described in chapter 8 of the revised *Microbiological Laboratory Guide (27)* were adapted for meat products, and those in the *Bacteriological Analytical Manual (30)* were adapted for nonmeat products.

Sample screening. Each sample was divided into two portions for screening and enumeration. For screening, approximately half (up to 100 g) of a sample was aseptically transferred to a sterile stomacher bag and blended with an equal amount of enrichment broth. For Maryland samples, University of Vermont broth 2 (UVM-2 broth) was used as the enrichment broth for luncheon meats, smoked seafood, and seafood salads; phosphate-buffered *Listeria* enrichment broth was used for the other product types. For northern California samples, demi-Fraser broth was used as the enrichment broth for all products. After blending, 50 g of the homogenate was added to 200 ml of enrichment broth and stomached for 1 min. This procedure resulted in a detection sensitivity equivalent to that of the current regulatory methods (i.e., 1 CFU/25 g). The initial sample-blending step was performed to account for the potential heterogeneous distribution of *L. monocytogenes* in the sample. The 250-ml enrichment was incubated at 35°C for 24 ± 2 h

step was confirmed to be an *L. monocytogenes* colony, the isolate was retained. When no isolate for a sample was obtained from the MPN or the direct plating step, the MOX or OXA agar plate held from the screening step was used to recover an *L. monocytogenes* isolate.

The agar plate was examined for suspected *L. monocytogenes* colonies (on a MOX agar plate, distinctive 1- to 2-mm round colonies surrounded by darkened zones of esculin hydrolysis; on an OXA plate, distinctive 1- to 2-mm round colonies surrounded by a black halo) at 24 h and then at 48 h. If suspected colonies were present on a plate obtained from the MPN step or the screening step, up to 20 colonies were picked (by running a loop through them), and streaked onto a horse blood agar plate. Suspected colonies on a plate from the direct plating step were individually picked and point transferred onto a horse blood agar plate. This plate was incubated at 35 ± 2 °C for 19 ± 3 h and examined for the presence of translucent colonies surrounded by a small zone of beta hemolysis. When necessary, colonies from the horse blood plate were restreaked onto a second horse blood plate to obtain isolated colonies. A clearly isolated beta-hemolytic colony, if present, was subjected to further biochemical confirmation. If it was confirmed to be an *L. monocytogenes* colony, all of the beta-hemolytic colonies were considered *L. monocytogenes* colonies. If it was confirmed not to be an *L. monocytogenes* colony, up to two more beta-hemolytic colonies were subjected to biochemical confirmation analysis. If all three colonies were confirmed not to be *L. monocytogenes* colonies, none of the beta-hemolytic colonies were considered *L. monocytogenes* colonies.

Biochemical confirmation was carried out with the use of the API *Listeria* ID strip (bioMérieux, Inc., Hazelwood, Mo.) or the Micro ID *Listeria* kit (Organon Teknica Corp., Durham, N.C.) according to the manufacturer's procedures. Northern California samples collected prior to October 2000 were confirmed by the Micro ID method, and all other samples were confirmed by the API method. Confirmed isolates were retained on Trypticase soy agar with yeast extract slants and sent to the NFPA laboratory in Washington, D.C., for archiving.

Statistical analysis. Contingency table analysis *(15, 22)* was used to determine whether *L. monocytogenes* prevalence levels for the eight product categories differed significantly. The contingency table analysis was based on the chi-square distribution and tested the null hypothesis that percentages of positive samples did not differ significantly among the product categories. For luncheon meat, deli salad, and seafood salad samples, we used a similar approach, the chi-square test for homogeneity, to test the null hypothesis that prevalence did not differ between samples packaged by manufacturers and those packaged in-store. This analysis was performed for percentages for combined Maryland and northern California samples. Chi-square tests were also performed for each of the eight product types to compare prevalence levels for Maryland samples with those for northern California samples. The χ^2 statistic for the ≤5% level of significance was used for the tests.

RESULTS AND DISCUSSION

Sample collection and temperature control. Deli salad and luncheon meat samples were collected over 23 months, whereas samples of the other products were collected over 14 months. A total of 31,705 product samples were tested for *L. monocytogenes*. The desired numbers of seafood salad and smoked-seafood samples were not always available, reflecting the small market for these products in the sampling regions. Consequently, fewer samples of these products than planned were collected for the study. Data on blue-veined and soft mold-ripened cheese samples, which were collected as a single category, were tabulated and analyzed separately because the two cheeses have different characteristics that could have effects on *L. monocytogenes.*

Temperatures experienced by the samples during collection and transport to the laboratories were within the expected range and were, to a certain degree, consistent with what products might experience during a consumer's grocery-shopping trip. Typical temperatures during transportation and temperatures recorded at the laboratory were <5°C. During the first few hours of sample collection, temperatures recorded by the logger were typically <10°C. Occasionally, the logger indicated that the cooler's environment was at 10 to 15°C for a period (less than a few hours, mostly during shopping) but then cooled to <5°C during transport. Such circumstances applied to samples that tested negative as well as to those that tested positive. In the few events in which the temperature exceeded 10°C, the exposure time and the temperature were not likely to allow the growth of more than one generation of *L. monocytogenes* in the products, even if the organism had been present and in a physiological state beyond the lag phase *(5, 28)*. Therefore, the numbers reported here are likely to be equal to or lower than those experienced by consumers, given the home refrigerator temperatures reported in a 1999 Audits International survey (http://www.foodriskclearinghouse.umd.edu/).

Prevalence. Of the 31,705 samples analyzed, 577 tested positive (a 1.82% prevalence rate). Table 1 shows a breakdown of positive samples by product and sampling region. The highest rates of positive samples were those for seafood salads (4.7%) and smoked seafood (4.3%). The prevalence rate for smoked-seafood samples was similar to that reported by Loncarevic et al. *(16)*, who found that 4 of 92 smoked-fish samples tested positive. Higher prevalence rates have been reported for smoked fish obtained from processing plants (7.3% *(19)* and 79% *(6)* in the United States, 34 to 60% in Denmark *(14)*, and about 20% in Italy *(4)*). A prevalence rate of about 22% was reported for smoked fish from retail outlets in Spain *(33)*. For seafood salads, a prevalence rate of 16% was reported for samples from markets in Iceland *(10)*, and a rate of 27% was reported for samples from supermarkets in Belgium *(31)*. In these studies, fewer samples (about 50 to 400 samples) were analyzed. In addition to the fact that the RTE products in our study and the products used in these studies were collected from different regions and at different times, differences in food production and handling practices as well as differences in detection methods may also account for some of the differences in prevalence rates.

The lowest prevalence rates were those for fresh soft cheese (0.17%) and bagged salads (0.74%). Percentages of *L. monocytogenes*–positive samples of deli salads and sliced luncheon meats were considerably lower than expected, at 2.4% and 0.89%, respectively. This compares

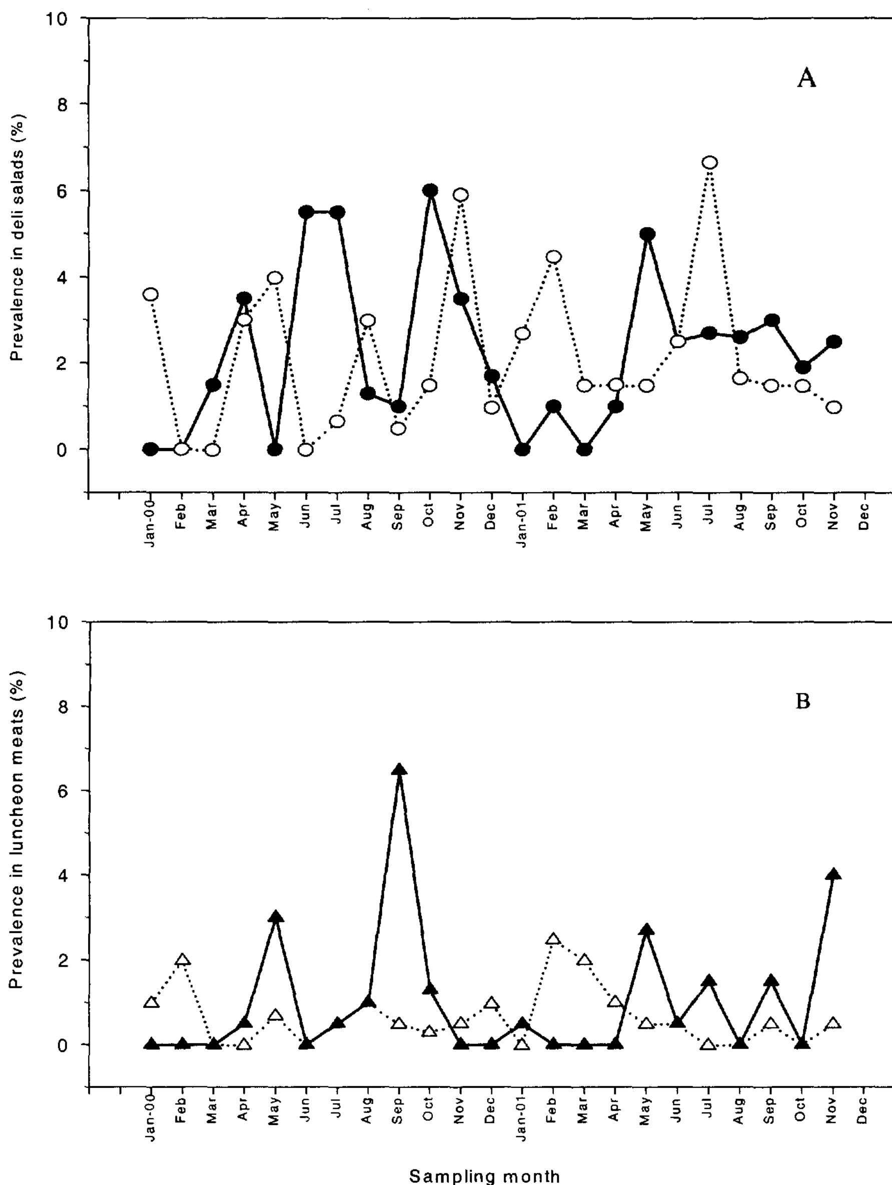

FIGURE 2. *Prevalence of* L. monocytogenes *in deli salads (A) and luncheon meats (B) by month. In both panels, solid and open symbols represent samples collected from the Maryland and northern California FoodNet sites, respectively.*

numbers of positive samples with levels of $>10^2$ CFU/g were those for luncheon meats and smoked seafood (eight and nine samples, respectively). The deli salads, seafood salads, and bagged salads accounted for four more positive samples with levels of $>10^2$ CFU/g. Of the 21 samples with concentrations of $>10^2$ CFU/g, 10 were from northern California and 11 were from Maryland. Only 2 of the 31,705 samples, one smoked-seafood sample from Maryland and one smoked-seafood sample from northern California, had levels of $>10^4$ CFU/g.

In previously published studies, most often only prevalence levels have been reported. For studies in which enumeration was carried out, Uyttendaele et al. *(31)* reported that *L. monocytogenes* was generally detected in small numbers (<10 CFU/g) for processed meat products, while larger numbers of *L. monocytogenes* (>10 CFU/g) were reported for fish and shrimp salads from supermarkets in Belgium. Levels of $>10^2$ CFU/g were reported for 14 of 199 *L. monocytogenes*–positive RTE products from retail displays in Northern Ireland *(32)*. For several studies, levels of contamination under various storage conditions have been reported. In a study on ready-to-use vegetables obtained from a processor in Canada *(21)*, levels of $>10^2$ CFU/g were found for 8 of 120 samples stored at 10°C for up to 11 days, while 5 of these samples had levels of $<10^2$ CFU/g. In the same study, none of 175 samples stored at 4°C after 7 days contained levels of $>10^2$ CFU/g. Contamination levels of $>10^3$ CFU/g were reported for vegetables stored at 10°C *(21)* and levels of $>10^4$ CFU/g were reported for cooked meat products *(32)*. Jørgensen and Huss *(14)* reported that of 76 positive smoked-fish samples, 12 contained 10^2 to 10^3 CFU/g and 4 contained $>10^3$ CFU/g after 14 days of storage at 5°C. *L. monocytogenes* levels as high as 10^5 CFU/g have been reported for smoked fish *(16)*.

Seasonality. The present study provided an opportunity to examine how seasonality affects the occurrence of *L. monocytogenes* in RTE foods. Figure 2 shows a breakdown of *L. monocytogenes* prevalence in deli salads and luncheon meats by month. No obvious seasonality was ob-

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Listeria monocytogenes: Low Levels Equal Low Risk

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ABSTRACT

Because of the public health significance of *L. monocytogenes,* U.S. regulatory agencies established a policy whereby ready-to-eat foods contaminated with the organism at a detectable level are deemed adulterated. This "zero tolerance" policy, however, makes no distinction between foods contaminated at high and low levels. We have reported elsewhere that a survey of over 31,000 ready-to-eat retail food samples, representing eight product categories, showed an overall prevalence rate of 1.82% for these foods. In this study, we used the food survey data in combination with concurrent data regarding illness in the population consuming the foods, together with other variable factors, to derive a dose-response model. The confidence interval for prevalence was 1.68 to 1.97%. *L. monocytogenes* levels, which ranged from −2 to 6 log CFU/g, were adequately described by the distribution beta (0.29, 2.68, −1.69, 6.1). An exponential dose-response model was obtained, with an *R* value (essentially the probability of a single cell causing illness) of 1.76×10^{-10} for the population at the highest risk. A microbial risk assessment based on the model shows that an alternative to the zero tolerance strategy has a greater risk reduction potential and suggests that a management strategy focusing on the concentration of *L. monocytogenes* rather than its presence alone may have a greater impact on the improvement of public health by facilitating the development of control measures to limit the maximum levels of *L. monocytogenes* in foods.

Listeriosis, an infection caused by *Listeria monocytogenes,* occurs relatively infrequently. The Centers for Disease Control and Prevention (CDC) have estimated that 2,500 cases occur each year (5 cases per million people), compared with, for example, 1,400,000 cases of salmonellosis *(28).* More recently, on the basis of data from the FoodNet active surveillance program, the CDC reported a listeriosis frequency of 3 cases per million people for 2000 and 2001 *(7–9).* However, although the incidence of listeriosis cases is comparatively low, the listeriosis case fatality rate of 20% is one of the highest for a foodborne illness *(28)*; thus, it is clearly important to develop appropriate risk management strategies for *L. monocytogenes.*

Almost all listeriosis is foodborne *(28).* One of the factors that makes *L. monocytogenes* particularly difficult to control in foods is that, unlike most foodborne pathogens, it can grow at refrigeration temperatures. *L. monocytogenes* is considered ubiquitous in the environment and has been isolated from a wide variety of foods, including dairy products, meat and poultry products, vegetables, seafood, and other products *(32, 39).* This organism has been isolated from food-processing environments *(2, 14, 17),* from retail products *(16, 33),* and from consumers' homes *(3, 11). L. monocytogenes* has also been isolated from the intestinal tracts of normal, healthy humans *(34).* Although listeriosis can occur in apparently healthy individuals, it is primarily pregnant women and their neonates, elderly people, and immunocompromised individuals who are considered to be at the highest risk *(34).*

Because of the public health significance of *L. monocytogenes,* U.S. regulatory agencies established a policy whereby ready-to-eat (RTE) foods contaminated with the organism at a detectable level are deemed adulterated. Since the establishment of this "zero tolerance" policy in the 1980s, the food industry has made major changes in an effort to eradicate the organism from RTE products and processing environments *(35, 36).* The prevalence of *L. monocytogenes* in certain products has been reduced *(26).* However, data suggest that *L. monocytogenes* cannot be eliminated from the environment or from all food products, and it continues to contaminate RTE products periodically despite the implementation of extensive control measures *(35).* The negative impact of a zero tolerance policy on efforts to control *L. monocytogenes* has recently been described *(35).*

One of the goals of the Healthy People 2010 initiative *(38)* is to reduce illnesses caused by *L. monocytogenes* by 50%. The regulatory approach currently being taken to meet this goal concentrates on further reducing the prevalence of *L. monocytogenes* in RTE foods and continues the zero tolerance standard for all RTE foods. Here, we report findings from a microbial risk assessment that suggest that an alternative to this management strategy may have a greater impact on the improvement of public health by facilitating the development of more effective control measures to achieve the objective.

MATERIALS AND METHODS

Determination of probabilities of illness for different dose levels. A major difficulty in undertaking a microbial risk assessment for *L. monocytogenes* has been the determination of the in-

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fectious dose required to cause illness in humans. An infectious dose is typically determined by feeding studies, i.e., feeding known quantities of a microorganism to a subject to determine the level required to cause illness *(20)*. Owing to the high case fatality rate for listeriosis, human feeding trials carry an unacceptably high risk. Therefore, direct measurement of the infectious dose of *L. monocytogenes* for humans has not been undertaken. As a result, some dose-response assessments have relied on animal data *(6, 20)*. The combination of contamination levels determined in food surveys and data on illnesses determined in epidemiological investigations provides an alternative derivation of a dose-response relationship based on data that are more directly relevant to humans.

In theory, it is conceivable that any dose level can cause illness in the susceptible population. Assuming that a single ingested *L. monocytogenes* cell is capable of causing infection and that when N organisms are consumed each of them has the same probability of causing illness results in an exponential dose-response model *(5, 6, 18, 19)*:

$$P(I) = 1 - e^{-RN} \qquad (1)$$

where $P(I)$ is the probability of listeriosis at dose N, and R is the model parameter specific to the pathogen of concern. According to equation 1, the probability of acquiring listeriosis increases exponentially as the number of cells consumed increases. When a single organism's probability of causing infection is small, this probability is approximately equal to the value of the model parameter, R. As described below, the model parameter is estimated so as to provide a purposely conservative model *(5, 6)*, thereby resulting in an overall conservative assessment of risk in our study.

The model parameter was derived on the basis of the levels of *L. monocytogenes* contamination in foods, the number of listeriosis cases in the population consuming the foods, the size of the population (only individuals at higher risk were considered), the number of servings consumed, and the serving size. The parameter R was determined according to the approach described by Buchanan et al. *(6)*. The prevalence of *L. monocytogenes* in the foods consumed and the shape of the concentration distribution defined the fractions of servings that were contaminated at various levels. In deriving the R value with the use of a spreadsheet in Excel (Microsoft Corp., Redmond, Wash.), calculations were repeated until the actual number of listeriosis cases was predicted on the basis of all other input variables, including prevalence and concentration distribution.

Data used in the assessment. Since almost all listeriosis is foodborne *(28)*, we made the conservative assumption that RTE foods are a primary source of consumer exposure to *L. monocytogenes*. The levels of *L. monocytogenes* contamination in foods were obtained from a food survey reported elsewhere *(16)*. Food samples collected in that survey represented eight RTE product categories: luncheon meats, deli salads, fresh soft cheeses, bagged leafy vegetable salads, blue-veined cheeses, soft mold-ripened cheeses, smoked seafood, and seafood salads. The data from the food survey were used to quantify the prevalence and concentration distribution of *L. monocytogenes* in the foods consumed.

The number of listeriosis cases was obtained from the CDC *(1)*. In order to relate listeriosis to *L. monocytogenes* exposure, we used illness data from the Maryland and northern California FoodNet sites at which the food survey was conducted *(16)*. The food survey was carried out over 2 years (2000 and 2001) when the CDC were conducting a listeriosis case-control study at the FoodNet sites. There were 53 listeriosis cases reported for the FoodNet sites in 2000 and 2001, which would result in an estimated 106 cases for the 2-year sampling period given a twofold multiplier for underreporting *(28)*.

According to Census 2000 *(37)*, the size of the population of the United States was 288,800,000, and the sizes of the populations for the Maryland and California sampling sites where the food survey was conducted were 4,620,000 and 2,220,000, respectively. Therefore, 2.37% of the U.S. population resided in the sampling regions. The size of the higher-risk population was estimated to be 25% of the U.S. population *(15, 29)*, a percentage that also applied to the populations in the regions in which the food survey was conducted and in which illness data were obtained.

The number of servings consumed by the higher-risk U.S. population was estimated to be 1.11×10^{10} per annum for the eight product categories included in the food survey on the basis of national consumption data used in the U.S. Food and Drug Administration–Food Safety and Inspection Service draft *L. monocytogenes* risk assessment *(39)*. These data were adjusted to reflect the actual population in 2000. The number of servings consumed by the higher-risk population in the sampling regions over the 2-year sampling period was calculated as $(1.11 \times 10^{10}) \times 2.37\% \times 2 = 5.26 \times 10^{8}$. Serving size was given a value of 50 g on the basis of the weighted median serving size calculated from the fraction of servings and the median serving size for each of the eight RTE product categories (data not shown).

Assumptions. The theoretical assumption underlying the exponential model is that a single *L. monocytogenes* cell is capable of causing infection (i.e., listeriosis) in a consumer upon ingestion given that the cell is pathogenic and the host is susceptible *(6, 40)*. This assumption also underlies another nonthreshold model established to describe the dose-response relationship for *L. monocytogenes (13)*. The exponential model is also based on the assumptions that each member of the susceptible population responds the same and that the effect of each organism is independent of that of others. Although the exponential model is an expression of the binomial probability of illness, the model is inherently limited in that no experimental data involving *L. monocytogenes* in humans were used in its selection. Unlike non–life-threatening foodborne pathogens, *L. monocytogenes* has not been subjected to human volunteer feeding studies because of its high hospitalization and case fatality rates *(6, 28)*. While the exponential model is one of the well-recognized models, when the mathematical relationship (equation 1) is extrapolated to describe the probability that illness will be caused by low dose levels, consistency between theory and reality has not been experimentally proven.

For this study, we further assumed that all *L. monocytogenes* organisms in foods are pathogenic, consistent with current regulatory policy. We made the conservative assumption that all listeriosis cases at the Maryland and California FoodNet sites resulted from the consumption of the eight product categories by the higher-risk population. Some factors that may influence risk that were not explicitly modeled in our risk assessment include variability in virulence among *L. monocytogenes* subtypes *(41)* and food matrix effect.

Determination of prevalence uncertainty and concentration distribution. The *L. monocytogenes* prevalence used in the risk assessment was the overall observed prevalence and the 2.5th and 97.5th percentiles of the prevalence uncertainty distributions. Data used for calculating prevalence uncertainty levels were the total number of samples (n) and the number of positive samples (s) obtained in the food survey *(16)*. The uncertainty distributions

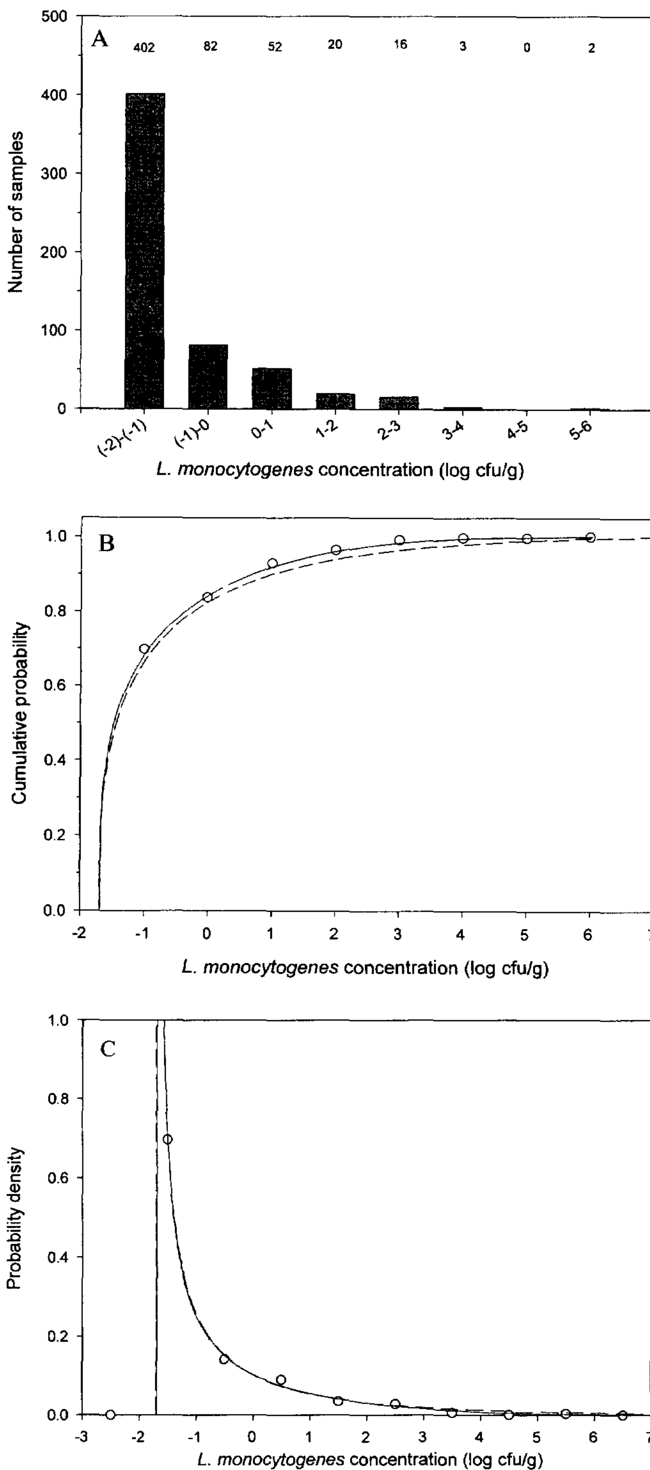

FIGURE 1. *Distribution of* L. monocytogenes *concentrations in samples of ready-to-eat foods in which the organism was detected. (A) Numbers above bars are the numbers of samples in each concentration range (totaling 577 positive samples). (B) Open circles represent observed cumulative frequencies at or below the concentration indicated. (C) Open circles represent observed frequencies in each of the 1-log concentration ranges. In panels B and C, solid and dashed lines represent the probability distribution functions beta (0.29, 2.68, −1.69, 6.1) and gamma (0.33, 2.96) −1.70, respectively.*

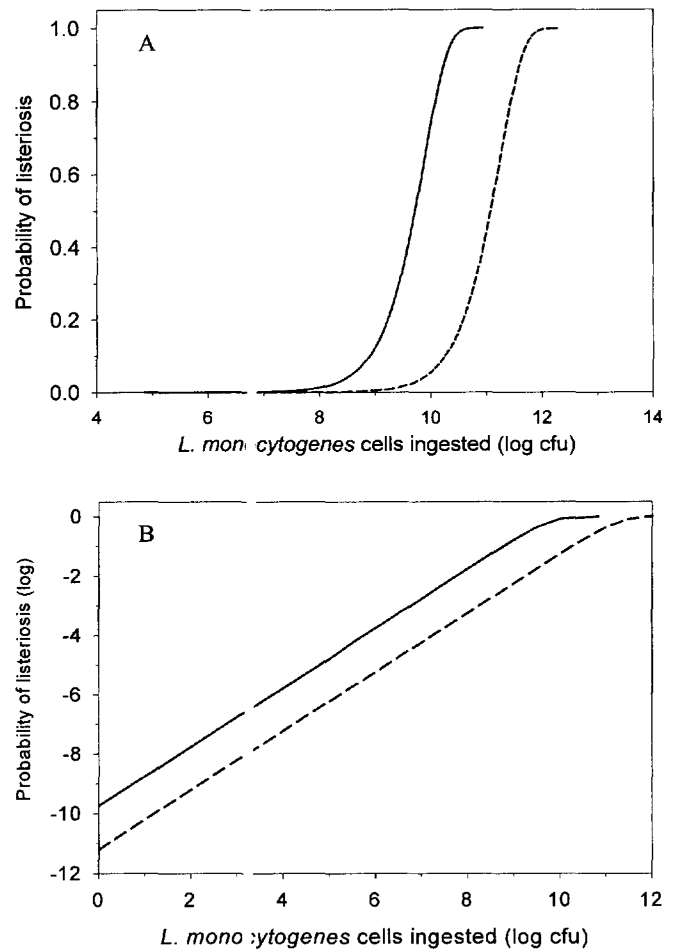

FIGURE 2. *Exponentic ' dose-response models derived from concurrent food survey ar 1 illness data collected at Maryland and northern California Fo ›dNet sites. (A) The models defined by the respective* R *values: 1. 6 × 10^{-10} (solid curve, based on the beta concentration model) c 1d 7.80 × 10^{-12} (dashed curve, based on the gamma concentrati ›n model). (B) Extrapolation of the dose-response models to lo› concentration levels by the plotting of log probability versus log :FU. Solid and dashed lines represent the models with* R *values c f 1.76 × 10^{-10} and 7.80 × 10^{-12}, respectively.*

served maximum le' el (5.18 log CFU/g *(16)*). The maximum concentration esulting from the gamma distribution was, in truncation, 7.)0 log CFU/g. We chose the beta model to represent the (istribution of *L. monocytogenes* concentrations in contai 1inated RTE foods in the subsequent analyses. The 95th-p :rcentile concentration level represented by the beta distri ›ution (1.67 log CFU/g) was used for the analysis underta :en to derive an exponential dose-response model.

Dose-response ınalysis. With the use of the dose-response analysis appr ›ach described by Buchanan et al. *(5)*, a median prevalenc‹ of 1.82%, and the concentration distribution represented by the beta model, our data produced an R value of 1.76 × 10^{-10}. If we used the gamma model instead to represent tl e concentration distribution for *L. monocytogenes* in the fo ›ds, we obtained an R value of 7.80 × 10^{-12}. The dose-res ›onse curves are shown in Figure 2. Although the beta di tribution provided the best fit, analysis

TABLE 1. *Contributions of RTE food servings contaminated at or below various levels to listeriosis cases at two FoodNet sites*[a]

L. monocytogenes level (logCFU/g) in servings	Contribution of servings to listeriosis (cases/year)[b]
0.0	0.0063
1.0	0.034
2.0	0.22
3.0	1.3
4.0	7.5
5.0	29
6.03 (maximum)	106

[a] A total of 53 cases were reported in Maryland and northern California in 2000 and 2001. The number was doubled to 106 to account for potential underreporting as per Mead et al. *(28)*.

[b] Cumulative number of cases for servings contaminated at or below the indicated level, based on a median 1.82% prevalence, the baseline concentration distribution, and the exponential dose-response curve with an R value of 1.76×10^{-10}.

basis of the beta model for concentration distribution, the lower bound and upper bound prevalences would predict that 0.191 and 0.225% of the cases, respectively, would result from the consumption of servings contaminated at concentrations of $\leq 10^2$ CFU/g (Table 2).

Preliminary data from the CDC *(1)* indicate that the frequency of listeriosis cases in the regions comprising the FoodNet sites and during the 2 years of our food survey *(16)* was consistent with a national estimate of 1,700 to 2,500 cases per annum. With the use of the dose-response analysis approach, an estimate of 1,700 cases per annum, a national consumption estimate of 1.11×10^{10} servings for the eight product categories, and the beta concentration model to represent *L. monocytogenes* concentrations, we obtained an R value of 1.34×10^{-10}. If we used an estimate of 2,500 cases per annum instead, we obtained an R value of 1.97×10^{-10}. On a national scale, the relative contribution to illness by food servings contaminated at concentrations of $\leq 10^2$ CFU/g was similar to that obtained for the two FoodNet sites (i.e., 0.19 to 0.22% of the cases occurring each year in the United States would be attributed to such levels of exposure [data not shown]).

It is clear that the most effective efforts to reduce the risk of listeriosis in RTE foods will involve targeting the food servings that are heavily contaminated, even though the fraction of those servings is very small. For example, on the basis of a 1.82% overall prevalence, 0.091% of the servings ($1.82\% \times (1 - 0.95)$) would be contaminated at a concentration above the 95th-percentile level (estimated to be 1.67 ± 0.26 log CFU/g), and an even more minute fraction would be contaminated at higher concentrations (Fig. 1B).

Taking the analysis one step further, we assessed the level of risk reduction that would result from the application of various risk management approaches. The zero tolerance strategy is in essence a prevalence-oriented approach that does not distinguish foods contaminated at high concentrations from those contaminated at low concentrations. With the concentration distribution unchanged, a 50% reduction in prevalence would result in a 50% risk reduction, e.g., from 106 to 53 cases (Table 3). Alternatively, with the prevalence unchanged, a control strategy that stipulates a maximum *L. monocytogenes* concentration of 10^4 CFU/g for all servings would reduce risk by 89%, e.g., from 106 to <12 cases, on the basis of the beta concentration distribution and the associated exponential model parameter. The targeting of a maximum concentration at a lower level would achieve an even higher level of risk reduction than a 50% reduction in prevalence would. A control strategy that stipulates a maximum *L. monocytogenes* concentration of 10^2 CFU/g for all servings would result in a 99.5% risk reduction, e.g., from 106 cases to <1 case (Table 3). With the food survey data being extrapolated to a national scale, on the basis of 1,700 cases per annum and the *L. monocytogenes* concentrations represented by the beta distribution, the stipulation of a maximum *L. monocytogenes* concentration of 10^2 CFU/g for all servings consumed by the higher-risk U.S. population would also result in a risk reduction of 99.5%, from 1,700 to <9 cases (data not shown). Clearly, a risk management approach that actively manages the levels of *L. monocytogenes* can have a greater impact on the reduction of cases of listeriosis than sole reliance on the reduction of the organism's prevalence (i.e., zero tol-

TABLE 2. *Influence of prevalence uncertainty on estimated listeriosis cases resulting from the consumption of RTE food servings contaminated with* L. monocytogenes *at or below 10^2 CFU/g at two FoodNet sites*

Prevalence (%)	Number (%) of cases (n = 106)
1.68 (lower bound)	0.202 (0.191)
1.82 (median)	0.219 (0.207)
1.97 (upper bound)	0.238 (0.225)

TABLE 3. *Predicted numbers of cases for all servings under various scenarios*

	Prevalence[b]		
L. monocytogenes concn[a]	Baseline[c]	Decreased by 50%	Increased by 100%
Baseline (beta distribution)	106	53	212
Decreased to a maximum of 10^4 CFU/g	11.9	—	—
Decreased to a maximum of 10^2 CFU/g	0.55	—	1.10

[a] Concentrations are levels given by the baseline distribution or by the same distribution truncated at the set maximum level, e.g., 10^2 CFU/g.

[b] Prevalence baseline is the observed frequency, which is allocated across the concentration spectrum of the beta distribution (shown in Fig. 1B). When a maximum concentration is set, the fraction representing servings contaminated at a higher level is added to the fraction at the set level, e.g., 10^2 CFU/g. A total of 53 cases were reported at two U.S. FoodNet sites (Maryland and northern California) in 2000 and 2001. The number was doubled to 106 to account for potential underreporting as per Mead et al. *(28)*. —, not calculated.

[c] Overall prevalence rate, 1.82%.

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