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Quantitative Risk Assessment of *Listeria monocytogenes* in French Cold-Smoked Salmon: II. Risk Characterization

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A model for the assessment of exposure to *Listeria monocytogenes* from cold-smoked salmon consumption in France was presented in the first of this pair of articles (Pouillot *et al*., 2007, Risk Analysis, 27:683–700). In the present study, the exposure model output was combined with an internationally accepted hazard characterization model, adapted to the French situation, to assess the risk of invasive listeriosis from cold-smoked salmon consumption in France in a second-order Monte Carlo simulation framework. The annual number of cases of invasive listeriosis due to cold-smoked salmon consumption in France is estimated to be 307, with a very large credible interval ([10; 12,453]), reflecting data uncertainty. This uncertainty is mainly associated with the dose-response model. Despite the significant uncertainty associated with the predictions, this model provides a scientific base for risk managers and food business operators to manage the risk linked to cold-smoked salmon contaminated with *L. monocytogenes.* Under the modeling assumptions, risk would be efficiently reduced through a decrease in the prevalence of *L. monocytogenes* or better control of the last steps of the cold chain (shorter and/or colder storage during the consumer step), whereas reduction of the initial contamination levels of the contaminated products and improvement in the first steps of the cold chain do not seem to be promising strategies. An attempt to apply the recent risk-based concept of FSO (food safety objective) on this example underlines the ambiguity in practical implementation of the risk management metrics and the need for further elaboration on these concepts.

KEY WORDS: *Listeria monocytogenes*; risk assessment; second-order Monte Carlo simulations

1. INTRODUCTION

During the 1980s, several listeriosis outbreaks linked to the consumption of cheese and raw vegetables led to the recognition of human listeriosis as a foodborne disease.(1) Noninvasive listeriosis is a mild form disease that leads to febrile gastroenteritis, whereas invasive listeriosis is a systemic, life-threatening disease that particularly affects persons with underlying conditions that impair their immune response. Patients demonstrate increased risk if they are pregnant, elderly, or have an underlying pathology, such as cancer, blood malignancy, organ transplant, chronic hemodialysis, liver failure, diabetes, or AIDS.(2) The present study considered only invasive listeriosis, hereinafter referred to simply as listeriosis.

Since 1999, surveillance of human listeriosis in France has included mandatory notification of cases, and the sensitivity of this surveillance system

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for reporting diagnosed cases of listeriosis is high (estimated 0.87 in 2001).[2] The annual incidence of diagnosed and reported listeriosis in France declined from approximately 4.5 cases/million persons during the 1999–2000 period (i.e., approximately 265 cases/year) to approximately 3.5 cases/million persons during the 2001–2005 period (i.e., approximately 220 cases/year), paralleling a substantial reduction in the proportion of *Listeria monocytogenes* contaminated products.[3,4] In 2006, the annual incidence increased to 4.6 cases/million persons (i.e., 290 cases).[4] This tendency was confirmed in January–June 2007 and was observed throughout Europe.[4,5] Although rare, listeriosis is very severe and has a high case-fatality rate, thus accounting for a significant part of foodborne disease-related deaths: 14–34% in France (second to salmonellosis),[6] 11% in England and Wales (fourth after salmonellosis, botulism, and campylobacteriosis),[7] and 28% in the United States (second to salmonellosis).[8]

The foods that could be associated with listeriosis transmission are mostly ready-to-eat (RTE) foods that support *L. monocytogenes* growth,[5] notably cold-smoked salmon (CSS), as highlighted by earlier quantitative risk assessments (QRA).[9–12] Exposure to *L. monocytogenes* per serving of CSS in France was modeled in our first study,[13] based on specifically acquired data describing the French situation in the early 2000s.[14–17] The present study combines these previous outputs with frequency consumption data[18,19] and a dose-response model based on an internationally accepted hazard characterization model[10] and specific French epidemiological data. The potential uses of this risk assessment are then discussed in terms of epidemiology and risk management.

2. MODEL AND DATA

Risk characterization is performed by combining the results of an exposure assessment with a dose-response model (hazard characterization).[20]

2.1. Scope and Hazard Identification

The scope of the present risk assessment is the hazard—food combination *L. monocytogenes*—CSS, under baseline conditions (i.e., only sporadic contaminations are taken into account) in France in the early 2000s. For an extensive hazard identification, we refer to the recent opinion of the European Food Safety Agency.[5]

2.2. Exposure Assessment

2.2.1. Exposure per Serving

The exposure assessment was fully described in the first article of the pair.[13] This study led to the assessment of exposure to *L. monocytogenes* per serving of CSS in France in the early 2000s. The general modeling framework was a second-order (or two-dimensional) Monte Carlo simulation[21,22] that allowed for separated characterization of the uncertainty and variability in the exposure estimates. The procedure is fully described and illustrated in the first article of the pair.[13] Briefly, input parameters were classified as reflecting either uncertainty or variability, and these two kinds of variations were transferred separately throughout the model within the Monte Carlo simulation until outputs.[13] The model accounted for competitive bacterial growth between *L. monocytogenes* and food flora for the part of the process ranging from the end of the production line to the consumer phase. The final output was the two-dimensional (i.e., uncertain and variable) exposure to *L. monocytogenes* from consumption of a CSS serving (E_{serv}, $\log_{10}$ cfu/serving).

2.2.2. Number of Servings per Year

The number of servings per year was assessed in the present study based on two consumption surveys. "*INCA2*" was conducted in France between December 2005 and May 2007 on 4,079 individuals aged 3 years and older, with all food intakes reported during a seven-day period.[18] "*Bébés 2005*" was conducted in France between January 2005 and March 2005 on 706 infants aged 0–36 months, with all food intakes reported during a three-day period.[19] Specific records of the consumption of CSS extracted from these studies are provided in Table I. Based on these results, the mean annual number of CSS servings per inhabitant was estimated at $S = 6.4$, after correcting for the proportion of each age category in the French population. An uncertainty distribution around this estimate was derived using the parametric bootstrap method,[21] assuming a binomial distribution of the number of days with CSS consumption within each age category. The 95% credible interval (CI95, evaluated from the 0.025th quantile and 0.975th quantile of the uncertainty distribution) of this statistic was estimated to be [5.9; 7.1].

Table I. Consumption Data Used to Evaluate the Number of Cold-Smoked Salmon Servings per Year

Age Category	Proportion of the French Population (%)[56]	Individual × Days of Study	Individual × Days with One CSS Serving	Annual Intake of CSS Servings[a]	Reference
<3 years old	2.66	2,118	5	0.86	19
[3–14]	15.20	7,109	89	4.57	18
[15–64]	65.48	18,665	356	6.96	18
≥ 65 year old	16.66	2,395	46	7.01	18

[a](individual × days with one CSS serving)/(individual × days of study) × 365.
CSS = cold-smoked salmon.

2.3. Hazard Characterization

The exponential dose-response equation[23] was selected as the dose-response model. The single parameter of this equation characterizes the bacterium-host interaction and is believed to differ between subpopulations.[10] We considered four exclusive subpopulations: (i) the "pregnant" subpopulation; (ii) the "susceptible" subpopulation, defined as the subpopulation of individuals with one of the following risk factors: cancer (all types), dialysis, transplant, liver cirrhosis, AIDS, and diabetes (all types), regardless of age; (iii) the ≥65 years of age subpopulation with none of the preceding risk factors, denoted as the "elderly" subpopulation; and (iv) the <65 years of age subpopulation with none of the preceding risk factors, denoted as the "reference" subpopulation.

2.3.1. Hazard Characterization for the "Reference" Subpopulation

For the "reference" subpopulation, the exponential dose-response equation is:

$$R_{i\in r} = 1 - \exp\left(-r_r \times 10^{E_{\text{serv}_i}}\right),$$

where $R_{i\in r}$ is the probability of invasive listeriosis for individuals issuing from the "reference" subpopulation after exposure to a product with an expected contamination of $10^{E_{\text{serv}_i}}$ cells, assuming a Poisson distribution of the cells between servings. Here, r_r is the probability of invasive listeriosis from exposure to one cell for the reference subpopulation.[23]

The parameter r_r was estimated based on methodology and data proposed in the Food and Agriculture Organization/World Health Organization (FAO/WHO) "Risk assessment of *L. monocytogenes* in ready-to-eat foods" report.[10] In this report, *r*-values were derived from estimates of the frequency and distribution of consuming *L. monocytogenes*[11] and estimated number of cases of listeriosis[8] in the United States. As no such exposure data are available in France, these U.S. data were used to derive r_r, assuming that the "reference" subpopulation in France as defined above and the "healthy" population as defined in the FAO/WHO report share the same susceptibility. The estimation procedure proposed in the FAO/WHO report was implemented while taking into account the uncertainty in the original data and assuming that all contamination levels contributed to the cases of listeriosis (see the Appendix).

2.3.2. Relative Risk of Listeriosis in the Subpopulations

For the "pregnant," "susceptible," and "elderly" subpopulations, the model was anchored to French epidemiological data such that the risk of listeriosis from consumption of CSS in these subpopulations relative to the "reference" subpopulation was equal to the relative risk of listeriosis observed in France for these categories.

Mandatory notifications of invasive listeriosis cases reported to the national public health institute (Institut de Veille Sanitaire) from 2001 to 2004 were reviewed. Numbers of cases were tabulated based on age and underlying conditions. Among the 853 cases reported during the four-year period, $C_p = 195$ cases were associated with pregnancy (i.e., pregnant women, miscarriage, stillbirth, or newborn <1 month old); $C_s = 385$ cases were observed in the "susceptible" subpopulation as defined above; $C_e = 181$ cases were observed in the "elderly" subpopulation; and $C_r = 92$ cases were observed in the remaining "reference" subpopulation. From these data, the number of cases expected in each subpopulation for one case in the "reference" subpopulation may be calculated

as follows: $RC_p = 195/92 = 2.1$ cases in the "pregnant" subpopulation, $RC_s = 385/92 = 4.2$ cases in the "susceptible" subpopulation, and $RC_e = 181/92 = 2.0$ cases in the "elderly" subpopulation. The mean numbers of individuals in each subpopulation during the studied period were estimated at $N_p = 798{,}000$ for pregnant women, $N_s = 3{,}987{,}000$ for the "susceptible" subpopulation, $N_e = 7{,}749{,}000$ for the "elderly" subpopulation, and $N_r = 49{,}090{,}000$ for the "reference" subpopulation. These data led to the following relative risks of invasive listeriosis compared to the reference subpopulation during this four-year period: $RR_p = \frac{C_p}{4\times N_p} / \frac{C_r}{4\times N_r} = \frac{195}{4\times 798{,}000} / \frac{92}{4\times 49{,}090{,}000} = 130$ for the "pregnant" subpopulation and, using a similar formula, $RR_s = 52$ for the "susceptible" subpopulation, and $RR_e = 12$ for the "elderly" population. It was not possible to evaluate the uncertainty around the demographic statistics N_p, N_s, N_e, and N_r. Uncertainty distributions were derived for these statistics (RC_x and RR_x) using the parametric bootstrap method[21] assuming that cases are sporadic and independent (Poisson process)[22] and no uncertainty in the number of individuals in each subpopulation. This procedure leads to CI95s equal to [103; 168], [42; 66], and [9.7; 16] for RR_p, RR_s, and RR_e, respectively. We assumed that RR_p, RR_s and RR_e do not depend on the consumed product, i.e., that the relative risks observed for all sources of *L. monocytogenes* are equal to the relative risks linked to the consumption of CSS.

2.4. Risk Characterization

2.4.1. Main Outputs

The mean risk of invasive listeriosis due to consumption of a CSS serving in the "reference" subpopulation, denoted R_r, was estimated in a second-order Monte Carlo simulation framework [21,22] as:

$$R_r = \lim_{n\to\infty} \frac{\sum_{i=1}^{n} 1 - \exp\left(-r_r \times 10^{E_{\text{serv}_i}}\right)}{n},$$

where E_{serv_i} is the two-dimensional output of the exposure assessment,[13] and r_r is the one-dimensional (uncertain) parameter of the dose-response. This estimation was performed within each variability dimension, i.e., independently for each set of uncertain parameters.[13] This allowed for estimation of the uncertainty in R_r. The mean risks for invasive listeriosis from consumption of a CSS serving in the "pregnant," "susceptible," and "elderly" subpopulations, and the overall population, respectively, denoted R_p, R_s, R_e, and R, are then estimated using

$$\begin{cases} R_p = R_r \times RR_p \\ R_s = R_r \times RR_s \\ R_e = R_r \times RR_e \\ R = \dfrac{N_r \times R_r + N_p \times R_p + N_s \times R_s + N_e \times R_e}{N_r + N_p + N_s + N_e} \end{cases}$$

in a one-dimensional Monte Carlo simulation, taking into account the uncertainty in the R_r and RR_x parameters. The expected number of cases in each subpopulation is estimated as

$$\begin{cases} n_r = N_r \times R_r \times S \\ n_p = n_r \times RC_p \\ n_s = n_r \times RC_s \\ n_e = n_r \times RC_e \\ n = n_r + n_p + n_s + n_e \end{cases}$$

in a one-dimensional Monte Carlo simulation, taking into account the uncertainty in the R_r, S, and RC_x parameters.

2.4.2. Ranking of Uncertainty Sources

A sensitivity analysis was performed on the Monte Carlo simulation results to evaluate how the uncertainty of the input parameters influences the uncertainty of n_r, the predicted number of cases in the "reference" population. For input parameters that were uncertain and variable, the variability dimension was collapsed into two summary statistics calculated over the variability dimension: the mean and the standard deviation. An analysis of variance (ANOVA) was performed after partitioning continuous parameters into 10 levels based on evenly spaced percentiles.[24] The first ANOVA model included all uncertain parameters used to derive n_r, including inputs for the exposure assessment model detailed in the first article.[13] A procedure using backward and forward selection of variables based on the Akaike information criteria (AIC) was used to obtain the final ANOVA model.[25]

2.4.3. Ranking of Mitigation Strategies ("What-If" Analyses)

Seventeen mitigation strategies were proposed to test the influence of risk management measures on the estimated number of cases. The six alternative models presented in the first article of the pair[13] were tested, as well as 11 new models. These risk

mitigation strategies are plausible measures concerning (i) the shelf-life or the limit time of storage at home; (ii) the prevalence or the initial contamination at the factory; and (iii) the storage temperature in the retail display cabinet and in domestic refrigerators. One mitigation strategy modeled the impact of a prevention campaign that would lead to a decrease in CSS consumption in subpopulations with increased susceptibility. We assumed that CSS consumption would be reduced by a factor of 50% in the "susceptible," "pregnant," and "elderly" subpopulations. For each of these 17 strategies, the efficacy of the mitigation strategy was assessed by calculating the ratio between the total number of listeriosis cases in the "what-if" scenario and the baseline method within each uncertainty simulation of the Monte Carlo simulation.

The entire procedure was developed using the R software (© The R Core Team),[26] and is available on request to the corresponding author. Each scenario was tested on a set of 10,000 variability iterations and 10,000 uncertainty iterations.

3. RESULTS

Table II presents the results for the estimated mean risk and the expected number of cases in each subpopulation. According to our best estimates [with 95% credibility intervals], one case of invasive listeriosis is expected per

- either 73,000 [1,800 to 2,310,000] CSS servings consumed by "pregnant" women;
- or 180,000 [4,600 to 5,800,000] CSS servings consumed by "susceptible" individuals (individuals with cancer, dialysis, transplants, liver cirrhosis, diabetes, or AIDS);
- or 760,000 [19,000 to 24,000,000] CSS servings consumed by "elderly" individuals (individuals $\geq$65 years of age with none of the cited pathologies);
- or 9,500,000 CSS servings [240,000 to 300,000,000] CSS servings consumed by "reference" individuals (individuals <65 years of age with none of the cited pathologies);
- or 1,300,000 [32,000 to 40,000,000] CSS servings consumed overall in France.

These estimates lead to wide credibility intervals associated with the number of estimated cases, for an overall prediction of 307 [10; 12,453] annual cases of invasive listeriosis linked to the consumption of CSS in France.

The results of the ANOVA exploring the sources of uncertainty in the model are expressed in Table III. For some factors, the p-value was extremely low. Indeed, this parameter value has no meaning, since p-values are determined by the number of iterations, 10,000 in this analysis: p-value levels should only be considered relative to the level observed for other factors. These results clearly indicate that the uncertainty in r_r, the parameter for the dose-response model, was the main factor influencing the uncertainty in the predicted number of cases in the "reference" population. Other influential factors were predictive microbiology model parameters, in particular the maximum achievable density of bacteria in CSS. Nevertheless, a large part of the variance remains unexplained using this ANOVA method.

Table IV shows the results obtained by modeling the various risk mitigation strategies. These 17 strategies are ranked according to the decreasing number of predicted listeriosis cases. This table also quotes the ranking of the six mitigation strategies tested in the first article, ordered based on decreasing 99th percentile of exposure and decreasing probability of observing a serving containing $>10^8$ cfu of *L. monocytogenes*.[13] Obviously, the risk mitigation strategies lead to lower exposure than the baseline model and, consequently, to lower risk. The strategies leading to the lowest risks were essentially those concerning the consumer phase, reducing either duration (by shortening shelf-lives or establishing a

Subpopulation	Mean Risk per Serving	Predicted Number of Cases per Year
Pregnant	1.4×10^{-5} [4.3×10^{-7}; 5.5×10^{-4}]	70 [2; 2,866]
Susceptible	5.4×10^{-6} [1.7×10^{-7}; 2.2×10^{-4}]	139 [4; 5,653]
Elderly	1.3×10^{-6} [4.1×10^{-8}; 5.3×10^{-5}]	65 [2; 2,651]
Reference	1.0×10^{-7} [3.3×10^{-9}, 4.3×10^{-6}]	33 [1; 1,345]
Overall	7.8×10^{-7} [2.5×10^{-8}, 3.1×10^{-5}]	307 [10; 12,453]

Table II. Mean Risk of Invasive Listeriosis per Serving and Expected Number of Cases per Year from Consumption of Cold-Smoked Salmon in France, According to the Considered Subpopulations [95% Credibility Interval]

Table III. Results of the ANOVA Evaluating the Impact of Uncertain Parameters on the Predicted Number of Listeriosis Cases in the Reference Population

Parameter	Total Variance (%)	*df*	*p*-value
r parameter of the dose-response model in the reference population (r_r)	15.7	9	$<10^{-300}$
Standard deviation of the maximum achievable bacterial population density in CSS (*MPD*)	4.9	9	1.8×10^{-137}
Mean growth rate of *L. monocytogenes* in a CSS at a reference temperature of 25°C ($\mu_{\text{ref,Lm}}$)	3.7	9	1.1×10^{-101}
Mean of MPD	2.8	9	3.5×10^{-76}
Mean of the minimal temperature for growth of *L. monocytogenes* in CSS ($T_{\text{min,Lm}}$)	0.5	9	4.7×10^{-12}
Mean growth rate of the food flora in a CSS at a reference temperature of 25°C ($\mu_{\text{ref,ff}}$)	0.4	9	1.8×10^{-8}
Prevalence of contaminated CSS	0.3	9	1.2×10^{-6}
Standard deviation of $T_{\text{min,Lm}}$	0.3	9	3.2×10^{-6}
Mean of the minimal temperature for growth of food flora in CSS ($T_{\text{min,ff}}$)	0.2	9	3.1×10^{-4}
*S*tandard deviation of $T_{\text{min,ff}}$	0.2	9	4.7×10^{-4}
Number of CSS servings per year	0.2	9	1.1×10^{-2}
Standard deviation of the serving size	0.1	9	8.8×10^{-2}
Residuals	70.7	9,891	

CSS = cold-smoked salmon.

Table IV. Risk Mitigation Strategies Ranked According to the Predicted Number of Listeriosis Cases Due to Consumption of Cold-Smoked Salmon in France

Risk Mitigation Strategy	Value in the Baseline Model	Rank Based on the 99th Percentile of Exposure at Consumption[13]	Rank Based on the Probability of Exposure $>10^8$ cfu/Serving[13]	Predicted Listeriosis Cases Compared to a Base 100 for the Baseline Model
Baseline model		1	1	100
Initial contamination: <10 cfu/g	No limit			100 [94; 100]
Effective shelf-life: 25 days	Up to 32 days			93 [56; 100]
Initial contamination: <1 cfu/g	No limit	2	2	92 [73; 99]
Mean retail temperature: N (3.6°C, 2.2°C)	N (4.6°C, 2.2°C)			80 [53; 93]
Shelf-life: 21 days	Up to 32 days			73 [27; 93]
Mean retail temperature: 4°C	N (4.6°C, 2.2°C)	5	3	67 [31; 92]
Mean retail temperature: N (2.6°C, 2.2°C)	N (4.6°C, 2.2°C)			66 [35; 87]
Consumed within 10 days after purchase	Up to 32 days			58 [18; 84]
Initial contamination: <1 cfu/50 g	No limit			55 [24; 80]
Reduced CSS consumption: 50% in increased susceptibility subpopulations				55 [54; 56]
Prevalence: 0.5 baseline model	1	7	4	50 [50; 50][a]
Mean refrigerator temperature: N (5.0°C, 3.0°C)	N (7.0°C, 3.0°C)			49 [18; 76]
Consumed within 7 days after purchase	Up to 32 days	4	5	37 [9; 67]
Mean refrigerator temperature: N (4.0°C, 3.0°C)	N (7.0°C, 3.0°C)			34 [10; 64]
Prevalence: 0.25 baseline model	1			25 [25; 25][a]
Shelf-life: 15 days	Up to 32 days	3	6	23 [4; 56]
Mean refrigerator temperature: 4°C	N (7.0°C, 3.0°C)	6	7	23 [5; 53]

[a]Note that these results are trivial according to the model, and can be directly extrapolated to other decreases in prevalence.

maximum duration between purchase and consumption) or temperature (by lowering the mean temperature in home refrigerators by 2°C or 3°C). We further observed that the predicted impact of the risk mitigation on risk in terms of the number of listeriosis cases is clearly correlated to the frequency of exposure $>10^8$ cfu/serving, whereas it is less correlated to the 99th percentile of exposure.

4. DISCUSSION

The proposed risk assessment model accounts for the uncertainty and variability associated with exposure to *L. monocytogenes* from CSS consumption and of susceptibilities to this hazard on the basis of data obtained in France specifically during the early 2000s.

Table V. Comparison Between the Results of the Present Study and Other Estimations Present in the Literature

QRA: Food, Country[reference]	Dose-Response Model (Reference)	Mean Risk per Serving (Overall Population)
Smoked (and gravad) seafood		
Smoked and gravad salmon and trout, Sweden[12]	12	2.6×10^{-5}[a]
Same study[12]	9	5.6×10^{-6}[a]
CSS, France, present study	Adapted from[10]	7.8×10^{-7}
Smoked fish, Germany[9]	9	1.3×10^{-7}[a]
Cold-smoked fish, world[10]	10	5.3×10^{-8}
Smoked seafood, United States[11]	11	6.2×10^{-9}
Other seafood ready-to-eat products		
Cooked ready-to-eat crustaceans, United States[11]	11	5.1×10^{-9}
Preserved fish, United States[11]	11	2.3×10^{-11}
Raw seafood, United States[11]	11	2.0×10^{-11}
Meat-based ready-to-eat products		
Deli meats, United States[11]	11	7.7×10^{-8}
Frankfurters (not reheated), United States[11]	11	6.5×10^{-8}
Pâté and spread meats, United States[11]	11	3.2×10^{-8}
Dry cured ham, Italy[57]	An earlier version of[10]	Between 4.7×10^{-10} (normal adult population) and 6.1×10^{-7} (most susceptible subpopulation)
Frankfurters (reheated), United States[11]	11	6.3×10^{-11}
Dry/semidry fermented sausages, United States[11]	11	1.7×10^{-11}
Fermented meat products, world[10]	10	2.1×10^{-12}
Dairy ready-to-eat products		
Unpasteurized milk, United States[11]	11	7.1×10^{-9}
Pasteurized milk, world[10]	10	5×10^{-9}
High fat and other dairy products, United States[11]	11	2.7×10^{-9}
Various cheese categories, United States[11]	11	From 1.8×10^{-9} to 4.5×10^{-15} (depending on the category)
Pasteurized milk, United States[11]	11	1.0×10^{-9}
Brie (soft cheese), France[58]	10	3.5×10^{-11}
Ice cream, world[10]	10	1.4×10^{-11}
Camembert (soft cheese), France[58]	10	5.1×10^{-12}
Ice cream and frozen dairies, United States[11]	11	4.9×10^{-14}
Cultured milk products, United States[11]	11	3.2×10^{-14}
Other ready-to-eat products		
Fruits, United States[11]	11	1.9×10^{-11}
Vegetables, United States[11]	11	2.8×10^{-12}
Deli-type salads, United States[11]	11	5.6×10^{-13}

[a]Mean risks per serving were given in the original publications[9,12] for the high-risk population (20% of the overall population), assuming that only members of the high-risk population become ill. In this table, the published risks were multiplied by 0.20 to obtain risks in the overall population.
QRA = quantitative risk assessments.

Validation of this QRA model may be attempted by comparing the obtained results to other published QRA and to available epidemiological data. The estimated mean risk of listeriosis per CSS serving obtained in this study is on the order of magnitude of that obtained in other studies (Table V). Observed differences within the category of seafood RTE products may be explained by (i) differences in the scopes of the assessments (e.g., only CSS vs. all smoked seafood); (ii) differences between the modeled food chains (e.g., initial contamination, time-temperature profiles), which reflect intercountry variability; and (iii) differences in modeling options (e.g., different growth models, different dose-response models), which reflect modeling uncertainty. Despite these differences, our results confirm that the estimated

risk associated with consumption of seafood RTE is generally higher than that for other products, reflecting the prevalence and ability to grow of *L. monocytogenes* in these products.

The results obtained from QRA models should not be in contradiction with epidemiological data observed in the same area during the same period of time, if available. The number of listeriosis cases in France due to CSS consumption is unknown. Indeed, source attribution is difficult for foodborne diseases. This is specifically the case for listeriosis, which is predominantly sporadic.[5] With a very crude analysis of epidemiologic data, the range of plausible values for the annual incidence of listeriosis due to CSS in France can be roughly estimated as follows: in the early 2000s, the incidence of diagnosed listeriosis in France with regard to all exposure sources was approximately 220 cases per year.[3,4] Assuming that 10% of listeriosis cases are not diagnosed and that 87% of diagnosed cases are reported,[2] the maximum annual incidence, including undiagnosed cases and diagnosed but unreported cases, would be approximately 280 cases per year. Given that 33% of patients have consumed smoked fish at least once during the two months preceding the symptoms,[27] the maximum boundary for plausible values of the annual incidence of listeriosis due to CSS would be approximately 100 cases. The minimal boundary would be 0, as the link between listeriosis and CSS consumption has never been clearly established for any of these French cases. Thus, the range of plausible estimates of the incidence of listeriosis in France due to CSS per year from epidemiological data is [0; 100]. This range overlaps the credible interval [10; 12,453] obtained through QRA modeling, but is clearly narrower.

In fact, the uncertainty expressed through credible intervals of the estimated mean risk and credible intervals of the expected number of cases linked to CSS consumption is extremely wide. This reflects key data gaps in the inputs. A large part of the uncertainty cannot be explained on the basis of an ANOVA, illustrating the difficulty of studying this nonlinear model.[28] It is difficult to test the normality and homoscedasticity assumptions needed for the ANOVA validity when the number of data points is 10,000, as determined by the number of iterations. Nevertheless, such a model-free and global method is recommended as the most appropriate choice for sensitivity analysis because of typical characteristics of exposure and risk assessment model.[28] This analysis indicates that the dose-response model parameter and some bacterial growth model parameters are key issues in such models, in comparison to, e.g., consumption parameters. Indeed, the exponential dose-response model parameter is obviously a fundamental parameter in the model. Using our hazard characterization based on the assumptions from the FAO/WHO report,[10] the probability of contracting invasive listeriosis for the reference population following the consumption of a very high number of *Listeria* cells, e.g., 10^{10}, is estimated with a credible interval $[1.4 \times 10^{-5}, 2.6 \times 10^{-3}]$. This probability is thus known within a scale of uncertainty of 1 to approximately 190. Further refinement of the *L. monocytogenes* dose-response should be developed to provide more accurate estimates of *L. monocytogenes*-associated risks. Among the predictive microbiology parameters, the maximum population density achievable in CSS seems influential, indicating both its importance and the lack of knowledge about it. A better knowledge of this parameter is essential for evaluating such hazards, for which infectious cases are mainly due to consumption of products with a level of bacteria reaching or approaching this level.[10,29] In the present model, the impact of this parameter was reinforced by the use of the "Jameson effect," originally defined in enrichment broths,[30–33] and recently extended to foods and in particular to CSS.[17,34–38] Thus, we modeled the interaction between *Listeria* and the background flora, assuming a simultaneous halt in the growth of *Listeria* and the background flora as soon as one or the other reaches this maximum level.[15,17] This maximum population density is rarely specifically studied in the predictive microbiology literature. The present uncertainty analysis highlights the necessity of gaining greater knowledge about this parameter.

One should keep in mind that only parameter uncertainty, i.e., a small part of the global uncertainty, was evaluated by the procedure used in our modeling framework. The global uncertainty should also include scenario uncertainty (descriptive errors, aggregation errors, errors in professional judgment, incomplete analysis), as well as model uncertainty (uncertainty due to necessary simplification of real-world processes, incorrect specification of the model structure, model misuse, use of inappropriate surrogate variables).[39,40] These other sources of uncertainty may be qualitatively explored through the main assumptions of the exposure assessment model[13] and two additional assumptions of this risk characterization.

First, we assumed that the value of the relative risk of listeriosis observed for all sources of *L. monocytogenes* could be used for the relative risk of listeriosis from CSS consumption only. This assumption may be justified if variations both in the exposure to *L. monocytogenes* according to the subpopulations and in the hazard characterization according to the subpopulations do not depend on the food considered. This latter hypothesis is very commonly assumed in QMRA. Nevertheless, it may be contradicted by studies suggesting an effect of foods on the *in vitro* virulence-associated phenotype levels of different *L. monocytogenes* strains.[34]

Second, the "reference" subpopulation in this study was assumed to share the same susceptibility as the "healthy" population defined in the FAO/WHO report[10] in the absence of available contamination data in France. This assumption is strong, and the relatively high number of cases predicted in our model could be linked to an overestimation of the r_r parameter. Nevertheless, it is justified by the similar definitions of the reference populations in both studies. Statistically speaking, the "healthy" population corresponds to 80–85% of the U.S. population for 80–98% of the cases,[10] while our "reference" subpopulation corresponded to 80% of the French population for 89% of the cases.

In conclusion, for this discussion about uncertainty, the present study suggests that the precision of risk estimates in a QRA on *Listeria* could currently be impaired due to the overly large uncertainty in the dose-response model and in some predictive microbiology parameters. We chose to explore the uncertainty of the output by studying the uncertainty of the various inputs in greater detail[17,41] and by transferring this uncertainty throughout the model using a second-order Monte Carlo simulation. Another common approach to managing these very uncertain parameters is to calibrate the model. Model calibration is the process of modifying these uncertain parameters until the model's output matches an observed set of data or a prior knowledge of its scale. This procedure is regularly employed in published risk assessment.[11,42] Finally, an intermediate method to deal with parameter uncertainty could have been to introduce epidemiological and expert data through prior distribution of parameters in a Bayesian network.[43] In our example, epidemiological data for the number of expected CSS-related listeriosis cases (i.e., [0; 100] expected cases per year) could be used, along with some additional data on natural contamination of CSS at the retail and consumption steps, through a prior distribution. The procedure would have refined the distribution of uncertain parameters, e.g., the dose-response factor. Despite some remaining technical difficulties, a global Bayesian framework could be very promising in QRA applied to food safety.[43,44]

It is obvious that the QRA model is not conceived to precisely estimate the number of cases of listeriosis, as epidemiology-based estimates are intrinsically more precise. Indeed, the main objective and interest of QRA modeling is mostly directed toward the identification of key factors influencing the risk. Assuming, based on comparison of our results with other studies and epidemiological data, that the structure of the model is satisfactory, it is possible to gain a clear understanding of the effect of interventions on outcomes, even in the presence of great uncertainty in the parameters. Indeed, the efficacy of the mitigation strategies was assessed by calculating *within each uncertainty simulation*, i.e., all uncertain parameters being fixed,[13] the ratio of the total number of listeriosis cases in the "what-if" scenario versus the baseline method. This procedure naturally reduces or even cancels the impact of most parameters' uncertainty,[45,46] e.g., the uncertainty linked to the dose-response model.

The question remains as to which risk mitigation strategy would best reduce the number of listeriosis cases due to CSS consumption. The FAO/WHO[10] suggested that mitigation strategies that reduce the highest exposures to *Listeria* are to be promoted. Our results show that the predicted number of cases is linked to the probability of obtaining a large exposure ($>10^8$ cfu/serving, representing the 0.1–0.2% highest percentiles of exposure)[13] rather than to the 99th percentile of exposure (Table IV). In fact, as regularly observed in *Listeria* QRA[10–12,29,47,48] and confirmed herein, the expected number of cases is linked to the very rare occurrence of very risky situations, i.e., a combination of events that leads to the consumption of highly contaminated products. We demonstrate here that "the highest percentiles"[10] requiring consideration may not be the 5% or 1% highest, but only the very few most extreme values. One difficulty of such QRA is then to model extreme but realistic situations.[22] We cared to model realistic situations, e.g., by limiting the storage of the product at home (shelf-life printed on the CSS pack plus five days as the maximum storage length) or modeling the interaction between *L. monocytogenes* and the food flora.[13] Classical methods recommended for QRA, including Monte Carlo simulations, are

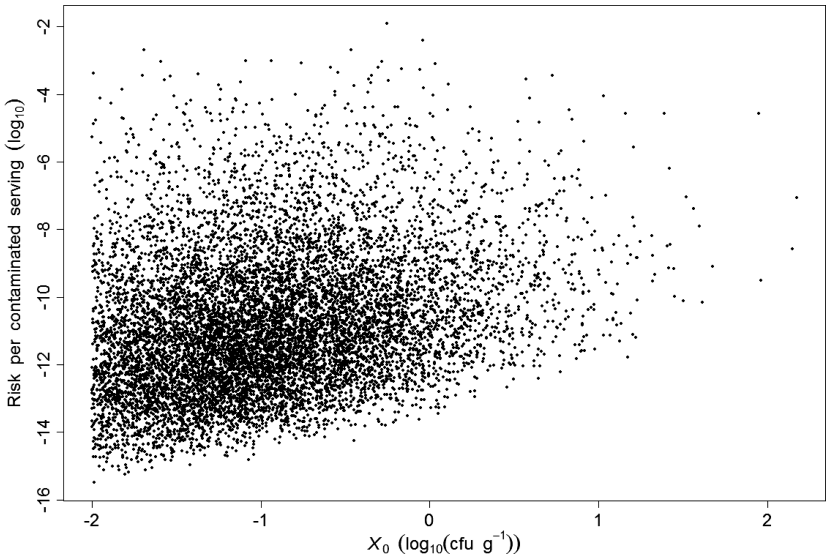

Fig. 1. Scatter plot of the density of *L. monocytogenes* in contaminated CSS at the end of the production step (X_0, $\log_{10}(\text{cfu g}^{-1})$) vs. the risk per contaminated serving of CSS in the reference population ($\log_{10}$).

probably not the most efficient framework to model the very rare events. Consequently, some new modeling techniques must be developed and promoted to assess such hazards.[49]

Results obtained from tested mitigation strategies nevertheless suggest that the consumer stage is a key step: some relatively rare contaminated products, even if the initial level of contamination is controlled (e.g., <1 cfu/g at the end of the production step), may lead to very high risk of listeriosis if the product is stored for a long time at abuse temperature. According to our results, managing the initial level of contamination would not efficiently manage the risk linked to *Listeria* in RTE foods that support growth. This confirms the results of the exposure assessment[13] and may be illustrated by the weak link observed between the density of *L. monocytogenes* in contaminated CSS at the end of the production and the risk per contaminated serving (Fig. 1). This conclusion applies for the studied situation, i.e., sporadic contaminations and storage conditions as observed in France in the early 2000s. It is also partly influenced by one of our modeling hypotheses in the exposure assessment,[13] the absence of a lag phase, which was justified by the observation of nil or very short lag times in the experimental data when the chosen preincubation temperature was realistic. However, these observations were obtained in challenge tests, with initial levels between 50 and 3×10^4 cfu/g,[14–17] and longer lag phases or even no growth might be expected at very low contamination levels, such as a few stressed cells per package, due to a stochastic effect.[50,51] This may partly explain discrepancies between growth in artificially versus naturally contaminated products.[15] The impact of the stochastic effect is nevertheless nuanced by the results of François *et al.*,[51] who observed that the individual cell lag phase variability was overruled by the global variability of their exposure assessment framework. To conclude, taking into account the individual cell lag times in the growth model may have led us to slightly different conclusions regarding the impact of very low initial contaminations. Finding the most efficient control measures would require evaluating the cost, feasibility, and effectiveness of each of these measures, which is outside the scope of this research. For example, the effectiveness of a prevention campaign on increased susceptibility population is *a priori* unknown: the corresponding "what-if" scenario was proposed here with a theoretical efficiency of 50% as a reminder of the impact potentially incurred by such a strategy. The results shown in Table IV should then be interpreted using the principle of equivalence. Five scenarios would lead to a reduction in the risk by a factor of 2: (i) all products consumed within 10 days after purchase; (ii) all products initially contaminated at a level below 1 cfu/50 g; (iii) prevalence divided by 2; (iv) the mean refrigerator temperature set 2°C lower than that currently observed; and (v) a 50% decrease in CSS consumption among subpopulations with increased susceptibility. Similarly, three scenarios would divide the risk by 4: (i) prevalence divided by 4; (ii) shelf-life of 15 days; and (iii) a mean refrigerator temperature of exactly 4°C. One can hypothesize that control measures concerning the consumer

phase, even if they appear to be the most promising, are the most difficult to implement, as this phase is not under direct regulatory control. Risk mitigation involving the consumer should thus mostly rely on improving the information available to the consumer (through, e.g., education campaigns, refrigerators user guides, and labeling of food). If the consumer step cannot be controlled, reducing the prevalence of contaminated products, and/or lowering the ability of the food to support growth through modification of physical and chemical characteristics likely remain the most efficient risk management strategies for these RTE foods that support *Listeria* growth.

Finally, we will illustrate how this model may be used by the competent authority to derive risk management metrics, such as the Food Safety Objective (FSO), with regard to *L. monocytogenes* in CSS. The current definition for an FSO is "the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP)."[20] It is now accepted that this definition must be operationalized, and the use of a high percentile of variability distribution in contamination levels at the time of consumption has been suggested to stand for "the maximum concentration" in the original definition.[52–55] To illustrate the use of this model to derive FSO, let us suppose that the number of listeriosis cases due to CSS in France in the early 2000s, regardless of the true level, is chosen as an ALOP for this commodity, and that the consumption and dose-response features are fixed. Our exposure model[13] can then be used to derive high percentiles (97th, 99th, etc.) for the simulated distribution of *L. monocytogenes* densities in CSS (both contaminated and noncontaminated) at the time of consumption. The FSO-values corresponding to each operationalization are then $FSO_{97th\ perc.} = 200$ cfu/g, with a CI95 of [20; 3,800] cfu/g; $FSO_{99th\ perc.} = 17{,}000$ cfu/g, with a CI95 of [730; 880,000] cfu/g. Higher percentiles entail a wider range of uncertainty.

An alternative would be to consider the frequency of contamination exceeding a given level (e.g., 10^6 cfu/g). Then, the phrase "the maximum frequency and/or concentration of a hazard" in the original definition of the FSO would be operationalized into "the maximum frequency above a given concentration of a hazard." This would intuitively combine the prevalence and distribution of the concentrations, and thus stress the importance of considering both,[53–55] and would also be more interpretable in terms of risk (number of listeriosis cases), as it focuses on the extreme tail. Taking the hypotheses of the example above, this leads to an $FSO_{>10^6\ cfu/g}$ of 0.34%, with a CI95 of [0.09%; 0.97%].

Choosing an operationalization of the original definition of FSO (and of PO, performance objective) may first appear unproblematic, but actually has large effects: (i) on the precision of the FSO (or PO) estimation based on MRA, as illustrated above by the various uncertainty intervals on each FSO estimation, (ii) on the comparison of different public health targets, as illustrated in our model by the observed differences between rank mitigation strategies (see Table IV), and (iii) on the adequacy of monitoring strategies to verify compliance to these targets, as discussed at length in the literature.[52] There is still a need for further elaboration on these concepts to avoid ambiguity in practical implementation.

5. CONCLUSION

Despite the important uncertainty associated with the predictions, this model provides a scientific base for risk managers and food business operators to gain a better understanding of the prevention of listeriosis due to CSS. The model confirms that the majority of cases are due to a very high level of contamination at the time of consumption linked to time-temperature abuse during the consumer step, rather than high initial levels, and points out that risk management options should be based on reducing the frequency of these high exposures, which may not be linked to the classical 95th or 97th percentiles.

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APPENDIX: DOSE-RESPONSE MODEL IN THE REFERENCE SUBPOPULATION

An exponential dose-response model was used to link exposure to *L. monocytogenes* with the probability of invasive listeriosis in the FAO/WHO "risk assessment of *L. monocytogenes* in ready-to-eat foods" report.[10] Two different methods were used to estimate the *r*-value in this report.

(i) The "single-dose *r*-value" was used to estimate r_h, the *r* parameter in the "healthy" population, and its uncertainty. This method assumes that all cases are attributed to servings contaminated by a specified "maximum dose level" of *L. monocytogenes*. Thus, r_h is derived from U.S. exposure data and U.S. epidemiological data by solving the equation:

$$C \times p_h = S_M \times P_h \times \left(1 - \exp\left(-r_h \times 10^M\right)\right),$$

where C is the annual number of cases of invasive listeriosis in the United States, p_h is the proportion of cases that occurs in the "healthy" population, S_M is the annual number of servings at the maximum dose level M consumed in the United States, and P_h is the proportion of the U.S. population considered as "healthy."

Uncertainty in r_h was estimated by calculating r_h within a Monte Carlo simulation using an uncertainty distribution for the healthy population percentage in the United States ($P_h \sim Unif(0.80, 0.85)$, where $Unif(min, max)$ is the uniform distribution with minimum (min) and maximum (max), an uncertainty distribution for the percentage of cases issued from the healthy population in the United States ($p_h \sim Unif(0.80, 0.98)$), an uncertainty distribution for the total number of listeriosis cases in the United States ($C \sim Unif(1888, 3148)$), and an uncertainty distribution for the maximum dose level in a serving ($M \sim Discrete(7.5; 8; 8.5; 9; 9.5; 10; 10.5)$, where $Discrete(\boldsymbol{X})$ is the uniform discrete distribution for all values of $\boldsymbol{X}$).

(ii) The "multiple-dose *r*-value" was used for r_h point estimates. This method uses a more feasible assumption that all dose levels, discretized from -1.5 $\log_{10}$ cfu/serving to the maximum dose level M by step of 1 $\log_{10}$ cfu/serving, contributed to cases of listeriosis. r_h is then derived from the equation:

$$C \times p_h = \sum_{i=-1.5}^{M} \left(S_i \times P_h \times \left(1 - \exp\left(-r_h \times 10^i\right)\right)\right),$$

where S_i is the annual number of servings at a dose level i consumed in the United States. In the FAO/WHO report, no uncertainty was assumed when this method was applied.

In this article, r_r and its uncertainty were derived in a Monte Carlo simulation framework using the multiple-dose equation above (as in option (ii)), with an uncertainty distribution for C, p_h, P_h, and M (as in option (i)). The data and uncertainty distributions used were similar to those used in the FAO/WHO report in option (i).

This procedure led to a mean estimate of r_r of 4.7×10^{-14}, a median of 1.7×10^{-14}, and a CI95 of $[1.4 \times 10^{-15}; 2.6 \times 10^{-13}]$. This estimate was slightly lower than the one obtained in the FAO/WHO report[10] with option (i) (median: 2.4×10^{-14}). The use of this procedure in the French context assumes that the "healthy" population, as defined,[10] and the French "reference" population have the same probability of developing invasive listeriosis from consumption of one *L. monocytogenes* cell.

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