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Abstract

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. 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). 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 for reducing the estimated risk. 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 *Listeria moncytogenes* and to apply the recent risk-based concept of risk management metrics illustrated here by the FSO (food safety objective).

Keywords

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 have led to the recognition of human listeriosis as a foodborne disease.[1] Non-invasive listeriosis is a mild form disease that leads to febrile gastroenteritis, whereas invasive listeriosis is a systematic, 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 for reporting diagnosed cases of listeriosis is high (estimated 0.87 in 2001).[2] The annual incidence of diagnosed and declared listeriosis in France declined from approx. 4.5 cases/million persons during the 1999–2000 period (i.e., approx. 265 cases / year) to approx. 3.5 cases/million persons during the 2001–2005 period (i.e., approx. 220 cases / year), paralleling a substantial reduction in the proportion of *L. 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 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 Exposure assessment

2.1.1 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. 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).

2.1.2 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 4079 individuals aged 3 years and older, with all food intakes reported during a 7-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 3-day period.[19] Specific records of the consumption of CSS extracted from these studies are provided in Table 1. 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 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 0.025^{th} quantile and 0.975^{th} quantile of the uncertainty distribution) of this statistic was estimated to be [5.9; 7.1].

2.2 Hazard characterization

The exponential dose-response equation[23] was selected as the dose-response model. The single parameter of this equation characterizes the host susceptibility and is believed to differ between sub-populations.[10] We considered four exclusive sub-populations: *i*) the "pregnant" sub-population; *ii*) the "susceptible" sub-population, defined as the sub-population 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 sub-population with none of the preceding risk factors, denoted as the "elderly" sub-population, and *iv*) the <65 years of age sub-population with none of the preceding risk factors, denoted as the "reference" sub-population.

2.2.1 Hazard characterization for the "reference" sub-population

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

$$R_{i\in r} = 1 - \exp\left(-r_r \times 10^{E_{serv_i}}\right).$$

$R_{i\in r}$ is the probability of invasive listeriosis for individuals issuing from the "reference" sub-population after exposure to a product with an expected contamination of $10^{E_{serv_i}}$ 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 sub-population.[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 (U.S.). As no such exposure data are available in France, these U.S. data were used to derive r_r, assuming that the "reference" sub-population 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 Appendix).

2.2.2 Relative risk of listeriosis in the sub-populations

For the "pregnant", "susceptible", and "elderly" sub-populations, the model was anchored to French epidemiological data such that the risk of listeriosis from consumption of CSS in these sub-populations relative to the "reference" sub-population 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 4 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" sub-population as defined above; C_e = 181 cases were observed in the "elderly" sub-population, and C_r = 92 cases were observed in the remaining "reference" sub-population. From these data, the number of cases expected in each sub-population for one case in the "reference" sub-population may be calculated as follows: RC_p = 195 / 92 = 2.1 cases in the "pregnant" sub-population, RC_s = 385 / 92 = 4.2 cases in the "susceptible" sub-population, and RC_e = 181 / 92 = 2.0 cases in the "elderly" sub-population. The mean numbers of individuals in each sub-population during the studied period were estimated at N_p = 798 000 for pregnant women, N_s = 3 987 000 for the "susceptible" sub-population, N_e = 7 749 000 for the "elderly" sub-population, and N_r = 49 090 000 for the "reference" sub-population. These data led to the following relative risks of invasive listeriosis, compared to the reference sub-population during this four-year period: $RR_p = \frac{C_p}{4\times N_p} \Big/ \frac{C_r}{4\times N_r} = \frac{195}{4\times 798000} \Big/ \frac{92}{4\times 49090000} = 130$ for the "pregnant" sub-population and, using a similar formula, RR_s = 52 for the "susceptible" sub-population, 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 parametric bootstrap method[21] assuming a Poisson process of the occurrence of cases and no uncertainty in the number of individuals in each sub-population. 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 and RR_s 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.

2.3 Risk characterization

2.3.1 Main outputs

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

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

from E_{serv_i}, the two-dimensional output of the exposure assessment,[13] and r_r, the one-dimensional (uncertain) parameter of the dose-response. This estimation was performed within each variability dimension, which 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" sub-populations 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 sub-population is estimated as

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

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

2.3.2 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]

2.3.3 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 conservation 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 sub-populations with increased susceptibility. We assumed that CSS consumption would be reduced by a factor of 50% in the "susceptible", "pregnant", and "elderly" sub-populations. 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] Each scenario was tested on a set of 10 000 variability iterations and 10 000 uncertainty iterations.

3 Results

Table 2 presents the results for the estimated mean risk and the expected number of cases in each sub-population. According to our best estimates, one case of invasive listeriosis is expected per either 73 000 CSS servings consumed by pregnant women, 180 000 CSS servings consumed by individuals with cancer, dialysis, transplants, liver cirrhosis, diabetes or AIDS, 760 000 CSS servings consumed by individuals ≥65 years of age with none of the cited pathologies, 9 500 000 CSS servings consumed by individuals <65 years of age with none of the cited pathologies, or 1 300 000 CSS servings consumed overall in France. Considerable uncertainties are associated with these estimates. Indeed, the associated CI95 leads to one expected case per:

- [1 800 to 2 310 000] CSS servings consumed by "pregnant" women;
- [4 600 to 5 800 000] CSS servings consumed by "susceptible" individuals;
- [19 000 to 24 000 000] CSS servings consumed by "elderly" individuals;
- [240 000 to 300 000 000] CSS servings consumed by "reference" individuals;
- [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 3. For some factors, the *p*-value was extremely low. Indeed, this observation has no meaning, since *p*-values are influenced by the number of data points, which was artificially 10 000 in this analysis: *p*-values 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 4 shows the results obtained by modeling the various risk mitigation strategies. These 17 strategies are ranked according to decreasing number of predicted listeriosis cases. This table also recalls the ranking of the six mitigation strategies tested in the first paper, ordered based on decreasing 99th percentile of exposure and decreasing probability of observing a serving containing >10^8 cfu of *Listeria 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 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 linked to the frequency of exposure >10^8 cfu/serving rather than 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.

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 5). Observed differences within the category of seafood ready-to-eat (RTE) products may be explained by: *i*) differences in the scopes of the assessments (e.g., only CSS versus all smoked seafood); *ii*) differences between the modeled food chains (e.g., initial contamination, time-temperature profiles), which reflects inter-country variability, and *iii*) differences in modeling options (e.g., different growth models, different dose-response models), which reflects 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 global high prevalence 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 food-borne 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 approx. 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 approx. 280 cases per year. Given that 33% of patients have consumed smoked fish at least once during the 2 months preceding

the symptoms,[27] the maximum boundary for plausible values of the annual incidence of listeriosis due to CSS would be approx. 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, confirming the difficulty in evaluating such non-linear models.[28] Nevertheless, 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 the assumptions from the FAO/WHO report[10] implies that the probability of contracting invasive listeriosis following the consumption of, e.g., 10^{10} *Listeria* cells, is known within a scale of uncertainty of 1 to approx. 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" to model 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.[30-32] This maximum population density is rarely specifically studied in the predictive microbiology literature. This 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).[33, 34] These other sources of uncertainty may be qualified through the main assumptions of the exposure assessment model[See 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 sub-populations and in the hazard characterization according to the sub-populations 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" sub-population 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" sub-population 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, 35] 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 consists of changing model input parameter values in an attempt to match the model's output. This procedure is regularly employed in published risk assessment.[11, 36] 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.[37] 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.[37]

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 towards 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,[38, 39] 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 to 0.2% highest percentiles of exposure)[13] rather than to the 99th percentile of exposure (Table 4). In fact, as regularly observed in *Listeria* QRA[10-12, 29, 40, 41] 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 lead 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. Classical methods recommended for QRA, including Monte-Carlo simulations, are probably not the most efficient framework to model these very rare events. Consequently, some new modeling techniques must be developed and promoted to assess such hazards.[42]

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 ready-to-eat foods that support growth. This conclusion is 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 pre-incubation 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.[43, 44] Taking this into account in the growth model may have led us to slightly different conclusions regarding the impact of very low initial contaminations, unless the stochastic effect was overruled by the global variability of the exposure assessment framework, as concluded by François et al.[44]

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 4 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 sub-populations 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 ready-to-eat 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.[45-48] 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 (97^{th}, 99^{th}, etc.) for the simulated distribution of *L. monocytogenes* densities in CSS (both contaminated and non-contaminated) at the time of consumption. The FSO-values corresponding to each operationalization are then: $FSO_{97^{th}perc.}$ = 200 cfu/g, with a CI95 of [20; 3800] cfu/g; $FSO_{99^{th}perc.}$ = 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,[46-48] 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 PO) 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 4), and (*iii*) on the adequacy of monitoring strategies to verify compliance to these targets, as discussed at length in the literature.[45] There is still a need for further elaboration on these concepts to avoid ambiguity in practical implementation.

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 95^{th} or 97^{th} percentiles.

Appendix. Dose response model in the reference sub-population

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 U.S., 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 U.S., 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 U.S. ($P_h \sim Unif(0.80, 0.85)$, 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 U.S. ($p_h \sim Unif(0.80, 0.98)$), an uncertainty distribution for the total number of listeriosis cases in the U.S. ($C \sim Unif(1888, 3148)$), and an uncertainty distribution for the maximum dose level in a serving (*M* ~ *Discrete*(7.5; 8; 8.5; 9; 9.5; 10; 10.5), where *Discrete*(***X***) is the uniform discrete distribution for all values of ***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 dose level *i* consumed in the U.S. 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, with an uncertainty distribution for *C*, p_h, P_h, and *M*. 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 [1.4×10^{-15}; 2.6×10^{-13}]. It assumes that the "healthy" population, as defined in[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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Tables

Table 1: Consumption data used to evaluate the number of cold-smoked salmon servings per year.

age category	proportion of the French population (%)[49]	individual×days of study	individual×days with one CSS serving	Annual intake of CSS servings[a]	Ref.
<3 years old	2.66	2118	5	0.86	[19]
[3 – 15[	15.20	7109	89	4.57	[18]
[15 – 65[	65.48	18 665	356	6.96	[18]
> 65 year old	16.66	2395	46	7.01	[18]

[a]: (individual × days with one CSS serving) / (individual × days of study) × 365.

Table 2: 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 sub-populations [95% credibility interval].

Sub-population	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; 5653]
Elderly	1.3×10^{-6} [4.1×10^{-8}; 5.3×10^{-5}]	65 [2; 2651]
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 3: Results of the ANOVA evaluating the impact of uncertain parameters on the predicted number of listeriosis cases in the healthy population.

Parameter	% of total variance	d.f.	*p*
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 ($\mu_{ref,Lm}$)	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 ($T_{min,Lm}$)	0.5	9	4.7×10^{-12}
Mean growth rate of the food flora in a CSS at a reference temperature of 25°C ($\mu_{ref,ff}$)	0.4	9	1.8×10^{-8}
Prevalence of contaminated CSS	0.3	9	1.2×10^{-6}
Standard deviation of $T_{min,Lm}$	0.3	9	3.2×10^{-6}
Mean of the minimal temperature for growth of food flora in CSS ($T_{min,ff}$)	0.2	9	3.1×10^{-4}
Standard deviation of $T_{min,ff}$	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	

Table 4: 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/50g	No limit			55 [24; 80]
Reduced CSS consumption: 50% in increased susceptibility sub-populations				55 [54; 56]
Prevalence: 0.5 baseline model	1	7	4	50 [50; 50]*
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]*
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]

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

Table 5: Comparison between the results of the present study and other estimations present in the literature.

QRA: Food, country, (reference)	Dose response model	Mean risk per serving (overall population)
Smoked (and gravad) seafood		
Smoked and gravad salmon and trout, Sweden, (12)	(12)	1.3×10^{-4}
Same study, (12)	(9)	2.8×10^{-5}
CSS, France, **present study**	Adapted from (10)	$\mathbf{7.8\times10^{-7}}$
Smoked fish, Germany, (9)	(9)	6.3×10^{-7}
Cold-smoked fish, world, (10)	(10)	5.3×10^{-8}
Smoked seafood, U.S., (11)	(11)	6.2×10^{-9}
Other seafood ready-to-eat products		
Cooked ready-to-eat crustaceans, U.S., (11)	(11)	5.1×10^{-9}
Preserved fish, U.S., (11)	(11)	2.3×10^{-11}
Raw seafood, U.S., (11)	(11)	2.0×10^{-11}
Meat-based ready-to-eat products		
Deli meats, U.S., (11)	(11)	7.7×10^{-8}
Frankfurters (not reheated), U.S., (11)	(11)	6.5×10^{-8}
Pâté and spread meats, U.S., (11)	(11)	3.2×10^{-8}
Dry cured ham (50)	An earlier version of (10)	Between 4.7×10^{-10} (normal adult population) and 6.1×10^{-7} (most susceptible sub-population)
Frankfurters (reheated), U.S., (11)	(11)	6.3×10^{-11}
Dry/semi-dry fermented sausages, U.S., (11)	(11)	1.7×10^{-11}
Fermented meat products, world, (10)	(10)	$2.1\ 10^{-12}$
Dairy ready-to-eat products		
Soft cheese, France, (10-12, 29, 40, 41)	Farber et al. 1996	2.3×10^{-8}
Unpasteurized milk, U.S., (11)	(11)	7.1×10^{-9}
Pasteurized milk, world, (10)	(10)	5×10^{-9}
High fat and other dairy products, U.S., (11)	(11)	2.7×10^{-9}
Various cheese categories, U.S., (11)	(11)	From 1.8×10^{-9} to 4.5×10^{-15} (depending on the category)
Pasteurized milk, U.S., (11)	(11)	1.0×10^{-9}
Brie (soft cheese), France, (51)	(10)	3.5×10^{-11}
Ice cream, world, (10)	(10)	1.4×10^{-11}
Camembert (soft cheese), France, (51)	(10)	5.1×10^{-12}
Ice cream and frozen dairies, U.S., (11)	(11)	4.9×10^{-14}
Cultured milk products, U.S., (11)	(11)	3.2×10^{-14}
Other ready-to-eat products		
Fruits, U.S., (11)	(11)	1.9×10^{-11}
Vegetables, U.S., (11)	(11)	2.8×10^{-12}
Deli-type salads, U.S., (11)	(11)	5.6×10^{-13}