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Running title: *L. monocytogenes* risk assessment

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Quantitative microbiological risk assessment as a tool to obtain useful information for risk managers – Specific application to *Listeria monocytogenes* and ready-to-eat meat products

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Abstract

The presence of *Listeria monocytogenes* in a sliced cooked, cured ham-like meat product was quantitatively assessed. Sliced cooked, cured meat products are considered as high risk products. These ready-to-eat, RTE, products (no special preparation, e.g. thermal treatment, before eating is required), support growth of pathogens (high initial pH=6.2-6.4 and water activity=0.98-0.99) and has a relatively long period of storage at chilled temperatures with a shelf life equal to 60 days based on manufacturer's instructions. Therefore, in case of post-process contamination, even with low number of cells, the microorganism is able to reach unacceptable levels at the time of consumption. The aim of this study was to conduct a Quantitative Microbiological Risk Assessment (QMRA) on the risk of *L. monocytogenes* presence in RTE meat products. This may help risk managers to make decisions and apply control measures with ultimate objective the food safety assurance. Examples are given to illustrate the development of practical risk management strategies based on the results obtained from the QMRA model specifically developed for this pathogen/food product combination.

Keywords: Food safety; *Listeria monocytogenes*; meat products; risk assessment; risk management

1. Introduction

Listeria monocytogenes is a Gram-positive bacterium capable of growing at refrigeration temperatures. The microorganism is difficult to be controlled in foods because of its ubiquity in the environment, tolerance to unfavorable environmental conditions, such as low pH and high sodium chloride levels, and ability to survive on equipment (i.e. biofilm formation) contaminating, in this way, the end-products. Several foods (e.g. dairy, meat and vegetables) have been implicated in food-borne outbreaks associated with this pathogen. *L. monocytogenes* is a significant hazard particularly for elderly, immunocompromised people, infants and pregnant women (ICMSF, 1996; NZFSA, 2008).

The aim of applying the Appropriate Level of Protection (ALOP) is the decrease of the number of food-borne cases per pathogen and per year to a pre-determined level which constitutes the appropriate or acceptable level of protection. For instance, in US the decrease of listeriosis cases by 50% has been set as target [from 0.50 reported cases (number of culture-confirmed cases of illness caused by *L. monocytogenes* reported to CDC) /year/100000 population to 0.25 cases/year/100000 population) by the end of 2010. Based on statistical data, this goal has almost been achieved since the listeriosis cases for 2007 were 0.27 cases/year/100000 population (CDC, 2008). Similar objectives have been set for other pathogens like *Salmonella* spp., *Campylobacter* spp. and *Escherichia coli* O157:H7. Food Safety Objectives (FSOs) determine the maximum frequency and/or concentration of a hazard in a food at the time of consumption that provides or contributes to the ALOP. FSOs constitute the link between the ALOP and food industries (ICMSF, 2002). To achieve the FSOs, Performance Objectives (POs) [maximum frequency of occurrence (%) and/or concentration (cfu/g) of a pathogen] at other stages, Performance Criteria (PC) [change (i.e. reduction or maximally allowed increase) in frequency of occurrence and/or concentration of a pathogen that should be achieved during processing or implementation of control measures], Process and Product Criteria (PrC) (conditions required to achieve the desired PO/PC, e.g. time-temperature combination, or pH) should be established in the process prior to consumption. Governmental risk managers are responsible for establishing ALOP and FSOs whereas industrial risk managers should design production processes to meet the FSOs (Walls, 2006).

Compliance of ALOP, FSO, POs or PC should be based on data and findings originated from scientific resources and/or studies (e.g. Quantitative Microbiological Risk Assessment – QMRA). A QMRA study produces a wealth of information useful for risk assessors and risk managers. It can be used as

tool to collect information regarding the microbiological hazard under study. Afterwards, Food Safety Management Systems (FSMS) executives can benefit from this information in terms of design of production process, application of control measures and risk management in general (Perez-Rodriguez et al., 2007). However, it should be taken in mind that so far no FSOs have been set by food safety managers.

The objective of the present study was not to simply give an additional risk assessment model to already existing ones [e.g. the quantitative risk assessment model for *L. monocytogenes* and deli meats (FDA/USDA, 2003)] but demonstrate how the QMRA can produce useful information for risk managers. Extracting useful information from a risk assessment model, practical risk management strategies and intervention steps might be developed for reducing listeriosis cases, in this particular example, or any other illness, in general, based each time on the pathogen/food product combination of concern. It is questionable whether it is possible to further reduce listeriosis but it might be that the few cases that do occur are related to infrequent high levels, which could be prevented. The QMRA model developed incorporates factors that influence the final risk estimation such as Jameson effect, food structure, temperature during distribution, storage and retail display as well as during storage in domestic refrigerators.

2. Materials and Methods

Before conducting a quantitative risk assessment, risk profiles may be constructed as a preliminary task in order the QMRA study to be orientated to a specific food product/pathogen combination. By developing risk profiles for the food product of concern all the related possible microbiological hazards are identified and prioritized. The prioritization helps to identify the food product/hazard combination with the higher food safety risk for which a further risk process model may be developed for fully quantitative and accurate estimation of the risk (Ross and Sumner, 2002). Such risk profiles have been developed for pork and poultry industry in a recent review by Mataragas et al. (2008). The authors found that *L. monocytogenes*/RTE meat products combination constitute high risk for specific groups of the population (elderly, immunocompromised people, infants and pregnant women – high risk population), whereas for the rest of population (healthy adults and children – low risk population) the risk is medium (mild or asymptomatic infection). The present study was concentrated on the high risk

101 population since these groups are very susceptible to listeriosis and also have been associated with high
102 number of cases (EFSA, 2008).
103 According to the industry, shelf life of the product studied in this work (i.e. sliced cooked, cured ham-
104 like meat product) at 4^{o}C is 60 days. Consequently, in case of post-process contamination of the
105 product with *L. monocytogenes*, even with low number of cells, the microorganism is capable of
106 reaching high numbers at the time of consumption, because of its ability to grow at common
107 refrigeration temperatures. Furthermore, the intrinsic factors of the product such as pH (6.2-6.4), water
108 activity (0.98-0.99) and sodium chloride content (approximately 2%) are not prohibitive to pathogen
109 growth. More information on product composition can be found in Mataragas et al. (2006a). Outbreaks
110 of listeriosis are predominantly associated with RTE foods and they have been found to be related with
111 listeriosis cases more than any other RTE food (ILSI, 2005; Sofos, 2008).
112 A QMRA study includes the assessment of the microbiological hazards severity and its likelihood of
113 appearance (i.e. frequency) following the approach form farm to fork. However, this approach has
114 practical difficulties owned to its complexity and the need for an enormous amount of data. Therefore,
115 it is sometimes more effective to focus the exposure assessment to a part of the food supply chain only.
116 For instance, the most common reason of the presence of *L. monocytogenes* in RTE cooked meat
117 products is their post-process (i.e. after the cooking step) contamination (ICMSF, 1996, 2002). In the
118 present study, the quantitative risk assessment was focused on the exposure assessment and risk
119 characterization stages from the manufacturing of the product, especially after the cooking and slicing
120 steps, up to the time of consumption, e.g. retail and consumer (FDA/USDA, 2003). A product pathway-
121 type QMRA study was developed to identify factors that influence the risk and evaluate the
122 effectiveness of potential interventions or mitigation strategies, using the Modular Process Risk Model
123 approach (Nauta et al., 2003; Dennis et al., 2008; Nauta, 2008).
124 The model developed was a second-order risk process model taking into account, separately, variability
125 and uncertainty of certain parameters of the model (Vose, 2000; Nauta, 2007). The model parameters
126 (input variables) were described by probability distributions. The data for the input variables were
127 collected from literature and interviews with experts (Worsfold and Griffith, 1997; Jay et al., 1999;
128 Nauta et al., 2003; FDA/USDA, 2003; Marklinder et al., 2004; Kennedy et al., 2005; Nauta, 2005;
129 Mataragas et al., 2006a,b; SMAS, 2006; Kim, 2006; Anonymous, 2007b; FAOSTAT, 2007). Factors
130 known to influence the final risk estimation such as data on explicit factors (i.e. temperature during

distribution, storage and retail display, and storage in domestic refrigerators), knowledge of spoilage bacteria (i.e. modeling spoilage microorganisms growth in parallel with pathogen growth), food structure (growth models of both spoilage and pathogen microorganisms developing in the food product and validated under constant and fluctuating temperature conditions) and Jameson effect (when one species reaches its maximum population density other species stop growing as well, at whatever population density they have achieved to that time) (Ross, 2008). The results obtained from the exposure assessment were combined with a dose-response relationship (i.e. exponential dose-response model) to characterize the final risk (Buchanan et al., 1997):

$$P_{ill} = 1 - \exp(-r \times D)$$

where P_{ill}, is the probability of illness; r, the probability of illness after the consumption of one *L. monocytogenes* cell; and D, the dose consumed (number of cells per serving). The dose is given by the following equation:

$$D = C \times S$$

where C, the concentration of the pathogen (number of cells/g); and S, the serving size consumed during a meal (g).

The model predicted the probability of illness for the high risk population (20-25%) (Buchanan et al., 1997). The percentage of 20% was further considered as the fraction of the total population being at high risk. Afterwards, the risk, expressed as number of listeriosis cases per year, was determined using a probabilistic approach (Perez-Rodriguez et al., 2007):

$$Risk = P_f \times D \times r \times S_{all} \quad (1)$$

where *Risk*, the total number of listeriosis cases per year in high risk population; P_f, the prevalence of *L. monocytogenes* at the time of consumption (%); and S_{all}, the total annual number of servings consumed by high risk population. The P_f parameter represents the unspoiled-unsafe fraction at the time of consumption assuming that some contaminated products will be spoiled before their consumption and therefore not all the contaminated products will be consumed. Unspoiled-unsafe products were considered as the products in which Specific Spoilage Organisms (SSOs) were below the spoilage level of 10^9 cfu/g (Mataragas et al., 2006a) and/or purchase day lower than shelf life of 60 days and at the same time *L. monocytogenes* population was above the microbiological criterion of 10^2 cfu/g (Anonymous, 2005, 2007a). Although, levels below 100 cfu/g may lead to illness the cut-off level of 100 cfu/g was used, according to EC Regulation 2073/2005 and its amendment 1441/2007

(Anonymous, 2005, 2007a), referring to that *L. monocytogenes* growth should not exceed 100 cfu/g throughout the shelf life in products supporting its growth. People often are exposed to levels lower than 100 cfu/g without getting ill. However, infective dose is influenced by the susceptibility of the high risk individuals and the ability of the microorganism to cause illness but, in general it can be assumed that *L. monocytogenes* levels ≥ 100-1000 cfu/g can cause listeriosis in high risk groups (NZFSA 2008). The parameter S_{all} was determined based on the frequency of consumption of RTE meat products by the total population in European Union, approximately 467000000 (Kim, 2006; Anonymous, 2007b; FAOSTAT, 2007). It was further assumed that the frequency of consumption of such products is similar between high risk groups and general population (Buchanan et al., 1997). The predicted listeriosis cases per year were compared with the reported cases (EFSA, 2008) for the reliability of the model. Listeriosis cases occurring in elderly people were considered because of their higher association with this particular group (FDA/USDA, 2003). Finally, the risk factors influencing the output of the model (i.e. listeriosis cases) and their threshold values, above of which a sharp increase of listeriosis cases is observed, were determined by the application of crude and advanced sensitivity analysis (Vose, 2000; Perez-Rodriguez et al., 2007). Crude sensitivity analysis is referred to the correlation coefficients between the model inputs and output as given by the simulation software used. Advanced sensitivity analysis is referred to the construction of the Tornado and sensitivity graphs. These graphs were constructed by testing the following cumulative probabilities (1, 5, 25, 50, 75, 95 and 99%) of the input distributions identified by the crude sensitivity analysis. Each input distribution was replaced by the corresponding percentile at a time allowing the others to vary and the output statistic of interest (i.e. mean of the listeriosis cases per year) was recorded. The model was developed in the Excel program and simulated using the @Risk 4.5 software (Palisade Corp., New York, USA). Ten thousands (10000) repetitions (iterations) in each simulation of the model were conducted whereas the simulation was repeated 100 times (uncertainty realizations) to take into account separately the variability and uncertainty of the model and model inputs.

To simplify the procedure of risk estimation and calculate the pathogen population at the time of consumption as accurate as possible, the food supply chain was divided into 3 sub-modules: the industry, the retail and the consumer (Nauta et al., 2003; Nauta, 2008) (Tables 1-3). In Tables 1-3 only the model for *L. monocytogenes* is presented but a similar model was constructed for SSOs. The Gompertz equation as modified by Zwietering et al. (1994) was used to calculate population changes

(i.e. increase) at various stages of the food chain. To calculate the kinetic parameters (μ_{max} and t_{lag}), the equations of square root for *L. monocytogenes* and Arrhenius for lactic acid bacteria (LAB) were used (Mataragas et al., 2006a,b). Also, only for *L. monocytogenes*, a second order equation was used (the equation was incorporated into the Gompertz equation) to calculate the maximum population density as function of temperature since it has been found that *L. monocytogenes* population was not the same at all temperatures examined (from 4 to 16^{o}C) during its growth in inoculated samples of a sliced cured cooked meat product (Mataragas et al., 2006b). A detailed demonstration of the use of the kinetic behavior models of both microorganisms can be found in Mataragas and Drosinos (2007).

Initial *L. monocytogenes* population in the sliced product (log cfu/g) (industry sub-module) was described by a Discrete distribution combining initial prevalence and concentration of the pathogen (Table 1). Prevalence in the following sub-modules (i.e. retail and consumer) was assumed to remain unchanged since cross-contamination of the product during its distribution and storage (retail and home) is not likely (vacuum-packaged product). In the consumer sub-module, product shelf life given by the industry (60 days) was combined with purchase day (purchase day = storage time until retail + transportation time from industry to retail + retail storage + transportation time from retail to home) to exclude the products exceeding shelf life at the time of consumption because it is unlikely these products to be consumed or purchased (Nauta et al., 2003).

3. Results and Discussion

The kinetic growth models used in the exposure assessment step predicted the *L. monocytogenes* or LAB growth as function of temperature (Mataragas et al., 2006a,b). Spoilage (SSOs growth) and shelf life duration were considered to estimate risk at the time of consumption based on the unspoiled-unsafe products. This fraction of the products poses a health risk for the consumers. If *L. monocytogenes* is present in the product, assuming *P* equal to the mean value of the Beta distribution in Table 1 (1.91%), the fractions considered at the time of consumption were: spoiled-unsafe, 0.38%; unspoiled-safe, 0.95%; spoiled-safe, 0.30%; and unspoiled-unsafe, 0.28%, representing 19.8, 49.8, 15.9 and 14.5%, respectively, of the contaminated products (i.e. 1.91%). Their values were obtained after applying Monte Carlo simulation running in parallel the growth of *L. monocytogenes* and SSOs.

The results showed that the *L. monocytogenes* dose consumed (log cfu/serving size) is described by a distribution with a mean value of 2.42 log cfu/serving size and 95% confidence interval from -0.34 to

221 6.65 log cfu/serving size (Fig. 1a). Fig. 1a shows that low prevalent high doses were responsible for the
222 highest number of listeriosis cases (the high bars in Fig. 1b was the result of the very low frequent high
223 exposures). Cross-contamination before eating was taken into consideration but sensitivity analysis
224 showed that it had a small contribution to the final risk. Therefore, in terms of simplicity this step was
225 not included in the final model. Besides, simple is not always wrong and complex always right
226 (Zwietering, 2009). Moreover, potential growth of the pathogen during storage of products, which may
227 lead to infectious doses at the time of consumption, is more important than the potential cross-
228 contamination during preparation (Yang et al., 2006). Indeed, the results of this study showed that
229 doses above 10^6-10^7 cfu/serving size at the time of consumption were responsible for 95% of the
230 simulated listeriosis cases (Fig. 1b).
231 Correlation coefficients, between inputs and output of the model, of the crude sensitivity analysis
232 showed that variables such as pathogen concentration at retail (0.67), storage duration (0.51) and
233 temperature (0.37) at retail, storage temperature (0.30) and duration (0.21) at industry, transport time to
234 home (0.22) and to the retailers (0.20), storage time at home (0.19), ambient temperature during
235 transport to home (0.17), amount of the product consumed (0.16) and temperature of home refrigerators
236 (0.10) had the greatest influence on the number of listeriosis cases per year. The remaining inputs of
237 the model had a correlation coefficient lower than 0.1 and, therefore, were not considered further (Fig.
238 2a). To have a more extended insight of the variability in parameters on the output of the model,
239 techniques like advanced sensitivity analysis (Fig. 2b) and sensitivity graphs (Figs 3a-f) were used
240 (Vose 2000; Perez-Rodriguez et al., 2007). Home fridge temperature and retail temperature, population
241 at retail, serving size consumed, storage time at home and retail were the most important parameters
242 from the set of those identified by the crude sensitivity analysis (Fig. 2b). Sensitivity graphs (Figs 3a-f)
243 display the changes in the number of listeriosis cases per year as function of the parameters identified
244 by advanced sensitivity analysis. The value at which a sharp increase (or a discrete inflexion point) in
245 the number of listeriosis cases is observed is known as threshold value.
246 The developed model was validated by comparing the predicted (mean value: 155 cases in high risk
247 population and 90% confidence interval: 0.0004 to 692) with observed (recorded) listeriosis cases (94
248 total cases in elderly people) (EFSA, 2008). Recorded cases were calculated using the equation:
249 $(Cases_{100000/RTE\ meat\ products} \times C_{65} \times PO_{total} \times PO_{high})/100000$, where $Cases_{100000/\ RTE\ meat\ products}$, the recorded
250 cases per 100000 of total population attributable to RTE meat products (0.18 cases); C_{65}, the recorded

cases occurred in individuals of age above 65 (56%); PO_{total}, the total population considered (467000000); and PO_{high}, the fraction of the high risk population (20%). Listeriosis cases attributable to RTE meat products were calculated according to FSIS (2008). *L. monocytogenes* illnesses due to consumption of meat and poultry products were equal to 66%. *L. monocytogenes* illnesses from meat and poultry products due to consumption of RTE meat products were equal to 91.2%. Thus, the 66×0.912 = 60% of *L. monocytogenes* illnesses was due to consumption of RTE meat products. Then, recorded cases attributable to RTE meat products: $Cases_{100000/RTE\ meat\ products} = Cases_{100000} \times Cases_{RTE\ meat\ products} = 0.3 \times 0.60 = 0.18$, where $Cases_{100000}$, the recorded cases per 100000 of total population (0.3 cases) (EFSA, 2008).

An important parameter, other than concentration of the pathogen at the time of consumption, is the prevalence of the pathogen. There are various combinations of concentration and prevalence that lead to similar probability of illness at the time of consumption (Havelaar et al., 2004). In Fig. 4, the $P_f - D$ equivalence curve representing the different combinations of prevalence-dose at the time of consumption that lead to similar risk (i.e. number of listeriosis cases per year) is given according to the developed model. The curve distinguishes the region of tolerable combinations of prevalence – dose from the intolerable region. So, the efficiency of any control measure applied for risk reduction can be evaluated using this graph. The implementation of control measures alters pathogen concentration and/or prevalence. These two parameters can be estimated from the developed model and thereafter to test if the simulated combination of prevalence – dose lies inside the tolerable region.

According to the advanced sensitivity analysis, significant parameters influencing the final risk estimation were mainly related with retail and home storage. Given the fact that cross-contamination of the products during their retail is unlikely, prevention or at least reduction of cross-contamination during their manufacturing becomes extremely important. At temperatures higher than 7-9^{o}C (threshold values according to Fig. 3d and 3f) a sharp increase in listeriosis cases occurs. Therefore, storage of the products at temperatures below this level could contribute to listeriosis cases reduction because the extended growth of the pathogen is inhibited. Consequently, there is the need of training of the people involved in transportation, distribution and storage of the products, including consumers, in the basic measures of food safety [low temperatures (3-4^{o}C), adequate cooking, separation of fresh products from RTE products, adequate/good cleaning of hands, equipment, tools and other utensils] (Sofos, 2008).

The results obtained from the QMRA study could be directly 'translated' in POs (Fig. 5). In the present study, a PO could be the prevalence and/or the concentration of the pathogen that should not be exceeded at the time of consumption. For instance, in Fig. 4 the equivalence curve (baseline model) was estimated when P=1.91% (Table 1). Therefore, this value could be considered as the PO that should not be exceeded because higher values could lead to $P_f - D$ combinations outside the tolerable region. This PO could be also placed in the industry sub-module since cross-contamination of the products during distribution is unlikely or if it happens at consumer level is not as important as the growth of the pathogen (Yang et al., 2006). Furthermore, pathogen concentration should not exceed a specified level (Fig. 5) in order the final $P_f - D$ combination to be in the tolerable region (Fig. 4). Therefore, final products should be analyzed by the manufacturer to verify or confirm such low values of prevalence and concentration. For this purpose, microbiological criteria (MC) are applied to ensure that POs are not being exceeded. MC is one of the potential control measures to reduce risk (Reij and Zwietering, 2008). When the distribution of the pathogen of concern is known (e.g. from a QMRA study), industry-specific MC, aimed to verify compliance with a PO, could be developed using statistical methods.

Based on the QMRA results obtained in the present study an example is given. Knowledge of pathogen distribution within the lot and its expected standard deviation (s.d.) is important in order to develop a MC. This information could be experimentally determined from the QMRA study (i.e. intermediate output of the industry sub-module). After performing Monte Carlo simulation, the mean and s.d. of the output distribution ($N_{0,s}$ parameter in Table 1) were -3.08 and 1.02 log cfu/g, respectively. It was further assumed that *L. monocytogenes* log counts follow within the lot a normal distribution with these characteristics in order to determine the MC. Log-normal distribution of a pathogen in food is usually assumed and it provides the basis for establishing a mathematical relationship between PO and MC (van Schothorst et al., 2009). The s.d. of 1.02 log cfu/g indicates a rather non-homogeneously distribution of the microorganism within the lot which is usually the case for solid foods. The aim of the MC is to decide whether a food lot is acceptable or unacceptable. This is a two-class attribute test characterized by the number of samples to be analyzed (n), the number of samples that are allowed to exceed the test criteria (c) (for pathogens, c is usually zero), the lower limit of detection for the test (m) and the confidence level (e.g. 95% or 99%) that the test will identify and reject a non-conforming or unacceptable lot (i.e. consumer Acceptable Level for Safety – consumer ALS) (Whiting et al., 2006).

For this example, a consumer ALS of 95% was assumed. Usually, microbiological testing protocols involve enrichment of 25g of food product (analytical units) and presence/absence testing of the pathogen on selective media. To estimate the number of analytical units (i.e. samples) that need to be tested, a modified procedure (i.e. Poisson-log-normal distribution) for determining the effectiveness of enrichment tests was followed (van Schothorst et al., 2009). The results showed that the probability of acceptance of a lot (with mean -3.08 and s.d. 1.02), based on a single sample, was 1 – 0.1091 = 0.8909 (the probability that a cell is present in the sample taken and leads to detection of a positive was 0.1091). Consequently, more negative samples are required to reach 95% confidence. Taking 25 or 26 samples, the probability that all samples are acceptable was $(0.8909)^{25}$ = 0.0557 and $(0.8909)^{26}$ = 0.0496, respectively. Given this calculation scheme, 26 negative samples (n = 26 and c = 0) are required to reject with more than 95% certainty a lot that has log mean concentration and s.d. greater than the corresponding determined values (i.e. the parameters of normal distribution) because taking 25 samples for analysis the confidence level was still below 95%. Another decision that must be made is to determine the safety level that is required (i.e. maximum frequency and/or concentration of the hazard) and its corresponding relationship to the lot mean. This safety limit comprises the PO. As described above, based on the QMRA results, a PO could be the *L. monocytogenes* prevalence ≤ 1.91%. Given the distribution of *L. monocytogenes* in the lot, the proportion of the allowable defective units (i.e. 1.91%) can be translated into an estimation of the maximum concentration of the pathogen in the lot that should not be exceeded (Fig. 5). The latter was calculated as follows: *L. monocytogenes* mean concentration of -3.08 log cfu/g and prevalence of 1.91% are the maximum values that can be tolerated because this combination is located on the $P_f - D$ equivalence curve (Fig. 4). The PO is determined by adding a certain number of s.d. to the hazard maximum tolerable concentration so that the required percentage of the lot will have concentrations below PO. The required number of s.d. is termed the *z* score. Therefore, in order 98.09% of the units to be at or below the target PO (or 1.91% of the units to be above the target PO), the number of s.d. that should be added is 2.07 (-3.08 + 2.07*1.02 = -0.97 log cfu/g) (Whiting et al., 2006; van Schothorst et al., 2009). The curve in Fig. 5 with a mean of -3.08 log cfu/g, s.d. of 1.02 log cfu/g and PO at -0.97 log cfu/g was assigned as the ‘just unacceptable lot’ that the MC should reject in 95 times out 100 (Whiting et al., 2006). Distributions with lower mean values will have higher probability of acceptance. Therefore, for this specific example the developed MC, for 95% confidence of lot rejection when it has more than 1.91% of the units above the PO or

341 contamination is greater than or equal to the lot mean, would be: Lot mean = -3.08 log cfu/g, s.d. =
342 1.02 log cfu/g, *m* = absence in 25g, *c* = 0, *n* = 26 and PO = -0.97 log cfu/g. Finally, an operating
343 characteristic curve (OC-curve) can be constructed to characterize the performance of the developed
344 MC (Fig. 6a) or relate the OC-curve to the mean pathogen concentration to obtain the consumer and
345 producer ALSs (Fig. 6b) (ICMSF, 2002; van Schothorst et al., 2009).
346 The QMRA study revealed areas on which to focus efforts to reduce listeriosis: reformulation of
347 products, the product is able to support growth of *L. monocytogenes*, thus, industry could reduce the
348 risk by reformulating the product so it no longer supports pathogen growth or through treatment after
349 packaging; review of product shelf life, product shelf life can be reassessed by taking into account *L.*
350 *monocytogenes* growth during storage; sufficient sanitation practices in industry to reduce cross-
351 contamination; surveillance of microbiological status of products, microbiological criteria and
352 sampling plans could be established in industry to meet pre-defined pathogen levels (i.e. POs) or to set
353 stringency of a food control system; improved control of temperature during distribution and storage.
354 This can be achieved through training of the people involved in these processes; and risk
355 communication messages/programs to consumers, educational messages/programs for consumers to
356 note the need of keeping refrigerator temperatures at or below 4-5^{o}C. Actually, the consumer should
357 also contribute to the safety of a product. This could also be emphasized via the FSO concept (FSO=at
358 consumption), so growth in last part is in the consumers' hand.
359
360 **4. Conclusions**
361 A QMRA study may give valuable information regarding the presence and development of a
362 microbiological hazard in a food product. This information is "translated" in: 1) identification of risk
363 factors contributing to occurrence of clinical manifestations due to consumption of products
364 contaminated with a pathogen, 2) determination of threshold values of the risk factors above which a
365 sharp increase in the number of infection cases is observed and 3) application of control measures to
366 reduce illness (i.e. risk management) (Zwietering and Nauta, 2007) (Fig. 7). The people involved in
367 food safety may use this information to draw conclusions, publish directives relative to risk
368 management or establish POs and/or PC. The QMRA model can be used as baseline to evaluate the
369 effectiveness of different risk management options or control measures (i.e. "what-if" scenarios).
370 Examples of such control measures, for this specific combination of *L. monocytogenes* and RTE meat

products, could be: the likelihood of antimicrobials addition (e.g. lactate, di-acetate, etc.) during product manufacturing or product immersion in a solution containing antimicrobial compounds (Lianou et al., 2007), the suggestion of thermal treatment of the final product with steam or hot water before consumption, the application of high hydrostatic pressure or irradiation (ILSI, 2005). Application of antimicrobial agents or a final process step with antimicrobial activity, have been integrated in regulations specifically published for the control of *L. monocytogenes* in RTE meat products (FSIS, 2003). Another control measure, as indicated by this study, could be the decrease of product shelf life at or close to its threshold value (i.e. 18-20 days) (Fig. 3). This will lead to a lower dose at the time of consumption and the resulting $P_f - D$ combination will be inside the tolerable region (Fig. 4). Product shelf life should be determined taking into account the potential growth of the pathogen during storage. In this manner, safety-based "use-by" date labels for refrigerated RTE foods could be developed (NACMCF, 2005). If shelf life studies indicate that a level of 100 cfu/g is likely to be exceeded before the end of the set shelf life, then shelf life or food safety management procedures should be reviewed (e.g. review of the implemented MC to ensure *L. monocytogenes* presence below a pre-specified level, i.e. the PO). Finally, equal approaches can be used for other deli meats or even other RTE foods. If specific parameters values and specific particularities of the product and process are taken into account equal types of analysis can be helpful in evaluating the risk and potential effects of interventions.

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528

Figure Captions

Fig. 1. Relationship a) frequency of doses at the time of consumption and b) dose at the time of consumption with number of listeriosis cases.

Fig. 2. Crude and advanced sensitivity analysis with a) Correlation coefficients (crude) and b) Tornado graph (advanced) displaying the most important factors contributing to model output.

Fig.3 Sensitivity graphs showing the threshold values of the parameters identified by the advanced sensitivity analysis. Solid square points indicate the 1, 5, 25, 50, 75, 95 and 99% cumulative probabilities of the input distributions.

Fig. 4. Prevalence-dose ($P_f - D$) combinations at the time of consumption that lead to similar risk or same number of listeriosis cases per year (solid line) according to the QMRA model developed. During construction of the $P_f - D$ equivalence curve, consumption patterns and fraction of unspoiled-unsafe products were taken into consideration. The equivalence curve defines the limit between tolerable and intolerable region. The equation (1) was used to determine the equivalence curve [to build the curve, values were drawn from the dose (D) distribution (Fig. 1a), e.g. 100 doses corresponding to the 100 simulations performed, and for each dose value (the mean from 10000 iterations performed at each simulation) the corresponding P_f value was calculated using for the remaining parameters of *Risk*, S_{all} and r the mean values of their distributions]: *Risk*, 157 listeriosis cases per year (the mean of the output distribution in Table 3); S_{all}, 1.4×10^{9} servings/year (as calculated in Table 3 using the mean (50g) of the Pert distribution by which the s parameter was described); r, (2.6×10^{-10}) (the mean of the Pert distribution in Table 3). (●) $P_f - D$ values estimated by the model at the time of consumption [current situation according to the baseline model developed; P=1.91% (the mean of the Beta distribution in Table 1), $P_{unsp\text{-}unsf}$=0.28% (P_f=P×14.5%, see Table 3) and D=2.42 log cfu], (▲) $P_f - D$ values estimated by the model at the time of consumption after the implementation of control measures to reduce prevalence of the pathogen in the industry sub-module (e.g. if GMP and GHP properly and effectively applied it could be P=1.00% then, according to the model, P_f=0.15% and D=2.53 log cfu), (♦) $P_f - D$ values estimated by the model at the time of consumption after the implementation of control measures to reduce concentration of the pathogen in the industry sub-module (e.g. application of MC to reject lots with pathogen population above a pre-specified level such as -3.08 log cfu/g. P=1.91% and distribution of *L. monocytogenes* equal to producer ALS, i.e. Normal(-5.26, 1.02) log cfu/g then, according to the model, P_f=0.28% and D=1.39 log cfu) and (■/□)$P_f - D$ values estimated by the model

559 at the time of consumption after the reduction of product shelf life from its actual value of 35 days (■;
560 P=1.91%, P_f=0.28% and D=3.35 log cfu) to 18 days (□; P=1.91%, P_f=0.28% and D=2.25 log cfu).
561 Shelf life of 35 days is based on industry data (i.e. returns) and studies dealing with shelf life
562 establishment of this particular product (Mataragas et al., 2006a). The shelf life of 60 days given by the
563 industry has been determined at constant temperature conditions (4^{o}C).
564 Fig. 5. Construction of 'just unacceptable lot' that MC should reject with 95% confidence.
565 Distributions with lower mean values will have higher probability of acceptance and distributions with
566 higher mean values will be rejected by the MC (> 95% probability of rejection).
567 Fig. 6. OC-curve that relates the probability of accepting a lot to a) defective proportion based on the
568 number of samples tested (n) and samples in excess of m (c, m = absence in 25g) and b) mean pathogen
569 population displaying the consumer and producer ALSs.
570 Fig. 7. Relationship between risk management and exposure assessment, dose-response and risk
571 characterization. Pathogen final concentration (N_t) is determined by initial contamination of products
572 (N_0), potential cross-contamination during and/or after processing (CC), increase (I), survival (S) and/or
573 reduction due to inactivation (R) during processing. Parameter (N_t) should be lower or at least equal to
574 the FSO. Dose (D) consumed at the time of consumption, which is the final population (N_t) multiplied
575 by the serving size (SS) consumed, combined with a dose-response model provide the risk per serving
576 (RpS). The risk is converted into probability of illness (P_{ill}) or number of cases based on the total
577 number of servings consumed in a year (S_{all}). The final risk is compared to the ALOP. To meet the
578 FSO, establishment of *POs* [maximum frequency of occurrence (%) and/or concentration (cfu/g) of a
579 pathogen], *PC* [change (i.e. reduction or tolerated increase) in frequency of occurrence and/or
580 concentration of a pathogen that should be achieved during processing or implementation of control
581 measures] and *PrC* (conditions required to achieve the desired *PO/PC*, e.g. time-temperature
582 combination) prior to consumption is necessary. Finally, compliance with *PO/PC*, and consequently
583 with *FSO*, is verified by the application of *MC* (level and/or frequency of occurrence of a pathogen
584 detected by the implementation of specific analytical method and sampling plan) [adapted from
585 Zwietering and Nauta (2007); Whiting and Buchanan (2008)].
586

587 Table 1. Industry sub-module[a]

Parameters	*Units*	*Notation*	*Description*	*Inputs*
Prevalence	%	P	Beta(645, 33180)[b]	Uncertainty of *L. monocytogenes* prevalence
New prevalence	%	P_{new}	Sampling from Beta distribution	
Prevalence of samples under detection limit	%	P_{neg}	$1 - P_{new}$	
Concentration in positive samples	log cfu/g	C	Cumulative(-1.4, 3, {-1.4, -1, 0, 0.7, 1, 1.7, 2, 3}, {0.83, 0.90, 0.93, 0.95, 0.96, 0.98, 0.99, 1})[b]	Variability of *L. monocytogenes* concentration in positive samples
New concentration	log cfu/g	C_{new}	Sampling from Cumulative distribution	
Concentration of samples under detection limit	log cfu/g	C_{neg}	Uniform(-4.83, -1.40)[c]	Uncertainty associated with the mean value of *L. monocytogenes* concentration in negative samples
Initial population in finished sliced product	log cfu/g	$N_{0,s}$	Discrete(C_{new}:C_{neg}, P_{new}:P_{neg})	
Storage temperature	^{o}C	T_0	Pert(0, 2, 4)	Uncertainty of storage temperature modeling of experts opinion
Storage time until retail (sliced product)	days	$t_{0,s}$	Pert(0.1, 1, 3)	Uncertainty of storage time until retail modeling of experts opinion
Population in sliced product after storage	log cfu/g	$N_{1,s}$	modified Gompertz equation[d]	
Transport time to the retailers	days	t_1	Pert(0.05, 0.15, 0.5)	Uncertainty of transport time to the retailers modeling of experts opinion
Transport temperature	^{o}C	T_1	Pert(5, 7, 12)	Uncertainty of transport temperature modeling of experts opinion
Population in sliced product after transport	log cfu/g	$N_{2,s}$	modified Gompertz equation[d]	

588 [a] After slicing
589 [b] Values of prevalence (33823 total samples analyzed, 644 positive) and concentration were taken from
590 the FDA/USDA risk assessment study regarding the *L. monocytogenes* presence in RTE foods (2003)

[c] To give a mean value equal to -3.11 log cfu/g which was an estimation of the pathogen concentration of samples under detection limit. The mean concentration of samples under detection limit was calculated by the equation (Jarvis, 2000): $\boldsymbol{mean = -\left(2.303 / AUs\right) \times \log\left(S_{neg} / S_{total}\right)}$, where *mean*, the mean concentration in cfu/g; *AUs*, the analytical units tested (e.g. 25g); S_{neg}, the number of samples tested as negative (33179); and S_{total}, the total number of samples analyzed (33823) (FDA/USDA, 2003)

[d] Kinetic parameters (μ_{max} and t_{lag}), were determined using the secondary models of the square root (*L. monocytogenes*) and Arrhenius (lactic acid bacteria) (Mataragas et al., 2006b). Also, only for *L. monocytogenes*, a second order polynomial equation was used to calculate the maximum population density (N_{max}) (Mataragas et al., 2006b)

Table 2. Retail sub-module

Parameters	*Units*	*Notation*	*Description*	*Inputs*
Population in sliced product after transport	log cfu/g	$N_{2,s}$	From the Industry sub-module	
Retail temperature	^{o}C	T_2	Normal(5.44, 2.32)	Variability of retail temperature
Storage time at retail[a]	days	$t_{2,95\%}$	Uniform(0, 45)	Uncertainty about the mean of storage time at retail from 0 to 45 days
Storage time at retail[a]	days	$t_{2,5\%}$	45+Uniform(0, 15)	Uncertainty about the mean of storage time at retail from 45 to 60 days
Population in sliced product after retail storage	log cfu/g	$N_{3,s,95\%}$	modified Gompertz equation[b]	
Population in sliced product after retail storage	log cfu/g	$N_{3,s,5\%}$	modified Gompertz equation[b]	
Population in sliced product after retail storage	log cfu/g	$N_{3,s}$	Discrete($N_{3,s,95\%}$: $N_{3,s,5\%}$, 0.95:0.05)	
Ambient temperature[c]	^{o}C	T_A	Pert(0, 20, 40)	Uncertainty of ambient temperature modeling of experts opinion
Max change in temperature during transport[c]	^{o}C	ΔT_{max}	T_A-T_2	
Potential change in temperature during transport[c]	^{o}C	T_{pc}	Normal(3.72, 2.82)	Variability of potential change in temperature during transport
Change in temperature during transport[c]	^{o}C	T_c	IF($\Delta T_{max} \leq 0$, 0, T_{pc})	
Product temperature after transport[c]	^{o}C	T_p	T_2+T_c	
Average transport temperature[c]	^{o}C	T_m	Average(T_2, T_p)	
Transport time to home	days	t_3	Cumulative(15, 225, {15, 37.5, 52.5, 75, 135, 225}, {0.57, 0.77, 0.86, 0.95, 0.99, 1})/1440[d]	Variability of transport time to home

Population in sliced product after transport	log cfu/g	$N_{4,s}$	modified Gompertz equation[b]

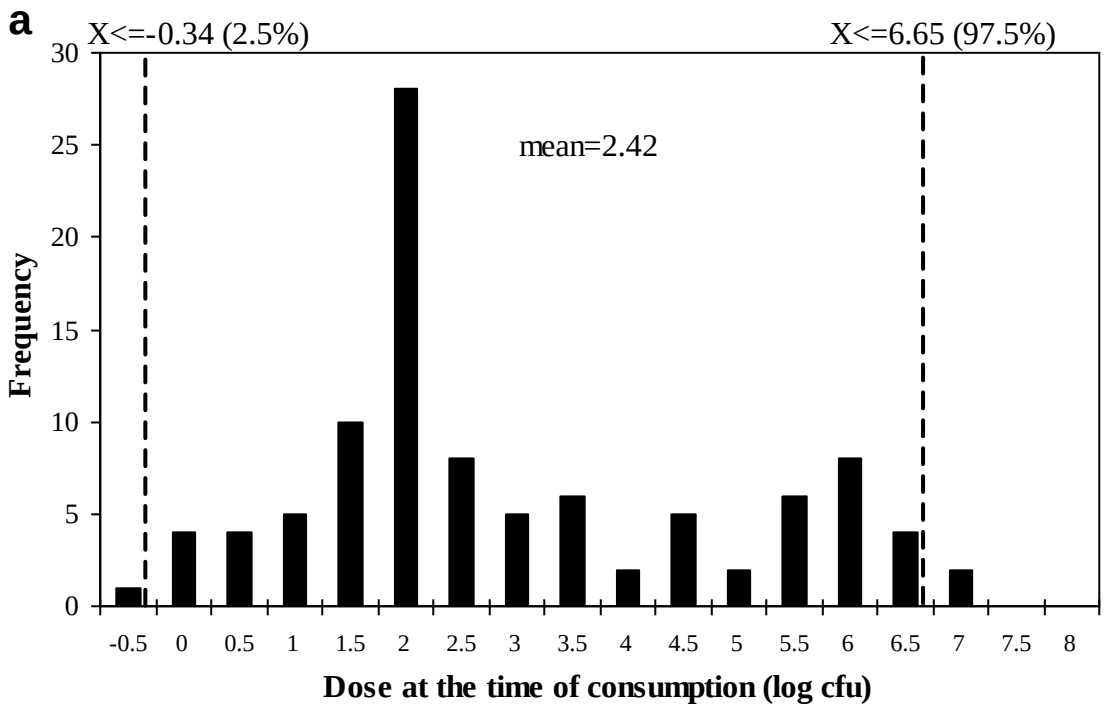
[a] Storage time at retail was estimated according to Nauta et al. (2003). Shelf life of products, given by industry, equal to 60 days. Percentage of products sold within the first 45 days (95%) and percentage of products sold the last 15 days of their shelf life (5%)
[b] See Table 1
[c] Changes in temperature during transport were estimated according to FDA/USDA (2003)
[d] Transport time in minutes converted to days (1 day = 1440 min)

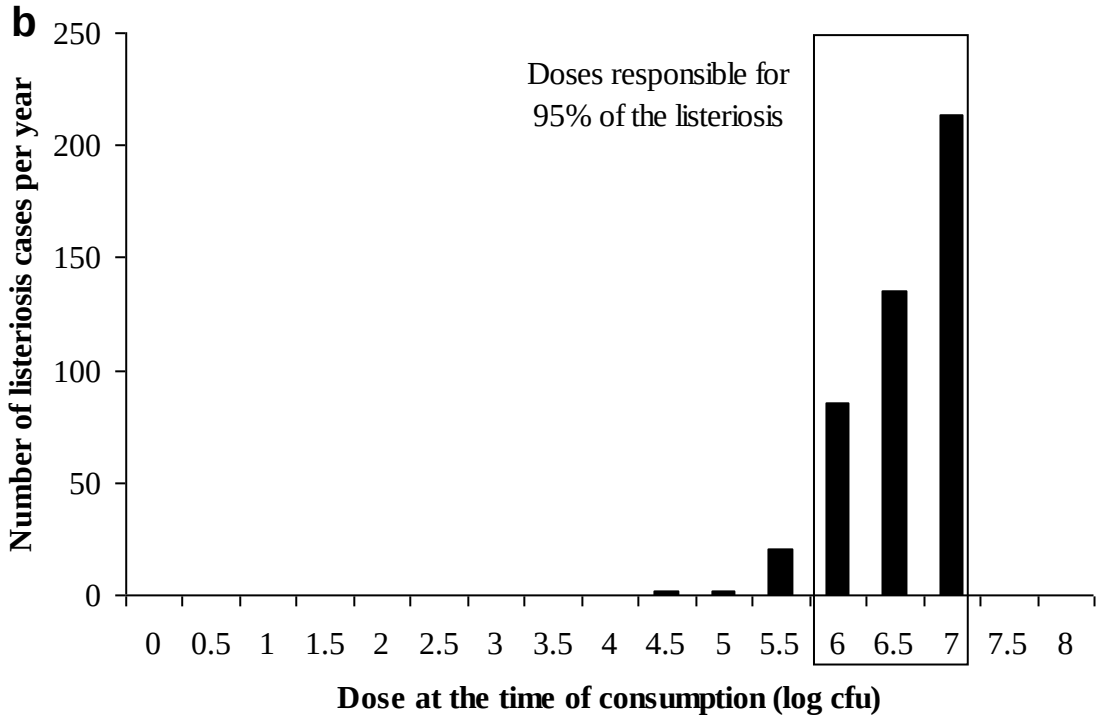
Table 3. Consumer sub-module

Parameters	*Units*	*Notation*	*Description*	*Inputs*
Population in sliced product after transport	log cfu/g	$N_{4,s}$	From the Retail sub-module	
Home fridge temperature	^{o}C	T_3	BetaGeneral(2.5282, 4.7672, 1.5501, 18.773)	Variability of home fridge temperature
Storage time at home	days	t_4	Cumulative(1, 49, {1, 2, 3.5, 5.5, 7, 14, 21, 35, 49}, {0.02, 0.77, 0.39, 0.50, 0.76, 0.78, 0.84, 0.97, 0.99})	Variability of storage time at home
Day of purchase[a]	days	$PD_{95\%}$	$t_{0,s}+t_1+t_{2,95\%}+t_3$	
Day of purchase[a]	days	$PD_{5\%}$	$t_{0,s}+t_1+t_{2,5\%}+t_3$	
Day of purchase	days	PD	Discrete($PD_{95\%}$: $PD_{5\%}$, 0.95:0.05)	Uncertainty associated with day of purchase
Shelf life indicated by the manufacturer	days	SL	60	
Population in sliced product after home storage	log cfu/g	$N_{5,s}$	IF($t_4+PD>SL$, 0, modified Gompertz[b])	
Weight of slice	g	W_s	20	
Serving size consumed	g	S	Pert(0, 50, 100)	Uncertainty of serving size modeling of experts opinion
Population at the time of consumption (dose)	log cfu	$N_{6,s}$ (D)	$\log(10^{N5,s}\times S)$	
Total population	-	PO_{total}	467000000	
High risk population	%	PO_{high}	20	
No. of servings consumed per person	slices	S_p	S/W_s	
No. of servings consumed by high risk population	slices	S_{high}	$S_p\times PO_{total}\times(PO_{high}/100)$	
Frequency of consumption (consumption on monthly basis by half of the population)[c]	per year	F	0.5×12	
No. of slices consumed/year by high risk population	-	S_{all}	$S_{high}\times F$	

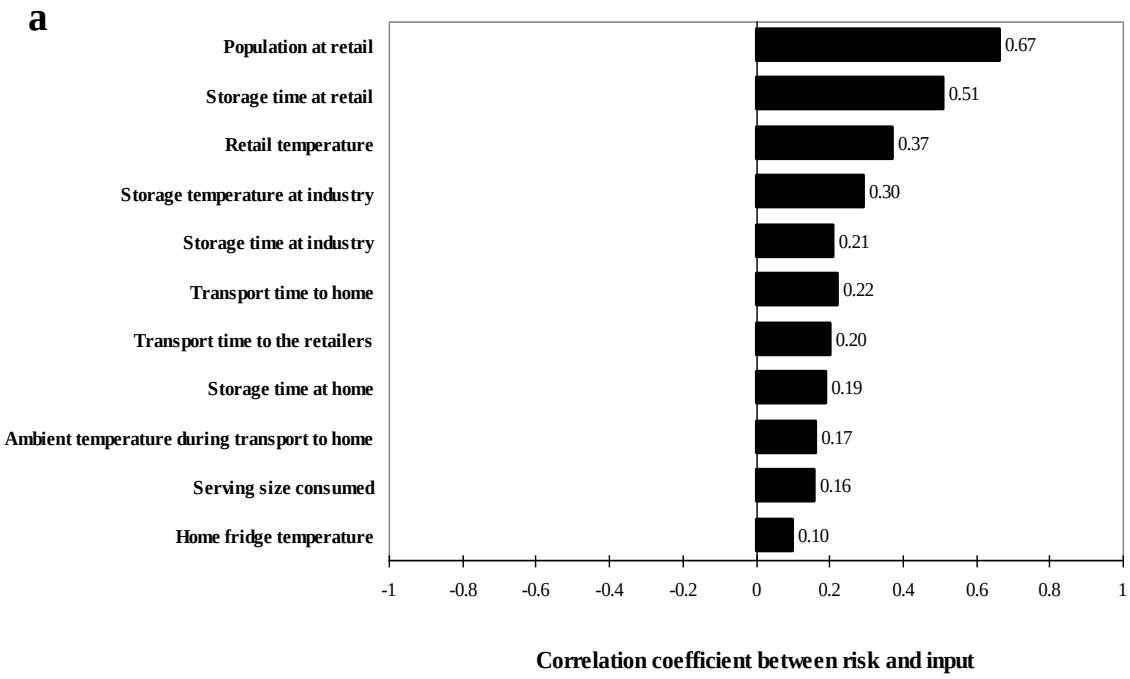
Unspoiled-Unsafe fraction[d]	%	P_f	$14.5 \times P$	
r-parameter[e]	-	*r*	Pert(1.11×10^{-15}, 4.47×10^{-11}, 1.36×10^{-9})	Uncertainty about the *r* value
Risk (annual cases)	-	*Risk*	$S_{all} \times r \times 10^{D} \times (P_f/100)$	

[a] Day of purchase was estimated according to Nauta et al. (2003)
[b] See Table 1
[c] Not all the people consume RTE meat products. Frequency of consumption of RTE meat products was estimated based on FAOSTAT (2007) data (Mataragas et al., 2008)
[d] This was calculated taking also into account SSOs growth. Assuming *P* equal to the mean value of the Beta distribution in Table 1 (1.91%) the fractions considered at the time of consumption were: spoiled-unsafe, 0.38%; unspoiled-safe, 0.95%; spoiled-safe, 0.30%; and unspoiled-unsafe, 0.28%, representing 19.8, 49.8, 15.9 and 14.5%, respectively, of the contaminated products (i.e. 1.91%)
[e] Simulation of the *r* parameter, using the equation: $r = -[\ln(1-P_{ill})]/D$, where P_{ill}, the probability of illness for the elderly people (high risk population) according to the QMRA study of *L. monocytogenes* presence in deli meats conducted by FDA/USDA (2003) (5×10^{-9}); and *D*, the dose at the time of consumption, and application of the bootstrap technique to determine the *min*, *most likely* and *max* values of the Pert distribution

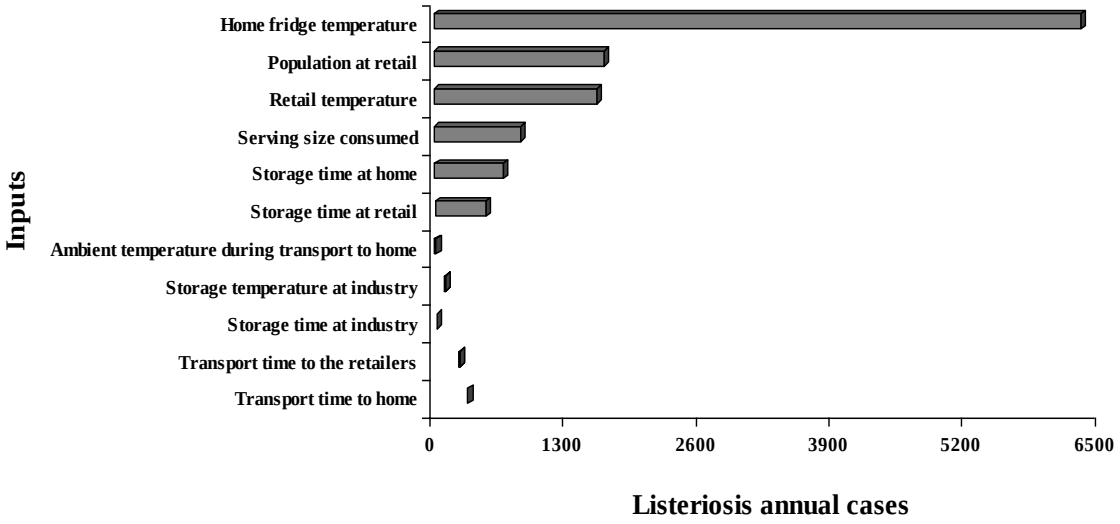

a
X<=-0.34 (2.5%)
X<=6.65 (97.5%)
mean=2.42
Frequency
30
25
20
15
10
5
0
-0.5 0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8
Dose at the time of consumption (log cfu)

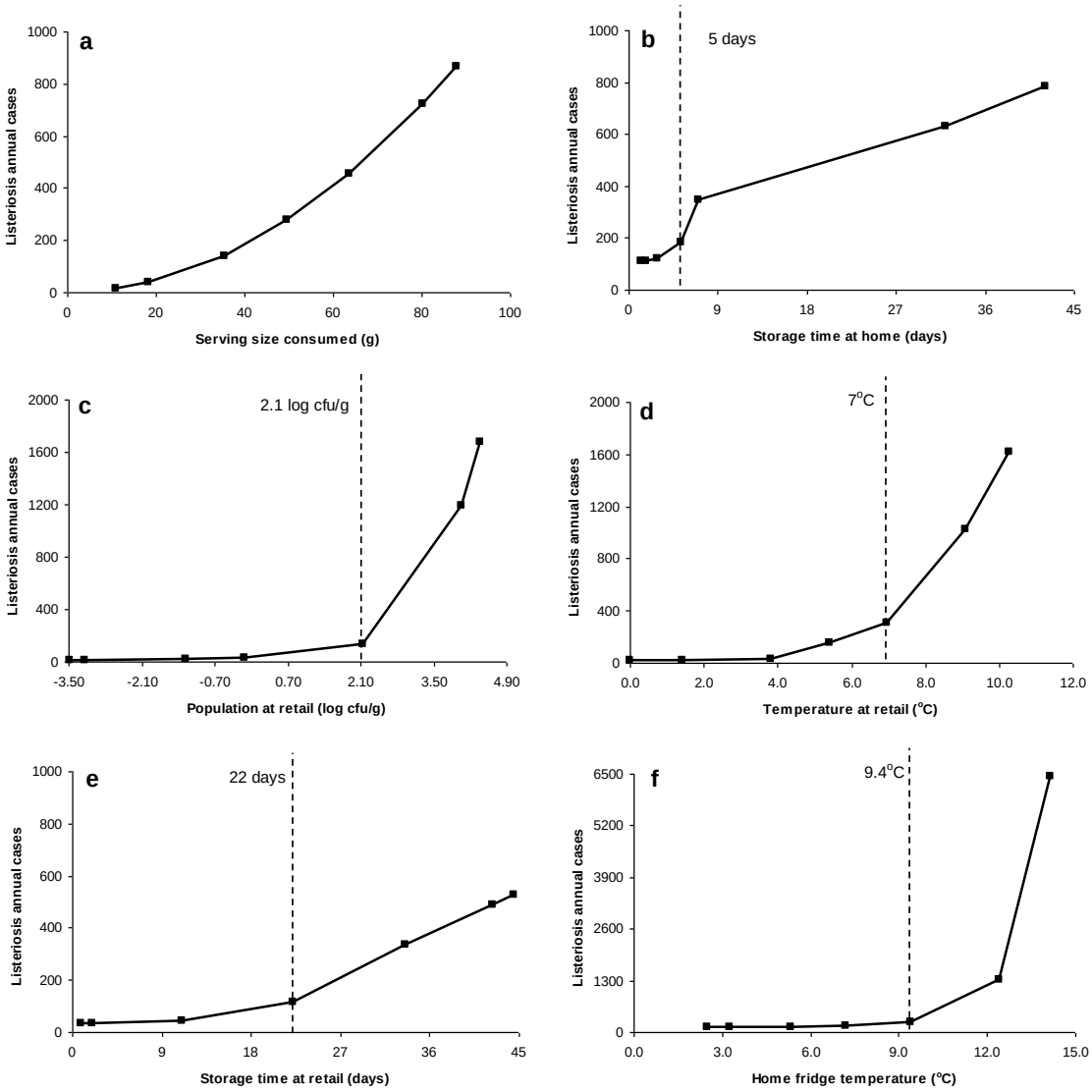

b
Doses responsible for
95% of the listeriosis
Number of listeriosis cases per year
250
200
150
100
50
0
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5 5.5 6 6.5 7 7.5 8
Dose at the time of consumption (log cfu)

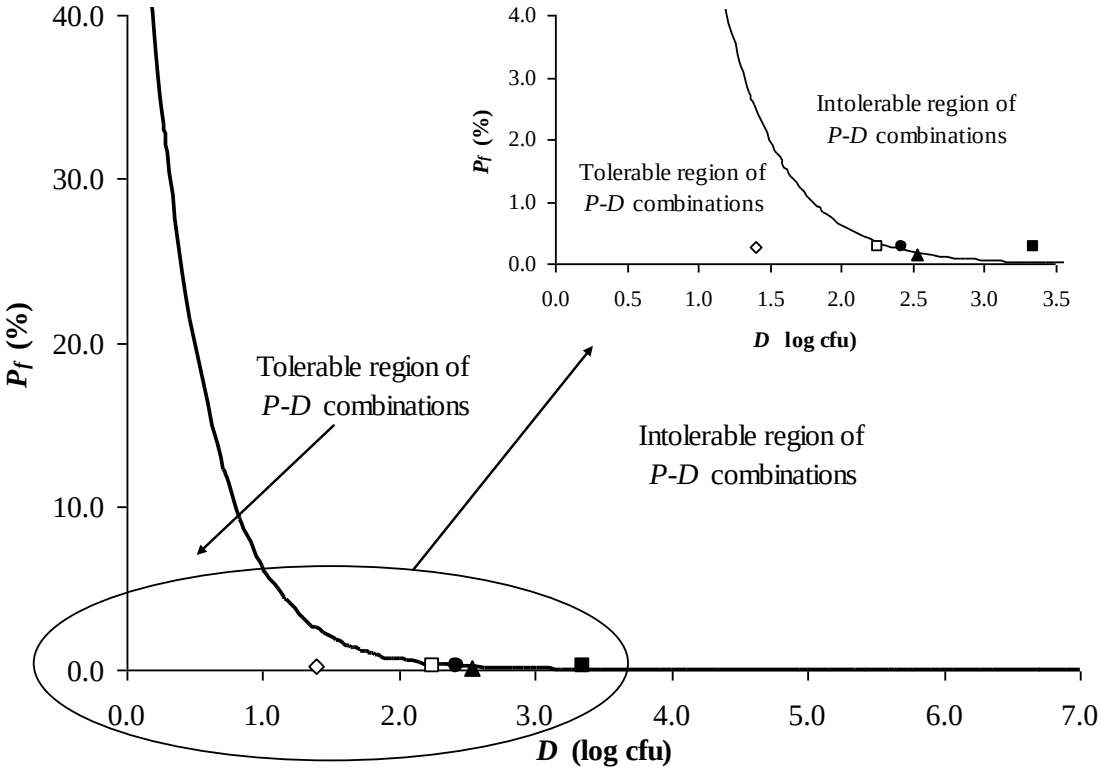
a

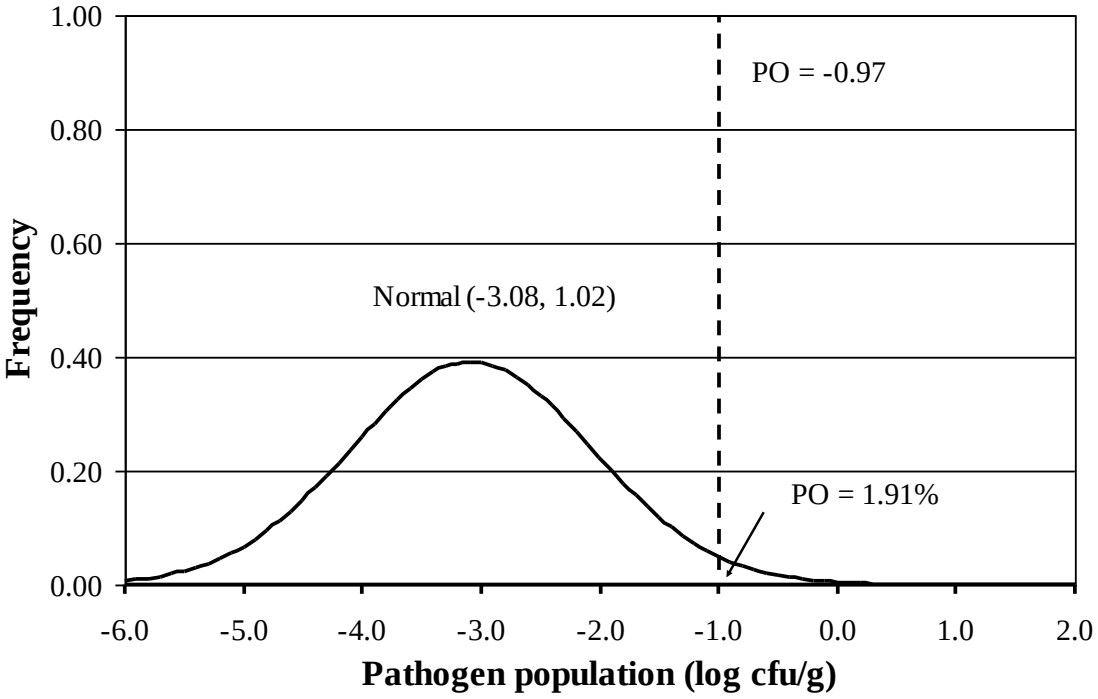

Population at retail
Storage time at retail
Retail temperature
Storage temperature at industry
Storage time at industry
Transport time to home
Transport time to the retailers
Storage time at home
Ambient temperature during transport to home
Serving size consumed
Home fridge temperature
0.67
0.51
0.37
0.30
0.21
0.22
0.20
0.19
0.17
0.16
0.10
-1
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8
1
Correlation coefficient between risk and input

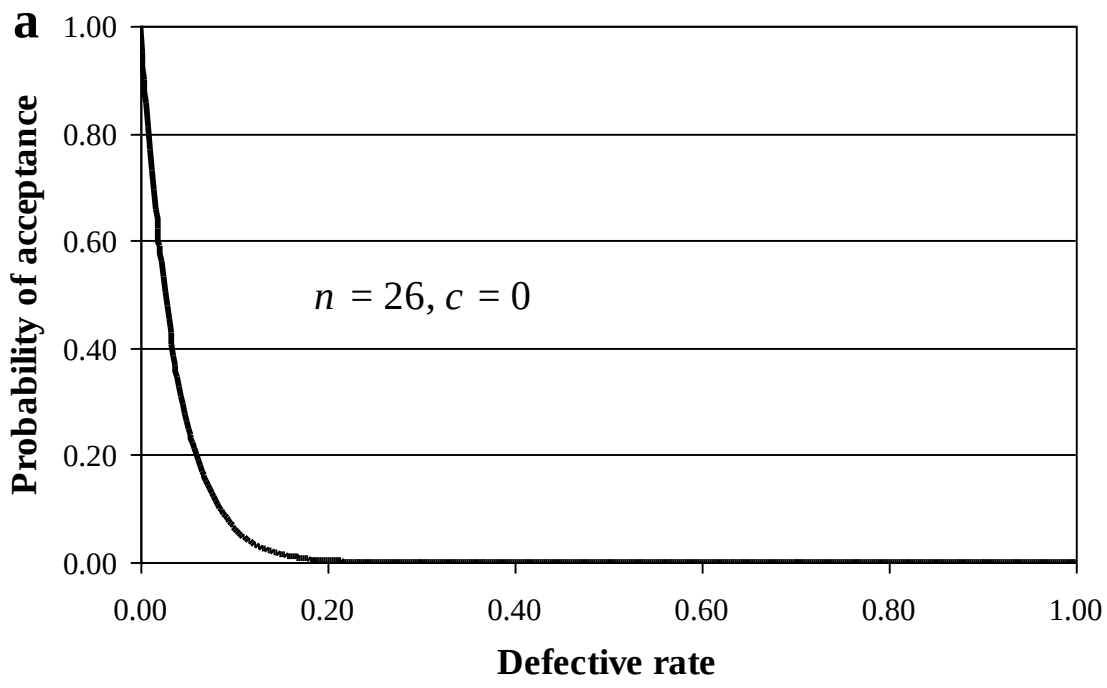
b

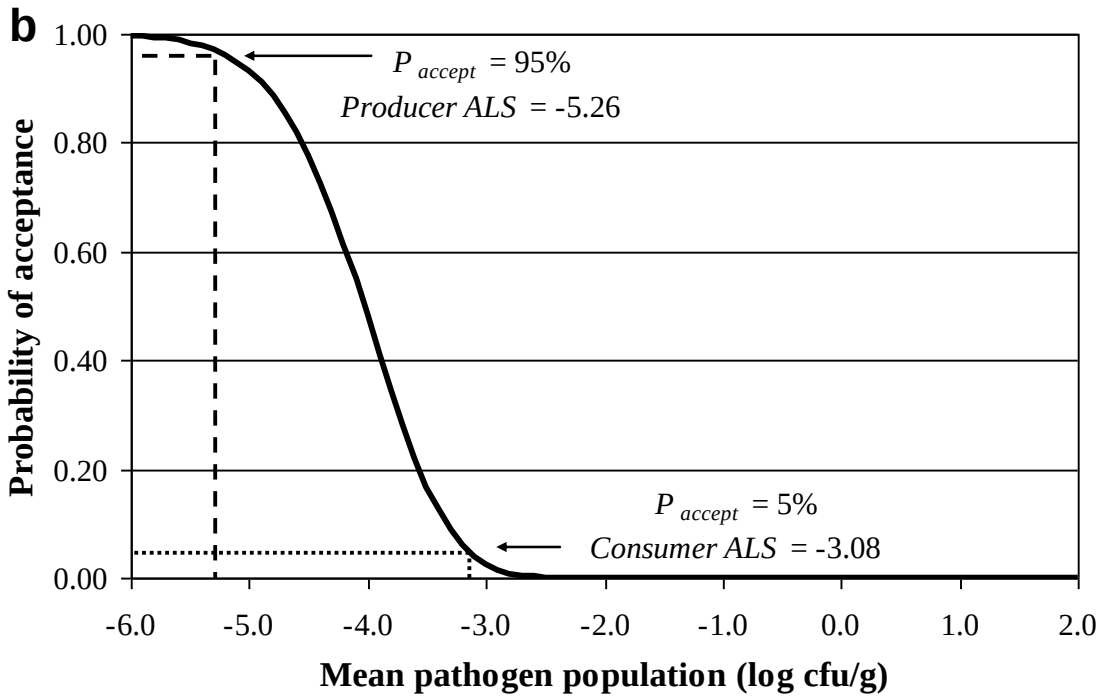

Inputs
Home fridge temperature
Population at retail
Retail temperature
Serving size consumed
Storage time at home
Storage time at retail
Ambient temperature during transport to home
Storage temperature at industry
Storage time at industry
Transport time to the retailers
Transport time to home
0
1300
2600
3900
5200
6500
Listeriosis annual cases

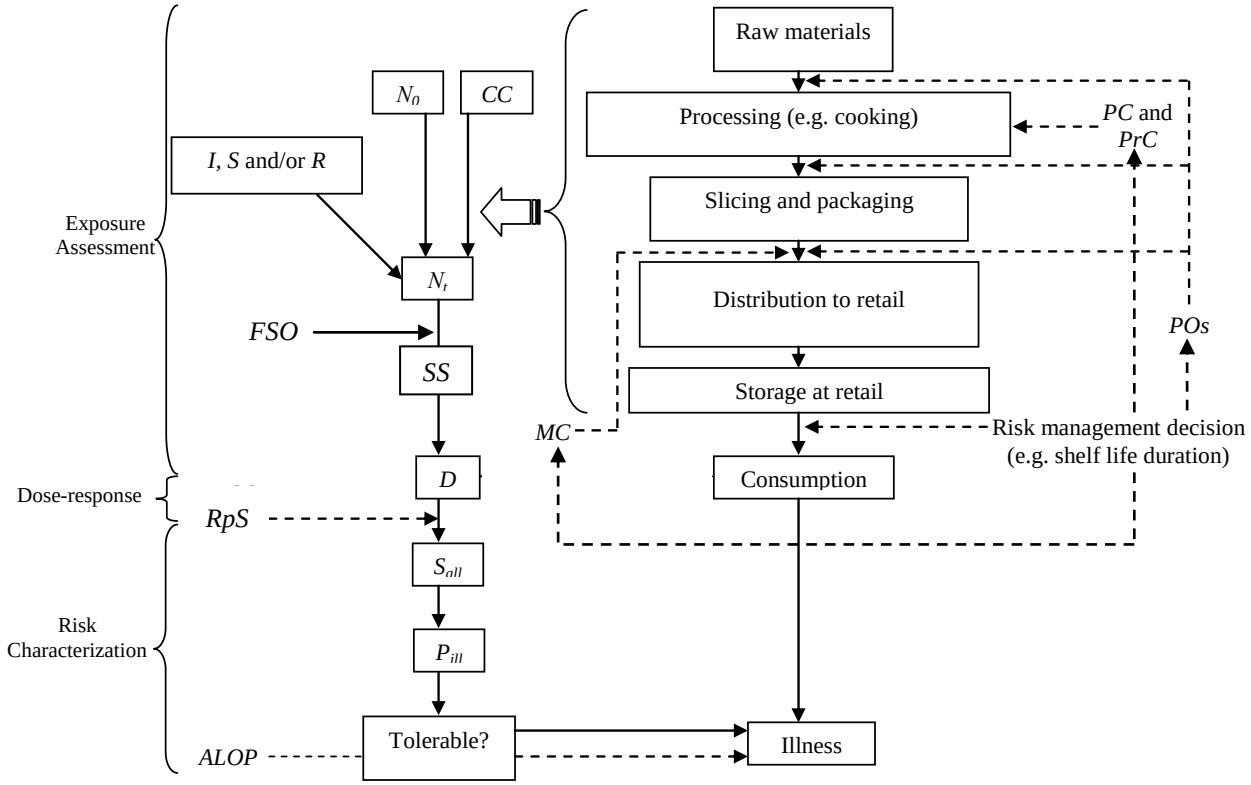

a
Listeriosis annual cases
Serving size consumed (g)
b
5 days
Storage time at home (days)
c
2.1 log cfu/g
Population at retail (log cfu/g)
d
7°C
Temperature at retail (°C)
e
22 days
Storage time at retail (days)
f
9.4°C
Home fridge temperature (°C)


40.0
30.0
20.0
10.0
0.0
P_f (%)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
D (log cfu)
Tolerable region of *P-D* combinations
Intolerable region of *P-D* combinations
4.0
3.0
2.0
1.0
0.0
P_f (%)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
D log cfu)
Intolerable region of *P-D* combinations
Tolerable region of *P-D* combinations


1.00
0.80
0.60
0.40
0.20
0.00
Frequency
PO = -0.97
Normal (-3.08, 1.02)
PO = 1.91%
-6.0
-5.0
-4.0
-3.0
-2.0
-1.0
0.0
1.0
2.0
Pathogen population (log cfu/g)


a
Probability of acceptance
1.00
0.80
0.60
0.40
0.20
0.00
n = 26, c = 0
0.00
0.20
0.40
0.60
0.80
1.00
Defective rate


b
Probability of acceptance
1.00
0.80
0.60
0.40
0.20
0.00
P accept = 95%
Producer ALS = -5.26
P accept = 5%
Consumer ALS = -3.08
-6.0
-5.0
-4.0
-3.0
-2.0
-1.0
0.0
1.0
2.0
Mean pathogen population (log cfu/g)


Raw materials
N_0
CC
Processing (e.g. cooking)
PC and PrC
I, S and/or R
Slicing and packaging
Exposure Assessment
N_t
Distribution to retail
FSO
POs
SS
Storage at retail
MC
Risk management decision (e.g. shelf life duration)
D
Consumption
Dose-response
RpS
S_{all}
Risk Characterization
P_{ill}
Tolerable?
ALOP
Illness