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

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26 **Abstract**

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

39

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

41

42 1. Introduction

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

50 The aim of applying the Appropriate Level of Protection (ALOP) is the decrease of the number of
51 food-borne cases per pathogen and per year to a pre-determined level which constitutes the appropriate
52 or acceptable level of protection. For instance, in US the decrease of listeriosis cases by 50% has been
53 set as target [from 0.50 reported cases (number of culture-confirmed cases of illness caused by *L.*
54 *monocytogenes* reported to CDC) /year/100000 population to 0.25 cases/year/100000 population) by
55 the end of 2010. Based on statistical data, this goal has almost been achieved since the listeriosis cases
56 for 2007 were 0.27 cases/year/100000 population (CDC, 2008). Similar objectives have been set for
57 other pathogens like *Salmonella* spp., *Campylobacter* spp. and *Escherichia coli* O157:H7. Food Safety
58 Objectives (FSOs) determine the maximum frequency and/or concentration of a hazard in a food at the
59 time of consumption that provides or contributes to the ALOP. FSOs constitute the link between the
60 ALOP and food industries (ICMSF, 2002). To achieve the FSOs, Performance Objectives (POs)
61 [maximum frequency of occurrence (%) and/or concentration (cfu/g) of a pathogen] at other stages,
62 Performance Criteria (PC) [change (i.e. reduction or maximally allowed increase) in frequency of
63 occurrence and/or concentration of a pathogen that should be achieved during processing or
64 implementation of control measures], Process and Product Criteria (PrC) (conditions required to
65 achieve the desired PO/PC, e.g. time-temperature combination, or pH) should be established in the
66 process prior to consumption. Governmental risk managers are responsible for establishing ALOP and
67 FSOs whereas industrial risk managers should design production processes to meet the FSOs (Walls,
68 2006).

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

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

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

88

89 **2. Materials and Methods**

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

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

$$139 \quad P_{ill} = 1 - \exp(-r \times D)$$

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

$$143 \quad D = C \times S$$

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

146 The model predicted the probability of illness for the high risk population (20-25%) (Buchanan et al.,
 147 1997). The percentage of 20% was further considered as the fraction of the total population being at
 148 high risk. Afterwards, the risk, expressed as number of listeriosis cases per year, was determined using
 149 a probabilistic approach (Perez-Rodriguez et al., 2007):

$$150 \quad Risk = P_f \times D \times r \times S_{all} \quad (1)$$

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

161 (Anonymous, 2005, 2007a), referring to that *L. monocytogenes* growth should not exceed 100 cfu/g
162 throughout the shelf life in products supporting its growth. People often are exposed to levels lower
163 than 100 cfu/g without getting ill. However, infective dose is influenced by the susceptibility of the
164 high risk individuals and the ability of the microorganism to cause illness but, in general it can be
165 assumed that *L. monocytogenes* levels ≥ 100 -1000 cfu/g can cause listeriosis in high risk groups
166 (NZFSA 2008). The parameter S_{all} was determined based on the frequency of consumption of RTE
167 meat products by the total population in European Union, approximately 467000000 (Kim, 2006;
168 Anonymous, 2007b; FAOSTAT, 2007). It was further assumed that the frequency of consumption of
169 such products is similar between high risk groups and general population (Buchanan et al., 1997). The
170 predicted listeriosis cases per year were compared with the reported cases (EFSA, 2008) for the
171 reliability of the model. Listeriosis cases occurring in elderly people were considered because of their
172 higher association with this particular group (FDA/USDA, 2003). Finally, the risk factors influencing
173 the output of the model (i.e. listeriosis cases) and their threshold values, above of which a sharp
174 increase of listeriosis cases is observed, were determined by the application of crude and advanced
175 sensitivity analysis (Vose, 2000; Perez-Rodriguez et al., 2007). Crude sensitivity analysis is referred to
176 the correlation coefficients between the model inputs and output as given by the simulation software
177 used. Advanced sensitivity analysis is referred to the construction of the Tornado and sensitivity
178 graphs. These graphs were constructed by testing the following cumulative probabilities (1, 5, 25, 50,
179 75, 95 and 99%) of the input distributions identified by the crude sensitivity analysis. Each input
180 distribution was replaced by the corresponding percentile at a time allowing the others to vary and the
181 output statistic of interest (i.e. mean of the listeriosis cases per year) was recorded. The model was
182 developed in the Excel program and simulated using the @Risk 4.5 software (Palisade Corp., New
183 York, USA). Ten thousands (10000) repetitions (iterations) in each simulation of the model were
184 conducted whereas the simulation was repeated 100 times (uncertainty realizations) to take into
185 account separately the variability and uncertainty of the model and model inputs.

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

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

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

208

209 **3. Results and Discussion**

210 The kinetic growth models used in the exposure assessment step predicted the *L. monocytogenes* or
211 LAB growth as function of temperature (Mataragas et al., 2006a,b). Spoilage (SSOs growth) and shelf
212 life duration were considered to estimate risk at the time of consumption based on the unspoiled-unsafe
213 products. This fraction of the products poses a health risk for the consumers. If *L. monocytogenes* is
214 present in the product, assuming P equal to the mean value of the Beta distribution in Table 1 (1.91%),
215 the fractions considered at the time of consumption were: spoiled-unsafe, 0.38%; unspoiled-safe,
216 0.95%; spoiled-safe, 0.30%; and unspoiled-unsafe, 0.28%, representing 19.8, 49.8, 15.9 and 14.5%,
217 respectively, of the contaminated products (i.e. 1.91%). Their values were obtained after applying
218 Monte Carlo simulation running in parallel the growth of *L. monocytogenes* and SSOs.

219 The results showed that the *L. monocytogenes* dose consumed (log cfu/serving size) is described by a
220 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

251 cases occurred in individuals of age above 65 (56%); PO_{total} , the total population considered
252 (467000000); and PO_{high} , the fraction of the high risk population (20%). Listeriosis cases attributable to
253 RTE meat products were calculated according to FSIS (2008). *L. monocytogenes* illnesses due to
254 consumption of meat and poultry products were equal to 66%. *L. monocytogenes* illnesses from meat
255 and poultry products due to consumption of RTE meat products were equal to 91.2%. Thus, the
256 $66 \times 0.912 = 60\%$ of *L. monocytogenes* illnesses was due to consumption of RTE meat products. Then,
257 recorded cases attributable to RTE meat products: $Cases_{100000/RTE\ meat\ products} = Cases_{100000} \times Cases_{RTE\ meat}$
258 $products = 0.3 \times 0.60 = 0.18$, where $Cases_{100000}$, the recorded cases per 100000 of total population (0.3
259 cases) (EFSA, 2008).

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

270 According to the advanced sensitivity analysis, significant parameters influencing the final risk
271 estimation were mainly related with retail and home storage. Given the fact that cross-contamination of
272 the products during their retail is unlikely, prevention or at least reduction of cross-contamination
273 during their manufacturing becomes extremely important. At temperatures higher than 7-9°C (threshold
274 values according to Fig. 3d and 3f) a sharp increase in listeriosis cases occurs. Therefore, storage of the
275 products at temperatures below this level could contribute to listeriosis cases reduction because the
276 extended growth of the pathogen is inhibited. Consequently, there is the need of training of the people
277 involved in transportation, distribution and storage of the products, including consumers, in the basic
278 measures of food safety [low temperatures (3-4°C), adequate cooking, separation of fresh products
279 from RTE products, adequate/good cleaning of hands, equipment, tools and other utensils] (Sofos,
280 2008).

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

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

311 For this example, a consumer ALS of 95% was assumed. Usually, microbiological testing protocols
312 involve enrichment of 25g of food product (analytical units) and presence/absence testing of the
313 pathogen on selective media. To estimate the number of analytical units (i.e. samples) that need to be
314 tested, a modified procedure (i.e. Poisson-log-normal distribution) for determining the effectiveness of
315 enrichment tests was followed (van Schothorst et al., 2009). The results showed that the probability of
316 acceptance of a lot (with mean -3.08 and s.d. 1.02), based on a single sample, was $1 - 0.1091 = 0.8909$
317 (the probability that a cell is present in the sample taken and leads to detection of a positive was
318 0.1091). Consequently, more negative samples are required to reach 95% confidence. Taking 25 or 26
319 samples, the probability that all samples are acceptable was $(0.8909)^{25} = 0.0557$ and $(0.8909)^{26} =$
320 0.0496, respectively. Given this calculation scheme, 26 negative samples ($n = 26$ and $c = 0$) are
321 required to reject with more than 95% certainty a lot that has log mean concentration and s.d. greater
322 than the corresponding determined values (i.e. the parameters of normal distribution) because taking 25
323 samples for analysis the confidence level was still below 95%. Another decision that must be made is
324 to determine the safety level that is required (i.e. maximum frequency and/or concentration of the
325 hazard) and its corresponding relationship to the lot mean. This safety limit comprises the PO. As
326 described above, based on the QMRA results, a PO could be the *L. monocytogenes* prevalence \leq
327 1.91%. Given the distribution of *L. monocytogenes* in the lot, the proportion of the allowable defective
328 units (i.e. 1.91%) can be translated into an estimation of the maximum concentration of the pathogen in
329 the lot that should not be exceeded (Fig. 5). The latter was calculated as follows: *L. monocytogenes*
330 mean concentration of -3.08 log cfu/g and prevalence of 1.91% are the maximum values that can be
331 tolerated because this combination is located on the $P_f - D$ equivalence curve (Fig. 4). The PO is
332 determined by adding a certain number of s.d. to the hazard maximum tolerable concentration so that
333 the required percentage of the lot will have concentrations below PO. The required number of s.d. is
334 termed the z score. Therefore, in order 98.09% of the units to be at or below the target PO (or 1.91% of
335 the units to be above the target PO), the number of s.d. that should be added is 2.07 ($-3.08 + 2.07 \cdot 1.02$
336 $= -0.97$ log cfu/g) (Whiting et al., 2006; van Schothorst et al., 2009). The curve in Fig. 5 with a mean of
337 -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
338 lot' that the MC should reject in 95 times out 100 (Whiting et al., 2006). Distributions with lower mean
339 values will have higher probability of acceptance. Therefore, for this specific example the developed
340 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 = $-3.08 \log \text{ cfu/g}$, s.d. =
342 $1.02 \log \text{ cfu/g}$, $m = \text{absence in } 25\text{g}$, $c = 0$, $n = 26$ and PO = $-0.97 \log \text{ cfu/g}$. 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 $4\text{-}5^{\circ}\text{C}$. 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

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

389

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393

394 **References**

- 395 Anonymous, 2005. Commission Regulation (EC) No. 2073/2005 of 15 November 2005 on
396 microbiological criteria for foodstuffs. Official Journal of the European Union L338, 22/12/2005,
397 1-26.
- 398 Anonymous, 2007a. Commission Regulation (EC) No. 1441/2007 of 5 December 2007 an amendment
399 of the Commission Regulation (EC) No. 2073/2005 on microbiological criteria for foodstuffs.
400 Official Journal of the European Union L322, 7/12/2007, 12-29.
- 401 Anonymous, 2007b. Habits of the Greek consumer relative to meat consumption. Meat point, Issue 1,
402 January 2007. Athens, Greece.
- 403 Buchanan, R.L., Damert, W.G., Whiting, R.C., van Schothorst, M., 1997. Use of epidemiologic and
404 food survey data to estimate a purposefully conservative dose-response relationship for *Listeria*
405 *monocytogenes* levels and incidence of listeriosis. Journal of Food Protection 60, 918-922.
- 406 Centers for Disease Control and Prevention (CDC), 2008. Preliminary FoodNet data on the incidence
407 of infection with pathogens transmitted commonly through food – 10 States, 2007. Morbidity
408 Mortality Weekly Report, April 11, 2008 / 57 (14), 366-370.
409 <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5714a2.htm>. Accessed 15 May 2008.
- 410 Dennis, S.B., Kause, J., Losikoff, M., Engeljohn, D.L., Buchanan, R.L., 2008. Using risk analysis for
411 microbial food safety regulatory decision-making. In: Schaffner, D.W. (Ed.), Microbial risk
412 analysis of foods. ASM press, Washington, D.C., pp. 137-175.
- 413 European Food Safety Authority (EFSA), 2008. Zoonoses Data Collection Reports.
414 http://www.efsa.europa.eu/en/science/monitoring_zoonoses/reports.html. Accessed 15 May 2008.
- 415 FAOSTAT, FAO Statistics Division, 2007. <http://faostat.fao.org>. Accessed 25 April 2007.
- 416 Food and Agriculture Organization (FAO) and World Health Organization (WHO), 2002. Risk
417 assessments of *Salmonella* in eggs and broiler chickens. <http://www.fao.org/es/esn>. Accessed 14
418 March 2008.
- 419 Food Safety and Inspection Service (FSIS), 2003. Control of *Listeria monocytogenes* in ready-to-eat
420 meat and poultry products. Final Rule, Federal Register 68, 34208-34254.
- 421 Food Safety and Inspection Service (FSIS), 2008. Public Health Risk-based Inspection System for
422 Processing and Slaughter.

- 423 [http://www.fsis.usda.gov/Regulations_&_Policies/National_Advisory_Committee_on_Meat_&_P](http://www.fsis.usda.gov/Regulations_&_Policies/National_Advisory_Committee_on_Meat_&_Poultry/index.asp)
424 [oultry/index.asp](http://www.fsis.usda.gov/Regulations_&_Policies/National_Advisory_Committee_on_Meat_&_Poultry/index.asp). Accessed 08 October 2009.
- 425 Havelaar, A.H., Nauta, M.J., Jansen, J.T., 2004. Fine-tuning Food Safety Objectives and risk
426 assessment. *International Journal of Food Microbiology* 93, 11-29.
- 427 International Commission on Microbiological Specifications for Foods (ICMSF), 1996.
428 *Microorganisms in foods 5: microbiological specifications of food pathogens*. Blackie Academic
429 and Professional, London.
- 430 International Commission on Microbiological Specifications for Foods (ICMSF), 2002.
431 *Microorganisms in foods 7: microbiological testing in food safety management*. Blackie
432 Academic and Professional, London.
- 433 ILSI Research Foundation/Risk Science Institute Expert Panel on *Listeria monocytogenes* in foods,
434 2005. Achieving continuous improvement in reductions in foodborne listeriosis – a risk-based
435 approach. *Journal of Food Protection* 68, 1932-1994.
- 436 Jarvis, B., 2000. Sampling for microbiological analysis. In: Lund, B.M., Baird-Parker, T.C., Gould,
437 G.W. (Eds.), *The microbiological safety and quality of food*. Aspen Publishers, Maryland, pp.
438 1727-1728.
- 439 Jay, L.S., Comar, D., Govenlock, L.D., 1999. A national Australian food safety telephone survey.
440 *Journal of Food Protection* 62, 921-928.
- 441 Kennedy, J., Jackson, V., Blair, I.S., McDowell, D.A., Cowan, C., Bolton, D.J., 2005. Food safety
442 knowledge of consumers and the microbiological and temperature status of their refrigerators.
443 *Journal of Food Protection* 68, 1421-1430.
- 444 Kim, M.K., 2006. Impact of temperature and pH on the survival of *Listeria monocytogenes* in Souse
445 meat. Thesis for the Master of Science by North Carolina State University, Department of Food
446 Science, Raleigh, NC, USA. [http://www.lib.ncsu.edu/theses/available/etd-11012006-](http://www.lib.ncsu.edu/theses/available/etd-11012006-153559/unrestricted/etd.pdf)
447 [153559/unrestricted/etd.pdf](http://www.lib.ncsu.edu/theses/available/etd-11012006-153559/unrestricted/etd.pdf). Accessed 16 July 2008.
- 448 Lianou, A., Geornaras, I., Kendall, P.A., Belk, K.E., Scanga, J.A., Smith, G.C., Sofos, J.N., 2007. Fate
449 of *Listeria monocytogenes* in commercial ham, formulated with or without antimicrobials, under
450 conditions simulating contamination in the processing or retail environment and during home
451 storage. *Journal of Food Protection* 70, 378-385.

- 452 Marklinder, I.M., Lindblad, M., Eriksson, L.M., Finnson, A.M., Lindqvist, R., 2004. Home storage
453 temperatures and consumer handling of refrigerated foods in Sweden. *Journal of Food Protection*
454 *67*, 2570-2577.
- 455 Mataragas, M., Drosinos, E.H., Vaidanis, A., Metaxopoulos, I., 2006a. Development of a predictive
456 model for spoilage of cooked cured meat products and its validation under constant and dynamic
457 temperature storage conditions. *Journal of Food Science* *71*, M157-M167.
- 458 Mataragas, M., Drosinos, E.H., Siana, P., Skandamis, P., Metaxopoulos, I., 2006b. Determination of
459 the growth limits and kinetic behavior of *Listeria monocytogenes* in a sliced cooked cured meat
460 product: Validation of the predictive growth model under constant and dynamic temperature
461 storage conditions. *Journal of Food Protection* *69*, 1312-1321.
- 462 Mataragas, M., Drosinos, E.H., 2007. Shelf life establishment of a sliced, cooked, cured meat product
463 based on quality and safety determinants. *Journal of Food Protection* *70*, 1881-1889.
- 464 Mataragas, M., Skandamis, P.N., Drosinos, E.H., 2008. Risk profiles of pork and poultry meat and risk
465 ratings of various pathogen/product combinations. *International Journal of Food Microbiology*
466 *126*, 1-12.
- 467 National Advisory Committee on Microbiological Criteria for Foods (NACMCF), 2005.
468 Considerations for establishing safety-based consume-by date labels for refrigerated ready-to-eat
469 foods. *Journal of Food Protection* *68*, 1761-1775.
- 470 Nauta, M.J., 2005. Microbiological risk assessment models for partitioning and mixing during food
471 handling. *International Journal of Food Microbiology* *100*, 311-322.
- 472 Nauta, M.J., 2007. Uncertainty and variability in predictive models of microorganisms in food. In:
473 Brul, S., van Gerwen, S., Zwietering, M.H. (Eds.), *Modelling microorganisms in food*. CRC
474 press, Boca Raton, pp. 44-66.
- 475 Nauta, M.J., 2008. The Modular Process Risk Model (MPRM): a structured approach to food chain
476 exposure assessment. In: Schaffner, D.W. (Ed.), *Microbial risk analysis of foods*. ASM press,
477 Washington, D.C., pp. 99-136.
- 478 Nauta, M.J., Litman, S., Barker, G.C., Carlin, F., 2003. A retail and consumer phase model for
479 exposure assessment of *Bacillus cereus*. *International Journal of Food Microbiology* *83*, 205-218.

- 480 New Zealand Food Safety Authority (NZFSA), 2008. Microbial pathogen data sheets-*Listeria*
481 *monocytogenes*. <http://www.nzfsa.govt.nz/science/data-sheets/index.htm>. Accessed 14 March
482 2008.
- 483 Perez-Rodriguez, F., van Asselt, E.D, Garcia-Gimeno, R.M., Zurera, G., Zwietering M.H., 2007.
484 Extracting additional risk managers information from a risk assessment of *Listeria*
485 *monocytogenes* in deli meats. *Journal of Food Protection* 70, 1137-1152.
- 486 Reij, M.W., Zwietering, M.H., 2008. Integrating concepts: a case study using *Enterobacter sakazakii* in
487 infant formula. In: Schaffner, D.W. (Ed.), *Microbial risk analysis of foods*. ASM press,
488 Washington, D.C., pp. 177-204.
- 489 Ross, T, 2008. Translating knowledge of microbial ecology into risk assessment models. In: Schaffner,
490 D.W. (Ed.), *Microbial risk analysis of foods*. ASM press, Washington, D.C., pp. 51-97.
- 491 Ross, T., Sumner, J., 2002. A simple, spreadsheet-based, food safety risk assessment tool. *International*
492 *Journal of Food Microbiology* 77, 39-53.
- 493 Safety Monitoring and Assurance System for chilled meat products (SMAS), 2006. Project running
494 from 2003-2006 under the key action of Food, Nutrition and Health of the “Quality of Life and
495 Management of Living Resources” thematic programme and the “Quality Monitoring and
496 Traceability throughout the Food Chain” thematic priority. <http://smas.chemeng.ntua.gr>. Accessed
497 14 March 2008.
- 498 Sofos, J.N., 2008. Challenges to meat safety in the 21st century. *Meat Science* 78, 3-13.
- 499 U.S. Food and Drug Administrator (FDA)/U.S. Department of Agriculture (USDA), 2003. Quantitative
500 assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among
501 selected categories of ready-to-eat foods. <http://www.foodsafety.gov/~dms/lmr2-toc.html>.
502 Accessed 14 March 2008.
- 503 van Schothorst, M., Zwietering, M.H., Ross, T., Buchanan, R.L., Cole, M.B., International
504 Commission on Microbiological Specifications for Foods, 2009. Relating microbiological criteria
505 to food safety objectives and performance objectives. *Food Control* 20, 967-979.
- 506 Vose, D., 2000. *Risk analysis: A quantitative guide*. John Wiley and Sons, Chichester.
- 507 Walls, I., 2006. Role of quantitative risk assessment and food safety objectives in managing *Listeria*
508 *monocytogenes* on ready-to-eat meats. *Meat Science* 74, 66-75.

- 509 Whiting, R.C., Rainosek, A., Buchanan, R.L., Miliotis, M., LaBarre, D., Long, W., Ruple, A., Schaub,
510 S., 2006. Determining the microbiological criteria for lot rejection from the performance objective
511 or food safety objective. *International Journal of Food Microbiology* 110, 263-267.
- 512 Whiting, R.C., Buchanan, R.L. 2008. Using risk assessment principles in an emerging paradigm for
513 controlling the microbial safety of foods. In: Schaffner, D.W. (Ed.), *Microbial risk analysis of*
514 *foods*. ASM press, Washington, D.C., pp. 29-50.
- 515 Worsfold, D., Griffith, C.J., 1997. Assessment of the standard of consumer food safety behavior.
516 *Journal of Food Protection* 60, 399-406.
- 517 Yang, H., Mokhtari, A., Jaykus, L., Morales, R.A., Cates, S.C., Cowen, P., 2006. Consumer phase risk
518 assessment for *Listeria monocytogenes* in deli meats. *Risk Analysis* 26, 89-103.
- 519 Zwietering, M.H., 2009. Quantitative risk assessment: Is more complex always better? Simple is not
520 stupid and complex is not always more correct. *International Journal of Food Microbiology*, 134,
521 57–62
- 522 Zwietering, M.H., Cuppers, H.G., de Wit, J.C., van't Riet, K., 1994. Evaluation of data transformations
523 and validation of a model for the effect of temperature on bacterial growth. *Applied*
524 *Environmental Microbiology* 60, 195-203.
- 525 Zwietering, M.H., Nauta, M.J., 2007. Predictive models in microbiological risk assessment. In: Brul,
526 S., van Gerwen, S., Zwietering, M.H. (Eds.), *Modelling microorganisms in food*. CRC press,
527 Boca Raton, FL, pp. 110-125.
- 528

529 **Figure Captions**

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

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

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

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

<i>Parameters</i>	<i>Units</i>	<i>Notation</i>	<i>Description</i>	<i>Inputs</i>
Prevalence	%	P	Beta(645, 33180) ^b	Uncertainty of <i>L. monocytogenes</i> 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 <i>L. monocytogenes</i> 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 <i>L. monocytogenes</i> concentration in negative samples
Initial population in finished sliced product	log cfu/g	$N_{0,s}$	Discrete($C_{new} \cdot C_{neg}, P_{new} \cdot P_{neg}$)	
Storage temperature	°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	°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 slicing589 ^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)

591 ^c To give a mean value equal to -3.11 log cfu/g which was an estimation of the pathogen concentration
592 of samples under detection limit. The mean concentration of samples under detection limit was
593 calculated by the equation (Jarvis, 2000): $mean = -\left(\frac{2.303}{AUs}\right) \times \log\left(\frac{S_{neg}}{S_{total}}\right)$, where *mean*, the
594 mean concentration in cfu/g; *AUs*, the analytical units tested (e.g. 25g); *S_{neg}*, the number of samples
595 tested as negative (33179); and *S_{total}*, the total number of samples analyzed (33823) (FDA/USDA,
596 2003)
597 ^d Kinetic parameters (μ_{max} and t_{lag}), were determined using the secondary models of the square root (*L.*
598 *monocytogenes*) and Arrhenius (lactic acid bacteria) (Mataragas et al., 2006b). Also, only for *L.*
599 *monocytogenes*, a second order polynomial equation was used to calculate the maximum population
600 density (N_{max}) (Mataragas et al., 2006b)
601

Table 2. Retail sub-module

<i>Parameters</i>	<i>Units</i>	<i>Notation</i>	<i>Description</i>	<i>Inputs</i>
Population in sliced product after transport	log cfu/g	$N_{2,s}$	From the Industry sub-module	
Retail temperature	°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	°C	T_A	Pert(0, 20, 40)	Uncertainty of ambient temperature modeling of experts opinion
Max change in temperature during transport ^c	°C	ΔT_{max}	$T_A - T_2$	
Potential change in temperature during transport ^c	°C	T_{pc}	Normal(3.72, 2.82)	Variability of potential change in temperature during transport
Change in temperature during transport ^c	°C	T_c	IF($\Delta T_{max} \leq 0$, 0, T_{pc})	
Product temperature after transport ^c	°C	T_p	$T_2 + T_c$	
Average transport temperature ^c	°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
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^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

<i>Parameters</i>	<i>Units</i>	<i>Notation</i>	<i>Description</i>	<i>Inputs</i>
Population in sliced product after transport	log cfu/g	$N_{4,s}$	From the Retail sub-module	
Home fridge temperature	°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	$N_{6,s} (D)$	$\log(10^{N_{5,s}} \times S)$	
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)	-	$Risk$	$S_{all} \times r \times 10^D \times (P_f / 100)$	

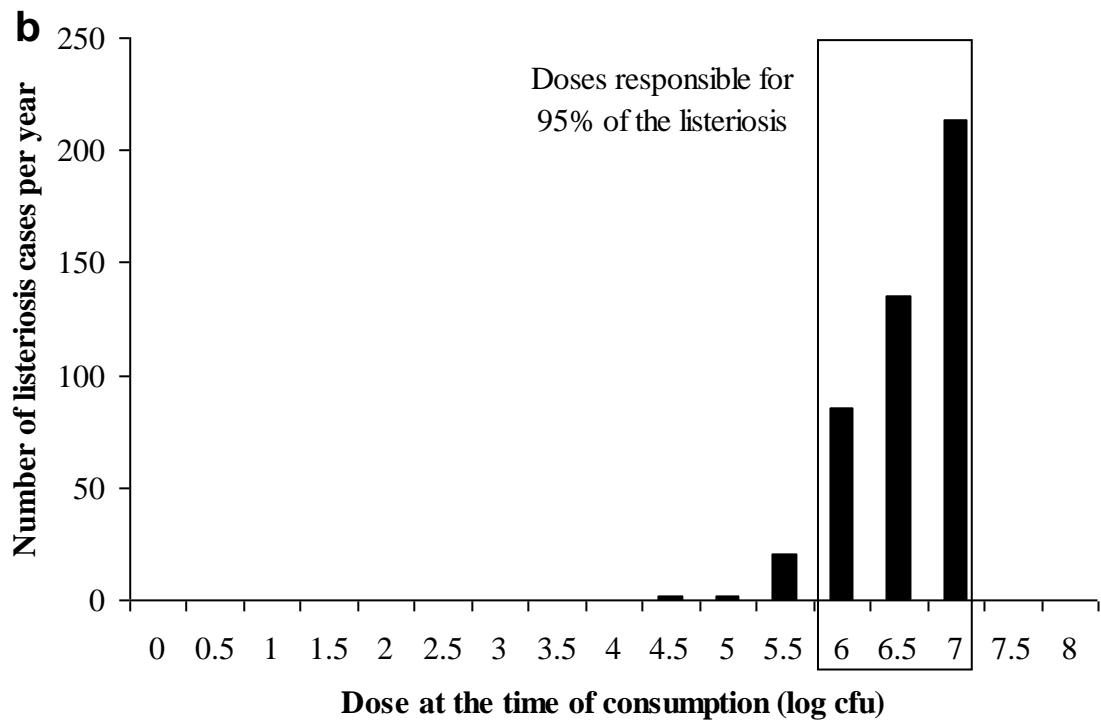
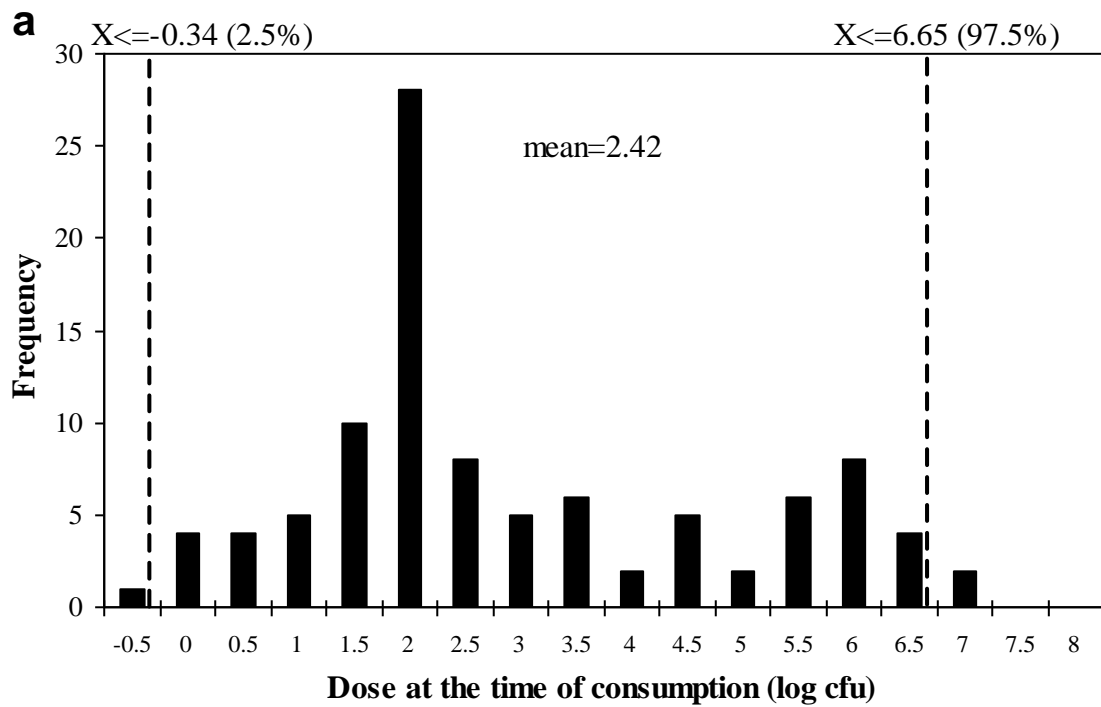
^a Day of purchase was estimated according to Nauta et al. (2003)

^b See Table 1

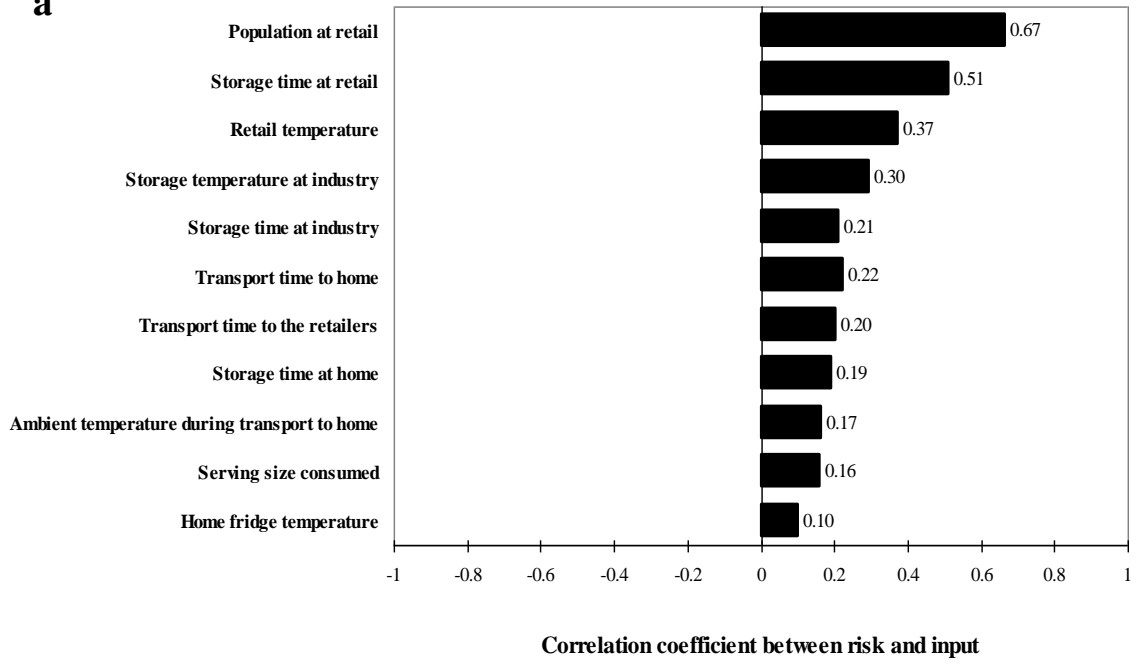
^c Not all the people consume RTE meat products. Frequency of consumption of RTE meat products was estimated based on FAOSTAT (2007) data (Mataragas et al., 2008)

^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



b

