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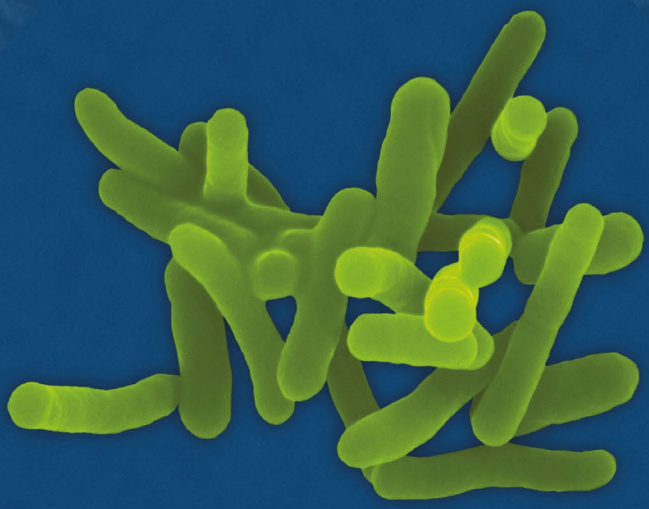
Risk assessment of *Listeria monocytogenes* in foods Part 2: Risk assessment

Meeting report



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MICROBIOLOGICAL RISK ASSESSMENT SERIES

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World Health Organization
Food and Agriculture Organization of the United Nations

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The preparatory work and expert meeting convened to prepare this report was coordinated by the Secretariat of the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA).

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Declaration of interests

All participants completed a Declaration of Interests form in advance of the meeting. Four of the experts declared interest in the topic under consideration: Ursula Gonzales-Barron, Vasco Cadavez, Laurent Guillier, and Régis Pouillot each had a closely related involvement in this work. It could not be excluded that the declared interests may be perceived as potential conflicts of interest. Therefore, while the four persons mentioned above were invited to the meeting, they participated as technical resource people only and were excluded from the decision-making process regarding final recommendations. All remaining experts were not considered by FAO and WHO to have declared any interest that may be perceived as a potential conflict regarding the objectives of the meeting.

All the declarations, together with any updates, were made known and available to all the participants at the beginning of the meeting.

All the experts participated in their individual capacities and not as representatives of their countries, governments, or organizations.

Abbreviations

AFFI	American Frozen Food Institute
CAC	Codex Alimentarius Commission
CC	clonal complexes
CCFH	Codex Committee on Food Hygiene
CFU	colony forming unit
DR	dose-response
EFSA	European Food Safety Agency
EGR	exponential growth rate
FAO	Food and Agriculture Organization of the United Nations
FBO	food business operators
ICMSF	International Commission on Microbiological Specification for Foods
JEMRA	Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment
MC	microbiological criteria
MPD	maximum population density
MPN	most probable number
MRA	microbiological risk assessment
LAB	lactic acid bacteria
LMIC	low- or middle-income countries
REF	reference scenario
RR	relative risk
RTE	ready-to-eat
WGS	whole genome sequencing
WHO	World Health Organization

Executive summary

BACKGROUND

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have undertaken risk assessments of *Listeria monocytogenes* in various foods since 1999. This work has provided scientific insights into the risk characterization of *L. monocytogenes* through food consumption, with the consideration of the susceptibility of different populations (MRA4 and MRA5) (FAO & WHO, 2004a; 2004b). In 2020, a virtual meeting of the Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) of *L. monocytogenes* in ready-to-eat (RTE) food: attribution, characterization and monitoring, recommended expanding future risk assessments of *L. monocytogenes* in RTE food to diverse commodity subgroups, incorporating a production to consumption perspective, and reviewing groupings of susceptible populations (MRA38) (FAO & WHO, 2022a). Therefore, the 52nd Session of the Codex Committee on Food Hygiene (CCFH) requested JEMRA to undertake full production-to-consumption risk assessments of *L. monocytogenes* in foods to inform a possible revision of the *Guidelines on the application of general principles of food hygiene to the control of Listeria monocytogenes in foods* (FAO & WHO, 2007). In response to this request, JEMRA convened two meetings, one each in 2022 and 2023, for the preparation and development of risk assessments of *L. monocytogenes* in various foods. In the first meeting (hereafter Part 1 expert meeting), the expert group elaborated formal models for the risk assessment of *L. monocytogenes* for lettuce, cantaloupe, frozen vegetables, and RTE fish, and it was concluded that these models should be programmed, tested, and reviewed.[1] During this second meeting (Part 2 expert meeting), several risk assessment models were developed and evaluated to characterize the risk of listeriosis due to the consumption of diced RTE cantaloupe, frozen vegetables, and cold-smoked RTE fish. However, the model for RTE lettuce was not ready for the expert group to evaluate.

[1] Summary and conclusions: https://www.fao.org/3/cc2966en/cc2966en.pdf or https://www.who.int/news-room/events/detail/2022/10/24/default-calendar/joint-fao-who-expert-meeting-on-microbiological-risk-assessment-of-listeria-monocytogenes-in-foods

SCOPE AND OBJECTIVES

The FAO/WHO Joint Expert Meeting on Microbiological Risk Assessment (JEMRA) held a meeting in Geneva, Switzerland, from 29 May to 2 June 2023, with the scope of performing a risk assessment of *L. monocytogenes* in selected foods using models developed in response to the Part 1 expert meeting. The objectives were: i) to test and evaluate the full production-to-consumption models for the selected commodities, ii) to use the models with different scenarios to provide recommendations to risk managers to control *L. monocytogenes*, and iii) based on the findings, to inform risk managers of possible updates of the *Guidelines on the application of general principles of food hygiene to the control of Listeria monocytogenes in foods* (CAC/GL 61 – 2007) (FAO & WHO, 2007).

The expert group agreed:

- to evaluate the models by comparing the structures suggested from the Part 1 expert meeting with the functions implemented in the new models and testing models' flexibility to change data inputs;
- to evaluate the outputs of models when tested with reference scenarios, by comparing with published results and/or expert experience, and to optimize parameterization of models if needed;
- to use a fit-for-purpose approach to determine which food commodities and processes to evaluate;
- that the models used for the scenario evaluation were for the diced RTE cantaloupe, frozen vegetables, and cold-smoked RTE fish;
- to use an updated dose-response model;
- that the outputs to evaluate were the average per-serving risk for the susceptible population using the JEMRA dose-response model developed in 2004 (FAO & WHO, 2004b);
- the reference scenario for each food commodity resulted from consensus by the expert panel. It was set as a departure point to assess the impact of a possible intervention described by a modification in one or several input parameters;
- that alternative scenarios were selected to accomplish the objectives in alignment with the recommendations from the Part 1 expert meeting, spanning different stages from primary production to consumption, and addressing factors such as time-temperature control, cross-contamination, environmental hygiene practices, water management, and climate change;
- that modelling results are subject to assumptions and context, making relative indicators more appropriate;

- that the relative impact of different factors and interventions along the production to consumption continuum was estimated by comparing the alternative scenarios to the reference scenario; and
- to evaluate/illustrate the impact of virulence and susceptibility on risk with the updated dose-response model distinguishing classes of *L. monocytogenes* strains of different virulence.

CONCLUSIONS

The expert group concluded the following:

Risk assessment models

- The processes and the steps from the Part 1 expert meeting and the structure and functions in the evaluated risk assessment models were consistent.
- The functions and parameters of the models as provided can be modified to evaluate the model performance and evaluate different scenarios.
- The outputs generated by the models were consistent with expert experiences. Based on the evaluation process that could be achieved during the meeting, the risk assessment models were considered useful and fit-for-purpose.
- The dose-response model can be further improved by considering additional factors, such as underlying health conditions.
- There is a need for representative data on *L. monocytogenes* in the food chain to better inform *L. monocytogenes* occurrence, virulence, and dose-response, so that a risk assessment for different classes of virulence of *L. monocytogenes* strains can be performed.
- The models should remain available as open-source tools.

Conclusions from elaboration of the risk assessment models

These conclusions are based on the conditions, data and practices simulated and evaluated in the scenarios during the meeting. Further, applicability and implications depend on specific conditions and individual production practices.

Diced RTE cantaloupe

- The model considered a full production-to-consumption chain representing preharvest, harvest and storage, cleaning and washing, processing, cold-chain storage, and consumer handling practices.
- The use of fit-for-purpose water in primary production was shown to reduce the risk.

- The use of an irrigation system that avoids the contact between water and the edible part of the crop also reduced the risk.
- Poor management of wash water increased the risk. The magnitude of the effect is dependent on the level of contamination in the wash water and the amount of water deposited on the product.
- Poor management of environmental hygiene during processing increased the risk.
- Climate change can considerably increase the risk as a result of its impact on different stages of the production-to-consumption chain, as tested in the model by assuming an increase of the prevalence of *L. monocytogenes* in soil, an increase of the quantity of soil transferred to produce, a decrease of the agricultural water quality, and an increase of storage temperature.

Frozen vegetables

- The stages represented in the model considered preconditioned vegetables as the raw material and included processing (blanching and packaging), environmental contamination, and consumer handling practices (defrosting and cooking).
- Blanching reduced the risk of *L. monocytogenes*. However, post-blanching contamination and growth of *L. monocytogenes* may occur.
- Poor environmental hygiene management increased the risk.
- If non-RTE frozen vegetables are consumed without adequate cooking, then defrosting practices increase the risk.

Cold-smoked ready-to-eat (RTE) fish

- The model considered a full production-to-consumption chain including primary processing, secondary processing, cold chain, and consumer handling.
- Considering the presence of the naturally occurring lactic acid bacteria (LAB) in the predictive growth model resulted in a reduction in estimated risk.
- Increased *L. monocytogenes* levels on incoming fish increased the risk.
- Poor environmental hygiene practices at filleting and slicing increased the risk.
- The elevated level of *L. monocytogenes* in brine solutions increased the risk.
- The addition of lactic acid and diacetate or LAB culture lowered the risk due to the reduced growth of *L. monocytogenes*.
- The potential effect of climate change, evaluated by assuming an increase in the initial levels of *L. monocytogenes* in the raw fish and in the temperature during the shelf-life of the product, increased the risk.

Dose-response (DR) model

- An updated DR model was developed to take class of strain virulence and age-gender group into account, as a surrogate for susceptibility, as determined by underlying health conditions.
- The updated model allowed for improved risk estimation for different classes of strain virulence.
- The updated DR model resulted in greater relative risk between the most extreme DR curves, based on age-gender group and class of strain virulence, compared with other age-gender based DR models.
- The updated model still lacks the actual information on susceptibility (as determined by underlying health conditions), and its potential interaction with defined classes of strain virulence. This information would make the DR model more specific and globally relevant.

Testing

- End-product sampling and microbiological testing on its own as a control measure had little effect on reducing the risk, even when applied to every lot produced (i.e. for lot release). However, there is a value in sampling and testing to verify that other control measures are effective, as described in MRA24 (FAO & WHO, 2016).

RECOMMENDATIONS AND CONSIDERATIONS FOR THE REVISION OF GUIDELINES RELATED TO CONTROL OF *L. MONOCYTOGENES*

General

- The ability of a food to support growth of *L. monocytogenes* and the likelihood of its consumption without further processing or treatment might depend on consumers' practices, which may deviate from the intended use of the food. Hence, caution should be used when classifying foods into distinct categories, e.g. as supportive or non-supportive of the growth of *L. monocytogenes,* or as RTE or non-RTE.
- The potential effects of climate change, such as due to increased temperatures and contamination, should be assessed by food business operators (FBO) and effective control measures should be implemented if needed.

Primary production

- Control of *L. monocytogenes* at the primary production can reduce the risk.

Processing

- The impact on the predicted risk of contamination during processing highlights the need for effective management of environmental hygiene practices.
- An important value of end-product sampling, environmental sampling and microbiological testing is to verify the effectiveness of implemented control measures.

Product information and consumer awareness

- The impact of non-intended use of RTE food highlights the need for improved food labelling about intended preparation and use.
- Consumer education on safe food preparation, food storage, and intended use should be enhanced.
- FBOs should provide clear messages to consumers for intended food use (e.g. website and social media).

1

Introduction

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have undertaken risk assessments of *Listeria monocytogenes* in various foods since 1999. The 2004 FAO/WHO risk assessment on *L. monocytogenes* (FAO & WHO, 2004a; 2004b) provided scientific insights into the risk characterization of *L. monocytogenes* through food consumption, with the consideration of the susceptibility of different populations (MRA4 and MRA5). The risk assessment models were limited to a select range of ready-to-eat (RTE) foods known to cause human listeriosis, including pasteurized milk, ice cream, cold-smoked fish, and fermented meats. These models addressed risks from the point of distribution to consumption. However, since the publication of the 2004 risk assessment, listeriosis outbreaks and related mortality have continued to occur, spanning different geographical regions and implicating a more diverse group of products than those considered in the original risk assessment.

In 2020, a virtual meeting of the Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) of *L. monocytogenes* in ready-to-eat (RTE) food: attribution, characterization, and monitoring, recommended expanding future risk assessments on *L. monocytogenes* in RTE food to diverse commodity subgroups, incorporating a production-to-consumption perspective, and reviewing groupings of susceptible populations (MRA38) (FAO & WHO, 2022a). Therefore, the 52nd Session of the Codex Committee on Food Hygiene (CCFH) requested JEMRA to undertake full production to consumption risk assessments of *L. monocytogenes* in foods to inform a possible revision of the *Guidelines on the Application of General Principles of Food Hygiene to the Control of Listeria monocytogenes in Foods* (CXG 61 – 2007) (FAO & WHO, 2007).

In response to the request from the 52nd Session of the CCFH, JEMRA convened two meetings, one each in 2022 and 2023, for the preparation and development

of risk assessments of *L. monocytogenes* in various foods. In the first meeting (hereafter Part 1 expert meeting), the expert group elaborated formal models for the risk assessment of *L. monocytogenes* for lettuce, cantaloupe, frozen vegetables and RTE fish and concluded that these models should be programmed, tested, and reviewed (FAO & WHO, 2022b).

The second JEMRA meeting (hereafter Part 2 expert meeting) was held in Geneva, Switzerland, from 29 May to 2 June 2023, with the scope of performing risk assessment of *L. monocytogenes* in selected foods using models developed in response to the Part 1 expert meeting. The main objectives of the meeting were: i) to test and evaluate the full production-to-consumption models for the selected commodities, ii) to use the models with different scenarios to provide recommendations to risk managers to control *L. monocytogenes*, and iii) based on the findings, to inform risk managers from Member States of possible updates of the *Guidelines on the application of general principles of food hygiene to the control of Listeria monocytogenes in foods* (CAC/GL 61 – 2007) (FAO & WHO, 2007).

One of the recommendations of the previous meeting was to update the dose-response model by using the existing models considering the susceptibility of the population and the virulence of the hazard based on genomic data. Section 3 of the report discusses the updated dose-response model. Section 4 also documents the effects of the microbiological sampling and testing of *L. monocytogenes*.

Three case-studies were developed considering the risk of listeriosis associated with different scenarios from production to consumption of RTE diced cantaloupe (Section 5), frozen vegetables (Section 6), and RTE cold-smoked fish (Section 7). Finally, the recommendations and considerations for the revision of guidelines related to the control of *L. monocytogenes* are also discussed (Sections 8 and 9).

2

Methodology

The risk assessment models (Objective 1) and the ways in which various factors and processes affect the estimated risk (Objective 2) were evaluated at using case studies for three types of food and "what-if" scenarios. For each food commodity being evaluated, a reference scenario was developed and run. The expert group's consensus guided the evaluation of the chain's stages in a reference scenario. It was set as a starting point to assess the impact of factors and processes, described by modification in one or several input parameters, on the estimated risk. The reference scenarios were used to evaluate the models developed by the technical group (see below) and for comparison with the alternative "what-if" scenarios with parameters defined by the expert group.

2.1 MODELLING APPROACH AND EVALUATION OF RISK ASSESSMENT MODELS

2.1.1 Modelling approach

The food commodities underwent the application of full primary production-to-consumption risk assessment models. The models incorporated a modular approach with flexible functions that can be reused between the evaluated foods and for other similar food commodities. The technical group considered recommendations from the Part 1 expert meeting into the various modules developed prior to the Part 2 expert meeting. Four meetings were organized prior to the Part 2 expert meeting to address data gaps and to refine the risk assessment models. The different modules included in the risk assessment models for the food groups proposed by the expert group were implemented using open-source software (R software version 4.4.0) and, after the expert meeting as a web tool.

Graphical interfaces were developed to facilitate more accessible uptake among member countries. These models were detailed in peer-reviewed publications for RTE diced cantaloupe (Guillier et *al.*, 2025), frozen vegetables (Gonzales-Barron *et al.*, 2024b) and smoked and graved fish (Gonzales-Barron *et al.*, 2024c).

The parameters and associated distributions mentioned in this section were selected based on the decisions from the Part 1 expert meeting (FAO & WHO, 2004). The list of the references and scenario parameters was agreed upon among the expert group of the Part 2 expert meeting and can be consulted in the annexes.

2.1.2 Evaluation of risk assessment models

The full production-to-consumption models for the selected commodities were tested and evaluated to assure that they were fit-for-purpose considering the recommendations of the Part 1 expert meeting and the mandate and objectives of the Part 2 expert meeting. First, the proposed models and the processes outlined in the flow charts from the previous meeting report were compared with the functions implemented in the new models. Second, their flexibility was tested to accommodate and allow the entry of new data and parameters. Finally, the output of the models when tested with the reference scenarios were compared with published results and expert experience, and parameters in the models were optimized and aligned with data/experience, if needed.

2.2 EVALUATION OF "WHAT-IF" SCENARIOS

In view of the resources available for the assessments, the expert group agreed to apply only a subset of the commodity models available and to assess the risks associated with suitable "what-if" scenarios. The "what-if" scenarios illustrated the impact of different factors or evaluated the effect of different interventions and sampling schemes by comparisons with reference scenarios. "What-if" scenarios were developed taking the recommendations of the Part 1 meeting and the mandate of the Part 2 expert meeting into consideration. The scenarios are described in detail in the respective commodity sections.

Uncertainty in risk assessment models stems from numerous potential sources, including limitations in available knowledge, data constraints, assumptions, and the specific context or scenario of the model that affect answers to an assessment question (EFSA, 2018). "Available knowledge" refers to the evidence and data available to the experts at the time of the assessment, within the agreed time and resource constraints. Acknowledging this, the estimated risks in the alternative scenarios were expressed relative to the reference scenario as a relative risk. This

approach acknowledges the inherent uncertainties and considers relative indicators more appropriate for modelling results.

2.3 Estimation of the risk

The risk of listeriosis per serving of the food commodities was estimated by considering both within-lot and between-lot variability, as shown in Table 1.

TABLE 1 Estimation of within- and between-lot risk

LOT NUMBER	SERVING NUMBER						RISK PER SERVING PER LOT[A]	*RISK PER SERVING FOR ALL THE LOTS*[a]
	1	*2*	. . .	*j*	. . .	*J*		
1	Rs_{11}	Rs_{12}	. . .	Rs_{1j}	. . .	Rs_{1J}	$Rs_{1.}$	R..
2	Rs_{21}	Rs_{22}	. . .	Rs_{2j}	. . .	Rs_{2J}	$Rs_{2.}$	
. . .	. . .	. . .		. . .		. . .	. . .	
i	Rs_{i1}	Rs_{i2}	. . .	Rs_{ij}	. . .	Rs_{iJ}	$Rs_{i.}$	
. . .	. . .	. . .		. . .		. . .	. . .	
I	Rs_{I1}	Rs_{I2}	. . .	Rs_{Ij}	. . .	Rs_{IJ}	$Rs_{I.}$	

[a] Each simulation considers **I** lots, and for each lot, **J** servings are simulated (representing the lot size). Rs_{ij} denotes the risk associated with a single serving j (where $j = 1, 2, \ldots, J$) from lot i (where $i = 1, 2, \ldots, I$).

$Rs_{i.} = \frac{Rs_{i1} + Rs_{i2} + \ldots + Rs_{iJ}}{J}$ and $\frac{Rs_{..}}{} = \frac{Rs_{1.} + Rs_{2.} + \ldots + Rs_{I.}}{I}$

First, the model generated a matrix of contaminated lots using parameters from a beta distribution, attributing random values for the concentration and prevalence of *L. monocytogenes*. By default, 1 000 lots values were generated. The parameters of a normal distribution (mean, standard deviation) were used to represent the total variability in microbial concentration in contaminated units (log_{10} colony forming unit [CFU/g]). Each row of the matrix corresponds to a production lot, which is further divided into units represented by the columns. For each unit, a risk was estimated and represented the within-lot variability. The risk per serving per lot was calculated by determining the mean risk of the units per lot (by row). Subsequently, the mean or the median risk per serving for all lots was estimated,

accounting for variability between lots. These statistics were used for evaluation of the "what-if" scenarios. The mean risk per serving of all lots was used as a statistic for comparison, and the term "risk per serving" was employed in this report. When the median is used instead of the mean risk per serving of all lots, this is specified in the report. The estimation of relative risk was based on the ratio between the mean risk per serving of the "what-if" scenario and the reference scenario.

The dose-response model used in this report was the FAO/WHO model from 2004 (FAO & WHO, 2004b) for populations with increased susceptibility, and it is referred to as the JEMRA model in this report.

3

Case study 1 – Ready-to-eat diced cantaloupe

3.1 DESCRIPTION AND JUSTIFICATION OF THE CASE STUDY

The Codex General Principles of Food Hygiene (CXC 1-1969) provide principles for ensuring food is produced hygienically (FAO & WHO, 2023a). It is recognized that even with the preventive measures applied (i.e. Good Agricultural Practices, Good Handling Practices and Good Manufacturing Practices) microbiological contamination in the agricultural and processing settings cannot be completely avoided, notably due to the lack of a killing step. For cantaloupes, contamination caused by *L. monocytogenes* can occur at any step of the production chain, including primary production in the field, post-harvest processing, retail and storage as well as consumers' practices. Pathogens introduced onto the product can later experience growth because of handling practices, including consumers' preparation at home. In 2022, the JEMRA expert group meeting on the "Prevention and Control of Microbiological Hazards in Fresh Fruits and Vegetables. Part 4: Commodity-Specific Interventions", discussed interventions for four subdivided commodity groups, including melons. They highlighted the importance of hygienic handling and hygiene control, including environmental monitoring and water management, as well as decontamination treatments for improving the microbiological safety of melons (FAO & WHO, 2023b). In addition, specific to *L. monocytogenes* control, recommendations from a 2020 expert group meeting (MRA38) highlighted the need for the development of a full production-to-consumption risk assessment model that includes the introduction and fate of *L. monocytogenes* on and in

cantaloupe (FAO & WHO, 2022a). In 2022, during Part 1 of the expert meeting on Microbiological Risk Assessment of *L. monocytogenes* in Foods, the framework for the risk assessment model was developed for both whole cantaloupe (intact) and RTE diced cantaloupe fruit (FAO & WHO, 2024).

3.2 FLOWCHART OF THE MODULES IN THE CASE STUDY MODEL

In response to the recommendations from the 2020 JEMRA expert group (MRA38), a cantaloupe risk assessment model was developed for various stages of the production of RTE diced and whole cantaloupe. The models are the same for the preharvesting, harvesting, cleaning and washing steps, where the conditions apply to both products. Following these steps, processing, cold-chain storage and consumer handling at home are considered for RTE diced cantaloupe (Figure 1), while retail, transportation, and consumer handling are considered for whole cantaloupe. In this assessment the production and processing chain with more steps (i.e. RTE diced cantaloupe) is addressed.

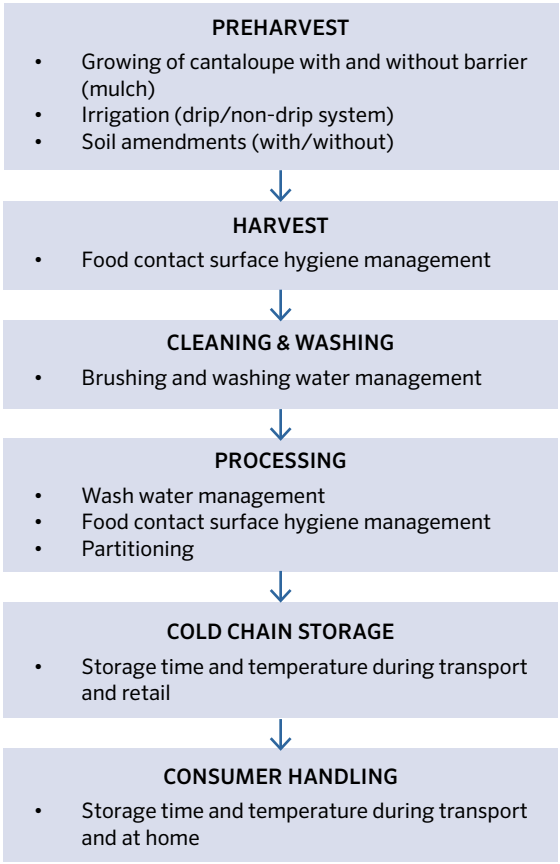
3.3 DESCRIPTION OF THE MODULES IN THE CASE STUDY PROCESS MODEL AND REFERENCE PARAMETERS

The reference scenario in this case study represents a situation where the different stages of the cantaloupe production have been sufficiently managed to reduce the contamination of the final product. In the reference scenario, the conditions and parameters are based on the data available in the literature and/or on expert knowledge. The overall process is shown in Figure 1 and described in the following section. The details of the parameters used as a reference scenario and comparative scenarios are available in Table A1.1 (Annex 1).

Module 1: Preharvest of cantaloupes

At the primary production stage or during the farming of cantaloupes (preharvest), agricultural practices such as the use of a soil barrier (mulch), and potential contamination with *L. monocytogenes* from soil, soil amendments and irrigation water (e.g. drip and nondrip [overhead, furrows] water application) were considered by the model. The baseline assumption for the reference input values for preharvest conditions were as follows:

FIGURE 1 Flowchart for the RTE diced cantaloupe production process



- Growth of cantaloupe with a soil barrier (mulch), with the assumption that there is 10% probability that soil is transferred, using a triangular distribution for the soil quantity (min = 0.05 g; mode = 0.5 g; max = 5 g) when the transfer occurs.
- The prevalence of *L. monocytogenes* in the soil was set at 9%, with soil contamination levels following a triangular distribution (min = -1 $\log_{10}$ CFU/g; mode = 0.6 $\log_{10}$ CFU/g; max = 1.48 $\log_{10}$ CFU/g) based on Dowe *et al.* (1997).
- Application of irrigation water using a drip system results in no transfer of the irrigation water to the cantaloupe (Hoelzer *et al.*, 2012) ($P_{irr} = 0$).

Module 2: Harvest of cantaloupes

During harvesting, contamination of cantaloupe from contact with the harvest container was considered and accounted for by assuming an one-way transfer (i.e. surface to cantaloupe) with a probability of cross-contamination (p = 0.25) and the transfer coefficient (mean = -1.42; standard deviation = 0.52) from Hoelzer *et al.* (2012). The surface contamination levels of harvest containers (e.g. crates) were found to be comparable to food contact surfaces in a processing facility with 9 CFU available for transfer. This value was determined considering the study of Gil *et al.* (2024), where the lowest levels of *Listeria* spp. on surfaces were between 0.2 and 0.3 CFU/site in zones in the plant in contact or near to the product, averaging to 0.25 CFU/site. Given a surface area of 25 cm^2 for the site, the quantity of *Listeria* spp. was calculated as $10^{0.25} \times 25 = 44.46$ CFU. Considering the contact surface area between a cantaloupe and the container is approximately 5 cm^2, the number of *L. monocytogenes* on food contact surfaces (such as conveyors or crates at harvest) touching the cantaloupes would be approximately 9 CFU (44.46/5).

Module 3: Preprocessing: cleaning and washing

During preprocessing, washing parameters, including the microbiological quality of process water, were the main variables considered. Since limited data exist on the effect of physical brushing (without water) to remove *L. monocytogenes*, this was not considered. It was assumed that the washing process is well managed, leading to no cross-contamination, and no increase in the *L. monocytogenes* counts on the melon (minimum and maximum efficiency of brushing = 0 $\log_{10}$). This corresponds with good microbiological quality of the process water used to washed melons.

Module 4: Processing: washing and sanitizing, dicing and partitioning

In this module the model simulates:

- a proportion of *L. monocytogenes* transferred to the rind of cantaloupe (cross-contamination) in the flume tank if the product is in direct contact with contaminated water;
- the possible transfer from the contaminated rind to the flesh during dicing; and
- the cross-contamination of cantaloupes when in direct contact with the dicing machine or knives, followed by the partitioning of all dices produced in one processing lot (sublot) into packed units.

In the reference scenario, it was assumed that the process water was well managed (not contaminated), and facilities manage environmental conditions well (e.g. effective cleaning and sanitizing and environmental monitoring programs). In these types of facilities, the assumption is that occasionally food contact surfaces will be positive for *L. monocytogenes*; however, cross-contamination of product from food contact surfaces will be low, with parameters set in the reference scenario as follows:

- Absence of contaminated water in the flume tank during washing of cantaloupes.
- Transfer from the rind to the flesh during dicing of cantaloupe at processing level was modelled using a PERT distribution (min = 0.089%, mode = 0.55%, max = 2.82%) from data extracted from studies by Ukuku and Fett (2002) and Ukuku *et al.* (2005).
- Food contact surface contamination during portioning stage is low: 75% no detection of *L. monocytogenes* and 25% at low contamination levels (9 cells present on the surface with a transfer coefficient from the environment to the cantaloupe [mean = -1.42; standard deviation = 0.25]).

Module 5: Cold chain during transport to retail (relevant for RTE diced cantaloupe)

Growth of *L. monocytogenes* was simulated during transport from processing plants to retail and was based on the Baranyi and Roberts' model (Baranyi and Roberts, 1997). The algorithm considers that RTE diced cantaloupe packs from every lot are subjected to the same initial lag phase (Q0), the same transportation temperature (min = 3 °C, mode = 5 °C, and max = 10.3 °C), and the same transportation time (min = 2 h, mode = 5 h, and max = 9 h). PERT distributions represent the lot-specific variability in transport time and temperature. The normal distribution with parameters of the mean natural log of Q0 and standard deviation of the natural log of Q0 represents the variability about natural log of Q0, a parameter related to the physiological state of cells in the Baranyi and Roberts' growth model. The exponential growth rate of *L. monocytogenes* in cantaloupe flesh at 5 °C is represented by a normal distribution (meanEGR5 = 0.04 $\log_{10}$ CFU/g/h, sdEGR5 = 0.004 $\log_{10}$ CFU/g/h and Tmin = -2.0196 °C).

Module 6: Consumer handling: transport to consumers' house and storage at home

The growth of *L. monocytogenes* in RTE diced cantaloupe during transport from retail to home was modelled with the following parameters:

- Gamma distribution for transport duration with shape 6.2 h and scale 8.2 h
- PERT distribution for temperature with min 7 °C, mode 15 °C, and max 30 °C.

Given the short shelf life of RTE cantaloupe, storage duration and storage temperature at home were assumed to have the following ranges:

- PERT distribution for storage duration with min 3 h, mode 24 h, and max 120 h
- PERT distribution for temperature with min 3.1 °C, mode 6.64 °C, and max 11.1 °C based on published studies (Guillier *et al.*, 2025).

3.4 SCENARIOS EVALUATED

A set of reference parameters were developed based on the existing data from published literature and/or expert elicitation. Various "what-if" scenarios (Figure 2) were included to evaluate the possible impact of different risk management options for cantaloupes from production to consumption. The simulated mean risk to the population with increased susceptibility (FAO & WHO, 2004b) in these scenarios was then compared to the risk in the reference scenario to determine the impact of the interventions expressed as a relative risk.

3.5 RISK CHARACTERIZATION

The results of each scenario are available in Annex 1, Table A1.2 and Table A1.3.

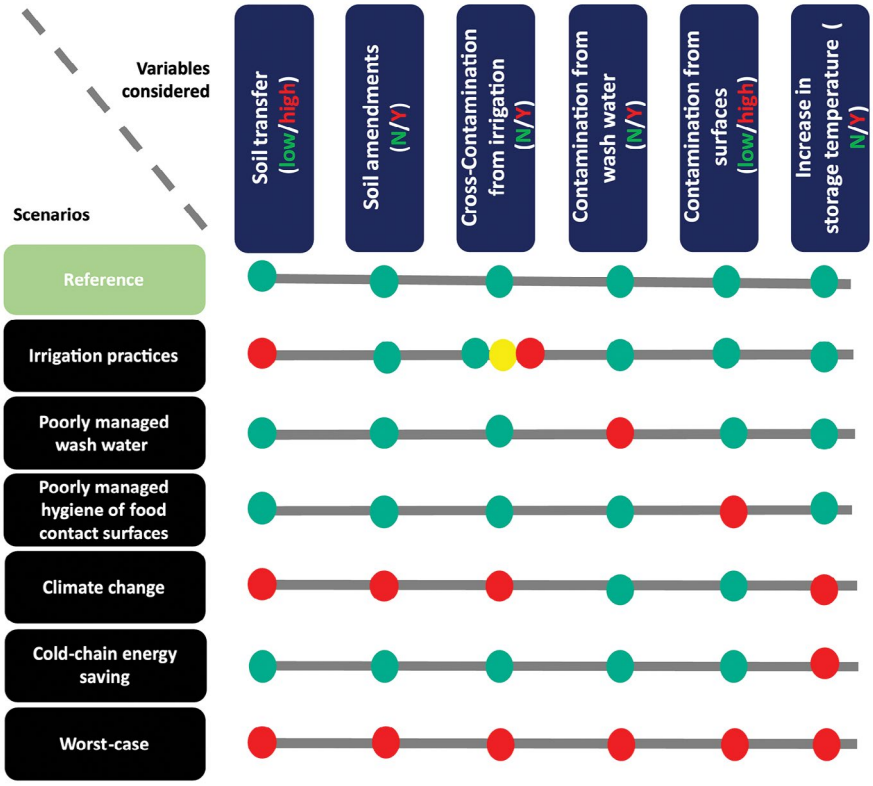
Scenario 1 – Irrigation practices

The quality of the irrigation water and the mode of application system are considered as important parameters for potential contamination of cantaloupes in the field. In this scenario, three different combinations of conditions are considered:

1a) Cantaloupes are grown **without** a soil barrier (mulch) resulting in soil transfer 100% of the time, using drip irrigation, and assuming that:

- The application of irrigation water using a drip system results in no transfer of the irrigation water to the cantaloupe.
- The contaminated soil transferred is described using a triangular distribution (min = 0.05 g; mode = 0.5 g; max = 5 g).
- No soil amendments are used.

FIGURE 2 Illustration of reference and "what-if" scenarios that were used to test the risk assessment model for RTE diced cantaloupe. Green lights indicate that the node's value aligns with the reference scenario, representing either low values or a "no" for the parameters in the respective boxes. Yellow lights signify medium values for the parameters within the respective boxes. Red lights denote high values or a "yes" for the parameters in the respective boxes.



1b) Cantaloupes are grown **without** a soil barrier (mulch) resulting in soil transfer 100% of the time, using non-drip irrigation categorized as low risk (e.g. furrow), and assuming that:

- The application of irrigation water using non-drip irrigation systems results in 0.4% of the irrigation water remaining on the cantaloupe.
- Low-risk water quality has the following characteristics:
  - > contamination concentration of irrigation water, estimated with a uniform distribution: min = -1.52 $\log_{10}$ CFU/L, max = 1.04 $\log_{10}$ CFU/L; and

    - prevalence of contamination in irrigation water: $P_{irrig} = 0.08$.
- The contaminated soil transferred is described using a triangular distribution (min = 0.05 g; mode = 0.5 g; max = 5 g).
- No soil amendments are used.

1c) Cantaloupes are grown **without** a soil barrier (mulch) resulting in soil transfer 100% of the time, using non-drip irrigation categorized as high-risk (e.g. overhead spray), and assuming that:

- Application of irrigation water using non-drip irrigation system results in 0.4% of the irrigation water being transferred onto the cantaloupe.
- High-risk water quality has the following characteristics:
    - contamination concentration of irrigation water, estimated with a uniform distribution: min = -1.52 $\log_{10}$ CFU/L, max = 4.75 $\log_{10}$ CFU/L; and
    - $P_{irrig} = 0.63$.
- The contaminated soil transferred is described using a triangular distribution (min = 0.05 g; mode = 0.5 g; max = 5 g).
- No soil amendments are used.

1d) Cantaloupes are grown **with** a soil barrier (mulch) resulting in soil transfer similar to the reference scenario 10% of the time, using non-drip irrigation categorized as high risk (e.g. overhead spray), and assuming that:

- Application of irrigation water using non-drip irrigation system results in 0.4% of the irrigation water being transferred onto the cantaloupe.
- High-risk water quality has the following characteristics:
    - contamination concentration of irrigation water, estimated with a uniform distribution: min = -1.52 $\log_{10}$ CFU/L, max = 4.75 $\log_{10}$ CFU/L; and
    - $P_{irrig} = 0.63$.
- The contaminated soil transferred is described using a triangular distribution (min = 0.05 g; mode = 0.5 g; max = 5 g) based on the assumption that there is a 10% probability that soil is transferred.
- No soil amendments are used.

Irrigation practices were estimated to have an impact on the risk compared to the reference scenario. The magnitude of the impact was primarily dependent on the quality and type of irrigation water used. Drip irrigation and non-drip irrigation low-risk practices (e.g. furrow) when low-risk water is used were comparable (*Scenario 1a* and *1b*), increasing the risk by approximately 450 to 537 times, corresponding to about 2.65 to 2.73 $\log_{10}$ increase, respectively, compared to the reference scenario. When high-risk water is applied using nondrip irrigation

high-risk practices such as overhead spray irrigation (*Scenario 1c*), the risk increased by about 4.3 $\log_{10}$ (around 22 000 times) compared with the reference scenario. The use of high-risk water in irrigation increased the risk by a similar level (4.2 $\log_{10}$, almost 17 000 times higher than the reference scenario) regardless of the use of a soil barrier (*Scenario 1d*).

Scenario 2 – Cross-contamination from water during washing step

In this scenario, the use of poorly managed wash water was explored, assuming:

- Cantaloupes are grown using a soil barrier (mulch) and drip irrigation, with the assumption that some soil (10%) is transferred, using the triangular distribution for the soil quantity (min = 0.05; mode = 0.5; max = 5 g).
- The prevalence of *L. monocytogenes* in the soil is set at 9%, with soil contamination levels following a triangular distribution (min = -1; mode = 0.6; max = 1.48 $\log_{10}$ CFU/g).
- No soil amendments are used.
- 0.4 percent of the wash water stays on the cantaloupe with the level of *L. monocytogenes* in 100 ml being dependent on the location, and in this scenario this level was derived from industrial data recently published by EFSA (EFSA, 2025).

When considering the impact of cross-contamination during washing, the use of contaminated and consequently poorly managed wash water increased the risk per serving by approximately 2.3 $\log_{10}$ (about 200 times higher than the reference scenario). The risk depends on the level of cross-contamination during the washing step, which is directly correlated to the concentration of *L. monocytogenes* in the wash water.

Scenario 3 – Cross-contamination from food contact surfaces

In this scenario, the effect of poorly managed food contact surfaces on the cross-contamination of diced cantaloupe is considered, assuming that in facilities with poorly managed environmental conditions (e.g. ineffective cleaning and sanitizing programs, lack of or inadequate environmental monitoring programs), high levels of *L. monocytogenes* contamination on food contact surfaces can lead to the probability of cross-contamination of product.

- Growth of cantaloupe with a soil barrier (mulch) and drip irrigation, with the assumption that some soil (10%) is transferred, using the distribution for the soil quantity (min = 0.05; mode = 0.5 g; max = 5 g).

- The prevalence of *L. monocytogenes* in the soil is set at 9%, with soil contamination levels following a triangular distribution (min = -1 $\log_{10}$ CFU/g; mode = 0.6 $\log_{10}$ CFU/g; max = 1.48 $\log_{10}$ CFU/g).
- No soil amendments are used.
- Wash water is properly managed, avoiding cross-contamination during washing.
- Food contact surfaces are poorly managed: 75% of surfaces with no *L. monocytogenes* detection and 25% with high levels (3 000 cells present on the surface multiplied by the transfer coefficient from the environment to the cantaloupe).

Cross-contamination from food contact surfaces had a similar impact to the wash water scenario under the assumptions of frequency and level of contamination explored in the current scenarios. Surface cross-contamination increased the mean per serving by approximately 2.5 $\log_{10}$ (approximately 290 times greater) compared to the reference scenario.

Scenario 4 – Climate change

Impact of climate change was explored using the following assumptions:

- To simulate heavy rains, it was assumed that approximately 0.4% of the water that is splashed onto the cantaloupe remains on the fruit. The inherent assumption here is that the splashing can increase the risk, comparable to the situation in which high-risk irrigation water is used. This corresponds to P_{irrig} = 0.63 and a prevalence following a uniform distribution (min = -1.52, max = 4.75 $\log_{10}$ CFU/L).
- Cantaloupes are grown without a soil barrier (mulch) with non-drip irrigation.
- Soil amendments are used.
- The amount of contaminated soil transferred is described using a triangular distribution (min = 0.05; mode = 0.5; max = 5 g).
- At the retail and consumer stages the maximum temperature in the refrigerator is assumed to be 10.8 °C and 11.6 °C, respectively.

As summarized above, climate change was assumed to have an influence on several parts of the production-to-consumption chain, such as heavy rains leading to splashing of high-risk irrigation water onto fruit, and increased temperatures at retail and in consumers' homes. This scenario was also coupled with other practices that may lead to higher risks, such as soil amendment usage and growing conditions (e.g. no soil barrier and non-drip irrigation). This combination of parameters resulted in a magnification of risk over the reference scenario of approximately 4.8 $\log_{10}$ (approximately 63 000 times higher). There is uncertainty, however, as to the extent of the influence of climate change along the production

chain. The combinations tested in the scenario were selected to represent the most likely stages along the production chain that can be impacted by climate change.

Scenario 5 – Cold-chain energy savings

To explore the impact of energy-saving strategies imposed on the cold chain on risk, all assumptions were kept consistent with the reference scenario except for the following:

- The maximum temperature at the retail stage would increase by 0.5 °C.
- The mode and maximum temperatures at the consumer stage are increased by 2 °C corresponding to a triangular distribution from min = 3.1 °C , mode = 8.64 °C , max = 13.1 °C to min = 3.1 °C , mode = 6.64 °C , max = 11.1 °C.

The impact of cold-chain energy savings strategies on the risk of listeriosis in this case study was relatively minor compared to other "what-if" scenarios. Risk was estimated to increase by approximately 1.6 $\log_{10}$ (43 times greater) over the reference scenario when considering the risk per serving.

Scenario 6 – Worst-case scenario

To establish a frame of reference to compare the risk per serving of RTE diced cantaloupe among different scenarios, a worst-case scenario was created combining all conditions that can increase the risk along the different stages of the production-to-consumption chain.

In this scenario, a combination of worst-case conditions was considered:

- Cantaloupes are grown without a soil barrier (mulch) with high-risk non-drip irrigation (e.g. overhead spray), assuming high-risk water quality (uniform distribution with min = -1.52 $\log_{10}$ CFU/L and max = 4.75 $\log_{10}$ CFU/L, P_{irrig} = 0.63).
- The application of irrigation water using a high-risk non-drip irrigation system results in 0.4% of the irrigation water being transferred onto the cantaloupe.
- Soil amendments are used.
- The transferred contaminated soil quantity follows a triangular distribution (min = 0.05 g; mode = 0.5 g; max = 5 g).
- 0.4 per cent of the wash water stays on the cantaloupe, taking into account the distribution of detected *L. monocytogenes* in 100 ml based on information previously published by EFSA (EFSA, 2025).
- Food contact surfaces are poorly managed: 75% of surfaces with no *L. monocytogenes* detection and 25% with high levels (3 000 cells present on the surface multiplied by the transfer coefficient from the environment to the cantaloupe).

- At the retail and consumer stage, assuming that the maximum temperature in the refrigerator will increase as for Scenario 5.

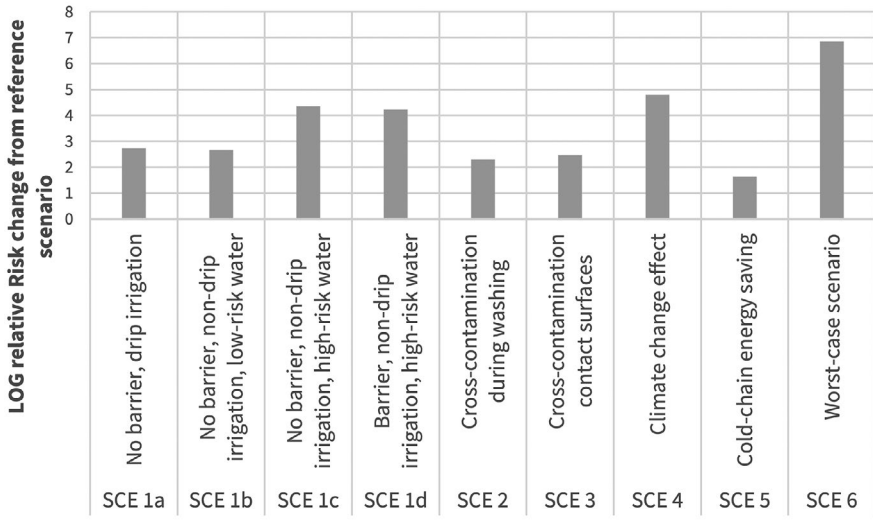
A worst-case scenario of risk assessment considers the most severe possible outcome that can reasonably be projected to occur in a given situation. The worst-case scenario showed that when all the key steps along the production and preparation of RTE dice cantaloupes are not well managed or are impacted by climate change, the risk is more than 7 million times greater than in the reference scenario (almost 7 logs) when compared with the reference scenario.

3.6 CONCLUSIONS

The model considered a full production-to-consumption chain representing preharvest, harvest and storage, cleaning and washing, processing, cold-chain storage, and consumer handling practices. Several scenarios were explored ranging from irrigation practices to cold-chain energy savings. The scenarios were evaluated on a relative risk basis by comparing the change in risk per serving to a reference scenario in which the system is relatively well controlled. A summary of these results is shown in Figure 3 below.

The use of fit-for-purpose water in primary production was estimated to reduce risk when using the low-risk water in comparison to when using the high-risk source,

FIGURE 3. Relative risk (log units) for six alternative scenarios compared to reference for RTE diced cantaloupe



with all other conditions remaining the same. This can be seen by comparing scenarios 1b and 1c. In addition, the difference between using non-drip and drip irrigation (*Scenario 1a* versus *1b*) is small when low contamination level water is used for non-drip irrigation – in fact, given the estimated magnitude they could be considered equivalent and differences likely a result of model variability.

The importance of water safety management practices with respect to wash water and environmental hygiene was reinforced by the results from scenarios 2 and 3. Poor management of wash water led to microbial contamination in the water and increased the risk of listeriosis, with the magnitude of the effect dependent on the level of contamination in the wash water and the amount of water deposited on the product.

Similarly, poor management of environmental hygiene during processing increased the risk, with the magnitude depending on the level of contamination assumed. The impact of slightly increasing storage temperatures, to approximate potential energy-saving policies (*Scenario 5*), was estimated to increase the risk, although to a lesser extent than the other scenarios (i.e. the smallest increase in risk over reference compared to all other scenarios).

Finally, climate change (*Scenario 4*) was estimated to affect the risk due to its potential impact at different stages of the production to consumption chain. In the current scenario it was assumed that there would be an increase in the prevalence of *L. monocytogenes* in soil, an increase of the quantity of soil transferred to the produce, a decrease of the agricultural water quality and an increase of storage temperature. It is uncertain if all those stages will be affected and by how much. However, even if only a few of those stages are affected, there is still likely to be an increase in risk, as can be inferred from the other scenarios and their exploration of changes at specific points in the process.

4

Case study 2 – Frozen vegetables

4.1 DESCRIPTION AND JUSTIFICATION OF THE CASE STUDY

Frozen vegetables include a large variety of vegetables (amongst others, leafy greens, carrots, broccoli, leeks, potatoes, pepper, onion, corn and eggplant). At the same time, frozen vegetables include whole products (not cut or shredded) and those that undergo size reduction operations (EFSA, 2018). Whenever possible, frozen vegetables are subjected to blanching to avoid quality deterioration during frozen storage. However, not all the vegetables can withstand blanching (e.g. peppers, tomatoes, winter squash, onions, potatoes, leeks and rutabaga).

The production of frozen vegetables is a complex process, where different activities are combined, creating different conditions at different stages of production. A general rule is that frozen vegetables are produced from fresh produce. Most commonly, freshly harvested vegetables are transported in bulk containers from the field to the freezing facility. The times required for post-harvest storage and transport to the freezing facility are usually short. Frozen vegetable-processing facilities typically receive raw materials directly from primary production. However, these facilities may also obtain preconditioned fresh produce from other processors. Preconditioning encompasses preliminary steps such as the removal of non-edible parts and the adjustment of the vegetable size to its final format, including peeling, cutting and washing. These preconditioning operations can be conducted at other processing facilities or directly on the farm. For instance, leeks can be cleaned in the field by removing their outer leaves and roots (EFSA, 2018). After preconditioning, the vegetables are blanched, frozen and packed before

distribution. Distribution can be done in bulk to other food business operators or in retail packages to the consumer.

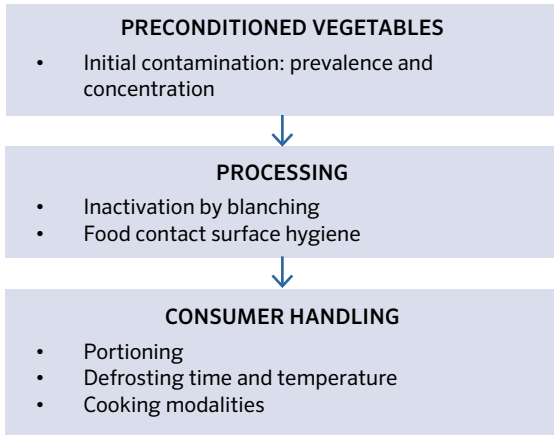
4.2 FLOWCHART OF THE MODULES IN THE CASE STUDY MODEL

Based on the recommendations from the 2020 JEMRA expert group (MRA38) and the discussion that took place during the Part 1 expert meeting, the frozen vegetables risk assessment model was developed to incorporate various stages of the production of these commodities. Figure 4 shows the most common processing steps included in a freezing plant considered by the risk assessment model.

The risk assessment considered here focuses on the activities in the freezing facility starting from blanching. It is assumed that all the raw material has been previously conditioned (trimming, cutting, peeling, washing, and so forth) and the freezing facility receive raw material with different microbiological quality. In addition to the assessment of the risk due to *L. monocytogenes* contamination during processing, the model also includes consumer handling practices since both processing and consumer handling have been identified as the important factors affecting the risk. The role of hygiene practices in the freezing plant on the risk of *L. monocytogenes* contamination of frozen vegetables is also considered.

Therefore, the current risk assessment started with preconditioned vegetables and the steps included were: i) blanching, ii) cross-contamination with the processing environment during freezing, partitioning and/or packaging, iii) defrosting and iv) cooking by the consumer.

FIGURE 4 Flowchart for the frozen vegetables considered by the model



4.3 DESCRIPTION OF THE MODULES INCLUDED IN THE CASE STUDY AND REFERENCE PARAMETERS

The reference scenario in this case study represents a situation where the different stages of the production chain were well managed to reduce the contamination of the final product until consumption. In this scenario, the conditions and parameters are based on the data available in the literature and/or on expert knowledge. The overall process is shown in Figure 4 and described in the following section. The details of the parameters used as a reference scenario and comparative scenarios are available in Table A2.1 (Annex 2).

Module 1: Reception of preconditioned vegetables

It was considered that the raw materials entering the freezing plant were preconditioned (e.g. cut, peeled, washed), and these activities were not included in this module.

The activities included in *Module 1* are specifically related to the freezing plant such as:

Reception of raw material

It is assumed that the preconditioned raw material arriving at the freezing plant is coming from different suppliers. A lot (or batch) size of 5 000 units was considered, each unit weighing 500 g, resulting in a lot weight of 2 500 kg. This initial assumption only represents one of the multiple possibilities that can be found in a freezing plant.

Initial contamination

As usually happens in the freezing facilities, it was considered that the processing plant receives preconditioned fresh produce with different microbiological quality. The initial contamination was based on the probability of individual lots being contaminated, and the concentration of *L. monocytogenes* in the contaminated product. Data on the occurrence of *L. monocytogenes* in vegetables was retrieved from relevant literature. The probability of contaminated lots[2] was modelled with a beta distribution, with $\alpha = 0.5112$, and $\beta = 1.99$, resulting in a mean prevalence of around 5%. The initial levels of contamination were also based on the available information in the literature, and specifically the data from minimally processed vegetables was considered (Jeyaletchumi *et al.*, 2011; Magdovitz *et al.*, 2021; Kuan *et al.*, 2017) with concentrations of *L. monocytogenes* between 3.0 to >100

[2] A lot is considered as contaminated if one unit is contaminated (N>0).

MPN/g in positive lots. The concentration in contaminated lots was modelled with a normal distribution with a mean concentration of 1.023 $\log_{10}$ CFU/g and a standard deviation 0.3267 $\log_{10}$ CFU/g.

Module 2: Processing in the freezing plant

Blanching

Blanching is performed for many commodities before freezing to inactivate the enzymes to prolong shelf-life, but it also has a significant impact on the microbiota of the product. The reference set of time and temperature for blanching was 83 °C and 0.75 min. It was assumed that the reduction in contamination accomplished by hot water blanching was variable. This variability was represented by a normal distribution for the logarithmic ($\log_{10}$) decimal reduction value (in minutes) at the reference temperature (70 °C), with a mean $\log_{10}$ of -1.78 min and a standard deviation $\log_{10}$ of 0.251 min. The function was based on the Bigelow model, which describes the decimal reduction time as a function of temperature, with parameters z (6.06 °C).

Cross-contamination from environment

The risk of post-blanching cross-contamination from equipment in contact with food was defined by the experts as high, and a probability of 25% was used for cross-contamination (Gil *et al.*, 2024). The cross-contamination accounted by the model can come from lots already contaminated to lots not contaminated or already contaminated. It can also consider no cross-contamination in lots that were or were not already contaminated. The variability of the transfer coefficient was represented by a normal distribution considering the $\log_{10}$ of the mean parameter corresponding to -0.44 and the $\log_{10}$ of the standard deviation parameter corresponding to 0.4 (Hoelzer *et al.*, 2012). Available data on *Listeria* spp. contamination on food contact surfaces of fresh-cut processing plants (Gil *et al.,* 2024) were used as a reference. Considering that the hygienic status of food contact surfaces in freezing facilities is usually lower than that of the fresh-cut processing plants, the reference levels of *Listeria* spp. found in fresh-cut processing plants were increased by a factor of 2 (i.e. 0.3 $\log_{10}$). Therefore, the low (0.4 $\log_{10}$ CFU/100 cm^2) and high (2.9 $\log_{10}$ CFU/100 cm^2) levels reported by Gil *et al.* (2024) were increased by 0.3 $\log_{10}$. The reference scenario assumed low levels (i.e. 4.5×10^3 CFU) of *L. monocytogenes* on the surface in contact with the product (assuming a lot contact surface of 90 000 cm^2, width and length of a conveyer belt of 60 cm and 1 500 cm, respectively).

Module 3: Consumer handling practices

Within this module the consumer practices at home (e.g. portioning, defrosting, storage and cooking) are considered.

Portioning

The model simulated the portioning of the pack (500 g) of a lot of frozen vegetables into smaller units (50 g), i.e. a serving. The microbial cells in the 500 g pack are distributed following a beta-binomial distribution with only one smaller 50 g unit per pack being modelled and retained, and this serving can be contaminated or not. The dispersion factor represents the extent of cell clustering in the frozen vegetables within the larger package. A dispersion factor of 1 represents moderate clustering of cells in the frozen vegetables within the 500 g pack and was chosen for the reference scenario.

Defrosting and storage after defrosting

Although it is well-known that *L. monocytogenes* cannot grow at freezing temperatures, they can still survive for extended periods of time in frozen vegetables (Pappelbaum *et al.,* 2008), and grow in defrosted products, needing a very short lag phase time at ambient temperature (Kataoka *et al.,* 2017). Consumer practices might include many different time and temperature combinations to thaw frozen vegetables including the counter-top at room temperature of 20 °C for 4 h, and several combinations of temperature/time in the refrigerator (6 °C, 24 h; 8 °C, 24 h; 8 °C, 72 h). In this case, the exponential growth rate (*EGR*) at the reference temperature of 5 °C (*EGR5*) was modelled, assuming a lognormal distribution with mean *EGR5* of 0.0117 $\log_{10}$ CFU/g/h and standard deviation of *EGR5* of 0.00816 $\log_{10}$ CFU/g/h,[3] and the minimum temperature for growth of *L. monocytogenes* in blanched vegetables of -1.18 °C. No lag phase was assumed and a maximum concentration of 8 $\log_{10}$ CFU/g was set. The *EGR* at abusive temperatures (*T*) was modelled as $\mathrm{EGR} = \mathrm{EGR5} \cdot \left(\frac{T - T_{min}}{5 - T_{min}}\right)^2$.

The reference scenario assumed no defrosting before cooking.

Cooking practices

This function simulates the reduction of *L. monocytogenes* in frozen or defrosted vegetables due to cooking. The intensity of the heat treatment can be different based on the method applied (e.g. microwave cooking or regular cooking). Although

[3] The mean EGR5 of 0.0117 log10 CFU/g/h and standard deviation EGR5 of 0.00816 log10 CFU/g/h are on a linear scale. The parameters of the lognormal distribution using these values are: lognormal mean = -4.646, and lognormal standard deviation = 0.6296.

recommendations indicate that frozen vegetables should be consumed cooked, consumption of uncooked frozen or defrosted vegetables has become more and more popular among consumers (EFSA, 2020). In this module, the use of frozen vegetables as cooked and an RTE product (uncooked) is included. Cooked vegetables were set in the reference scenario with a regular cooking (e.g. proper boiling). A medium cooking (e.g. fast boiling) and a microwave cooking were also included.

4.4 SCENARIOS EVALUATED

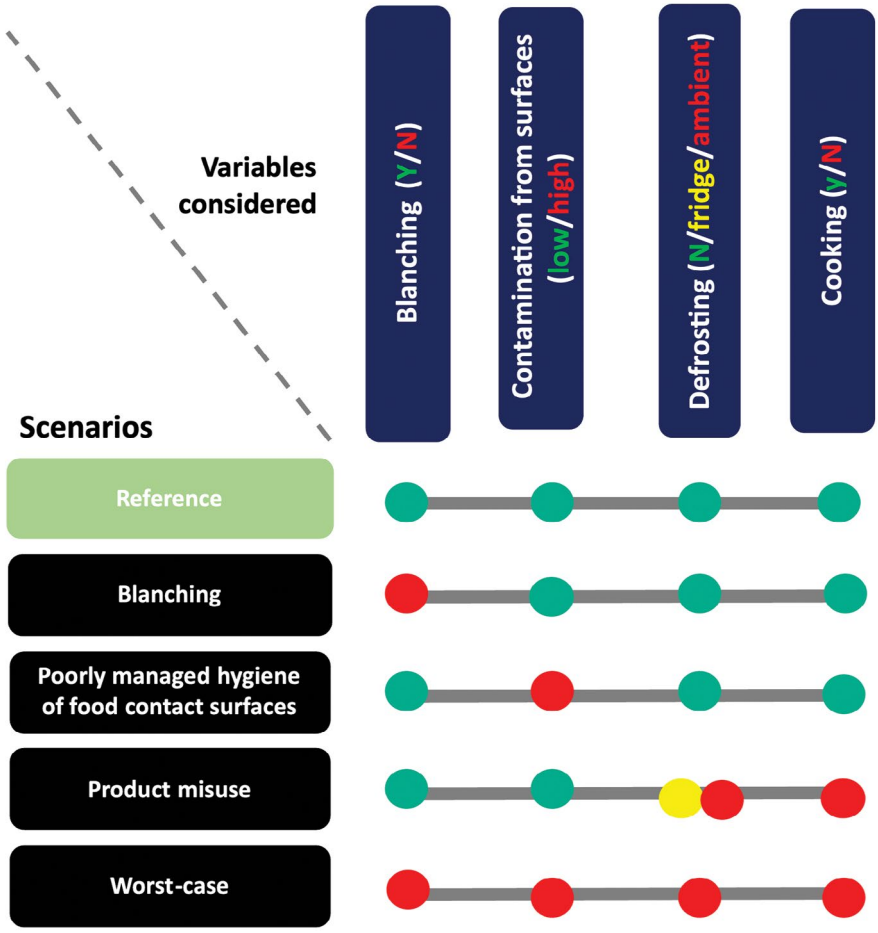
To evaluate the risk of listeriosis of non-RTE frozen vegetables per serving different scenarios based on expert knowledge were evaluated. Scenarios where frozen vegetables are consumed raw as RTE (without cooking) were deemed relevant to include due to increased reports of such uses by consumers. The predicted risk of the reference scenario for a population with increased susceptibility was compared with the predicted risk associated with the different scenarios (Figure 5).

The model aims to represent the initial contamination, cross-contamination, inactivation and potential growth of *L. monocytogenes* in non-RTE blanched and frozen vegetables, such as corn and peas, from processing to consumption. By assessing the change in risk of listeriosis per serving, the model measures and compares the effects of: i) specific processing activities that might impact the microbial reduction (e.g. blanching, or any additional inactivation step, pre- or post-processing), ii) different degrees of environmental contamination likely due to different hygienic practices, and iii) a comparison of the effects of consumer handling practices, such as product misuse (e.g. defrosting without subsequent cooking). This can inform (practical) recommendations to improve compliance with cooking non-RTE frozen foods, such as better labelling and enhanced consumer education. The different scenarios evaluated were as follows:

Scenarios evaluating blanching parameters

The first set of time and temperature, defined in the reference scenario and corresponding to 83 °C and 0.75 min, is a less stringent condition compared to that commonly applied by the industry (AFFI, 2018). The second set of time and temperature was 92 °C and 2 min (*Scenario 29* and *Scenario 30*), which corresponds to more stringent conditions compared with those in the reference scenario, which are commonly applied by the industry (AFFI, 2018). However, it should be noted that there are some specific commodities, which due to quality losses, cannot be subjected to blanching (e.g. onion and cucumber). This scenario has also been considered (*Scenario no blanching*).

FIGURE 5 Illustration of reference and "what-if" scenarios that were used to test the risk assessment model for frozen vegetables. Green lights indicate that the node's value aligns with the reference scenario in a well-managed scenario. Yellow lights and red lights denote the medium- or worst-case scenarios for the respective boxes.



Scenario evaluating poorly managed hygiene of food surfaces

Based on the available data, when cross-contamination occurred, the following low and high levels of cross-contamination were considered due to the contact of frozen vegetables with the contaminated surface of the production environment:

- Low level corresponds to a cross-contamination of 4.5×10^3 CFU.
- High level corresponds to a cross-contamination of 1.5×10^6 CFU.

The probability of cross-contamination of vegetables from production material and the transfer coefficient remained the same in all the "what-if" scenarios (cf. reference scenario description).

Scenarios evaluating product misuse

Within this module the consumer practices at home (e.g. defrosting, storage and cooking) are considered.

Defrosting and storage after defrosting:

Thawing frozen vegetables before consumption allows *L. monocytogenes* to grow. In this case study and based on the current consumer trends, different defrosting conditions were considered:

- defrosting at adequate refrigeration conditions in a refrigerator (24 h, 6 °C);
- defrosting at poor refrigeration conditions in a refrigerator (24 h, 8 °C);
- defrosting at the countertop in a kitchen at room temperature of 20 °C for 4 h to simulate quick defrosting; and
- defrosting for a longer time in a refrigerator at a poorly controlled temperature (72 h, 8 °C), mimicking the duration that the product might remain stored in the refrigerator for approximately three days while some product remains in the package. This reflects a scenario where the product is opened and stored in the refrigerator for approximately three days with some product remaining in the package.

Cooking practices:

Different cooking practices were considered, simulating the reduction of *L. monocytogenes* in the event of cooking using a triangular distribution in the model:

- regular cooking (reference): min = 1 $\log_{10}$, mode = 5 $\log_{10}$, min = 9 $\log_{10}$ reduction;
- microwave cooking: min = 0 $\log_{10}$, mode = 1 $\log_{10}$, max = 2 $\log_{10}$ reduction;
- reference cooking as used by EFSA (EFSA, 2020): min = 1 $\log_{10}$, mode = 2 $\log_{10}$, max = 3 $\log_{10}$ reduction; and
- no cooking and therefore no reduction.

Scenario evaluating the worst-case scenario

The worst-case scenario considered the worst situation that a frozen vegetable product can undergo during processing and consumer handling stages, leading to the highest risk for the consumer:

- no blanching procedure;
- high levels of contamination of the surface leading to cross-contamination (1.5×10^6 CFU);

- defrosting at ambient temperature (20 °C) for a short period of time (4 h) or defrosting and storing for 72 h under refrigerated conditions (8 °C); and
- no cooking before consumption.

4.5 RISK CHARACTERIZATION

The model was run using different inputs to illustrate the different situations that might happen at various stages of processing and handling of frozen vegetables. The simulated risk of the population with increased susceptibility (FAO & WHO, 2004b) in the scenarios was then compared to the risk in the reference scenario to determine the impact of the interventions expressed as a relative risk. The different scenarios are presented following the order presented in Figure 5. The results of each scenario are available in Annex 2, Table A2.2 and Table A2.3.

Impact of blanching

The reference scenario, which entails minimal cross-contamination from the processing environment and proper consumer handling practices – such as avoiding defrosting and ensuring adequate cooking (boiling) – was compared with a scenario lacking blanching while maintaining other conditions unchanged (*Scenario 1*). This comparison showed that blanching reduced the risk per serving by approximately 2.4 $\log_{10}$. It is important to highlight that, even though there was a significant reduction, the estimated risk of *L. monocytogenes* was very low (10^{-16} versus 10^{-14}) because post-blanching conditions were well controlled.

Two levels of blanching intensity were compared: the milder blanching treatment of the reference scenario (83 °C for 0.75 min) and a more rigorous approach (92 °C for 2 min) (*Scenario 29*), which aligns more closely with industrial practices (AFFI, 2018). Results showed limited differences between the two blanching processes in the final risk, highlighting that the reference blanching regime (83 °C, 0.75 min) had already achieved maximum reduction with the assumed initial contamination load.

The impact of blanching was further evaluated by comparing scenarios where conditions increase environmental cross-contamination (high-level cross-contamination), and proper consumer handling practices were not maintained. These scenarios included longer defrosting times (72 h at 8 °C) with no subsequent cooking (*Scenarios 3* and *4*), defrosting for a day (24 h at 6 °C) with no cooking (*Scenarios 9* and *11*), and countertop defrosting (4 hours at 20 °C) with no cooking (*Scenarios 8* and *10*). In these cases, blanching reduced the risk per serving by approximately 0.8 $\log_{10}$ (*Scenario 4* versus *Scenario 3*), 1.7 $\log_{10}$ (*Scenario 11* versus *Scenario 9*) and 1.7 $\log_{10}$ (*Scenario 8* versus *Scenario 10*), respectively.

Impact of poorly managed hygiene of food contact surfaces

The impact of the environmental cross-contamination on the final risk was evaluated in two different situations. In the first one it was assumed that the vegetables were subjected to blanching and combined with good handling practices by the consumer, which include no defrosting and cooking. Based on the results of the model, a high level of environmental cross-contamination in the processing plant increased the final risk per serving by about 1.1 $\log_{10}$ (*Scenario 2*) compared to a low level of environmental cross-contamination (*Reference scenario*). The impact of the environmental cross-contamination was also tested in scenarios with a different defrosting condition. In these scenarios, the impact of high- versus low-level environmental cross-contamination was combined with a defrosting period at ambient temperature (4 h, 20 °C) and no cooking (*Scenario 8* versus *Scenario 13*). This resulted in a similar risk increase of risk per serving by 1.2 $\log_{10}$.

Impact of the consumer handling practices

1) Defrosting and storage after defrosting

Thawing frozen vegetables before consumption allows *L. monocytogenes* to grow. In this case study and based on current consumer trends, different sets of time (4 h, 24 h and 72 h) and temperature (6 °C, 8 °C and 20 °C) combinations were tested in the scenarios.

The impact of the defrosting practices was evaluated considering that blanching was done, low-level cross-contamination occurred from contact surfaces, and the product was consumed raw. A long defrosting time (72 h) at 8 °C increased the final risk by about 7 $\log_{10}$ when compared to no defrosting (*Scenario 20* versus *Scenario 28*). This highlights the high impact that the defrosting practices might have on the risk of *L. monocytogenes* contamination of frozen vegetables that are consumed raw. When a quick (4 h) defrosting at room temperature was compared to no defrosting (*Scenario 13* versus *Scenario 28*), the risk increased by about 1.3 $\log_{10}$. On the other hand, when defrosting was done for 24 h at 6 °C, the risk increased by 0.6 $\log_{10}$ compared to no defrosting (*Scenario 12* versus *Scenario 28*). When the temperature was set to 8 °C instead of 6 °C during the 24 h of defrosting, the risk increased approximately 13 times (*Scenario 19* versus *Scenario 12*) (i.e. 1.1 $\log_{10}$ increase). Therefore, the temperature of the refrigerator and the time of defrosting have a clear effect on the risk.

2) Cooking

The impact of cooking before consumption was evaluated at three levels: regular cooking (e.g. boiling), microwave cooking, and EFSA reference cooking (EFSA, 2020).

Regular cooking was able to reduce the risk of *L. monocytogenes* in the final product by approximately 3 $\log_{10}$ (*reference scenario* versus *Scenario 28*), assuming that the product was blanched, the cross-contamination in the factory was low and the product was not defrosted before cooking. Similar risk reductions (3.0 to 3.5 $\log_{10}$) were observed when the product was defrosted (24 h, 6 °C [*Scenario 14* versus *Scenario 12*]; 4 h, 20 °C [*Scenario 15* versus *Scenario 13*]) before cooking. Also, when blanching was not performed, a high level of environmental cross-contamination was considered, and the product was defrosted (4 h, 20 °C); cooking reduced the risk per serving by 3.4 logs (*Scenario 16* versus *Scenario 10*).

The use of different cooking intensities (e.g. regular cooking, microwave cooking, EFSA reference cooking) also had an impact on the final risk. Using the microwave to cook defrosted vegetables reduced the risk by about 1 $\log_{10}$ (e.g. *Scenario 22* versus *Scenario 12*), while the medium cooking considered by EFSA (EFSA, 2020) reduced the risk by approximately 1.8 $\log_{10}$ compared to no cooking (*Scenario 27* versus *Scenario 28*). Based on the results, it can be concluded that regular cooking of frozen vegetables significantly reduces the risk but may not be sufficient to balance out very inadequate defrosting practices (72 h, 8 °C).

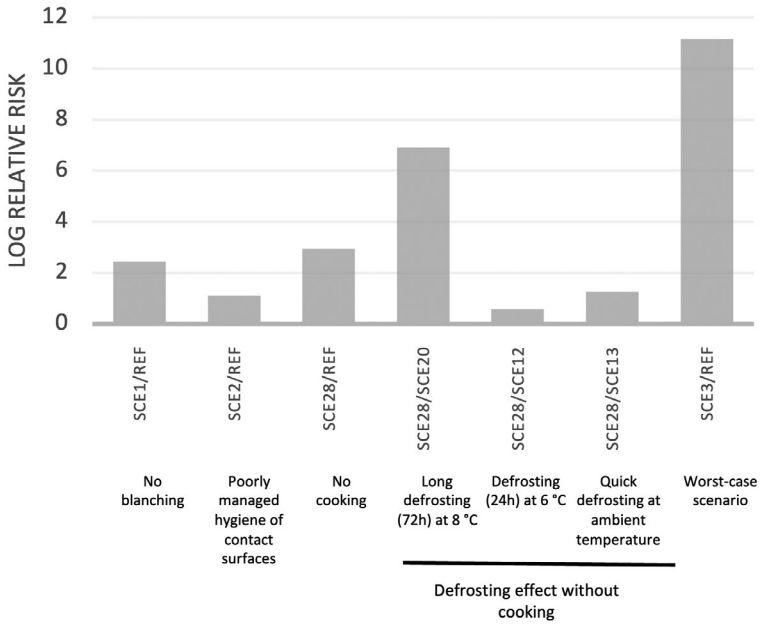
Worst-case scenario

To compare the predicted risk per serving among different scenarios, a worst-case scenario was created, considering all factors that could increase the risk at the different stages of processing. This worst-case scenario included unblanched vegetables, a high level of cross-contamination, and defrosting followed by storages for 72 h at 8 °C until the product is consumed raw. These defrosting and storage conditions were selected because they posed a higher risk than defrosting at room temperature (20 °C) for 4 h. Results showed that when all these steps were not well managed the risk increased by 11.2 $\log_{10}$ (*Scenario 3* versus *Reference scenario*) from 10^{-16} to levels of 10^{-5}.

4.6 CONCLUSIONS

The frozen vegetable chain was simulated from processing to consumer stage. Several scenarios were run to estimate the impact of different processing and handling practices, including blanching to reduce the initial contamination, the increase of risk due to poor hygiene management of food contact surfaces in the processing environment, and the impact of possible misuse of the frozen product at the consumer's home, such as defrosting of frozen vegetables and storage of thawed vegetables, and inadequate cooking practices.

FIGURE 6 Relative risk (log units) of intervention scenarios compared with the reference (REF) or risk factor scenarios for frozen vegetables. Worst-case scenario: no blanching, poorly managed hygiene, long defrosting (72 h) at 8 °C, no cooking



As shown in Figure 6, the relative risk comparisons based on the model assumptions revealed that blanching at the producer stage clearly reduces the risk. Contamination introduced due to contact with food surfaces increased the risk, underlining the need for effective management of environmental hygiene practices. Evidently, the impact of consumer practices on the final risk was high. Defrosting and storage of frozen vegetables significantly increased the risk, while proper cooking (e.g. boiling) considerably reduced the risk of listeriosis. This indicates that the labelling of frozen vegetables with adequate handling instructions is deemed important to control the risk.

5

Case study 3 – Ready-to-eat fish

5.1 DESCRIPTION AND JUSTIFICATION OF THE CASE STUDY

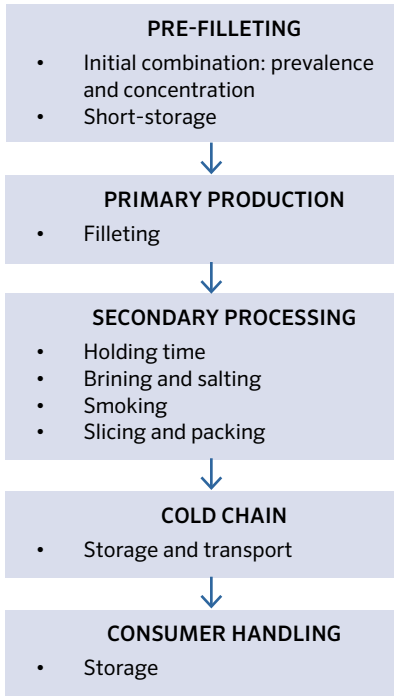
FAO and WHO previously developed a risk assessment model for *L. monocytogenes* in ready-to-eat foods (FAO & WHO, 2004a, b). In recent years, a need to update the existing model has emerged, driven by an increase in seafood-related outbreaks and an increasing consumption trend of smoked fish and other seafood products. Recent studies underscore the potential of *L. monocytogenes* to contaminate fish during sea-based harvesting and filleting processes prior to smoking, marinating or graving (FAO & WHO, 2024). Whole genome sequencing has revealed distinct genotypes in diverse environmental and processing contexts, both on land and at sea. Given the intricate production logistics entailing multiple facilities, suppliers, and international distribution networks, the probability of cross-contamination along the farm-to-fork chain may be substantial.

FAO and WHO experts of the Part 1 expert meeting in 2022 advocated for the development of a risk assessment model that can comprehensively describe the introduction and fate of *L. monocytogenes* from primary production to consumption. The model should exhibit flexibility to accommodate potential inclusion of other RTE fish products in the future, such as sashimi and ceviche. Key factors in the model developed encompass the initial contamination levels of raw materials, the contribution of cross-contamination, the time/temperature profiles across the entire supply chain (including processing, transport, retail, and consumer homes), the impact of improved practices, the use of preservatives, and the effectiveness of lactic acid bacteria (LAB) cultures employed for *L. monocytogenes* biocontrol.

5.2 FLOWCHART OF THE MODULES IN THE CASE STUDY MODEL

Many different industrial processes are involved in the production of different types of RTE fish. For example, brining or salting followed by smoking and maturation are key processing steps for the production of smoked fish, while maceration of fish previously coated with a mixture of ingredients are regular steps for the production of gravad fish. Figure 7 shows the different pathways of ready-to-eat (RTE) fish production. For the purpose of this work, models describing *L. monocytogenes* contamination during the production of RTE smoked fish, including a brining step, were applied.

FIGURE 7 Flowchart diagram of the production of RTE fish. Main operations and steps with an effect on the fate of *L. monocytogenes* are represented.



5.3 DESCRIPTION OF THE MODULES INCLUDED IN THE CASE STUDY AND REFERENCE PARAMETERS

A reference scenario was defined considering those inputs representing a well-managed and realistic process (with the exception of the contamination of the slicer at primary processing, that is set at 1 000 CFU for calibration purposes). The use of additives like diacetate or lactate acid were not considered in the reference scenario. The details of the parameters used as reference scenario and comparative scenarios are available in Table A3.1 (Annex 3).

Module 1: Pre-filleting

Initial contamination at processing plant

A lot (or batch) size of 100 units of fish was considered, with each unit containing 4 000 g. This initial assumption only represents one of the multiple possibilities that can be found in a processing plant.

The initial contamination was based on the probability of individual lots being contaminated, and the concentration of *L. monocytogenes* in the contaminated product. Data on the occurrence of *L. monocytogenes* in fish was retrieved from relevant literature. The probability of contaminated lots[4] was modelled with a beta distribution, with $\alpha = 0.874$, and $\beta = 5.88$. Based on literature data, initial counts of *L. monocytogenes* in fish were calculated from the prevalence information assuming a Poisson distribution, and they resulted in a concentration with mean and standard deviation values of -2.5 and 0.6 $\log_{10}$ CFU/g.

Storage before filleting

The growth of *L. monocytogenes* in whole fish before filleting was assumed to be log-linear until reaching the maximum population density (MPD) of 9.2 CFU/g. Two important kinetic parameters for the growth model – lag-phase duration and growth rate – were both quantified as a function of temperature as outlined by Jia *et al.* (2020). Pert distributions were employed to represent the lot-specific variability in holding time (min = 0.5 h; mode = 2 h; max = 6 h) and temperature (min = -2 °C; mode = 0 °C; max = 4 °C) of raw fish. These parameters were selected to ensure that the contamination level at the end of primary processing reached 1 000 CFU, aligning the risk estimate with values found in a recent EFSA report (EFSA, 2018).

[4] A lot is considered as contaminated if one unit is contaminated (N>0).

Module 2: Filleting

Each whole fish was divided into two slices, with the initial contamination value for the slicer in primary processing set at 1 000 CFU.

The model simulates the transfer of *L. monocytogenes* for every lot from the slicer to the product using the compartmental model published in Hoelzer *et al.* (2012), which is defined by two variability distributions:

- *a*, the transfer rate between slicer blade and product that follows a logistic distribution with the following reference scenario values: location parameter = 0.07; scale parameter = 0.03 and maximum value used to truncate the logistic distribution n = 0.5; and
- *e*, the transfer rate from the original contamination to the slicing system that follows a normal distribution at $\log_{10}$ scale with the following reference scenario values: mean = -2.12 and standard deviation = 0.85.

Module 3: Secondary processing

Holding time

The growth of *L. monocytogenes* during the holding time between filleting and brining was modelled using the same function as for the "Storage before filleting" in "*Module 1: Pre filleting*" with a change of the parameters on the holding time (min = 1 h; mode = 2 h; max = 6 h). Additionally, the models consider the new weight of the unit of fish being 1 300 g.

Brining and salting

The models simulated the potential internal or external cross-contamination of fish fillets during the process of salting, either by brine injection or dry salting. Each lot of fish fillets undergoes one type of salting method exclusively. In the reference scenario, it was assumed that all the fish filets were salted by brine injection, with a probability that the brine solution was contaminated by *L. monocytogenes* of 0.135 based on studies by Gudbjörnsdóttir *et al.* (2004) and Gudmundsdóttir *et al.* (2005). The concentration of *L. monocytogenes* in brine was assumed to follow a Pert distribution, with minimum, mode and maximum values of 0 CFU/ml, 0.0145 CFU/ml and 0.06 CFU/ml, respectively. Likewise, a Pert distribution was used to describe the volume injected, with minimum, mode and maximum values of 25 ml, 35 ml and 100 ml, respectively, in the reference scenario.

The cross-contamination of the product by *L. monocytogenes* during dry salting, or smearing the fillets with sugar/spices from tables or other surfaces was considered with the probability of cross-contamination 0.029 (Gudmundsdóttir *et al.,* 2005). Transfer coefficient parameters were modelled as a normal distribution (mean

= -0.29, standard deviation = 0.31) representing the variability in the log of the transfer coefficient of *L. monocytogenes,* sourced from Hoelzer *et al.* (2012). The contamination levels from tables or surfaces in contact with the product were assumed by experts to be 9 CFU available for transfer.

Smoking

If the product is intended to be smoked, the model simulates the effect of smoking brined or salted fish fillets and maturing them for 18–24 hours. Lot-specific $\log_{10}$ reduction values are sampled from normal distributions, depending on the type of salting (brining or dry salting). The reference scenario is set as:

- for brining: mean = 0.871 and standard deviation= 0.807, based on data from Eklund *et al.* (1995) and Porsby *et al.* (2008); and
- for dry salting: mean = 1.093, standard deviation = 0.532, based on data from Eklund *et al.* (1995), Neunlist *et al.* (2005) and Porsby *et al.* (2008).

Slicing and packing

After the maturation period, the product was sliced. The model simulates the transfer of the pathogen from the slicing machine using the compartmental model described in the filleting section. Per fillet, 40 slices were considered, and no initial contamination of the slicer was assumed in the reference scenario. After slicing, eight slices were placed in each package.

Module 4: Cold chain

In the reference scenario, the product was assumed to be stored at 4.6 °C for 144 h corresponding to the mode value of a PERT distribution (min = 12 h; max = 720 h). The growth of *L. monocytogenes* can be impacted by the level of LAB throughout the cold-chain period, which includes transport to retail and then to the consumer's home. The initial concentration of LAB was described by three parameters, including the minimum, mode and maximum values of -1 $\log_{10}$ CFU/g, 0.28 $\log_{10}$ CFU/g, and 1.6 $\log_{10}$ CFU/g, respectively, through a Pert distribution. Additionally, other factors also affect the growth of the pathogen such as pH, water activity and the level of NaCl. The kinetic parameters of growth for *L. monocytogenes* and LAB, and their interaction can be found in Table A3.1.

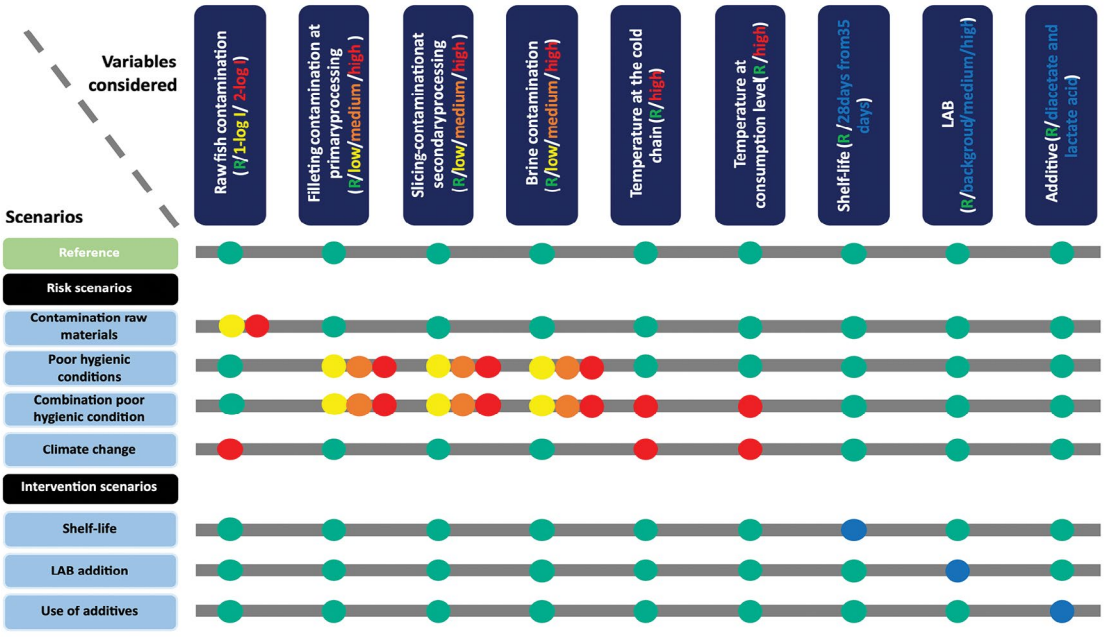
Module 5: Consumer handling

The storage time and temperature at home were also described by Pert distributions, with a maximum time of 35 days (35 x 24 h), and a mode and max temperatures of 7 °C and 12.9 °C, respectively. Below a description of different scenarios for the production of RTE smoked fish is presented.

5.4 SCENARIOS EVALUATED

To evaluate the impact of different factors and intervention measures on the risk of *L. monocytogenes* in RTE smoked fish, various scenarios were simulated incorporating various hypothetical situations from raw material, processing to consumption, based on published literature and/or existing data and expert knowledge. The factors (risk factor scenarios) and interventions (intervention scenarios) are summarized in Figure 8 and were evaluated by comparing them with the results of the reference scenario. Table A3.1 shows the different parameters and values considered in the different scenarios evaluated.

FIGURE 8 Illustration of reference, risk and intervention scenarios (left) used for testing the RTE smoked fish risk assessment model and evaluating factors and processes. Low, medium, and high represent low, medium, and high values of the parameters evaluated in the different stages of the production chain (upper dark blue). Green lights indicate that the node's value is consistent with the reference scenario's values (R), while yellow, orange, and red lights represent low, medium, and high values for the parameters in the respective boxes. Blue lights indicate that implementing certain intervention measures can reduce the final risk of listeriosis.



5.4.1 Risk factor scenarios

Contamination in slicing machine

The effect of the contamination concentration in the slicing machine used in secondary processing was evaluated as one risk factor. The reference scenario defined 1 000 CFU on the filleting slicer during primary processing and 0 CFU on the slicer at secondary processing. The investigation included three scenarios of levels of contamination at secondary processing: low contamination (*Scenario 1*, 100 CFU), medium-high contamination (*Scenario 2*, 10 000 CFU), and high contamination (*Scenario 3*, 1 000 000 CFU). Furthermore, to analyse the impact of no contamination on the slicer at primary processing but only at secondary processing, another scenario (*Scenario 19*) was conducted with the slicer at primary processing set to 0 CFU and the slicer at secondary processing set to 1 000 CFU.

Contamination in brining solution

After primary processing of the fish, it is necessary to prepare the fish for curing. This can be achieved by brining the fish in a saltwater solution for a certain period/concentration combination to achieve the required salt content for smoked fish products (Thomas *et al.*, 2012). Due to this process, the contamination of *L. monocytogenes* in the brining solution can affect the final risk of RTE smoked fish products. To assess this impact, three concentrations of *L. monocytogenes* in the brining solution were evaluated, i.e. a 10-fold, 100-fold, and 1 000-fold increase compared to the reference scenario (mode = 0.0145 CFU/ml; max = 0.06 CFU/ml), with other parameter values unchanged:

- *Scenario 4*: Assumption of a 10-fold increase, described as "low", mode = 0.145 CFU/ml, max = 0.6 CFU/ml;
- *Scenario 5*: Assumption of a 100-fold increase, described as "moderate", mode = 1.45 CFU/ml, max = 6 CFU/ml; and
- *Scenario 6*: Assumption of a 1 000-fold increase, described as "high", mode = 14.5 CFU/ml, max = 60 CFU/ml.

Raw fish contamination

Many studies report that the initial bacterial contamination level is one of the crucial factors influencing the final risk (Gonzales-Barron *et al.*, 2024a). In the reference scenario, the initial contamination levels were assumed to follow a Poisson distribution based on prevalence data from literature, with mean and standard deviation of the Poisson parameter (lambda) estimated as -2.5 and 0.6 $\log_{10}$ CFU/g, respectively. In the alternative scenarios, higher levels of contamination were evaluated, as described below:

- *Scenario 7*: Initial $\log_{10}$ counts as a normal distribution N(0,1), with other parameter values unchanged; this scenario examined the impact of a moderate increase in initial bacterial contamination in the fish on the final risk.
- *Scenario 8*: Initial $\log_{10}$ counts as a normal distribution N(1,1), with other parameter values unchanged; this scenario examined the impact of a high increase in initial bacterial contamination in the fish on the final risk.

Temperature increase during cold chain

To assess the impact of storage temperature in the cold chain on the final risk, an increased cold-chain temperature was assumed:

- *Scenario 9*: Assumption of an increased temperature of the cold chain described by temperature mode and maximum values of 5.6 °C and 10 °C, with other parameter values unchanged.

Temperature increase during storage at home

To evaluate the impact of storage temperature during home storage on the final risk, we assumed increased refrigerator temperatures as follows:

- *Scenario 10*: Assumption of an increased storage temperature at home described by temperature mode and maximum values of 8 °C and 14 °C, with other parameter values unchanged.

High environmental contamination

The hygienic conditions of the processing environment directly determine the potential to contaminate the final smoked fish product directly or indirectly. This scenario examined the impact of suboptimal hygiene conditions on the final risk:

- *Scenario 20*: The contamination level of the slicer for fish filleting in both primary and secondary processing was set at 10 000 CFU, with a 100-fold brining solution contamination compared to the reference scenario (mode = 1.45 CFU/ml, max = 6 CFU/ml). Other parameter values remained unchanged.

Very high environmental contamination

To examine the impact of extremely poor environmental hygiene conditions on the final risk the following scenario was designed:

- *Scenario 23*: The contamination level of the slicer in both primary and secondary processing was set at 1 000 000 CFU. The brining solution contamination was increased 1 000-fold compared to the reference scenario (mode = 14.5 CFU/ml, max = 60 CFU/ml), with other parameter values unchanged.

Climate change scenario with increased initial counts of L. monocytogenes and storage temperatures

- *Scenario 15*: This scenario consisted of a combination of *Scenario 7*, *Scenario 9* and *Scenario 10*. Briefly, initial $\log_{10}$ counts of the pathogen was described with a normal distribution N(0,1), and temperatures of the cold chain and home storage were (mode and max temperatures) 5.6 °C and 10 °C for the first, and 8 °C and 14 °C for the latter, with other parameter values unchanged; this scenario examined potential effects of global warming on the final risk.

Very low initial lactic acid bacteria

Compared to the reference scenario, a lower initial concentration of LAB was considered to represent a reduced competition by background microbiota:

- *Scenario 18*: Initial contamination by LAB defined as minimum = -3.0 $\log_{10}$ CFU/g, mode = -2.5 $\log_{10}$ CFU/g and max = -2.0 $\log_{10}$ CFU/g, with other parameter values unchanged.

5.4.2 Intervention scenarios

Reducing storage time at home

To evaluate the impact of home storage time on the final risk, we examined shorter home storage times as follows:

- *Scenario 11*: Assumption of home storage times described by a maximum value of 28 days × 24 h, with other parameter values unchanged.

Addition of lactic acid bacteria

The addition of LAB is considered a well-recognized strategy to limit *L. monocytogenes* development, with minimal interference with the sensory quality of the product. It should be noted that not all LAB have the same inhibitory effect on *L. monocytogenes*. To evaluate the impact of LAB, two different scenarios were simulated. *Scenario 12* examined the effect of a medium-high concentration of LAB on the final risk:

- *Scenario 12*: A 4-log addition of LAB compared to the reference scenario described by min = 5.5 $\log_{10}$ CFU/g, mode = 6.8 $\log_{10}$ CFU/g and max = 8.0 $\log_{10}$ CFU/g, with other parameter values unchanged; and
- *Scenario 13*: A 5-log addition of LAB concentration on the final risk, where the min = 6.5 $\log_{10}$ CFU/g, mode = 7.8 $\log_{10}$ g CFU/g and max = 8.0 $\log_{10}$ CFU/g, with other parameter values unchanged.

Use of preservatives

According to literature reports, organic acids, especially combinations of diacetate and lactate, have the potential to limit the growth of *L. monocytogenes* in various meat products (Mejlholm & Dalgaard, 2007). A scenario employing specific combinations of organic acids was simulated:

- *Scenario 14*: Concentrations of diacetate and lactate acid were set as follows:
  - > Diacetate acid: min = 500 ppm, mode = 1 500 ppm and max = 1 900 ppm
  - > Lactate acid: min = 6 000 ppm, mode= 12 000 ppm and max = 28 000 ppm

  with other parameter values unchanged.

The effect of starters (LAB) in scenarios with high environmental contamination

Scenario 21: *Scenario 20* was combined with *Scenario 13*, where the contamination level of the slicer in both primary and secondary processing was set at 10 000 CFU, and higher levels of *L. monocytogenes* in the brining solution (mode = 1.45 CFU/ml, max = 6 CFU/ml), and of LAB (min = 6.5 $\log_{10}$ CFU/g, mode = 7.8 $\log_{10}$ g CFU/g and max = 8.0 $\log_{10}$ CFU/g) compared to the reference scenario were assumed with other parameter values unchanged.

The effect of additives in scenarios with high environmental contamination

Scenario 22: *Scenario 20* was combined with *Scenario 14*, where the contamination level of the slicer in both primary and secondary processing was set at 10 000 CFU, the level of *L. monocytogenes* in the brining solution higher than in the reference scenario (mode = 1.45 CFU/ml, maximum = 6 CFU/ml), and diacetate and lactate acid were added at the following concentrations:

- Diacetate acid: min = 500 ppm, mode = 1 500 ppm and max = 1 900 ppm
- Lactate acid: min = 6 000 ppm, mode= 12 000 ppm and max = 28 000 ppm

with other parameter values unchanged.

The effect of starters (LAB) in scenarios with very high environmental contamination

Scenario 24: *Scenario 23* and *Scenario 13* were combined, where the contamination level of the slicer in both primary and secondary processing was set at 1 000 000 CFU. The brining solution contamination was 1 000-fold higher than in the reference scenario (mode = 14.5 CFU/ml, max = 60 CFU/ml), and a high concentration of LAB was assumed (min = 6.5 $\log_{10}$ CFU/g, mode = 7.8 $\log_{10}$ g CFU/g and max = 8.0 $\log_{10}$ CFU/g), with other parameter values unchanged.

The effect of additives in scenarios with very high environmental contamination

Scenario 25: *Scenario 23* and *Scenario 14* were combined, where the contamination level of the slicer in both primary and secondary processing was set at 1 000 000 CFU. The brining solution contamination was 1 000-fold higher than in the reference scenario (mode = 14.5 CFU/ml, max = 60 CFU/ml), and diacetate and lactate acid were added at the following concentrations:

- Diacetate acid: min = 500 ppm, mode = 1 500 ppm and max = 1 900 ppm
- Lactate acid: min = 6 000 ppm, mode= 12 000 ppm and max = 28 000 ppm

with other parameter values unchanged.

The effect of starters (LAB) in the climate change scenario

Scenario 17: Building upon *Scenario 15*, this scenario simulates an intervention strategy to counteract the potential adverse effects of climate change (higher initial counts and storage temperatures), consisting of the addition of starters, i.e. LAB, to the food (*Scenario 13*). Thus, the variable initial counts were described as a normal distribution N(0,1), and temperatures of the cold chain and home storage were (mode and max temperatures) 5.6 °C and 10 °C for the first, and 8 °C and 14 °C for the last. An addition of high levels of LAB was employed (min = 6.5 $\log_{10}$ CFU/g, mode = 7.8 $\log_{10}$ g CFU/g and max = 8.0 $\log_{10}$ CFU/g), with other parameters unchanged.

The effect of additives in the climate change scenario

Scenario 16: Building upon *Scenario 15*, this scenario simulates an intervention strategy to counteract the potential adverse effects of climate change (higher initial counts and storage temperatures), consisting of the addition of preservatives to the food (*Scenario 14*). Thus, the initial counts variable was described as a normal distribution N(0,1), and temperatures of the cold chain and home storage were (mode and max temperatures) 5.6 °C and 10 °C for the former, and 8 °C and 14 °C for the latter. Diacetate and lactate acid concentrations were set as follows:

- Diacetate acid: min = 500 ppm, mode = 1 500 ppm and max = 1 900 ppm
- Lactate acid: min = 6 000 ppm, mode= 12 000 ppm and max = 28 000 ppm

with other parameters unchanged.

5.5 RISK CHARACTERIZATION

The model was run using various inputs to evaluate the different scenarios reflecting diverse conditions at different stages of the processing and handling of RTE smoked fish. The simulated mean risk to the population with increased susceptibility (FAO & WHO, 2004b) in the scenarios was then compared to the risk in the reference scenario to determine the impact of the risk factors and interventions expressed as a relative risk. The results of all scenarios evaluated, including the Reference scenario, for the production of brine-salted cold-smoked fish, are shown in Table A3.2 and Table A3.3. Table A3.2 focuses on the results concerning contamination levels and prevalence of *L. monocytogenes* at the e*nd of processing* and the *time of consumption*. Concerning pathogen levels, the probability of having more than 10 CFU/g or more than 100 CFU/g was also calculated at both stages, although at the *end of processing*, the probability value is given *in a per pack* basis, while at time of consumption, *in a per serving* basis. Table A3.3 provides the main statistics of the risk of listeriosis per serving for the different scenarios.

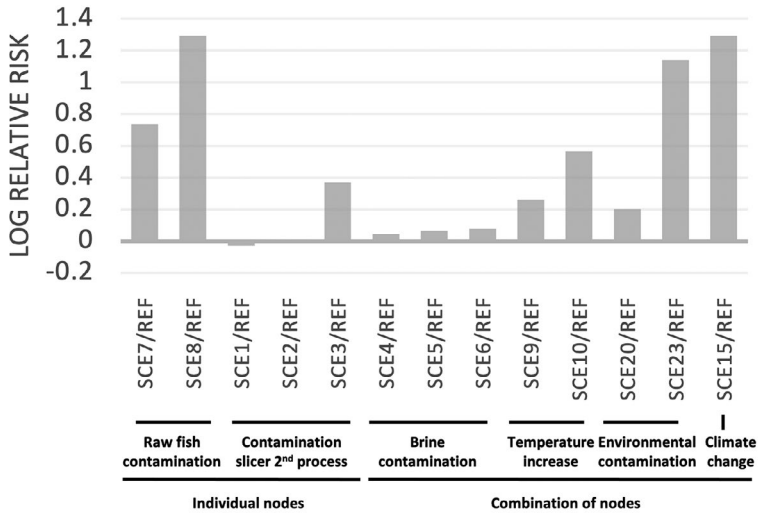
It can be observed that the results of the reference scenario are skewed (see Table A3.3). Regarding the levels of the pathogen, such skewness is denoted by the difference between the mean and median, taking into account the 2.5 and 97.5 percentiles for the concentration of *L. monocytogenes*, especially at the time of consumption (mean = 1 213 CFU/g; median = 0.0615 CFU/g; 2.5 percentile = 0.0140 CFU/g; 97.5 percentile = 93.40 CFU/g) (see Table A3.2). Similarly, skewed results were also obtained for the risk of listeriosis per serving (Table A3.3), with a median value = 1.039×10^{-12} and a mean value of 3.929×10^{-9}. This indicates that infrequent occurrences of high contamination events contribute the most to the mean risk per serving.

5.5.1 Risk factor scenarios

The relative risk between the risk per serving of a scenario and the reference scenario (REF), were, as expected, always positive, i.e. greater than in the REF. Figure 9 shows the relative risk in $\log_{10}$ of the risk factor scenarios. The scenarios simulating higher initial counts (*Scenario 8* and *Scenario 15*) resulted in the largest risk compared to the REF, especially when higher storage temperatures were considered (*Scenario 15*), with about 1.3 $\log_{10}$ increase of the risk (≈ 20 times higher than the REF). Very high environmental contamination (*Scenario 23*) and very low initial lactic acid bacteria levels follow (*Scenario 18*), with 1.1 $\log_{10}$ and 0.9 $\log_{10}$, respectively (≈14 and 7 times, respectively) higher than the REF.

Figure 10 (a and b) represent, respectively, the increment in the level of *L. monocytogenes* at the end of processing (CFU/g in a pack) and at the time of

FIGURE 9 Relative risk (log units) of risk factor scenarios compared with the reference scenario (REF) for RTE smoked fish

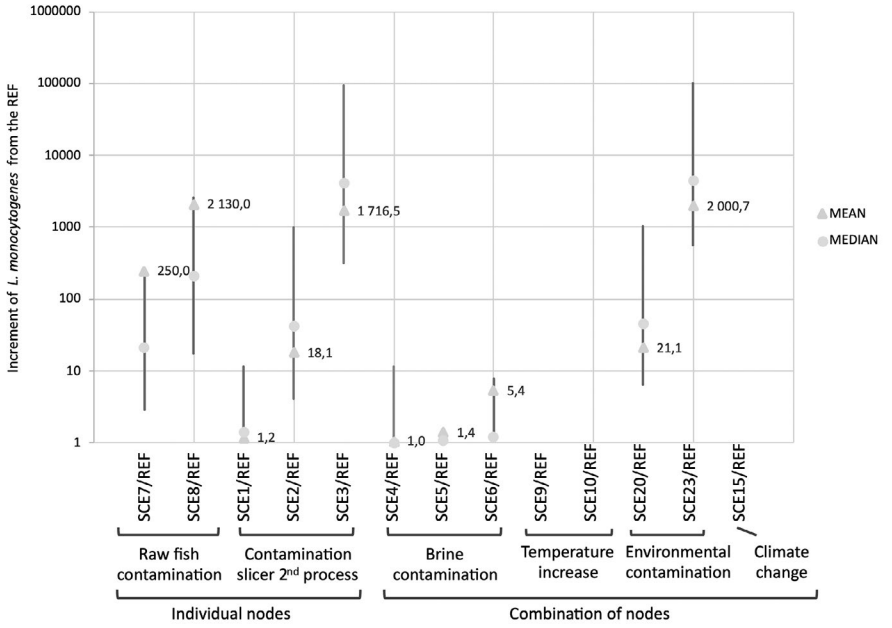


consumption (CFU/g in a serving) with respect to the REF. It was calculated as the ratio between the concentrations in arithmetic scale of the scenarios compared. As can be seen, the increment is much more noticeable at the end of processing than at the time of consumption. The largest increase in the level of the pathogen at the end of processing (Figure 10a is observed in two scenarios, i.e. high contamination of the slicer in secondary processing and very high environmental contamination, followed by high contamination of the raw fish. The smallest increment observed at the end of processing corresponded to brine contamination as well as to the low contamination of the slicer in secondary processing, and in fact, this trend was also observed at the time of consumption.

At the time of consumption (Figure 10b) the widest 2.5–97.5 inter percentile range of the increment of the levels, indicating the largest variability, is associated with the three scenarios with the highest increment found at the end of processing (high contamination of the slicer in secondary processing and very high environmental contamination), and also, with the climate change scenario. The upper extreme of the three scenarios corresponds to the 97.5 percentile. The increase of the median and mean levels of these three scenarios are calculated in a range of 4–7 $\log_{10}$ units, with the exception of the climate change scenario, with a mean value close to 11 $\log_{10}$ units. The scenarios involving medium raw fish contamination and high contamination of slicer in secondary processing follow, with the rest of scenarios at a lower increment in levels.

FIGURE 10 Increment of *L. monocytogenes* levels from the reference scenario (REF) a) at the end of processing and b) at the time of consumption. Line extremes represent 2.5 and 97.5 percentiles.

a)



b)

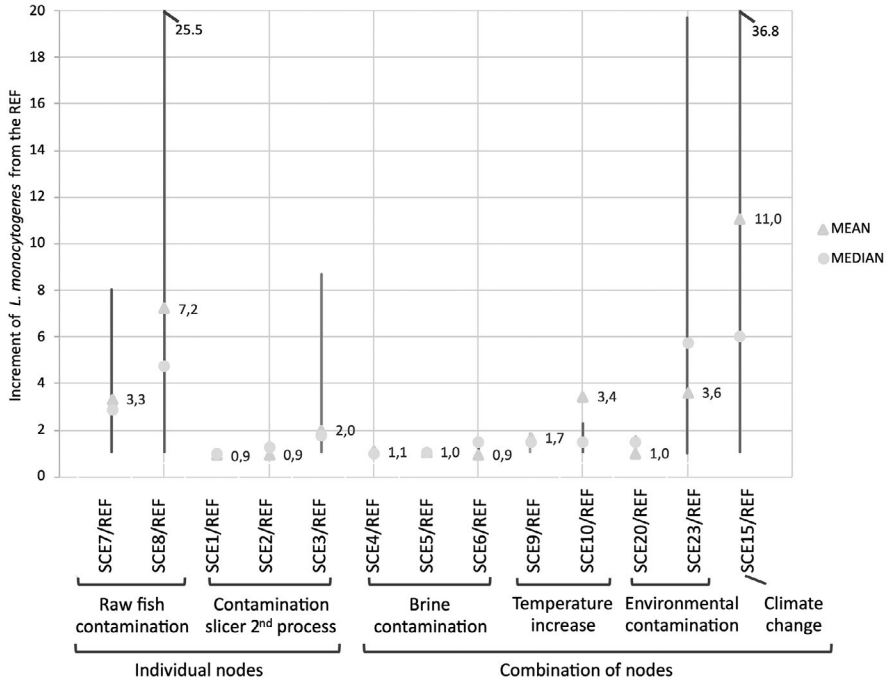


Figure 11 represents the increase in prevalence associated with the risk factor scenarios relative to the REF scenario. In general, it can be seen that the increase in prevalence is higher at the time of consumption than at the end of processing, probably due to the prevalence of calculations units, consisting of the proportion of contaminated servings at the time of consumption, while at the end of processing, the prevalence stands for the proportion of contaminated packs. The "partitioning" effect when passing from *pack* to *serving*, may have led to a spread of *L. monocytogenes*. The scenarios assuming very high environmental contamination and high contamination of raw fish are those exhibiting a major impact on the prevalence.

A similar shape as in Figure 11 is observed in Figure 12, where the increase in the probability of detecting a concentration greater than 10 and 100 CFU/g at the time of consumption is shown. Data at the end of processing was not included in the figure, as the probability of detecting a level greater than 10 and 100 CFU/g was 0 in the REF scenario, being also the case for many of the risk factor scenarios evaluated (see Table A3.2).

When trying to establish relationships between the different statistical measures of risk, a high correlation between the median value of the risk per serving and

FIGURE 11 Increase of *L. monocytogenes* prevalence from the reference scenario (REF) at the end of processing and at the time of consumption

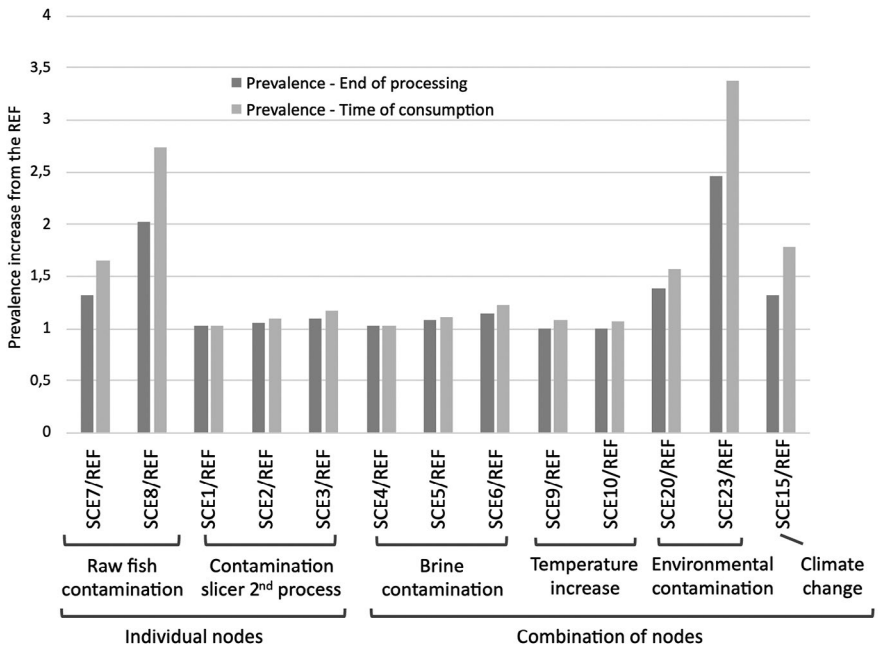
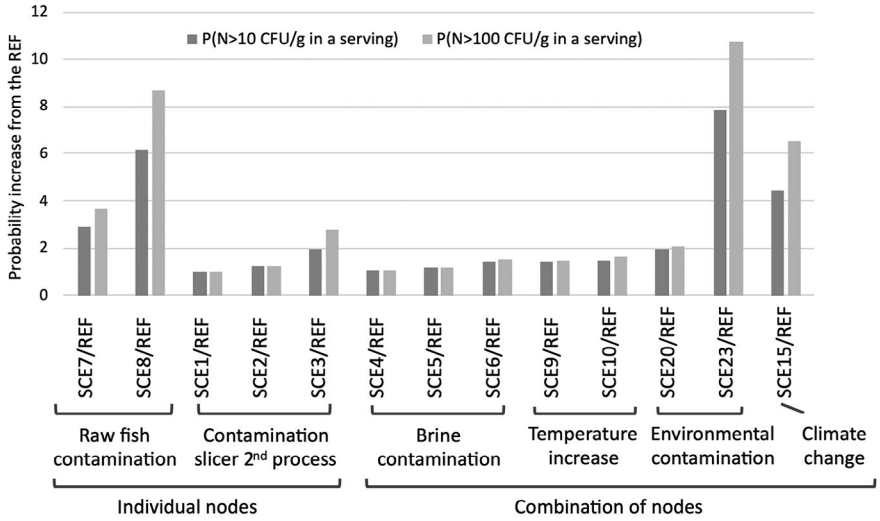


FIGURE 12 Increase in the probability of detecting more than 10 and 100 CFU/g of *L. monocytogenes* in a serving at the time of consumption compared to the REF



i) prevalence data, ii) P (N>10 CFU/g in a serving), and iii) P (N>100 CFU/g in a serving) at the time of consumption (Table 2) was found.

A scenario was designed to see the impact of contamination of the slicer in either primary or secondary production. Thus, the REF scenario, with a contamination of the slicer in primary processing established at 1 000 CFU (no contamination of slicer in secondary processing) was compared with *Scenario 19*, that is, contamination of the slicer in secondary processing established at 1 000 CFU (no contamination of slicer in primary processing), with the other variables left unchanged. Results in Table A3.3 indicate that, in general, the estimated risk statistics were higher in the REF scenario than in *Scenario 19*, so for an easier comparison, the ratio of change REF/*Scenario 19* was calculated for the different statistics, resulting in 1.05, 1.30, 0.05 and 1.30 for the mean, median, 2.5 percentile and 97.5 percentile, respectively. This result indicates that the contamination of the slicer in primary processing (REF) may result in a risk increase of up to 30% in relation to the contamination of the slicer in secondary processing (*Scenario 19*). According to the relative data presented in Table 3, it is interesting to note that the concentration values in the REF scenario are lower than those in *Scenario 19,* despite occurring at the end of processing. At the time of consumption, the probability of detecting more than

TABLE 2 Correlation coefficients between the median and the mean of the risk per serving, respectively, and various variables at the time of consumption in risk factor scenarios

	C MEDIAN	C MEAN	C 97.5	PREV	P (N>10)	P (N>100)
Median of the risk per serving of all the lots	0.68	0.16	0.48	0.85	0.84	0.83
Mean of the risk per serving of all the lots	0.89	0.86	0.96	0.75	0.83	0.86

C median: median concentration in contaminated servings (CFU/g)
C mean: mean concentration in contaminated servings (CFU/g)
C 97.5: 97.5 percentile concentration in contaminated servings (CFU/g)
Prev: prevalence of contaminated servings
P (N>10): probability of detecting more than 10 CFU/g in a serving
P (N>100): probability of detecting more than 100 CFU/g in a serving

10 or 100 CFU/g in a serving was higher in the REF scenario than in *Scenario 19*. This last finding would very likely account for the higher risk obtained in the REF scenario.

5.5.2 Intervention scenarios

A number of intervention scenarios including shelf-life management, the addition of LAB and the use of additives were implemented in order to evaluate their effect in minimizing the risk of listeriosis compared to the REF scenario, and compared to the risk factor scenarios tested previously.

Figure 13 shows the relative risk calculated in $\log_{10}$ units. By applying the different intervention strategies, the relative risk per serving was reduced by -0.6 to -4.1 $\log_{10}$. In comparison with the REF scenario, it is evident that the intervention consisting of shelf-life management (reduction of 20% of the parameter maximum time, that is, from 35 to 28 days in *Scenario 11*) resulted in the least mitigation effect, and the addition of LAB was the most effective intervention strategy (*Scenarios 12* and *13*).

In the case of the remediation effect against the risk factor scenarios, it can be observed that, in general, the efficacy of the intervention strategies depended on the factors contributing to the risk. Specifically, both the addition of LAB and the use of additives were more effective against high environmental contamination than against climate change scenarios, probably due to the fact that both intervention strategies perform during the shelf-life of the product. It should be noted that the "climate change" scenario simulated the abuse temperatures throughout the cold chain during the shelf-life of the product, while the "environmental contamination"

TABLE 3 Ratio (REF/*Scenario 19*) of the concentration, prevalence and probabilities of detecting different concentrations of *L. monocytogenes* at the end of processing or time of consumption

	C_R median	C_R mean	C_R 2.5	C_R 97.5	$Prev_R$	P_R (N>10)	P_R (N>100)
End of processing	0.21	0.41	0.01	0.90	1.09	Not applicable	Not applicable
Time of consumption	0.96	0.80	0.92	1.06	1.09	1.10	1.13

C_R median: ratio of change (REF/Sce 19) of median concentration in contaminated servings (CFU/g).
C_R mean: ratio of change (REF/Sce 19) of mean concentration in contaminated servings (CFU/g)
C_R 2.5: ratio of change (REF/Sce 19) of 2.5 percentile concentration in contaminated servings (CFU/g)
C_R 97.5: ratio of change (REF/Sce 19) of 97.5 percentile concentration in contaminated servings (CFU/g)
$Prev_R$: ratio of change (REF/Sce 19) of prevalence of contaminated servings
P_R (N>10): ratio of change (REF/Sce 19) of the probability of detecting more than 10 CFU/g in a serving
P_R (N>100): ratio of change (REF/Sce 19) of the probability of detecting more than 100 CFU/g in a serving

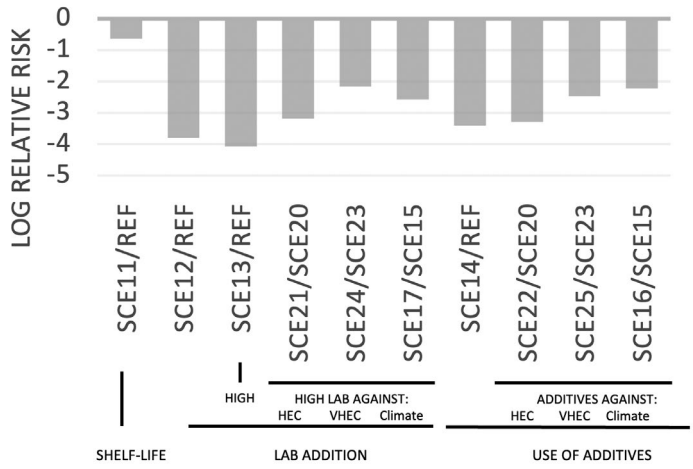
scenario simulated higher levels of the pathogen on slicers and in brine, during processing.

In order to get insight about the contribution of the decrease in levels and prevalence of *L. monocytogenes* on the observed decrease of risk, different relative measures were calculated at the time of consumption. At the end of processing, levels and prevalence of the pathogen when applying these measures were the same as in the scenarios under comparison (REF or risk factor scenarios), since these mitigation measures would take place after food processing, i.e. during storage and transport of the food (Table A3.2).

Figure 14 shows the relative levels of *L. monocytogenes* by the application of intervention measures at the time of consumption. As can be seen, from the REF scenario, the same trend was observed as for the estimated relative risk (Figure 13), that is, a low effect of shelf-life management, and a major effect was observed for the "addition of LAB" scenario in terms of the mean. The median, however, was greatly reduced in the case of the addition of additives.

Among the intervention scenarios used as remediation against risk factor scenarios, in general, a major effect can also be observed in the use of additives or the addition of LAB against high environmental contamination. And also, similar to the fact observed previously, the addition of LAB and the use of additives were more effective against the "environmental contamination" than against "climate change" scenarios when considering the mean.

FIGURE 13 Relative risk (log units) of intervention scenarios compared with the reference (REF) or risk factor scenarios for RTE smoked fish. LAB: lactic acid bacteria. HEC: high environmental contamination. VHEC: very high environmental contamination. Climate: climate change



Finally, a representation of the relative prevalence, and relative probability of detecting more than 10 or 100 CFU/g in a serving at the time of consumption is provided (Figure 15). As can be seen, the prevalence of contaminated servings was substantially reduced by the use of additives by nearly 50% when compared with the REF scenario. Also, the probability of detecting more than 10 or 100 CFU/g in a serving was reduced by the use of additives, similar to the addition of LAB, which also produced a substantial decrease when compared with the REF scenario (Sce 12/REF and Sce 13/REF).

Similar to the procedure followed for risk factor scenarios, different measures and statistics of intervention scenarios were evaluated in order to find relationships between them. A correlation was found between the median of the risk per serving and i) median concentration in contaminated servings, ii) prevalence data, iii) P(N>10 CFU/g in a serving), and iv) P (N>100 CFU/g in a serving) at the time of consumption (Table 4).

FIGURE 14 Relative level of *L. monocytogenes* at the time of consumption of intervention scenarios compared to the reference (REF) or risk factor scenarios. Lines represent the range between relative measures calculated for 2.5 and 97.5 percentiles of the levels of *L. monocytogenes*. LA: lactic acid bacteria. HEC: high environmental contamination. VHEC: very high environmental contamination. Climate: climate change

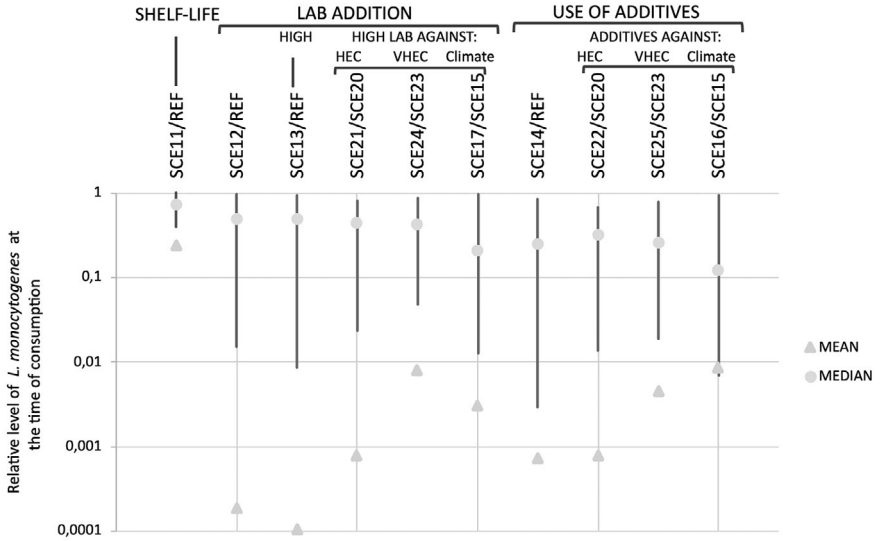
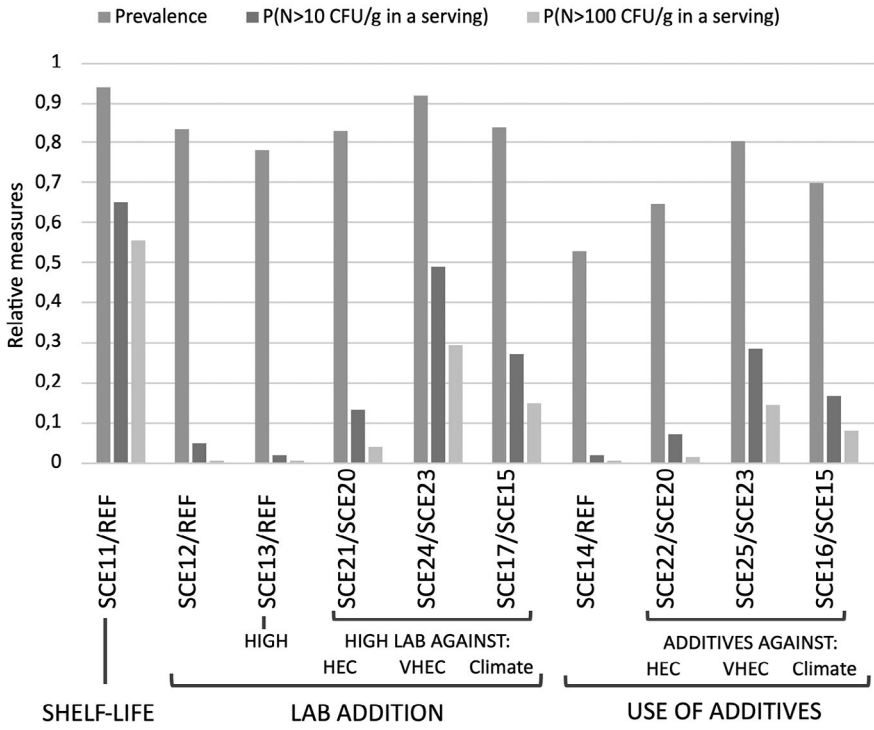


TABLE 4 Correlation coefficients between the median of the risk per serving (mean or median) and various variables at the time of consumption in intervention scenarios

	C MEDIAN	C MEAN	C 97.5	PREV	P (N>10)	P (N>100)
Median of the risk per serving for all the lots	0.91	-0.12	0.78	0.94	0.94	0.93
Mean risk per serving risk per serving for all the lots	0.30	0.95	0.56	0.18	0.32	0.35

C median: concentration median in contaminated servings (CFU/g)
C mean: concentration mean in contaminated servings (CFU/g)
C 97.5: concentration 97.5 percentile in contaminated servings (CFU/g)
Prev: prevalence of contaminated servings
P (N>10): probability of detecting more than 10 CFU/g in a serving
P (N>100): probability of detecting more than 100 CFU/g in a serving

FIGURE 15 Relative prevalence and relative probability of detecting more than 10 CFU/g or 100 CFU/g in a serving at the time of consumption. Relative measures are calculated for intervention scenarios compared to the reference (REF) or risk factor scenarios. LAB: lactic acid bacteria. HEC: high environmental contamination. VHEC: very high environmental contamination. Climate: climate change



5.6 CONCLUSIONS

The food chain of the production of smoked fish was simulated from primary production to consumption. The production of cold-smoked fish that have been brine salted was selected as one of the most relevant pathways. Nevertheless, a simulation regarding dry-salting of smoked fish was also performed and resulted in a risk per serving slightly lower than the dry brining scenario.

Different assumptions and "what-if" scenarios were considered taking into account probable routes of contamination and factors influencing the development of *L. monocytogenes* in the product. The risk assessment outputs and end-points included: concentration statistics (mean, median, 2.5 and 97.5 percentile), prevalence, and probability of exceeding 10 or 100 CFU/g in a serving at both the end of the process and time of consumption. Also, the risk per serving statistics (mean, median, 2.5 and 97.5 percentile) were calculated at the time of consumption.

Relative impacts on outputs and end-points were calculated for the risk factor scenarios evaluated as well as for the intervention scenarios. For the risk factor scenarios, under the assumptions and approaches adopted in this study, it can be concluded that the contamination of raw fish gains as much importance as the environmental contamination of the fish during processing, leading to high values of prevalence, concentration, and probability of detecting more than 10 or 100 CFU/g at the time of consumption. It is also worth mentioning that contamination coming from the slicer at primary processing results in a higher risk than when coming from the slicer at secondary processing, given equal levels of contamination. These findings highlight the need for a thorough control of the hygiene during the entire process. Also, the simulation of the climate change scenario demonstrated substantial effects, which was evaluated by assuming an increase in the contamination of raw fish together with a temperature increase in the cold chain and at the consumer level.

Intervention scenarios were also evaluated, measuring their relative impact on decreasing the risk of *L. monocytogenes*. With the data and variables assumed, it was found that the addition of LAB was moderately more effective than the use of additives. Nevertheless, this was very dependent on the levels used and the composition of the additives employed, and thus, this statement should be taken with caution. Simulations also showed that a 20% reduction of shelf-life was, under the conditions evaluated, less effective than the other two intervention strategies.

With regard to the model employed, associations between different variables of the model were explored in both risk and intervention scenarios. For example, in the risk scenario, high correlations values (>0.90) were found between the risk per serving and prevalence and the probability of exceeding 10 or 100 CFU/g in a serving at the time of consumption.

6

Dose-response model

6.1 DESCRIPTION AND JUSTIFICATION OF THE CASE STUDY

The dose-response (DR) model for any pathogen may be affected by the three aspects of the infectious disease triangle (FAO & WHO, 2021), namely the food matrix, the host susceptibility and the pathogen characteristics/virulence. However, in practice, most DR models are developed from limited data and cannot fully reflect all these effects. The DR model for *L. monocytogenes* is no exception in this respect (Pouillot *et al.*, 2024).

The FAO and WHO (2004b) DR models for *L. monocytogenes* were of the exponential form which had the "*r*-value" as its single parameter. The *r*-value denotes the probability that with exposure to each *L. monocytogenes* cell, a "single-hit" results in illness and is considered constant for all cells and independent from the presence of other cells (see also FAO & WHO, 2021). Differences between the general and susceptible populations could be accommodated by fitting different *r*-values for the susceptible populations (FAO & WHO, 2004b). This set of models has been developed for *L. monocytogenes* in RTE foods, based on the method described by Buchanan *et al.* (1997). Such studies are not feasible for pathogens that either have a significant risk of being life threatening or for which morbidity is primarily associated with high-risk populations (i.e. immunocompromised persons). The method involves fitting the DR model to surveillance data of *L. monocytogenes* contamination observed in the food supply and matching the expected versus actual total number of listeriosis cases annually to the extent possible. However, the approach relies on many untested assumptions (FAO & WHO, 2021; Pouillot *et al.*, 2024).

More recent DR models for *L. monocytogenes* were developed to better incorporate host susceptibility. The approaches include the use of animal model data (Roulo *et al*., 2014; Smith *et al*., 2003, 2008; Williams *et al*., 2007), outbreak data (Pouillot *et al*., 2016) and epidemiological surveillance data from France for 11 subpopulations that were defined according to underlying health conditions (Pouillot *et al*., 2015). A similar approach using European age-sex[5] subgroups as surrogates for underlying health conditions was used by the European Food Safety Agency (EFSA) (2018, 2020). Collectively these models suggest higher r-values than those used previously (FAO & WHO, 2004b). The effect of higher r-values is that they result in a left-shift of the DR curve, i.e. to lower doses, and therefore result in a lower Infectious Dose 50 (ID50), the dose at which the probability of illness equals 0.5 (or 50%).

In contrast to the work on host susceptibility, Fritsch, Guillier & Augustin (2018) focused on the pathogen and differences in virulence between different clonal complexes (CCs), using a similar approach to Pouillot *et al.* (2015). The researchers refined the DR model with parameters relevant to three different groups of virulence, which were based on CCs.

Finally, with respect to the food matrix and supply-chain history, FAO and WHO (2022a) concluded that not enough information was available to consider these food-related aspects as part of the DR model.

Nevertheless, incorporation of host and pathogen related information into the DR model was considered important by the previous JEMRA meetings (FAO and WHO, 2022a, 2022b). To this effect a new DR model was developed by integrating the EFSA model for age-sex subgroups together with the three classes of virulence characteristics (FAO and WHO, 2022a), giving a total of 42 DR curves, one for each age-sex and virulence class ("less virulent", "moderate virulent", and "more virulent") combination (Pouillot *et al*., 2024). It should be noted that the use of the EFSA subgroups as a surrogate for underlying health conditions in favour of actual health condition observations in France (Pouillot *et al*., 2015) was based on the fact that the EFSA data are more broadly representative geographically. Ideally, however, globally representative data on underlying health conditions, similar to those from France presented in Goulet *et al.* (2012), would be preferrable; this would also allow the DR model to be more globally relevant.

The mathematical details for this approach can be found in Annex 4 and the *r*-values for each curve are provided in Table A4.4 in Annex 4 as a reference. In the context of the work referred to in this report, Pouillot *et al.* published in 2024

[5] It should be noted that the EFSA panel used the term "age-gender". However, since the effects of the pathogen on the human body are likely to be related to a biological mechanism, the term "age-sex" has been used here.

a detailed description of the updated parameters for the *L. monocytogenes* dose-response model. This model takes into account the virulence of the pathogen as well as the age and sex of the consumer. The updated parameters were specifically developed to support expert discussions and the conclusions of this report.

6.2 DOSE-RESPONSE SCENARIOS

To illustrate and compare various DR models, several graphs and tables are presented below, along with key observations.

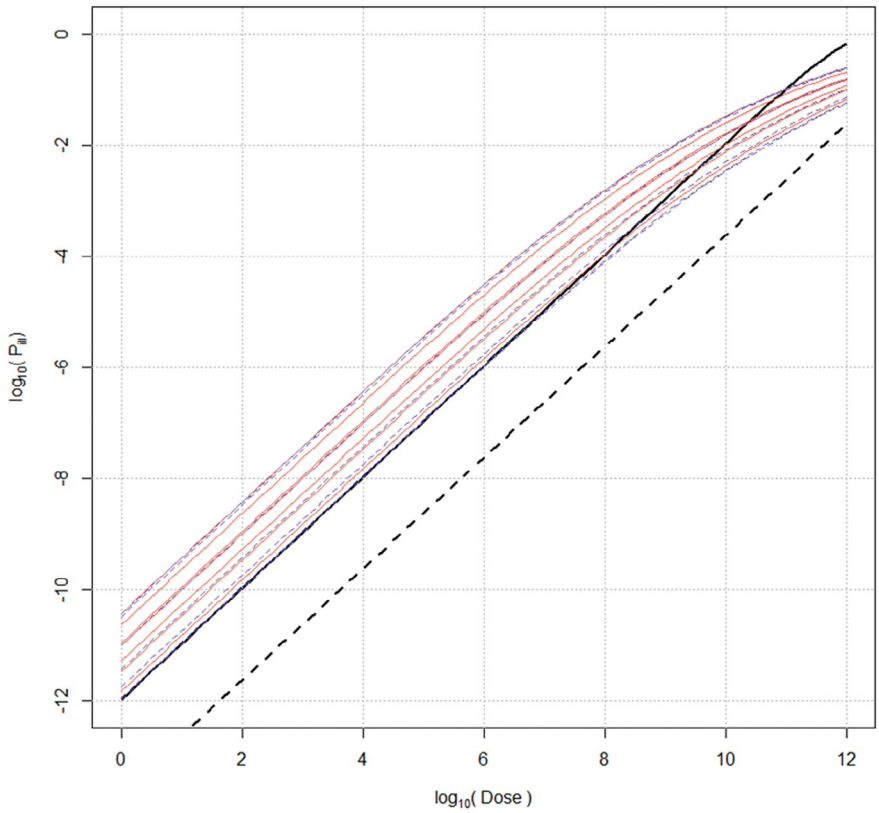
A plot of the EFSA DR curves (EFSA, 2018) for the 14 age-sex combinations versus the two FAO/WHO curves (FAO & WHO, 2004b) are shown in Figure 16. The shapes of the EFSA curves differ from the FAO/WHO curves due to the difference in the functional form of the model, that is, the lognormal-binomial model versus the binomial model,[6] respectively (see also Annex 4). In particular, the curvature of the DR curve, especially noticeable at the high-dose end (i.e. greater than 10^8 CFU), is because of the standard deviation of the lognormal distribution for r; a larger standard deviation results in a greater curvature while a standard deviation of 0 results in the exponential DR model.

From Figure 16, and considering a dose of 10^5 CFU, which lies within the linear part of the DR curves, for calculating the "relative risk" (RR), the following observations can be made (Pouillot *et al.*, 2024):

- For the FAO/WHO model, the RR between general and susceptible populations is 44.8, with the latter resulting in the higher probability of illness of 1.06×10^7.
- The RR between the least (Males between 15–24 years) and most (Females >75 years) susceptible EFSA populations was 29.8, with the latter resulting in a higher probability of illness of 2.86×10^{-6}.
- Within the linear part of the DR model, i.e. doses up to about 10^8, the RRs between the least (Males between 15–24 years) and most (Females >75 years) susceptible EFSA populations versus the general population of the FAO/WHO model were 40.5 and 1 209.4, respectively. At larger doses the RRs reduce, though the relative position of individual age-sex groups is maintained.
- In contrast, the RR between least (Males between 15–24 years) and most (Females >75 years) susceptible EFSA populations versus the susceptible

[6] The binomial model corresponds to the classical exponential dose-response model where the dose is the actual number of bacteria rather than a mean dose; similarly, the lognormal-binomial model corresponds to the lognormal-Poisson model (Pouillot *et al.*, 2015).

FIGURE 16 EFSA DR curves for 14 age-sex groups (Females = solid red lines; Males = dashed blue lines) and FAO/WHO's general (black solid line) and susceptible (black dashed line) population DR curves



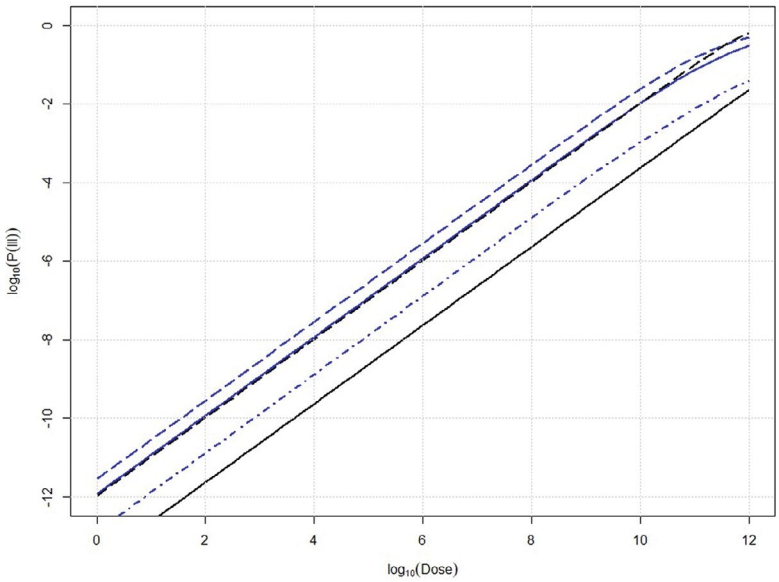
Source: Prepared by the authors based on the content in: EFSA, 2018; and WHO & FAO, 2004b.

population of the FAO/WHO model were 0.9 and 27.0, respectively. At greater doses ($>10^{11}$) the RRs reduce, and in fact reverse, i.e. the FAO/WHO susceptible population model predicts higher probability of illness compared with the EFSA models. As noted above, this is due to the mathematical form of the DR model and the variability of r parameter.

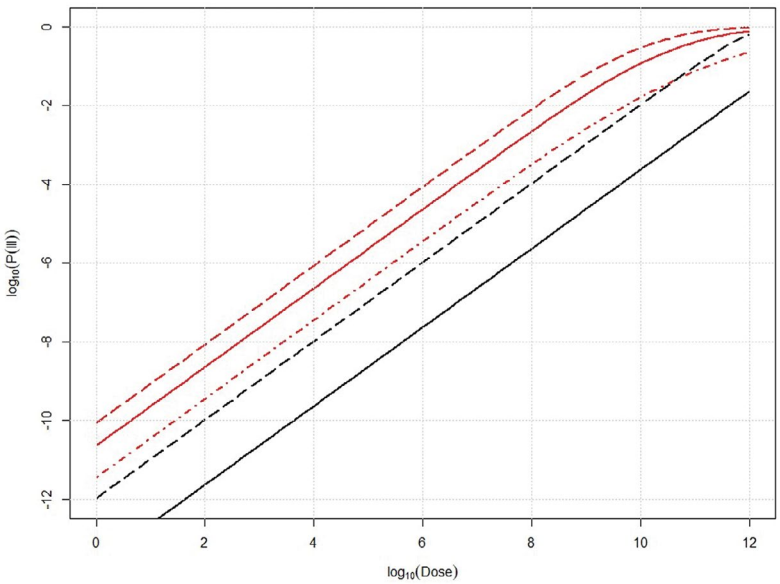
The new DR model takes three classes of strain virulence into account, in addition to the age-sex groups of the EFSA model, resulting in a total of 42 DR curves. Instead of displaying all of these, the least (Males between 15–24 years) and most (Females >75 years) susceptible EFSA populations were selected and displayed for each of the three virulence classes "less virulent", "virulent" and "more virulent".

FIGURE 17 New DR model curves for: a) the least susceptible population (Males between 15–24 years); and b) most susceptible population (Females >75 years). Virulence class of strains are represented: less virulent: dot-dash: virulent: plain: more virulent: dashed. FAO & WHO's DR (2004): general population (black solid line) and susceptible population (black dashed line)

a)



b)



Source: Prepared by the authors based on the content in FAO & WHO, 2004b.

The resulting plot is shown in Figure 16. It should be noted that when integrating the virulence class information with the EFSA model, females over 75 years of age are marginally more susceptible than males of the same age.

As before, considering a dose of 10^5 CFU, which lies within the linear part of the DR curves, for calculating the "relative risk" (RR), the following observations can be made from Figure 17 (Pouillot *et al.*, 2024):

- The RRs between the less virulent class and the more virulent class of *L. monocytogenes* strains depend on the population of interest, i.e. they are not constant. The RRs are 21.6 and 24.6 for the least susceptible population (Males between 15–24 years) and the most susceptible population (Females >75 years), respectively.
- Comparing the general and susceptible population FAO/WHO models with the new DR model, and using the "virulent" class of strains, the following RRs can be calculated:
  - > Susceptible FAO/WHO population versus the most susceptible population (Females >75 years) yields an RR of 21.9.
  - > General FAO/WHO population versus least susceptible population (Males between 15–24 years) yields an RR of 50.2.
- The RR between the least susceptible group exposed to the less virulent class of strains versus the most susceptible group exposed to the most virulent class of strains is 655.
- Taking class of strain virulence and age-sex subpopulations into account results in a wider range of possible probability of illness at a specific dose (RR = 655) compared with the range obtained from the previous FAO/WHO model (RR = 45).

To further contextualize various DR models, the risk of listeriosis per 1 billion servings, as predicted from the reference scenario for ready-to-eat smoked fish (see Section 5) was calculated. In particular, the model was run considering the strain virulence proportions observed in Europe by Møller Nielsen *et al.* (2017), i.e. 51.4% less virulent strains, 36.2% virulent strains and 14.4% more virulent strains. In addition, theoretical scenarios with 100% less virulent strains, 100% virulent strains, 100% more virulent strains, or with the European proportions after removal of the more virulent strains were explored.

The results are shown in Table 5. They confirm that the FAO/WHO 2004 dose-response model tends to underestimate the risk of listeriosis compared to the EFSA 2018 or the dose-response developed in this report.

Using these models and the ready-to-eat fish reference scenario, the 100% less virulent strain scenario leads to a risk per serving that is 7.9 times lower when

compared with the 100% virulent strain scenario, while the 100% more virulent strain scenario leads to a risk per serving that is 3.0 times higher. Clearly, using the European mixture of strains falls between the two extremes (100% less or more virulent strains); the estimate is marginally lower than the 100% virulent strain result due to the greater proportion (51.4%) of less virulent strains. Removing the 12.4% more virulent strains from smoked fish would reduce the risk of listeriosis per serving by 39% (23.52 versus 14.46 per billion servings).

TABLE 5 Estimated mean risk per 1 billion servings from various dose-response model, considering different populations and strain virulence (where applicable)

Dose-response model/population	Virulence strain proportions	Mean risk for 1 billion servings
FAO & WHO (2004), Susceptible population[a]	Not considered	3.93
FAO & WHO (2004), All populations[b]	Not considered	0.76
EFSA (2018), All populations	Not considered	13.78
New DR model from this report		
All populations	100% Less virulent (LV)	3.76
All populations	100% Virulent (V)	29.64
All populations	100% More virulent (MV)	87.46
All populations, European mixture of strains[c]	51.4% LV. 36.2% V. 12.4%MV	23.52
All populations, European mixture of strains without more virulent strains[d]	58.7% LV. 41.3%. 0% MV	14.46

[a] As used in this report to estimate the risk of listeriosis from various foods (see Section 5).
[b] All populations: marginal dose-response model over the various considered populations, weighted according to the proportion of each subpopulation as reported in FAO & WHO, 2004 or Møller Nielsen *et al.,* 2017.
[c] As reported in Moller Nielsen *et al.,* 2017.
[d] LV and V relative proportions similar to the one observed by Moller Nielsen *et al.,* 2017.

6.3 CONCLUSIONS

A new DR model has been developed, in response to a recommendation from the Part 1 expert meeting, to incorporate class of strain virulence and age-sex, as a surrogate for host susceptibility. This new model allows for better risk estimation but requires two additional pieces of information. Firstly, the number of exposed consumers in each age-sex subpopulation is needed to allow overall estimation of the number of illnesses in the total population of interest. Secondly, the

proportionality of the classes of strain virulence in the specific food of interest is needed.

Unfortunately, the current model still lacks specific information related to host susceptibility, as determined by underlying health conditions and by the potential interaction with strain virulence. Such information would result in a DR model that is more specific and more globally relevant. However, the expert group did not have data to support development of such a model at this stage.

7

Microbiological sampling and testing of ready-to-eat foods for *L. monocytogenes*

To inform decisions on potential updates of the Codex guidelines (CAC/GL 61-2007) the effect of sampling and microbiological criteria (MC) on risk was evaluated and discussed. Sampling food products and testing them for microbial contamination are the practices that have long been used by the food industry and regulatory agencies. The term "Microbiological Criteria" refers to the combination of the various aspects involved in these practices (FAO & WHO, 2013; FAO & WHO, 2016; ICMSF, 2002). It should be noted that the application of MC is generally accepted to be only a part of a comprehensive food control system, and MC can be applied to verify that other control measures are effective. The Codex Alimentarius Commission (CAC) points out the following in CAC/GL 21-1997 (FAO & WHO, 2013):

> *The microbiological safety of foods is managed by the effective implementation of control measures that have been validated, where appropriate, throughout the food chain to minimise contamination and improve food safety. This preventative approach offers more advantages than sole reliance on microbiological testing through acceptance sampling of individual lots of the final product to be placed on the market. However, the establishment of microbiological criteria may be appropriate for verifying that food safety control systems are implemented correctly.*

With respect to *L. monocytogenes*, CAC provides guidance in relation to sampling and testing of ready-to-eat (RTE) products. The suggested MC depend on whether the growth of *L. monocytogenes* can or cannot occur in the food product, as

determined by scientifically justified factors, such as pH and water activity. Table 6 provides the key parameters used for defining the sampling plans that are part of the MC for *L. monocytogenes* in RTE foods (FAO & WHO, 2013). These sampling plans are two-class plans (FAO & WHO, 2016) and can be applied at the end of manufacture or at port of entry (for imported products) through to the point of sale (FAO & WHO, 2013).

TABLE 6 Sampling plans for *L. monocytogenes* in RTE foods; based on Table A2.1 and A2.2 in Annex 2 of CAC/GL 21-1997

L. MONOCYTOGENES GROWTH CAN OCCUR IN FOOD PRODUCT?	SAMPLE SIZE (N)	ACCEPTANCE NUMBER (C)	MICROBIOLOGICAL LIMIT (M)
Yes	5	0	Absence in 25 g (i.e. < 0.04 CFU/g)
No	5	0	100 CFU/g

These plans were developed under the assumption that the distribution of *L. monocytogenes* in the food follows a $\log_{10}$ normal distribution with standard deviation of 0.25 $\log_{10}$ CFU/g. However, no information is provided in relation to the selection of this standard deviation, which might imply a very homogenous food product, such as a very well mixed liquid (van Schothorst *et al.*, 2009). The same authors also noted that a higher standard deviation of 0.8 $\log_{10}$ CFU/g may be more representative of a heterogeneous food product, such as solid foods.

The effects of different testing scenarios on reducing risk per serving, as quantified by calculating the relative risk, were evaluated using the reference scenario for RTE cold-smoked fish as described in Section 5 and Annex 3 (Table A3.1). This "test reference scenario" is an example of practices that resulted in a higher contamination of product compared with a corresponding scenario which would be based on good practices.

The expert group discussed evaluating different MCs in relation to strains of different virulence. However, it was ultimately decided not to include this factor in the assessment. Since all strains of *L. monocytogenes* have the potential to cause illness, it was agreed that the same corrective action should be applied upon detection of any *L. monocytogenes* in RTE food. No evaluation was conducted in relation to environmental testing.

The model enables the testing of *L. monocytogenes* in food unit samples taken randomly from each lot, according to a two-class sampling plan. In the two-class plan, *n* samples are randomly extracted and analysed per lot. For each sample,

a subsample of *g* grams is used in the enrichment essay and the lot is rejected if more than *c* samples are positive in detection (Gonzales-Barron *et al.*, 2024c). It is assumed that the contamination is homogeneous in each food unit and that the sensitivity and the specificity of the bacterial method are one. If a lot is rejected, it is replaced with a lot of the same production that tests negative.

7.1 THE EFFECTS OF TESTING USING CAC/GL 61

To evaluate the effects of applying different sampling plans for *L. monocytogenes* in the quantitative risk assessment, each lot was first evaluated for the risk per serving posed in the absence of testing. Subsequently, a specific sampling plan was applied to each lot, and the risk per serving was then recalculated, excluding those lots where *L. monocytogenes* was detected and thus removed from the simulation. The effect of a testing plan can then be quantified as the relative risk or percent reduction in risk by comparing the risk per serving with testing implemented versus that without testing.

Specifically, to evaluate the effect of product testing as described in CAC/GL 61, the sampling plan designated as "N5" ($n = 5$, $c = 0$, and m = absence in 25 g or 0.04 CFU/g) was applied to each lot. Smoked fish was used in the simulation as a representative RTE food, presenting a situation favourable for the growth of *L. monocytogenes*. In the simulation, the selected "test reference scenario" (without testing) is indicative of practices" that lead to higher levels of contamination compared to good practice scenarios. Thus, this evaluation serves as an illustration of the better outcomes achievable through testing, by employing higher contamination levels than the "best" situations where CAC/GL 61 guidelines are well implemented and effective.

The simulation results show that in this high contamination scenario, using the FAO/WHO 2004 dose-response for the increased susceptibility population and "no testing" as a reference, testing each lot for *L. monocytogenes* using N5 results in an RR of 0.68, i.e. the risk per serving was reduced by 32% across the susceptible population. However, it must be noted that this reduction relates to a very low absolute risk per serving. That is, the estimated risks per serving for no testing and N5 were 3.93×10^{-9} and 2.68×10^{-9}, respectively, which equate to one illness in approximately 250 000 000 and 370 000 000 servings, respectively.

The simulations used a lot size of 1 000 packages and, in this case, N5 testing results in the removal of about 8% of lots from commerce as a result of testing.

7.2 EFFECTS OF INCREASING THE NUMBER OF SAMPLES TESTED

To evaluate the effect of increasing the number of samples tested, sampling plans put forward by the International Commission on Microbiological Specification for Foods (ICMSF) were considered (ICMSF, 2018). The ICMSF suggests sample sizes according to hazard severity and whether the handling and consumption conditions, after the point of sampling, are: a) likely to decrease, b) not affected or c) likely to increase the level of concern related to the hazard (Chapter 8 in ICMSF, 2018). With respect to hazard severity, the ICMSF classify *L. monocytogenes* for the:

- general population as "II. Serious hazard; incapacitating but not life-threatening; sequelae infrequent; moderate duration"; and
- susceptible population as "III.B. Severe hazard for vulnerable populations, life-threatening or substantial chronic sequelae or long duration".

Two-class sampling plans are suggested by the ICMSF for the two populations, and these are listed in depending on the change in degree of concern after the point of sampling. For RTE smoked fish the growth of *L. monocytogenes* is possible and thus the degree of concern is likely to increase after the point of sampling. Ultimately, the risk manager needs to decide which stringency of sampling is appropriate, taking into account other considerations, such as costs, likely compliance, industry acceptance, and so on.

TABLE 7 Suggested sampling plans for the two populations, based on severity of *L. monocytogenes* in these populations, and conditions in which the food is usually expected to be handled and consumed after sampling. With n: sample size, c: acceptance number

POPULATION	REDUCED DEGREE OF CONCERN	NO CHANGE IN CONCERN	INCREASED DEGREE OF CONCERN
General	$n = 5, c = 0$	$n = 10, c = 0$	$n = 20, c = 0$
Susceptible	$n = 15, c = 0$	$n = 30, c = 0$	$n = 60, c = 0$

Source: Prepared by the authors based on the content in ICMSF, 2018.

Since any sampling plan can be considered as part of potential control measures, the various sampling plans detailed in Table 7 were evaluated. This was done in the context of the European population (as above). The relative risk applying the sampling plans detailed in Table 7 were quantified in relation to N5 as the reference scenario (rather than the no sampling and testing case), to evaluate the effect of "more sampling". The results are shown in Table 8.

TABLE 8 Summary of the evaluation of different two-class sampling plans (all with c = 0 and m = 0.04, i.e. absence in 25g)

Sample size n	5	10	15	20	30	60
Absolute risk	2.68×10^{-9}	2.07×10^{-9}	1.74×10^{-9}	1.53×10^{-9}	1.30×10^{-9}	1.00×10^{-10}
Relative risk* (%)	100 (ref)	77.3	64.8	56.9	48.5	37.5
Discarded lots	8%	13%	17%	19%	23%	30%

* Compared with $n = 5$

7.3 CONCLUSIONS

Class of strain virulence in the context of sampling and testing was considered but was not further pursued as any detection of *L. monocytogenes* in the food should result in the same corrective action, irrespective of the class of strain virulence, especially since consumers are unlikely to be guaranteed to be limited to a particular (sub-)population. In addition, the use of MC should be focused on verifying that other risk management measures are working as intended rather than for ensuring safety.

The results from the simulations used to evaluate different sampling and testing plans show large effects in terms of reducing risk, as shown by the relative risk per serving. However, these reductions are relative to small absolute risks posed by the RTE smoked fish scenario evaluated. However, at the same time the increase in sample size results in both additional costs from sampling and testing and considerable rejections of lots. Whether the benefits of such increases in sample size outweigh the collective costs remains for the risk manager to consider. No specific scenarios have been evaluated in relation to environmental testing.

8

Conclusions

RISK ASSESSMENT MODELS

The structure and functions in the risk assessment models evaluated in the Part 2 expert meeting remained consistent with the processes and the steps recommended during the Part 1 expert meeting. Regarding the model evaluation and implications, the following observations are indicated.

- The functions and parameters of the models as provided are adaptable for evaluating model performance and evaluating various scenarios.
- The outputs generated by the models were consistent with expert experiences. It was concluded that the risk assessment models were considered useful and fit-for-purpose based on the evaluation process during the meeting.
- Suggestions for improvement include enhancing the dose-response model by integrating additional factors such as underlying health conditions.
- There is a need for comprehensive data on *L. monocytogenes* in the food chain to better inform the characterization of occurrence, virulence, and dose-response for different virulence classes, facilitating the conduct of class-specific risk assessments.
- It is recommended that these models remain accessible as open-source tools.
- Conclusions drawn from the risk assessment models are contingent upon the simulated conditions, data and practices discussed during the meeting. Their applicability and implications depend on specific production conditions and practices.

APPLICATION OF MODELS TO SPECIFIC CASES

Diced RTE cantaloupe

- The model considered a full production-to-consumption chain representing preharvest, harvest and storage, cleaning and washing, processing, cold-chain storage, and consumer handling practices.
- Control measures in primary production such as the use of fit-for-purpose water and irrigation systems avoiding water contact with the edible part of the crop was shown to reduce the risk.
- Inadequate management of wash water and poor environmental hygiene during processing were shown to increase the risk.
- Climate change was identified as a risk factor affecting various stages along the production-to-consumption continuum, by assuming an increase of the prevalence of *L. monocytogenes* in soil, an increase of the quantity of soil transferred to produce, decreasing agricultural water quality, and increasing storage temperature.

Frozen vegetables

- The model addressed various stages from preconditioned vegetables, through processing (blanching and packaging), to consumer handling (defrosting and cooking).
- The model results highlight the importance of blanching in mitigating risk. However, the potential of post-blanching contamination coupled with the possibility of *L. monocytogenes* growth, and poor environmental hygiene management pose increasing risks.
- If non-RTE frozen vegetables are consumed without adequate cooking, improper defrosting practices increase the risk.

Cold-smoked RTE fish

- The model considered a full production-to-consumption chain including primary and secondary processing, cold-chain storage, and consumer handling.
- Consideration of the presence of the naturally occurring LAB in the predictive growth model resulted in a reduction in estimated risk.
- Elevated levels of *L. monocytogenes* on incoming fish, poor environmental hygiene practices during filleting and slicing, and higher concentrations of *L. monocytogenes* in brine solutions were identified as risk factors increasing the risk.

- Addition of lactic acid and diacetate or LAB culture were shown to lower the risk by inhibiting the growth of *L. monocytogenes*.
- The potential effect of climate change, evaluated by assuming an increase in the initial levels of *L. monocytogenes* on the raw fish and in the temperature during the shelf-life of the product led to an increase of risk.

Dose-response (DR) model

- An updated DR model was developed to incorporate both strain virulence classes and age-sex groups, serving as a surrogate for population susceptibility. This modification led to a greater relative risk between the most extreme DR curves, in comparison to other age-sex based DR models.
- However, to enhance the DR model's specificity and global relevance, further refinement is needed to incorporate data on susceptibility as determined by any underlying health conditions and considering potential interactions with defined classes of strain virulence.

Testing

- While end-product sampling and microbiological testing alone had limited risk reduction effects, they hold value in verifying the effectiveness of other control measures, as described in MRA24 (FAO & WHO, 2016).

9

Considerations and recommendations for the revision of guidelines related to control of *L. monocytogenes*

9.1 CONSIDERATIONS

The models developed in response to the Part 1 expert meeting and evaluated in this report are considered useful and fit-for-purpose. Thus, the functions and parameters of the models as provided were considered valid for evaluating the different scenarios developed to inform recommendations on revision of the *Guidelines on the application of general principles of food hygiene to the control of Listeria monocytogenes in foods* (CAC/GL 61-2007) (FAO & WHO, 2007). It is important to note that the modelling results are contingent upon assumptions and contextual factors, necessitating conclusions that are sensitive to the conditions, data and practices simulated and evaluated within the scenarios. Consequently, the practical applicability and implication of results will ultimately depend on specific conditions and individual production practices. In view of this, the availability of these models as open-source tools, implemented with user-friendly interfaces, stands as an important outcome of this work.

9.2 RECOMMENDATIONS

Based on the scenarios, and the data and practices simulated, evaluated and discussed during the meeting, the following recommendations were made.

General

- The classification of foods into distinct categories, such as supportive/non-supportive of *L. monocytogenes* growth or as RTE/non-RTE, should be approached with caution due to potential variations in consumer practices diverging from intended use.
- The potential effects of climate change, such as due to increased temperatures and contamination, should be assessed by food business operators (FBO) and effective control measures should be implemented if needed.

Primary production

- Implementing control measures for *L. monocytogenes* at the primary production can reduce the risk.

Processing

- The impact on the predicted risk of contamination during processing highlights the need for effective management of environmental hygiene practices.
- End-product sampling, environmental sampling, and microbiological testing serve as vital tools for verifying the effectiveness of implemented control measures.

Product information and consumer awareness

- The impact of non-intended use of RTE food highlights the need for improved food labelling about intended preparation and use.
- FBO should employ clear messaging across various platforms, including websites and social media, to communicate intended food usage to consumers.
- Consumer education on safe food preparation, storage practices, and intended use should be enhanced.

10

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Annexes

Annex 1

RTE diced cantaloupe risk assessment model

TABLE A1.1 Parameters of the risk assessment model of *L. monocytogenes* in RTE diced cantaloupe that were changed in the "what-if" scenarios

OBJECTIVE	SCENARIO	PRIMARY PRODUCTION	WASHING OF CANTALOUPES	DICING OF CANTALOUPE	DISPLAY AT RETAIL	HOME STORAGE
Reference	Reference	$P_{foil} = 1$ $Pi_{irrig} = 0$ $P_{manure} = 0$	$prob_{CCW} = 0$	N = 9 CFU	$Temp_{max} = 10.3$ °C	$Temp_{mode} = 6.64$ °C $Temp_{max} = 11.1$ °C
Greater contamination during primary production due to absence of protective soil barrier (mulch) and/or contaminated irrigation water	Scenario 1a	$P_{foil} = 0$	$prob_{CCW} = 0$	N = 9 CFU	$Temp_{max} = 10.3$ °C	$Temp_{mode} = 6.64$ °C $Temp_{max} = 11.1$ °C
	Scenario 1b	$P_{foil} = 0$ $P_{irrig} = 0.08$ $cIrrig_{LogMin} = -1.52$ log_{10} CFU/L $cIrrig_{LogMax} = 1.04$ log_{10} CFU/L	NA	NA	NA	NA
	Scenario 1c	$P_{foil} = 0$ $P_{irrig} = 0.63$ $cIrrig_{LogMin} = -1.52$ log_{10} CFU/L $cIrrig_{LogMax} = 4.75$ log_{10} CFU/L	NA	NA	NA	NA
	Scenario 1d	$P_{irrig} = 0.63$ $cIrrig_{LogMin} = -1.52$ log_{10} CFU/L $cIrrig_{LogMax} = 4.75$ log_{10} CFU/L	NA	NA	NA	NA
Contamination during washing in flume tank	Scenario 2	NA	$prob_{CCW} = 0.5$	NA	NA	NA

OBJECTIVE	SCENARIO	PRIMARY PRODUCTION	WASHING OF CANTALOUPES	DICING OF CANTALOUPE	DISPLAY AT RETAIL	HOME STORAGE
Higher contamination during dicing	Scenario 3	NA	NA	N = 3 000 CFU	NA	NA
Greater contamination during primary production and slightly higher maximum storage temperatures	Scenario 4	$P_{manure} = 1$ $P_{foil} = 0$ $P_{irrig} = 0.63$ $clrrig_{LogMin} = -1.52$ CFU/L $clrrig_{LogMax} = 4.75$ CFU/L	NA	NA	$Temp_{max} = 10.8$ °C	$Temp_{max} = 11.6$ °C
Higher maximum retail temperature and higher home storage temperature	Scenario 5	NA	NA	NA	$Temp_{max} = 10.8$ °C	$Temp_{mode} = 8.64$ °C $Temp_{max} = 13.1$ °C
Greater contamination during primary production, processing, and higher storage temperatures	Scenario 6	$P_{manure} = 1$ $P_{foil} = 0$ $P_{irrig} = 0.63$ $clrrig_{LogMin} = -1.52$ CFU/L $clrrig_{LogMax} = 4.75$ CFU/L	$prob_{CCW} = 0.5$	N = 3 000 CFU	$Temp_{max} = 10.8$ °C	$Temp_{mode} = 8.64$ °C $Temp_{max} = 13.1$ °C

Note: $clrrig_{LogMax}$: maximum value of the uniform distribution representing the contamination of irrigation water; $clrrig_{LogMin}$: minimum value of the uniform distribution representing the contamination of irrigation water; N: numbers of *L. monocytogenes* on the surface of the dicing machine ready to be transferred; P_{irrig}: prevalence of contamination in irrigation water; P_{foil}: proportion of fields grown in foil (e.g. plastic mulch); P_{manure}: proportion of fields using organic amendments; $prob_{CCW}$: probability that water of flume tank is contaminated; $Temp_{max}$: maximum value of the temperature of storage; $Temp_{mode}$: mode value of the temperature of storage.

TABLE A1.2 Simulation results for the reference and "what-if" scenarios from the exposure assessment model of *L. monocytogenes* in RTE diced cantaloupe

SCENARIO	END OF PROCESSING				POINT OF CONSUMPTION			
	COUNTS (CFU/G) IN CONTAMINATED LOTS (MEAN, MEDIAN, 95% CI)	PREVALENCE OF CONTAMINATED PACKS	P(N>10 CFU/G IN A PACK)	P(N>100 CFU/G IN A PACK)	COUNTS (CFU/G) IN CONTAMINATED SERVINGS (MEAN, MEDIAN, 95% CI)	PREVALENCE OF CONTAMINATED SERVINGS	P(N>10 CFU/G IN A SERVING)	P(N>100 CFU/G IN A SERVING)
Reference	3.81×10^{-6}; 2.11×10^{-6} [2.11×10^{-6}–1.26×10^{-5}]	1.42×10^{-7}	0	0	0.9974; 0.0850 [0.0250–3.9286]	1.42×10^{-7}	1.63×10^{-9}	1.88×10^{-10}
Scenario 1a	1.74×10^{-5}; 4.21×10^{-6} [2.11×10^{-6}–0.0002]	6.05×10^{-5}	0	0	1.2581; 0.0850 [0.0250–3.9450]	6.05×10^{-5}	6.36×10^{-7}	6.80×10^{-8}
Scenario 1b	1.67×10^{-5}; 4.21×10^{-6} [2.11×10^{-6}–0.0001]	6.71×10^{-5}	0	0	0.9522; 0.0850 [0.0250–4.0741]	6.71×10^{-5}	7.56×10^{-7}	7.23×10^{-8}
Scenario 1c	0.0001; 1.89×10^{-5} [2.11×10^{-6}–0.0008]	0.0029	0	0	1.0911; 0.0850 [0.0300–4.1350]	0.0029	3.32×10^{-5}	3.21×10^{-6}
Scenario 1d	0.0001; 1.47×10^{-5} [2.11×10^{-6}–0.0008]	0.0023	0	0	1.0517; 0.0850 [0.0300–4.2950]	0.0023	2.70×10^{-5}	2.65×10^{-6}
Scenario 2	1.36×10^{-5}; 2.11×10^{-6} [2.11×10^{-6}–0.0001]	2.37×10^{-5}	0	0	1.1858; 0.0850 [0.0250–3.8950]	2.37×10^{-5}	2.56×10^{-7}	2.59×10^{-8}
Scenario 3	0.0005; 0.0002 [2.11×10^{-5}–0.0024]	3.78×10^{-5}	0	0	1.0979; 0.0900 [0.0300–4.3550]	3.78×10^{-5}	4.54×10^{-7}	4.54×10^{-8}
Scenario 4	0.0001; 2.95×10^{-5} [2.11×10^{-6}–0.0008]	0.0057	0	0	1.5577; 0.0900 [0.0300–5.1400]	0.0057	8.34×10^{-5}	1.02×10^{-5}
Scenario 5	3.81×10^{-6}; 2.11×10^{-6} [2.11×10^{-6}–1.26×10^{-5}]	1.42×10^{-7}	0	0	42.83; 0.1650 [0.0300–55.95]	1.42×10^{-7}	8.95×10^{-9}	2.51×10^{-9}
Scenario 6	0.0003; 0.0001 [2.11×10^{-6}–0.0017]	0.0188	0	0	54.85; 0.1750 [0.0300–49.46]	0.0188	0.0012	0.0003

TABLE A1.3 Statistics of the risk of listeriosis for a population with increased susceptibility using FAO & WHO's dose-response (2004b) per serving of RTE diced cantaloupe for the reference and "what-if" scenarios and relative risks

SCENARIO	MEAN	MEDIAN	2.5 PCT	97.5 PCT	RELATIVE RISK
Reference	3.007×10^{-17}	2.666×10^{-18}	4.998×10^{-19}	1.444×10^{-16}	-
Scenario 1a	1.616×10^{-14}	4.698×10^{-16}	4.778×10^{-17}	9.308×10^{-14}	537.4
Scenario 1b	1.354×10^{-14}	5.753×10^{-16}	5.479×10^{-17}	9.904×10^{-14}	450.3
Scenario 1c	6.704×10^{-13}	3.544×10^{-14}	3.751×10^{-16}	4.464×10^{-12}	22 294.6
Scenario 1d	5.087×10^{-13}	2.204×10^{-14}	2.253×10^{-16}	3.566×10^{-12}	16 917.2
Scenario 2	5.956×10^{-15}	1.738×10^{-16}	2.425×10^{-17}	3.200×10^{-14}	198.1
Scenario 3	8.801×10^{-15}	2.631×10^{-15}	8.478×10^{-17}	5.497×10^{-14}	292.7
Scenario 4	1.896×10^{-12}	1.270×10^{-13}	9.171×10^{-16}	1.327×10^{-11}	63 052.9
Scenario 5	1.291×10^{-15}	6.415×10^{-18}	5.000×10^{-19}	2.368×10^{-15}	42.9
Scenario 6	2.192×10^{-10}	9.131×10^{-12}	3.731×10^{-15}	1.204×10^{-9}	7 289 657.5

Annex 2

Frozen vegetables risk assessment model

TABLE A2.1 Parameters of the risk assessment model of *L. monocytogenes* in non-RTE frozen vegetables that were changed in the "what-if" scenarios.

OBJECTIVE	SCENARIO	BLANCHING	CONTAMINATION FROM ENVIRONMENT AND PACKAGING	DEFROSTING	COOKING
Reference	Reference	Time = 0.75 min Temperature= 83 °C	n_{Equip} = 4.5 x 10^3 CFU	$p_{Defrost}$ = 0	p_{Cook} = 1 min_{Cook} = 1 log_{10} $mode_{Cook}$ = 5 log_{10} max_{Cook} = 9 log_{10}
No blanching	Scenario 1	Time = 0 min	NA	NA	NA
Higher contamination during processing	Scenario 2	NA	n_{Equip} = 1.5 x 10^6CFU	NA	NA
No blanching, high contamination, longer defrosting time and no cooking	Scenario 3	Time = 0 min	n_{Equip} = 1.5 x 10^6CFU	$p_{Defrost}$ = 1 Time = 72 h Temperature = 8 °C	p_{Cook} = 0
Higher contamination, longer defrosting time and no cooking	Scenario 4	NA	n_{Equip} = 1.5 x 10^6CFU	$p_{Defrost}$ = 1 Time = 72 h Temperature = 8 °C	p_{Cook} = 0
No blanching, higher contamination, longer defrosting time and no cooking	Scenario 5	Time = 0 min	n_{Equip} = 1.5 x 10^6CFU	$p_{Defrost}$ = 1 Time = 24 h Temperature = 8 °C	p_{Cook} = 0
Higher contamination, no cooking	Scenario 6	NA	n_{Equip} = 1.5 x 10^6CFU	NA	p_{Cook} = 0

OBJECTIVE	SCENARIO	BLANCHING	CONTAMINATION FROM ENVIRONMENT AND PACKAGING	DEFROSTING	COOKING
No blanching, higher contamination, longer defrosting time and fast cooking	Scenario 7	Time = 0 min	n_{Equip} = 1.5 x 10^6CFU	$p_{Defrost}$ = 1 Time = 24 h Temperature = 8 °C	min_{Cook} = 0 log_{10} $mode_{Cook}$ = 1 log_{10} max_{Cook} = 2 log_{10}
Higher contamination during processing, defrosting on countertop and no cooking	Scenario 8	NA	n_{Equip} = 1.5 x 10^6CFU	$p_{Defrost}$ = 1 Time = 4 h Temperature = 20 °C	p_{Cook} = 0
No blanching, higher contamination, defrosting in the fridge and no cooking	Scenario 9	Time = 0 min	n_{Equip} = 1.5 x 10^6CFU	$p_{Defrost}$ = 1 Time = 24 h Temperature = 6 °C	p_{Cook} = 0
No blanching, higher contamination, defrosting on countertop and no cooking	Scenario 10	Time = 0 min	n_{Equip} = 1.5 x 10^6CFU	$p_{Defrost}$ = 1 Time = 4 h Temperature = 20 °C	p_{Cook} = 0
Higher contamination during processing, defrosting in the fridge and no cooking	Scenario 11	NA	n_{Equip} = 1.5 x 10^6CFU	$p_{Defrost}$ = 1 Time = 24 h Temperature = 6 °C	p_{Cook} = 0
Defrosting in the fridge and no cooking	Scenario 12	NA	NA	$p_{Defrost}$ = 1 Time = 24 h Temperature = 6 °C	p_{Cook} = 0
Defrosting on countertop and no cooking	Scenario 13	NA	NA	$p_{Defrost}$ = 1 Time = 4 h Temperature = 20 °C	p_{Cook} = 0
Defrosting in the fridge	Scenario 14	NA	NA	$p_{Defrost}$ = 1 Time = 24 h Temperature = 6 °C	NA

OBJECTIVE	SCENARIO	BLANCHING	CONTAMINATION FROM ENVIRONMENT AND PACKAGING	DEFROSTING	COOKING
Defrosting on countertop	Scenario 15	NA	NA	$p_{Defrost} = 1$ Time = 4 h Temperature = 20 °C	NA
No blanching, higher contamination during processing, defrosting on countertop	Scenario 16	Time = 0 min	$n_{Equip} = 1.5 \times 10^6$ CFU	$p_{Defrost} = 1$ Time = 4 h Temperature = 20 °C	NA
No blanching, higher contamination during processing, defrosting on the fridge, fast cooking	Scenario 17	Time = 0 min	$n_{Equip} = 1.5 \times 10^6$ CFU	$p_{Defrost} = 1$ Time = 24 h Temperature = 6 °C	$min_{Cook} = 0$ $\log_{10}$ $mode_{Cook} = 1$ $\log_{10}$ $max_{Cook} = 2$ $\log_{10}$
No blanching, higher contamination during processing, higher time of defrosting, slow reduction	Scenario 18	Time = 0 min	$n_{Equip} = 1.5 \times 10^6$ CFU	$p_{Defrost} = 1$ Temperature = 24 h Temperature = 8 °C	$min_{Cook} = 0$ $\log_{10}$
Higher time of defrosting (1 day), no cooking	Scenario 19	NA	NA	$p_{Defrost} = 1$ Time = 24 h Temperature = 8 °C	$p_{Cook} = 0$
Higher time of defrosting (3 days), no cooking	Scenario 20	NA	NA	$p_{Defrost} = 1$ Time = 72 h Temperature = 8 °C	$p_{Cook} = 0$
Defrosting on the countertop and fast cooking	Scenario 21	NA	NA	$p_{Defrost} = 1$ Time = 4 h Temperature = 20 °C	$min_{Cook} = 0$ $\log_{10}$ $mode_{Cook} = 1$ $\log_{10}$ $max_{Cook} = 2$ $\log_{10}$
Defrosting on the fridge and fast cooking	Scenario 22	NA	NA	$p_{Defrost} = 1$ Time = 24 h Temperature = 6 °C	$min_{Cook} = 0$ $\log_{10}$ $mode_{Cook} = 1$ $\log_{10}$ $max_{Cook} = 2$ $\log_{10}$
Higher time of defrosting (1 day) and fast cooking	Scenario 23	NA	NA	$p_{Defrost} = 1$ Time = 24 h Temperature = 8 °C	$min_{Cook} = 0$ $\log_{10}$ $mode_{Cook} = 1$ $\log_{10}$ $max_{Cook} = 2$ $\log_{10}$

OBJECTIVE	SCENARIO	BLANCHING	CONTAMINATION FROM ENVIRONMENT AND PACKAGING	DEFROSTING	COOKING
Higher time of defrosting (3 days) and fast cooking	Scenario 24	NA	NA	$p_{Defrost} = 1$ Time = 72 h Temperature = 8 °C	$min_{Cook} = 0\ log_{10}$ $mode_{Cook} = 1\ log_{10}$ $max_{Cook} = 2\ log_{10}$
Higher time of defrosting (1 day) and slow decrease	Scenario 25	NA	NA	$p_{Defrost} = 1$ Time = 24 h Temperature = 8 °C	$min_{Cook} = 0\ log_{10}$
Higher time of defrosting (3 days) and slow decrease	Scenario 26	NA	NA	$p_{Defrost} = 1$ Time = 72 h Temperature = 8 °C	$min_{Cook} = 0\ log_{10}$
Fast cooking	Scenario 27	NA	NA	NA	$mode_{Cook} = 2\ log_{10}$ $max_{Cook} = 3\ log_{10}$
No cooking	Scenario 28	NA	NA	NA	$p_{Cook} = 0$
Stringent blanching and slow decrease	Scenario 29	Time = 2 min Temperature= 92 °C	NA	NA	$min_{Cook} = 0\ log_{10}$
Stringent blanching, higher contamination, defrosting at the countertop and no cooking	Scenario 30	Time = 2 min Temperature= 92 °C	$n_{Equip} = 1.5 \times 10^6$ CFU	$p_{Defrost} = 1$ Time = 4 h Temperature = 20 °C	$p_{Cook} = 0$

Note: max_{Cook}: maximum value of the triangular distribution representing the variability in the reduction of *L. monocytogenes* in the event of cooking; min_{Cook}: minimum value of the triangular distribution representing the variability in the reduction of *L. monocytogenes* in the event of cooking ; $mode_{Cook}$: mode value of the triangular distribution representing the variability in the reduction of *L. monocytogenes* in the event of cooking; n_{Equip}: numbers of *L. monocytogenes* cells on the surface of conveyors, freezer or packaging machine in contact with the product; p_{Cook}: probability of cooking the defrosted or frozen vegetables for consumption; $p_{Defrost}$: probability of defrosting.

TABLE A2.2 Simulation results for the reference and "what-if" scenarios from the exposure assessment model of *L. monocytogenes* in non-RTE frozen vegetables

SCENARIO	END OF PROCESSING				POINT OF CONSUMPTION			
	COUNTS (CFU/G) IN CONTAMINATED LOTS (MEAN, MEDIAN, 95% CI)	PREVALENCE OF CONTAMI-NATED PACKS	P(N>10 CFU/G IN A PACK)	P(N>100 CFU/G IN A PACK)	COUNTS (CFU/G) IN CONTAMINATED SERVINGS (MEAN, MEDIAN, 95% CI)	PREVALENCE OF CON-TAMINATED SERVINGS	P(N>10 CFU/G IN A SERVING)	P(N>100 CFU/G IN A SERVING)
Reference	3.38×10^{-3}; 1.59×10^{-3} [1.60×10^{-5};1.70×10^{-2}]	4.32×10^{-1}	0	0	3.43×10^{-6} ; 1.74×10^{-6} [1.38×10^{-8} ; 1.64×10^{-5}]	1.72×10^{-4}	0	0
Scenario 1	2.67; 1.22 [4.46×10^{-3}; $1.35 \times 10^{+1}$]	9.58×10^{-1}	6.33×10^{-2}	2.07×10^{-4}	9.27×10^{-4}; 6.05×10^{-4} [1.70×10^{-6}; 2.42×10^{-3}]	3.66×10^{-2}	7.44×10^{-6}	0
Scenario 2	5.73×10^{-2} ; 2.76×10^{-3} [1.60×10^{-5} ; 4.45×10^{-1}]	5.44×10^{-1}	0	0	4.30×10^{-5}; 3.12×10^{-6} [1.38×10^{-8}; 2.95×10^{-4}]	2.13×10^{-3}	0	0
Scenario 3	2.72; 1.27 [1.62×10^{-2}; $1.37 \times 10^{+1}$]	9.67×10^{-1}	6.43×10^{-2}	2.10×10^{-4}	$4.94 \times 10^{+5}$; $1.79 \times 10^{+1}$ [7.18×10^{-2}; $1.29 \times 10^{+5}$]	8.68×10^{-1}	6.25×10^{-1}	3.33×10^{-1}
Scenario 4	5.73×10^{-2}; 2.76×10^{-3} [1.60×10^{-5}; 4.45×10^{-1}]	5.44×10^{-1}	0	0	$8.33 \times 10^{+4}$; 1.26×10^{-1} [2.02×10^{-4} ; $1.41 \times 10^{+3}$]	2.62×10^{-1}	3.31×10^{-1}	1.43×10^{-1}
Scenario 5	2.72; 1.27 [1.62×10^{-2}; $1.37 \times 10^{+1}$]	9.67×10^{-1}	6.43×10^{-2}	2.10×10^{-4}	$1.18 \times 10^{+2}$; 1.55 [1.68×10^{-2}; $1.54 \times 10^{+2}$]	8.68×10^{-1}	2.54×10^{-1}	4.31×10^{-2}
Scenario 6	5.73×10^{-2}; 2.76×10^{-3} [1.60×10^{-5}; 4.45×10^{-1}]	5.44×10^{-1}	0	0	5.12×10^{-2}; 2.54×10^{-3} [1.20×10^{-5}; 4.81×10^{-1}]	2.62×10^{-1}	3.38×10^{-5}	0
Scenario 7	2.72; 1.27 [1.62×10^{-2}; $1.37 \times 10^{+1}$]	9.67×10^{-1}	6.43×10^{-2}	2.10×10^{-4}	$2.34 \times 10^{+1}$; 1.31×10^{-1} [3.44×10^{-3}; $2.18 \times 10^{+1}$]	7.56×10^{-1}	6.37×10^{-2}	8.13×10^{-3}
Scenario 8	5.73×10^{-2}; 2.76×10^{-3} [1.60×10^{-5}; 4.45×10^{-1}]	5.44×10^{-1}	0	0	8.79×10^{-1}; 8.33×10^{-3} [3.20×10^{-5}; 2.33]	2.62×10^{-1}	2.01×10^{-2}	2.27×10^{-3}
Scenario 9	2.72; 1.27 [1.62×10^{-2}; $1.37 \times 10^{+1}$]	9.67×10^{-1}	6.43×10^{-2}	2.10×10^{-4}	9.67; 9.19×10^{-1} [1.10×10^{-2} ; $6.01 \times 10^{+1}$]	8.68×10^{-1}	1.72×10^{-1}	1.48×10^{-2}

SCENARIO	END OF PROCESSING				POINT OF CONSUMPTION			
	COUNTS (CFU/G) IN CONTAMINATED LOTS (MEAN, MEDIAN, 95% CI)	PREVALENCE OF CONTAMINATED PACKS	P(N>10 CFU/G IN A PACK)	P(N>100 CFU/G IN A PACK)	COUNTS (CFU/G) IN CONTAMINATED SERVINGS (MEAN, MEDIAN, 95% CI)	PREVALENCE OF CONTAMINATED SERVINGS	P(N>10 CFU/G IN A SERVING)	P(N>100 CFU/G IN A SERVING)
Scenario 10	2.72; 1.27 [1.62 x 10^{-2}; 1.37 x 10^{+1}]	9.67 x 10^{-1}	6.43 x 10^{-2}	2.10 x 10^{-4}	4.57 x 10^{+1}; 1.33 [1.54 x 10^{-2}; 1.15 x 10^{+2}]	8.68 x 10^{-1}	2.30 x 10^{-1}	3.32 x 10^{-2}
Scenario 11	5.73 x 10^{-2}; 2.76 x 10^{-3} [1.60 x 10^{-5}; 4.45 x 10^{-1}]	5.44 x 10^{-1}	0	0	1.88 x 10^{-1}; 5.65 x 10^{-3} [2.21 x 10^{-5}; 1.33]	2.62 x 10^{-1}	6.44 x 10^{-3}	3.47 x 10^{-4}
Scenario 12	3.38 x 10^{-3}; 1.59 x 10^{-3} [1.60 x 10^{-5}; 1.70 x 10^{-2}]	4.32 x 10^{-1}	0	0	1.13 x 10^{-2}; 3.12 x 10^{-3} [2.21 x 10^{-5}; 5.54 x 10^{-2}]	1.03 x 10^{-1}	4.80 x 10^{-4}	7.44 x 10^{-6}
Scenario 13	3.38 x 10^{-3}; 1.59 x 10^{-3} [1.60 x 10^{-5}; 1.70 x 10^{-2}]	4.32 x 10^{-1}	0	0	5.54 x 10^{-2}; 4.49 x 10^{-3} [3.20 x 10^{-5}; 1.21 x 10^{-1}]	1.03 x 10^{-1}	2.98 x 10^{-3}	8.25 x 10^{-4}
Scenario 14	3.38 x 10^{-3}; 1.59 x 10^{-3} [1.60 x 10^{-5}; 1.70 x 10^{-2}]	4.32 x 10^{-1}	0	0	1.03 x 10^{-5}; 5.46 x 10^{-6} [4.44 x 10^{-8}; 4.72 x 10^{-5}]	5.14 x 10^{-4}	0	0
Scenario 15	3.38 x 10^{-3}; 1.59 x 10^{-3} [1.60 x 10^{-5}; 1.70 x 10^{-2}]	4.32 x 10^{-1}	0	0	1.84 x 10^{-5}; 9.61 x 10^{-6} [7.90 x 10^{-8}; 7.78 x 10^{-5}]	8.74 x 10^{-4}	7.46 x 10^{-6}	0
Scenario 16	2.72; 1.27 [1.62 x 10^{-2}; 1.37 x 10^{+1}]	9.67 x 10^{-1}	6.43 x 10^{-2}	2.10 x 10^{-4}	1.65 x 10^{-2}; 1.35 x 10^{-3} [5.63 x 10^{-5}; 8.35 x 10^{-3}]	7.30 x 10^{-2}	8.29 x 10^{-4}	1.71 x 10^{-4}
Scenario 17	2.72 ; 1.27 [1.62 x 10^{-2}; 1.37 x 10^{+1}]	9.67 x 10^{-1}	6.43 x 10^{-2}	2.10 x 10^{-4}	1.50; 7.34 x 10^{-2} [2.26 x 10^{-3}; 8.71]	7.20 x 10^{-1}	3.04 x 10^{-2}	1.57 x 10^{-3}
Scenario 18	2.72; 1.27 [1.62 x 10^{-2}; 1.37 x 10^{+1}]	9.67 x 10^{-1}	6.43 x 10^{-2}	2.10 x 10^{-4}	4.74 x 10^{-1}; 2.66 x 10^{-3} [2.18 x 10^{-4}; 8.28 x 10^{-2}]	1.28 x 10^{-1}	4.97 x 10^{-3}	8.43 x 10^{-4}
Scenario 19	3.38 x 10^{-3}; 1.59 x 10^{-3} [1.60 x 10^{-5}; 1.70 x 10^{-2}]	4.32 x 10^{-1}	0	0	1.46 x 10^{-1} ; 5.17 x 10^{-3} [3.31 x 10^{-5}; 1.71 x 10^{-1}]	1.03 x 10^{-1}	5.08 x 10^{-3}	1.53 x 10^{-3}
Scenario 20	3.38 x 10^{-3}; 1.59 x 10^{-3} [1.60 x 10^{-5}; 1.70 x 10^{-2}]	4.32 x 10^{-1}	0	0	2.40 x 10^{+4}; 4.85 x 10^{-2} [2.01 x 10^{-4}; 2.65 x 10^{+2}]	1.03 x 10^{-1}	2.04 x 10^{-1}	8.79 x 10^{-2}
Scenario 21	3.38 x 10^{-3}; 1.59 x 10^{-3} [1.60 x 10^{-5}; 1.70 x 10^{-2}]	4.32 x 10^{-1}	0	0	5.32 x 10^{-3}; 6.40 x 10^{-4} [5.02 x 10^{-6}; 9.82 x 10^{-3}]	4.49 x 10^{-2}	7.17 x 10^{-4}	1.39 x 10^{-4}

SCENARIO	END OF PROCESSING				POINT OF CONSUMPTION			
	COUNTS (CFU/G) IN CONTAMINATED LOTS (MEAN, MEDIAN, 95% CI)	PREVALENCE OF CONTAMI-NATED PACKS	P(N>10 CFU/G IN A PACK)	P(N>100 CFU/G IN A PACK)	COUNTS (CFU/G) IN CONTAMINATED SERVINGS (MEAN, MEDIAN, 95% CI)	PREVALENCE OF CON-TAMINATED SERVINGS	P(N>10 CFU/G IN A SERVING)	P(N>100 CFU/G IN A SERVING)
Scenario 22	3.38×10^{-3}; 1.59×10^{-3} [1.60×10^{-5}; 1.70×10^{-2}]	4.32×10^{-1}	0	0	1.20×10^{-3}; 4.77×10^{-4} [3.77×10^{-6}; 5.00×10^{-3}]	3.65×10^{-2}	8.16×10^{-5}	0
Scenario 23	3.38×10^{-3}; 1.59×10^{-3} [1.60×10^{-5}; 1.70×10^{-2}]	4.32×10^{-1}	0	0	1.45×10^{-2}; 7.28×10^{-4} [5.52×10^{-6}; 1.38×10^{-2}]	4.82×10^{-2}	1.09×10^{-3}	6.05×10^{-4}
Scenario 24	3.38×10^{-3}; 1.59×10^{-3} [1.60×10^{-5}; 1.70×10^{-2}]	4.32×10^{-1}	0	0	$3.40 \times 10^{+3}$; 4.72×10^{-3} [2.49×10^{-5}; $2.76 \times 10^{+1}$]	8.48×10^{-2}	9.65×10^{-2}	4.19×10^{-2}
Scenario 25	3.38×10^{-3}; 1.59×10^{-3} [1.60×10^{-5}; 1.70×10^{-2}]	4.32×10^{-1}	0	0	1.51×10^{-4}; 4.16×10^{-5} [3.46×10^{-7}; 3.04×10^{-4}]	3.48×10^{-3}	2.22×10^{-4}	5.38×10^{-5}
Scenario 26	3.38×10^{-3}; 1.59×10^{-3} [1.60×10^{-5}; 1.70×10^{-2}]	4.32×10^{-1}	0	0	$5.43 \times 10^{+1}$; 2.12×10^{-4} [1.76×10^{-6}; 1.78×10^{-2}]	1.49×10^{-2}	1.55×10^{-2}	7.78×10^{-3}
Scenario 27	3.38×10^{-3}; 1.59×10^{-3} [1.60×10^{-5}; 1.70×10^{-2}]	4.32×10^{-1}	0	0	4.57×10^{-5}; 2.31×10^{-5} [1.84×10^{-7}; 2.17×10^{-4}]	2.28×10^{-3}	0	0
Scenario 28	3.38×10^{-3}; 1.59×10^{-3} [1.60×10^{-5}; 1.70×10^{-2}]	4.32×10^{-1}	0	0	3.04×10^{-3}; 1.40×10^{-3} [1.20×10^{-5}; 1.57×10^{-2}]	1.03×10^{-1}	0	0
Scenario 29	6.65×10^{-4}; 5.73×10^{-4} [1.20×10^{-4}; 1.67×10^{-3}]	5.79×10^{-2}	0	0	1.31×10^{-6}; 1.14×10^{-6} [2.40×10^{-7}; 3.18×10^{-6}]	6.55×10^{-5}	0	0
Scenario 30	2.22×10^{-1}; 1.91×10^{-1} [3.86×10^{-2}; 5.55×10^{-1}]	2.46×10^{-1}	0	0	9.02×10^{-1}; 6.06×10^{-2} [5.97×10^{-3}; 2.08]	1.94×10^{-1}	2.66×10^{-2}	2.89×10^{-3}

TABLE A2.3 Statistics of the risk of listeriosis for a population with increased susceptibility using FAO & WHO's dose-response (2004b) per serving of non-RTE frozen vegetables for the reference and "what-if" scenarios and relative risks

SCENARIO	MEAN	MEDIAN	2.5 PCT	97.5 PCT	RELATIVE RISK
Reference	1.82×10^{-16}	9.20×10^{-17}	7.33×10^{-19}	8.67×10^{-16}	-
Scenario 1	4.91×10^{-14}	3.32×10^{-14}	8.99×10^{-17}	1.98×10^{-13}	269.8
Scenario 2	2.28×10^{-15}	1.64×10^{-16}	7.31×10^{-19}	1.54×10^{-14}	12.5
Scenario 3	2.61×10^{-5}	2.61×10^{-5}	3.41×10^{-6}	4.99×10^{-5}	143 406 593 406.6
Scenario 4	4.41×10^{-6}	1.37×10^{-6}	6.95×10^{-9}	1.88×10^{-5}	24 230 769 230.8
Scenario 5	6.26×10^{-9}	2.33×10^{-9}	2.51×10^{-11}	3.77×10^{-8}	34 395 604.4
Scenario 6	2.71×10^{-12}	1.47×10^{-13}	6.36×10^{-16}	2.10×10^{-11}	14 890.1
Scenario 7	1.24×10^{-9}	3.96×10^{-10}	2.54×10^{-12}	8.05×10^{-9}	6 813 186.8
Scenario 8	4.66×10^{-11}	2.57×10^{-12}	1.16×10^{-14}	3.68×10^{-10}	256 044.0
Scenario 9	5.13×10^{-10}	2.27×10^{-10}	1.86×10^{-12}	2.68×10^{-9}	281 8681.3
Scenario 10	2.42×10^{-9}	9.63×10^{-10}	1.06×10^{-11}	1.27×10^{-8}	13 296 703.3
Scenario 11	9.98×10^{-12}	5.37×10^{-13}	2.36×10^{-15}	7.64×10^{-11}	54 835.2
Scenario 12	5.98×10^{-13}	2.98×10^{-13}	2.37×10^{-15}	2.83×10^{-12}	3 285.7
Scenario 13	2.94×10^{-12}	1.45×10^{-12}	1.16×10^{-14}	1.44×10^{-11}	16 153.8
Scenario 14	5.48×10^{-16}	2.90×10^{-16}	2.36×10^{-18}	2.51×10^{-15}	3.0
Scenario 15	9.78×10^{-16}	5.29×10^{-16}	4.34×10^{-18}	4.40×10^{-15}	5.4
Scenario 16	8.76×10^{-13}	1.81×10^{-13}	3.09×10^{-15}	6.15×10^{-12}	4 813.2
Scenario 17	7.97×10^{-11}	3.21×10^{-11}	1.98×10^{-13}	4.35×10^{-10}	437 912.1
Scenario 18	2.51×10^{-11}	3.56×10^{-12}	2.63×10^{-14}	1.86×10^{-10}	137 912.1

SCENARIO	MEAN	MEDIAN	2.5 PCT	97.5 PCT	RELATIVE RISK
Scenario 19	7.74×10^{-12}	3.81×10^{-12}	3.07×10^{-14}	3.79×10^{-11}	42 527.5
Scenario 20	1.27×10^{-6}	7.98×10^{-7}	7.06×10^{-9}	4.93×10^{-6}	6 978 021 978.0
Scenario 21	2.82×10^{-13}	1.31×10^{-13}	1.05×10^{-15}	1.40×10^{-12}	1 549.5
Scenario 22	6.34×10^{-14}	3.27×10^{-14}	2.64×10^{-16}	2.94×10^{-13}	348.4
Scenario 23	7.70×10^{-13}	3.46×10^{-13}	2.79×10^{-15}	4.05×10^{-12}	4 230.8
Scenario 24	1.80×10^{-7}	1.11×10^{-7}	9.64×10^{-10}	7.15×10^{-7}	989 010 989.0
Scenario 25	8.01×10^{-15}	4.06×10^{-15}	3.31×10^{-17}	3.90×10^{-14}	44.0
Scenario 26	2.87×10^{-9}	1.76×10^{-9}	1.51×10^{-11}	1.15×10^{-8}	15 769 230.8
Scenario 27	2.42×10^{-15}	1.22×10^{-15}	9.76×10^{-18}	1.14×10^{-14}	13.3
Scenario 28	1.61×10^{-13}	8.03×10^{-14}	6.37×10^{-16}	7.52×10^{-13}	884.6
Scenario 29	6.95×10^{-17}	6.02×10^{-17}	1.27×10^{-17}	1.68×10^{-16}	0.4
Scenario 30	4.78×10^{-11}	3.71×10^{-11}	6.33×10^{-12}	1.53×10^{-10}	262 637.4

Annex 3

RTE fish risk assessment model

TABLE A3.1 Parameters of the risk assessment model of *L. monocytogenes* in RTE smoked fish that were changed in the "what-if" scenarios

OBJECTIVE	SCENARIO	INITIAL COUNTS IN FISH	FILLETING OF FISH	BRINING OR SALTING	SLICING	COLD CHAIN	HOME STORAGE
Reference	Reference	Poisson assumption	InitSlicer=10^3 CFU	$Cbrine_{mode}$ = 0.0145 CFU/ml $Cbrine_{max}$ = 0.06 CFU/ml	InitSlicer = 0 CFU	$Temp_{mode}$ = 4.6 °C $Temp_{max}$ = 7 °C DA_{min} = 0 ppm DA_{mode} = 0 ppm DA_{max} = 0 ppm LA_{min} = 0 ppm LA_{mode} = 0 ppm LA_{max} = 0 ppm N_0LAB_{min} = 1.5 log_{10} CFU/g N_0LAB_{mode} = 2.8 log_{10} CFU/g N_0LAB_{max} = 4.1 log_{10} CFU/g	$Temp_{mode}$ = 7 °C $Temp_{max}$ = 12.8 °C $Time_{max}$ = 35 x 24 h
Increasing contamination in slicing machine	Scenario 1	NA	NA	NA	InitSlicer = 10^2 CFU	NA	NA
	Scenario 2	NA	NA	NA	InitSlicer = 10^4 CFU	NA	NA
	Scenario 3	NA	NA	NA	InitSlicer = 10^6 CFU	NA	NA
Increasing contamination in brining solution	Scenario 4	NA	NA	10-fold increase: $Cbrine_{mode}$ = 0.145 CFU/ml $Cbrine_{max}$ = 0.6 CFU/ml	NA	NA	NA
	Scenario 5	NA	NA	100-fold increase: $Cbrine_{mode}$ = 1.45 CFU/ml $Cbrine_{max}$ = 6 CFU/ml	NA	NA	NA

OBJECTIVE	SCENARIO	INITIAL COUNTS IN FISH	FILLETING OF FISH	BRINING OR SALTING	SLICING	COLD CHAIN	HOME STORAGE
	Scenario 6	NA	NA	1000-fold increase: $Cbrine_{mode}$ = 14.5 CFU/ml $Cbrine_{max}$ = 60 CFU/ml	NA	NA	NA
Impact of initial counts as normal distribution	Scenario 7	C0 ~ N (0,1) log_{10} CFU/g	NA	NA	NA	NA	NA
	Scenario 8	C0 ~ N (1,1) log_{10} CFU/g	NA	NA	NA	NA	NA
Higher temperature during cold chain	Scenario 9	NA	NA	NA	NA	$Temp_{mode}$ = 5.6 °C $Temp_{max}$ = 10 °C	NA
	Scenario 9b	NA	NA	NA	NA	$Temp_{mode}$ = 4.6 °C $Temp_{max}$ = 10 °C	NA
	Scenario 9b (50 000 ite)[b]	NA	NA	NA	NA	$Temp_{mode}$ = 4.6 °C $Temp_{max}$ = 10 °C	NA
Higher home storage temperature	Scenario 10	NA	NA	NA	NA	NA	$Temp_{mode}$ = 8 °C $Temp_{max}$ = 14 °C
Lower home storage time	Scenario 11	NA	NA	NA	NA	NA	$Time_{max}$ = 28 x 24 h
Added lactic acid bacteria	Scenario 12	NA	NA	NA	NA	4-log addition of N_0LAB_{min} and N_0LAB_{mode}: N_0LAB_{min} = 5.5 log_{10} CFU/g N_0LAB_{mode} = 6.8 log_{10} CFU/g N_0LAB_{max} = 8.0 log_{10} CFU/g	NA
	Scenario 13	NA	NA	NA	NA	5-log addition of N_0LAB_{min} and N_0LAB_{mode}: N_0LAB_{min} = 6.5 log_{10} CFU/g N_0LAB_{mode} = 7.8 log_{10} CFU/g N_0LAB_{max} = 8.0 log_{10} CFU/g	NA

OBJECTIVE	SCENARIO	INITIAL COUNTS IN FISH	FILLETING OF FISH	BRINING OR SALTING	SLICING	COLD CHAIN	HOME STORAGE
Use of preservatives	Scenario 14	NA	NA	NA	NA	DA_{min} = 500 ppm DA_{mode} = 1 500 ppm DA_{max} = 1 900 ppm LA_{min} = 6 000 ppm LA_{mode} = 12 000 ppm LA_{max} = 28 000 ppm	NA
Higher initial counts and higher storage temperatures	Scenario 15	C0 ~ N (0,1) log_{10} CFU/g	NA	NA	NA	$Temp_{mode}$ = 5.6 °C $Temp_{max}$ = 10 °C	$Temp_{mode}$ = 8 °C $Temp_{max}$ = 14 °C
Effect of preservatives on Scenario 15	Scenario 16	C0 ~ N (0,1) log_{10} CFU/g	NA	NA	NA	$Temp_{mode}$ = 5.6 °C $Temp_{max}$ = 10 °C DA_{min} = 500 ppm DA_{mode} = 1 500 ppm DA_{max} = 1 900 ppm LA_{min} = 6 000 ppm LA_{mode} = 12 000 ppm LA_{max} = 28 000 ppm	$Temp_{mode}$ = 8 °C $Temp_{max}$ = 14 °C
Effect of added lactic acid bacteria on Scenario 15	Scenario 17	C0 ~ N (0,1) log_{10} CFU/g	NA	NA	NA	$Temp_{mode}$ = 5.6 °C $Temp_{max}$ = 10 °C N_0LAB_{min} = 6.5 log_{10} CFU/g N_0LAB_{mode} = 7.8 log_{10} CFU/g N_0LAB_{max} = 8.0 log_{10} CFU/g	$Temp_{mode}$ = 8 °C $Temp_{max}$ = 14 °C
Very low initial lactic acid bacteria (negligible Jameson effect)	Scenario 18	NA	NA	NA	NA	N_0LAB_{min} = -3.0 log_{10} CFU/g N_0LAB_{mode} =-2.5 log_{10} CFU/g N_0LAB_{max} = -2.0 log_{10} CFU/g	NA
	Scenario 18, (50 000 ite)[b]	NA	NA	NA	NA	N_0LAB_{min} = -3.0 log_{10} CFU/g N_0LAB_{mode}=-2.5 log_{10} CFU/g N_0LAB_{max} = -2.0 log_{10} CFU/g	NA

OBJECTIVE	SCENARIO	INITIAL COUNTS IN FISH	FILLETING OF FISH	BRINING OR SALTING	SLICING	COLD CHAIN	HOME STORAGE
No contamination from filleting but from slicing machines	Scenario 19	NA	InitSlicer = 0 CFU		InitSlicer = 10^3 CFU	NA	NA
High environmental contamination	Scenario 20	NA	InitSlicer = 10^4 CFU	$Cbrine_{mode}$ = 1.45 CFU/ml $Cbrine_{max}$ = 6 CFU/ml	InitSlicer = 10^4 CFU	NA	NA
High environmental contamination in RTE seafood with added lactic acid bacteria	Scenario 21	NA	InitSlicer = 10^4 CFU	$Cbrine_{mode}$ = 1.45 CFU/ml $Cbrine_{max}$ = 6 CFU/ml	InitSlicer = 10^4 CFU	N_0LAB_{min} = 6.5 $\log_{10}$ CFU/g N_0LAB_{mode} = 7.8 $\log_{10}$ CFU/g N_0LAB_{max} = 8.0 $\log_{10}$ CFU/g	NA
High environmental contamination in RTE seafood with added preservatives	Scenario 22	NA	InitSlicer = 10^4 CFU	$Cbrine_{mode}$ = 1.45 CFU/ml $Cbrine_{max}$ = 6 CFU/ml	InitSlicer=10^4 CFU	DA_{min} = 500 ppm DA_{mode} = 1 500 ppm DA_{max} = 1 900 ppm LA_{min} = 6 000 ppm LA_{mode} = 12 000 ppm LA_{max} = 28 000 ppm	NA
Very high environmental contamination	Scenario 23	NA	InitSlicer = 10^6 CFU	$Cbrine_{mode}$ = 14.5 CFU/ml $Cbrine_{max}$ = 60 CFU/ml	InitSlicer=10^6 CFU	NA	NA
Very high environmental contamination in RTE seafood with added lactic acid bacteria	Scenario 24	NA	InitSlicer = 10^6 CFU	$Cbrine_{mode}$ = 14.5 CFU/ml $Cbrine_{max}$ = 60 CFU/ml	InitSlicer=10^6 CFU	N_0LAB_{min} = 6.5 $\log_{10}$ CFU/g N_0LAB_{mode} = 7.8 $\log_{10}$ CFU/g N_0LAB_{max} = 8.0 $\log_{10}$ CFU/g	NA

OBJECTIVE	SCENARIO	INITIAL COUNTS IN FISH	FILLETING OF FISH	BRINING OR SALTING	SLICING	COLD CHAIN	HOME STORAGE
Very high environmental contamination in RTE seafood with added preservatives	Scenario 25	NA	InitSlicer = 10^6 CFU	$Cbrine_{mode}$ = 14.5 CFU/ml $Cbrine_{max}$ = 60 CFU/ml	InitSlicer=10^6 CFU	DA_{min} = 500 ppm DA_{mode} = 1 500 ppm DA_{max} = 1 900 ppm LA_{min} = 6 000 ppm LA_{mode} = 12 000 ppm LA_{max} = 28 000 ppm	NA

Note: C0: microbial concentration of contaminated units; Cbrinemax: maximum concentration of *L. monocytogenes* in contaminated brine solution; Cbrinemode: mode value of the concentration of *L. monocytogenes* in contaminated brine solution; DAmax: maximal value of diacetate concentration; DAmin: minimum value of diacetate concentration; DAmode: mode value of diacetate concentration; InitSlicer: initial contamination of the slicer; LAmax: maximum value for lactic acid concentration; LAmin: minimal value for lactic acid concentration; LAmode: mode value for lactic acid concentration; N0LABmax: maximum value number of LAB; N0LABmin: minimal value number of LAB; N0LABmode: mode value number of LAB; Tempmax: maximum value of storage temperature; Tempmode: mode value of storage temperature; Timemax: maximum value of storage time.

[b]50 000 ite: setting indicates that 50 000 lots were generated, as opposed to the default of 1 000.

TABLE A3.2 Simulation results for the reference and "what-if" scenarios from the exposure assessment model of *L. monocytogenes* in RTE smoked fish

SCENARIO	END OF PROCESSING				TIME OF CONSUMPTION			
	COUNTS (CFU/G) IN CONTAMINATED LOTS (MEAN, MEDIAN, 95% CI)	PREVALENCE OF CONTAMINATED PACKS	P(N>10 CFU/G IN A PACK)	P(N>100 CFU/G IN A PACK)	COUNTS (CFU/G) IN CONTAMINATED SERVINGS (MEAN, MEDIAN, 95% CI)	PREVALENCE OF CONTAMINATED SERVINGS	P(N>10 CFU/G IN A SERVING)	P(N>100 CFU/G IN A SERVING)
Reference	0.0022; 0.0009 [3.46 x 10^{-5}–0.0123]	0.0842	0	0	1213; 0.0615 [0.0140–93.40]	0.0459	0.0106	0.0036
Scenario 1	0.0026; 0.0013 [0.0004–0.0128]	0.0862	0	0	1096; 0.0615 [0.0143–89.93]	0.0472	0.0108	0.0036
Scenario 2	0.0399; 0.0390 [0.034 –0.0504]	0.0891	0.0010	0	1111; 0.0769 [0.0144–112.9]	0.0504	0.0134	0.0044
Scenario 3	3.7764; 3.8328 [3.2985–3.8513]	0.0918	0.0077	0.0039	2391; 0.1077 [0.0147–812.9]	0.0538	0.0209	0.0100
Scenario 4	0.0023; 0.0009 [0.0004–0.0128]	0.0862	0	0	1298; 0.0615 [0.0139–99.49]	0.0471	0.0111	0.0038
Scenario 5	0.0031; 0.0010 [4.23 x 10^{-5}–0.0194]	0.0905	0	0	1245; 0.0655 [0.0131–103.7]	0.0508	0.0123	0.0042
Scenario 6	0.0118; 0.0011 [4.23 x 10^{-5}–0.0968]	0.0965	0	0	1140; 0.0923 [0.0130–154.1]	0.0565	0.0151	0.0056
Scenario 7	0.5501; 0.0196 [0.0001–3.096]	0.1115	0.0051	0.0008	4023; 0.1762 [0.0150–752.3]	0.0755	0.0307	0.0133
Scenario 8	4.6861; 0.1922 [0.0006–32.08]	0.1706	0.0202	0.0050	8741; 0.2923 [0.0152–2378]	0.1258	0.0656	0.0313
Scenario 9	0.0022; 0.0009 [3.46 x 10^{-5}–0.0123]	0.0842	0	0	2028; 0.0923 [0.0147–170.2]	0.0497	0.0152	0.0054
Scenario 9b	0.0022; 0.0009 [3.46 x 10^{-5}–0.0123]	0.0842	0	0	2376; 0.0769 [0.0146–144.0]	0.0473	0.0130	0.0047

SCENARIO	END OF PROCESSING				TIME OF CONSUMPTION			
	COUNTS (CFU/G) IN CONTAMINATED LOTS (MEAN, MEDIAN, 95% CI)	PREVALENCE OF CONTAMINATED PACKS	P(N>10 CFU/G IN A PACK)	P(N>100 CFU/G IN A PACK)	COUNTS (CFU/G) IN CONTAMINATED SERVINGS (MEAN, MEDIAN, 95% CI)	PREVALENCE OF CONTAMINATED SERVINGS	P(N>10 CFU/G IN A SERVING)	P(N>100 CFU/G IN A SERVING)
Scenario 9b (50 000ite)C	0.0023; 0.0009 [3.46 x 10^{-5}–0.0127]	0.0847	0	0	1647; 0.0769 [0.0145–134.5]	0.0481	0.0131	0.0046
Scenario 10	0.0022; 0.0009 [3.46 x 10^{-5}–0.0123]	0.0842	0	0	4166; 0.0923 [0.0147–212.9]	0.0492	0.0155	0.0059
Scenario 11	0.0022; 0.0009 [3.46 x 10^{-5}–0.0123]	0.0842	0	0	297.8; 0.0462 [0.0141–37.54]	0.0431	0.0069	0.0020
Scenario 12	0.0022; 0.0009 [3.46 x 10^{-5}–0.0123]	0.0842	0	0	0.2328; 0.0308 [0.0134–1.4308]	0.0383	0.0005	1.8 x 10-5
Scenario 13	0.0022; 0.0009 [3.46 x 10^{-5}–0.0123]	0.0842	0	0	0.1300; 0.0308 [0.0130–0.8000]	0.0359	0.0002	3.34 x 10-6
Scenario 14	0.0022; 0.0009 [3.46 x 10^{-5}–0.0123]	0.0842	0	0	0.9043; 0.0154 [0.0118–0.2769]	0.0243	0.0002	2.00 x 10-5
Scenario 15	0.5501; 0.0196 [0.0001–3.0961]	0.1115	0.0051	0.0008	13384; 0.3692 [0.0153–3442]	0.0820	0.0474	0.0235
Scenario 16	0.5501; 0.0196 [0.0001–3.0961]	0.1115	0.0051	0.0008	115.47; 0.0462 [0.0141–23.43]	0.0572	0.0078	0.0019
Scenario 17	0.5501; 0.0196 [0.0001–3.0961]	0.1115	0.0051	0.0008	41.87; 0.0769 [0.0146–43.29]	0.0688	0.0128	0.0035
Scenario 18	0.0022; 0.0009 [3.46 x 10^{-5}–0.0123]	0.0842	0	0	8999; 0.0615 [0.0144–623.4]	0.0460	0.0127	0.0059
Scenario 18 (50 000ite)	0.0022; 0.0009 [3.46 x 10^{-5}–0.0127]	0.0847	0	0	8982; 0.0708 [0.0144–660.6]	0.0466	0.0132	0.0061

SCENARIO	END OF PROCESSING				TIME OF CONSUMPTION			
	COUNTS (CFU/G) IN CONTAMINATED LOTS (MEAN, MEDIAN, 95% CI)	PREVALENCE OF CONTAMINATED PACKS	P(N>10 CFU/G IN A PACK)	P(N>100 CFU/G IN A PACK)	COUNTS (CFU/G) IN CONTAMINATED SERVINGS (MEAN, MEDIAN, 95% CI)	PREVALENCE OF CONTAMINATED SERVINGS	P(N>10 CFU/G IN A SERVING)	P(N>100 CFU/G IN A SERVING)
Scenario 19	0.0054; 0.0043 [0.0036–0.0136]	0.0773	0	0	1269; 0.0766 [0.0152–88.30]	0.0420	0.0096	0.0032
Scenario 20	0.0465; 0.0421 [0.0360–0.0801]	0.1162	0.0010	0	1185; 0.0923 [0.0124–159.4]	0.0722	0.0208	0.0075
Scenario 21	0.0465; 0.0421 [0.0360–0.0801]	0.1162	0.0010	0	0.9470; 0.0414 [0.0100–3.6920]	0.0600	0.0027	0.0003
Scenario 22	0.0465; 0.0421 [0.0360–0.0801]	0.1162	0.0010	0	0.9486; 0.0305 [0.0084–2.1692]	0.0466	0.0015	0.0001
Scenario 23	4.4015; 4.1128 [3.5496–6.8070]	0.2067	0.0208	0.0043	4370; 0.3538 [0.0139–1841]	0.1551	0.0832	0.0387
Scenario 24	4.4015; 4.1128 [3.5496–6.8070]	0.2067	0.0208	0.0043	35.38; 0.1519 [0.0123–88.91]	0.1421	0.0406	0.0114
Scenario 25	4.4015; 4.1128 [3.5496–6.8070]	0.2067	0.0208	0.0043	20.07; 0.0923 [0.0108–34.70]	0.1247	0.0235	0.0056

TABLE A3.3 Statistics of the risk of listeriosis for a population with increased susceptibility using FAO & WHO's dose-response (2004b) per serving of RTE smoked fish for the reference and "what-if" scenarios and relative risks

SCENARIO	MEAN	MEDIAN	2.5 PCT	97.5 PCT	RELATIVE RISK
Reference	3.929×10^{-9}	1.039×10^{-12}	1.013×10^{-15}	2.763×10^{-9}	-
Scenario 1	3.668×10^{-9}	1.080×10^{-12}	4.770×10^{-15}	2.686×10^{-9}	0.93
Scenario 2	3.986×10^{-9}	3.007×10^{-12}	1.663×10^{-13}	3.646×10^{-9}	1.01
Scenario 3	9.174×10^{-9}	1.190×10^{-10}	1.152×10^{-11}	4.870×10^{-8}	2.33
Scenario 4	4.343×10^{-9}	1.103×10^{-12}	1.060×10^{-15}	2.900×10^{-9}	1.11
Scenario 5	4.550×10^{-9}	1.256×10^{-12}	1.416×10^{-15}	4.104×10^{-9}	1.16
Scenario 6	4.694×10^{-9}	1.512×10^{-12}	1.427×10^{-15}	7.168×10^{-9}	1.19
Scenario 7	2.140×10^{-8}	1.658×10^{-11}	6.079×10^{-15}	1.172×10^{-7}	5.45
Scenario 8	7.742×10^{-8}	1.426×10^{-10}	2.611×10^{-14}	6.445×10^{-7}	19.70
Scenario 9	7.133×10^{-9}	1.474×10^{-12}	1.060×10^{-15}	6.724×10^{-9}	1.82
Scenario 9b	7.960×10^{-9}	1.192×10^{-12}	1.037×10^{-15}	4.725×10^{-9}	2.03
Scenario 9b (50 000 ite)	5.599×10^{-9}	1.268×10^{-12}	1.060×10^{-15}	4.337×10^{-9}	1.43
Scenario 10	1.448×10^{-8}	2.391×10^{-12}	1.531×10^{-15}	4.953×10^{-8}	3.69
Scenario 11	9.075×10^{-10}	3.806×10^{-13}	5.948×10^{-16}	4.974×10^{-10}	0.23
Scenario 12	6.303×10^{-13}	4.076×10^{-14}	4.581×10^{-16}	4.398×10^{-12}	0.00
Scenario 13	3.292×10^{-13}	3.286×10^{-14}	3.817×10^{-16}	2.335×10^{-12}	0.00
Scenario 14	1.552×10^{-12}	1.908×10^{-14}	1.395×10^{-16}	1.210×10^{-12}	0.00

SCENARIO	MEAN	MEDIAN	2.5 PCT	97.5 PCT	RELATIVE RISK
Scenario 15	7.745×10^{-8}	5.378×10^{-11}	1.166×10^{-14}	6.212×10^{-7}	19.71
Scenario 16	4.640×10^{-10}	5.074×10^{-13}	1.055×10^{-15}	3.849×10^{-10}	0.12
Scenario 17	2.030×10^{-10}	8.541×10^{-13}	1.585×10^{-15}	4.145×10^{-10}	0.05
Scenario 18	2.923×10^{-8}	1.398×10^{-11}	1.059×10^{-15}	2.542×10^{-7}	7.44
Scenario 18 (50 000 ite)	2.961×10^{-8}	1.576×10^{-11}	1.116×10^{-15}	2.759×10^{-7}	7.54
Scenario 19	3.729×10^{-9}	8.000×10^{-13}	1.904×10^{-14}	2.124×10^{-9}	0.95
Scenario 20	6.241×10^{-9}	8.000×10^{-12}	2.451×10^{-13}	1.387×10^{-8}	1.59
Scenario 21	4.144×10^{-12}	1.062×10^{-12}	1.547×10^{-13}	2.519×10^{-11}	0.00
Scenario 22	3.226×10^{-12}	7.414×10^{-13}	1.235×10^{-13}	7.561×10^{-12}	0.00
Scenario 23	5.427×10^{-8}	6.214×10^{-10}	2.282×10^{-11}	4.456×10^{-7}	13.81
Scenario 24	3.721×10^{-10}	1.028×10^{-10}	1.487×10^{-11}	2.482×10^{-9}	0.09
Scenario 25	1.853×10^{-10}	7.105×10^{-11}	1.168×10^{-11}	5.575×10^{-10}	0.05

Annex 4

Dose-response model[7]

MODELS FROM THE LITERATURE

The dose-response models considered here link the ingested number of bacteria (as an actual number or as the mean of a Poisson distribution) with the marginal probability (over strains, over individuals of a given population) of developing an invasive case of listeriosis. Various dose-response models for *L. monocytogenes* are described in the literature and these are summarized below. Within this project, some functions were derived to implement some of those dose-response models.

The model used by FAO & WHO (2004b) is an exponential dose-response model (Haas, Rose and Gerba, 2014). In the exponential dose-response model, each ingested *L. monocytogenes* cell has a given and independent probability r (also known as the "r-value") to trigger invasive listeriosis. In this model, r is assumed to be constant within a given subpopulation. The probability of developing invasive listeriosis following the ingestion of exactly n bacteria is then

$$\text{Prob}(\text{ill} \mid r,n) = 1-(1-r)^n,$$

which is a "binomial dose-response model" as it follows a binomial process (Haas, 2002). In contrast, if the number of bacteria is expressed as the mean of a Poisson distribution of parameter d, then the dose-response, integrated over the (serving-to-serving) variability of the dose, is written as

$$\text{Prob}(\text{ill} \mid r,d) = 1 - \exp(-r \times d).$$

FAO & WHO (2004b) inferred sets of r parameters for two subpopulations. In their risk assessment of food examples, a median r value is 1.06×10^{-12} (equivalent to $10^{11.975}$) for "population with increased susceptibility" of their definition (JEMRA model, population 2 in Table A4.1), and 2.37×10^{-14} (equivalent to $10^{-13.625}$) for the "healthy population"(JEMRA model, population 1 in Table A4.1).

Pouillot *et al.* (2015) revisited this dose-response model and considered a model where r would not be considered constant but would follow a lognormal (base

[7] The information from this annex was extracted from Pouillot *et al.* 2024.

10) distribution.[8] The marginal (over strains and individuals from a given subpopulation) model can then be written:

$$\text{Prob (ill } | n,\theta) = 1 - \int_0^1 (1 - r)^n f(r,\theta)\, dr,$$

with the probability density function of r following a $\log_{10}$ normal distribution with parameter μ and σ, and n, the exact number of bacteria ingested, giving the "lognormal binomial" dose-response model. Alternatively, if the dose d is expressed as the mean of a serving-to-serving Poisson distribution, then the result is the "lognormal Poisson" dose-response model, given by:

$$\text{Prob (ill } | n,\theta) = 1 - \int_0^1 \exp(-r \times d)\, f(r,\theta) dr.$$

The parameters of the $\log_{10}$ normal distributions were estimated from Goulet *et al.*'s (2012) data of relative risk of listeriosis in 11 subpopulations in France (pregnancy, non-haematological cancer, other cancer, and so on) and exposure data estimated from the United States of America; estimates are provided in Table A4.1. Note that the classical exponential dose-response model is a special case of the lognormal Poisson model with $\sigma = 0$.

Fritsch, Guillier & Augustin (2018) proposed an updated version of the Pouillot *et al.* (2015) model, considering between strain virulence levels observed for different *L. monocytogenes* strains in mice. The median lethal doses (LD50) obtained by intraperitoneal infection route for these 26 strains (USFDA & FSIS, 2003) were first translated to r values, then clustered in 3 groups of virulence ("more virulent", "moderate virulent" and "less virulent"). Subsequently, three sets of $\log_{10}$ normal Poisson parameters were estimated to consider marginally over all populations (see Table A4.1).

Lastly, in 2018, the European Food Safety Agency (EFSA) published a report in which they derived 14 sets of $\log_{10}$ normal Poisson parameters for 14 subpopulations, the combinations of 2 sex and 7 age categories (EFSA, 2018). Those parameters were scaled to estimates of the exposure to *L. monocytogenes* from the consumption of fish products (3 subproducts, 2 different packaging types), meat products (3 subproducts, 2 different packaging types) and cheese (1 product, one packaging type) in the European Union and listeriosis cases estimated in the European Union. The resulting parameters are also provided in Table A4.1.

[8] The parametrization used here is as follows: $X \sim \log_{10}\text{Normal}(\mu, \sigma)$ if $\log_{10}(X) \sim \text{Normal}(\mu, \sigma)$.

TABLE A4.1 Models are from the literature considered in the report and the associated estimates of the mean and standard deviation of the $\log_{10}$ normal distribution of the r parameter; the classical exponential dose-response model is a special case of the lognormal Poisson model with $\sigma = 0$

MODEL	POPULATION	CHARACTERISTICS	μ	σ	COMMENT
JEMRA	1	Healthy population	-13.625	0.000	FAO&WHO (2004b, page 60)
JEMRA	2	Increased susceptibility	-11.975	0.000	
Pouillot	1	Less than 65 years old, no known underlying condition	-14.110	1.620	Pouillot *et al*. (2015, Table II)
Pouillot	2	More than 65 years old, no known underlying condition	-12.830	1.620	
Pouillot	3	Pregnancy	-11.700	1.620	
Pouillot	4	Non-haematological cancer	-12.110	1.620	
Pouillot	5	Haematological cancer	-11.020	1.620	
Pouillot	6	Renal or liver failure	-11.560	1.620	
Pouillot	7	Solid organ transplant	-11.510	1.620	
Pouillot	8	Inflammatory diseases	-12.080	1.620	
Pouillot	9	HIV/AIDS	-12.190	1.620	
Pouillot	10	Diabetes	-13.130	1.620	
Pouillot	11	Heart diseases	-13.300	1.620	
Fritsch	1	Highly virulent	-11.878	0.521	Fritsch *et al*. (2018)
Fritsch	2	Medium virulent	-13.991	0.632	
Fritsch	3	Hypovirulent	-16.707	1.121	
EFSA	1	Female 1–4 yo	-14.574	1.620	EFSA (2018, Table C.8)
EFSA	2	Male 1–4 yo	-14.467	1.620	
EFSA	3	Female 5–14 yo	-14.916	1.620	
EFSA	4	Male 5–14 yo	-15.005	1.620	
EFSA	5	Female 15–24 yo	-14.325	1.620	
EFSA	6	Male 15–24 yo	-15.036	1.620	

MODEL	POPULATION	CHARACTERISTICS	μ	σ	COMMENT
EFSA	7	Female 25–44 yo	-14.025	1.620	
EFSA	8	Male 25–44 yo	-14.764	1.620	
EFSA	9	Female 45–64 yo	-14.081	1.620	
EFSA	10	Male 45–64 yo	-14.045	1.620	
EFSA	11	Female 65–74 yo	-13.702	1.620	
EFSA	12	Male 65–74 yo	-13.560	1.620	
EFSA	13	Female >75 yo	-13.536	1.620	
EFSA	14	Male >75 yo	-13.536	1.620	

DOSE-RESPONSE MODEL DERIVED FOR THIS REPORT

The Part 1 expert group considered that the most appropriate DR model approaches would include variability in subpopulation susceptibility and variability in strain virulence and that these models should be implemented in the QRA models for the present work.

Thus, the Pouillot *et al.* (2015) model was considered, using the EFSA (2018) model of exposure, subdivided using the Fritsch *et al.* (2018) classification of strain virulence. The $\log_{10}$ normal Poisson model has two parameters, i.e. the μ and σ parameters of the normal distribution of $\log_{10}(r)$ parameter in the combination of subpopulations exposed and class of virulence that is being considered. The estimation process then involved estimating σ for each of the class of virulence, and then scaling the μ to European Union exposure and epidemiological data. For that purpose, the following information was needed:

i) a classification of *L. monocytogenes* strains as a function of their virulence;

ii) some estimates of the exposure of the 14 EFSA subpopulations to the three classes of virulence; and

iii) the corresponding number of cases.

The process is explained below and further information can be found in Pouillot *et al*. (2024).

Classification of strain virulence

Following Fritsch *et al.* (2018) and using the clinical frequency data of Maury *et al.* (2016, 2017a, 2017b) three groups of Sequence Type (ST) or Clonal Complexes

(CCs) were created (Table A4.2). For CCs/STs not present in that list, Fritsch *et al.* (2018) recommends classifying them in the "Virulent" group.

TABLE A4.2 Classification of strains in three classes of virulence

LESS VIRULENT	VIRULENT	MORE VIRULENT
CC121, CC204, CC31, CC9, CC193, CC19, ST214	CC14, CC155, CC177, CC18, CC20, CC21, CC26, CC3, CC37, CC379, C388, CC398, CC5, CC59, CC8, CC403 and all others	CC1, CC101, CC2, CC220, CC224, CC4, CC451, CC54, CC6, CC7, CC87

Proportion of various classes of virulence strain in European Union RTE and clinical cases

Using the Møller Nielsen *et al.* (2017) the comparison of isolates from different compartments along the food chain, and from humans using whole genome sequencing (WGS data collected in the European Union, the proportion of each of these virulence classes is estimated in seafood products, meat products, cheese, as well as in sporadic cases of invasive listeriosis). The results are provided Table A4.3. Note that the Others/unknown category will be considered as "Virulent".

TABLE A4.3 Proportion of the different classes of virulence in seafood, meats, cheese and dairy, and sporadic cases in the European Union

	More virulent	Virulent	Less virulent	Others/ unknown	n
RTE Seafood	12.4%	35.2%	51.4%	1.0%	290
RTE Meats	19.9%	19.9%	59.1%	1.1%	176
RTE Cheese and dairy	32.6%	47.2%	12.4%	7.9%	89
Sporadic	59.5%	29.0%	8.4%	3.1%	262

Source: Prepared by the authors based on the content in Møller Nielsen *et al.*, 2017. The comparison of isolates from different compartments along the food chain, and from humans using whole genome sequencing (WGS).

Exposure of the European Union population to various classes of virulence

To estimate the exposure of the European Union population to the various classes of virulence, the generic quantitative microbial risk assessment of EFSA (2018) was adapted. Briefly, the output of the original EFSA model was an empirical distribution function of the exposure to *L. monocytogenes* for each of the 14 subpopulations considered, from the consumption of 13 RTE foods (including heat-treated meat, smoked and gravad fish and soft and semi-soft cheeses). This model considered

the observed prevalence of *L. monocytogenes* (per food subcategory, data from the European Union-wide baseline survey), the concentration of *L. monocytogenes* at retail (based on the European Union-wide baseline survey complemented with United States of America data (Gombas *et al.*, 2003), the exponential growth rate at 5 °C (as a reference) of each food category, the storage time and the estimated consumption (per food subcategories and subpopulations). Food serving size and the number of servings per year were estimated from the European Union food consumption database. Maximum population density of *L. monocytogenes* in food of 6.23 $\log_{10}$ CFU/g for cooked meat and sausages, 7.28 $\log_{10}$ CFU/g for cheeses, 7.29 $\log_{10}$ CFU/g for fish products or 7.53 $\log_{10}$ CFU/g for pâté were used. See EFSA (2018) for additional details.

The R code (R Core Team, 2023) from EFSA was adapted to separate the estimated exposure to the various classes of strain virulence. For that purpose, the model was applied to the frequencies of the various virulence classes as observed in Table A4.4. This resulted in three empirical distribution functions for each of the 14 age-sex subpopulations.

Note that this evaluation considers, as underlying assumptions, that the initial concentration of *L. monocytogenes* in contaminated food is independent of the virulence class, and that the general behaviour, and growth in particular, of *L. monocytogenes* in all those products is also independent of the class of virulence.

Prevalence of invasive listeriosis of the European Union population according to classes of virulence

The proportion of each class of virulence observed on sporadic cases in the European Union was applied to the data from EFSA (2018 Table 1).

The assumption for this process was that the proportion of cases linked to "More Virulent", "Virulent" or "Less Virulent" strains is independent of the age, sex, European Union country, underlying conditions, and so forth. This assumption could be refined in the future if additional data regarding the cases of listeriosis per age, sex and country become available.

Estimate of the standard deviation of $\log_{10}(r)$ in a subpopulation/class of virulence

Pouillot *et al.* (2015) showed that the standard deviation of $\log_{10}(r)$ for a given subpopulation could be estimated from the interindividual variability in the susceptibility, σ_i, and the interstrain variability of virulence, σ_s. Similar to Fritsch *et al.* (2018) and EFSA (2018) the estimate of the interindividual variability in the susceptibility $\sigma_i = 0.55$ was used, as estimated by Pouillot *et al.* (2015). For the inter-strain variability of virulence, the experimental data on mice reported in Fritsch

et al. (2018), collected from USFDA & FSIS (2003), were used. From these the estimates of $\sigma_s = 0.52$, $\sigma_s = 0.63$ and $\sigma_s = 1.12$ were observed for the "More Virulent", "Virulent" and "Less Virulent" *L. monocytogenes* strains, respectively. Note that the larger standard deviation for the "Less Virulent" strains suggests greater variability in this category. These estimates can then be combined, assuming independence, e.g. for a subpopulation consuming strains of the "More Virulent" class:

$$sd = \sqrt{\sigma_i^2 + \sigma_s^2} = \sqrt{0.55^2 + 0.52^2} = 0.756.$$

The list of various standard deviation estimates for the different models is provided in Table A4.4.

Scaling of the model to epidemiological data

With these data, the mean of the $\log_{10}(r)$ population can be estimated, such that they match the model outputs with the epidemiological surveillance data for the European Union. The results are reported in Table A4.4.

TABLE A4.4 Models and their parameter estimates derived for this report

MODEL	STRAIN	POPULATION	CHARACTERISTICS	μ	σ
EFSAV	Virulent	1	Female 1–4 yo	-12,296	0,836
EFSAV	Virulent	2	Male 1–4 yo	-12,256	0,836
EFSAV	Virulent	3	Female 5–14 yo	-12,582	0,836
EFSAV	Virulent	4	Male 5–14 yo	-12,690	0,836
EFSAV	Virulent	5	Female 15–24 yo	-12,123	0,836
EFSAV	Virulent	6	Male 15–24 yo	-12,730	0,836
EFSAV	Virulent	7	Female 25–44 yo	-11,815	0,836
EFSAV	Virulent	8	Male 25–44 yo	-12,522	0,836
EFSAV	Virulent	9	Female 45–64 yo	-11,890	0,836
EFSAV	Virulent	10	Male 45–64 yo	-11,869	0,836
EFSAV	Virulent	11	Female 65–74 yo	-11,594	0,836
EFSAV	Virulent	12	Male 65–74 yo	-11,447	0,836
EFSAV	Virulent	13	Female >75 yo	-11,437	0,836
EFSAV	Virulent	14	Male >75 yo	-11,468	0,836
EFSAMV	More Virulent	1	Female 1–4 yo	-11,671	0,756

MODEL	STRAIN	POPULATION	CHARACTERISTICS	μ	σ
EFSAMV	More Virulent	2	Male 1–4 yo	-11,625	0,756
EFSAMV	More Virulent	3	Female 5–14 yo	-12,046	0,756
EFSAMV	More Virulent	4	Male 5–14 yo	-12,165	0,756
EFSAMV	More Virulent	5	Female 15–24 yo	-11,521	0,756
EFSAMV	More Virulent	6	Male 15–24 yo	-12,200	0,756
EFSAMV	More Virulent	7	Female 25–44 yo	-11,239	0,756
EFSAMV	More Virulent	8	Male 25–44 yo	-11,973	0,756
EFSAMV	More Virulent	9	Female 45–64 yo	-11,272	0,756
EFSAMV	More Virulent	10	Male 45–64 yo	-11,274	0,756
EFSAMV	More Virulent	11	Female 65–74 yo	-10,916	0,756
EFSAMV	More Virulent	12	Male 65–74 yo	-10,785	0,756
EFSAMV	More Virulent	13	Female >75 yo	-10,718	0,756
EFSAMV	More Virulent	14	Male >75 yo	-10,734	0,756
EFSALV	Less Virulent	1	Female 1–4 yo	-14,166	1,247
EFSALV	Less Virulent	2	Male 1–4 yo	-14,124	1,247
EFSALV	Less Virulent	3	Female 5–14 yo	-14,516	1,247
EFSALV	Less Virulent	4	Male 5–14 yo	-14,633	1,247
EFSALV	Less Virulent	5	Female 15–24 yo	-14,002	1,247
EFSALV	Less Virulent	6	Male 15–24 yo	-14,668	1,247
EFSALV	Less Virulent	7	Female 25–44 yo	-13,708	1,247
EFSALV	Less Virulent	8	Male 25–44 yo	-14,444	1,247
EFSALV	Less Virulent	9	Female 45–64 yo	-13,755	1,247
EFSALV	Less Virulent	10	Male 45–64 yo	-13,753	1,247
EFSALV	Less Virulent	11	Female 65–74 yo	-13,418	1,247
EFSALV	Less Virulent	12	Male 65–74 yo	-13,283	1,247
EFSALV	Less Virulent	13	Female >75 yo	-13,234	1,247
EFSALV	Less Virulent	14	Male >75 yo	-13,255	1,247

Annex 5

Supplementary material

R PACKAGES REPOSITORY

R packages were developed as part of this report that provides functions to apply to the quantitative risk assessment and dose-response models. All the data and code used to make those inferences are available on open source repositories:

- Quantitative risk assessment: https://github.com/WorldHealthOrganization/qraLM
- Dose-response model: https://github.com/WorldHealthOrganization/doseresponsemodels
- Risk assessment tool: https://github.com/WorldHealthOrganization/Shiny_qraLm

PARAMETERS

The parameters used in the assessment described in this report can be found at the following links:

- Cantaloupe: https://github.com/WorldHealthOrganization/Shiny_qraLm/blob/main/data/Cantaloupe_parametersbaseline.xlsx
- Frozen vegetables: https://github.com/WorldHealthOrganization/Shiny_qraLm/blob/main/data/FV_parametersbaseline.xlsx
- Ready-to-eat (RTE) fish: https://github.com/WorldHealthOrganization/Shiny_qraLm/blob/main/data/RTEFish_parametersbaseline.xlsx

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Microbiological Risk Assessment Series

The 52nd Session of the Codex Committee on Food Hygiene (CCFH) requested the Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) to undertake full production-to-consumption risk assessments of *Listeria monocytogenes* in foods to inform a possible revision of the *Guidelines on the application of general principles of food hygiene to the control of Listeria monocytogenes in foods*.

In response to this request, JEMRA convened a series of meetings, to prepare and develop risk assessments for *Listeria monocytogenes* in various foods. Several risk assessment models were developed and evaluated to characterize the risk of listeriosis due to the consumption of diced ready-to-eat cantaloupe, frozen vegetables, and cold-smoked ready-to-eat fish. Additionally, an updated dose–response model for *L. monocytogenes* was developed.

This report describes the output of this expert meeting and the advice herein is useful for both risk assessors and risk managers, at national and international levels and those in the food industry working to control the hazard in foods.

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